Section 5

Pyrethroid and endosulfan resistance: biology of resistant and susceptible larvae and pupae

Summary

Both pyrethroid and endosulfan resistant *Helicoverpa armigera* larvae were shown to have marginally longer development times. Pyrethroid resistant larvae were slower developers often requiring an extra moult before ultimately pupating to the same size pupa as susceptibles. There were no differences between pyrethroid resistant and susceptible pupal development times (male or female). Laboratory and field competition studies could demonstrate no selective advantage for either pyrethroid resistant or susceptible larvae or prepupae. Thus it would seem that the slightly longer larval development times do not manifest as significant biological deficits. This helps explain the gradually deteriorating pyrethroid resistance situation documented during evaluation of the Australian insecticide resistance management (IRM) strategy. There was no evidence of the selection of fitness modifiers (co-adaptation) to overcome the slower development of either pyrethroid or endosulfan resistant larvae. Endosulfan fitness disadvantages were not sufficiently researched in this study to discount the possibility of a fitness deficit contributing to the much more successful management of endosulfan resistance.

Introduction

It has been widely recognized that the key assumption underlying the success of an IRM rotation strategy is that fitness disadvantages, as pleiotropic effects of the resistant allele, will cause the decline of resistance in the absence of selection pressure (Georghiou, 1980, 1983; Georghiou *et al.*, 1983; Leeper *et al.*, 1986; Roush & McKenzie, 1987; Roush, 1989; Tabashnik, 1989). If there were no biological disadvantage to the resistant insect, then all that could be expected to be achieved by a rotation strategy would be to delay the problem and 'buy time' (Plapp *et al.*, 1989, Denholm *et al.*, 1990). The concept of primitive resistance mechanisms incurring substantial pleiotropic costs has been well entrenched in the literature (Georghiou & Taylor, 1976, 1986; Brown, 1977; Brown, 1982; Uyenoyama, 1986). However, in their landmark 1987 review, Roush & McKenzie challenged this traditional view stating that 'one generality emerging from fitness studies is that large disadvantages in resistant arthropods, unlike those in some other biological taxa, seem to be the exception, rather than the rule'.

As mentioned previously, the presence of a biological deficit is considered critical for the long-term success of an IRM rotation strategy. In the light of Roush & McKenzie's (1987) review, there was clearly a need to determine the pleiotropic costs of the pyrethroid and endosulfan resistance mechanisms for this particular case, rather than extrapolate from the general literature. This study aims to examine various larval and pupal development parameters in resistant and susceptible *Helicoverpa armigera* and to determine the impact of these on selection in field and laboratory competition studies. The data generated from this study were anticipated to be helpful in interpreting the resistance trends documented during evaluation of the Australian IRM strategy (Section 2).

Methods and materials

Early larval development

As well as proving extremely successful in documenting the impact of the strategy on pyrethroid and endosulfan resistance, the monitoring technique outlined in Section 2 (discriminating dose screening of larvae reared from field collected eggs) also allowed a direct comparison of the early larval development of resistant and susceptible *H. armigera*. As each sample site was processed individually and testing size larvae (30-40 mg) assessed daily, it was possible to calculate the time taken for individual field collected eggs to develop to 30-40 mg larvae. The resistance status of each individual larva was then assessed by its response to the fenvalerate (0.2 μg/larva) or endosulfan (10 μg/larva) discriminating dose. Larvae were classed simply as susceptible or resistant (killed by or surviving the discriminating dose, respectively). No attempt was made to partition the resistant larvae into homozygotes or heterozygotes due to the reasons discussed in Section 2 (i.e. unknown interaction of multiple resistance genes, etc.) so the resistant larvae are necessarily a variable mixture of genotypes while the susceptibles would also contain a small proportion of heterozygotes because of the variable overlap of the susceptible and heterozygote lines for the principal metabolic resistance mechanism (also discussed in Section 2). Hatched larvae from the Namoi/Gwydir and Inverell study areas were reared immediately on artificial diet at 25°C ± 2°C. However, samples from the remote Emerald study area were held initially at 12°C and then despatched at weekly intervals in insulated transportable coolers, to the central testing laboratory. These samples were exposed to ambient temperatures during the 2-3 day transit period but on receipt at the central Narrabri laboratory, were held at 25°C ± 2°C as for larvae from the other two study areas.

The average development time (field egg to 30-40 mg larva) was calculated for resistant and susceptible larvae for each Stage of the resistance management strategy for the three study areas. Numbers of larvae tested in each Stage are given in tables 17 and 18. Resistant and susceptible development times were compared using unpaired t tests and the differences (resistant minus susceptible) calculated (tables 17 and 18) and displayed graphically in figures 28 and 29.

Larval and pupal development: laboratory studies using field material

Many authors have advised against using artificially bred laboratory colonies in biology studies of resistant and susceptible insects. They instead recommend using field material from 'natural sources under natural conditions' (Boggild & Keiding, 1958; National Research Council, 1986; Roush & Croft, 1986; Uyenoyama, 1986). Thus a conscious effort was made to follow this advice and a technique was adapted from Pimentel *et al.*'s (1951) study on DDT resistant houseflies. Their study involved collecting a mixed (resistant/susceptible) population of housefly larvae, rearing them through to adults (while measuring development parameters) and then screening the adults to determine their resistance status. The study described here utilized a mixed (resistant/susceptible) natural infestation of *H. armigera* eggs collected off silks from a maize crop at Narrabri in December 1986. Eggs were collected from the field, brought back to the laboratory, held until they reached the 'brown ring' stage (i.e. just prior to eclosion), then placed on artificial diet and the ensuing larvae reared individually through to adults which were screened on emergence with the fenvalerate discriminating dose (2.0 µg/moth, topical eye test, from table 43) to determine pyrethroid resistance or susceptibility. Larvae and pupae were checked daily to determine the following development parameters (number and duration of larval instars, larval weights at 10, 12 and 14 days from hatching, pupal weight and duration). There were no significant differences for male and female development parameters, so data for males and females were pooled, except for pupal duration (males had a longer pupal period so these data were kept separate from the female data, table 19). Data on resistant and susceptible development parameters were obtained for 82 and 97 individuals, respectively, and compared using unpaired t tests (table 19).

Larval competition: laboratory study using field material

This study aimed to determine the competitive ability of pyrethroid resistant and susceptible *H. armigera* larvae in an artificial laboratory situation where external mortality factors such as insecticides, parasitoids, predators, pathogens, weather, etc. could be excluded. The study utilized a mixed (resistant/susceptible) natural infestation of *H. armigera* eggs collected off silks from a maize crop at Narrabri in December 1987. On hatching, larvae were reared on artificial diet at 25°C ± 2°C in 28 ml clear plastic pots (see Appendix 1) either individually or in groups of 10 larvae per pot. Larvae had access to approximately 20 ml of free space after allowing for the diet volume. The artificial diet was checked and replenished regularly to ensure that food was not a limiting factor. Cannibalistic competition in the collective rearing treatment resulted in the initial cohort size of 10 larvae being reduced to one sole surviving larva. The larvae from the singly and collectively reared treatments were reared through to adults and screened on emergence with the fenvalerate discriminating dose (2.0 µg/moth, topical eye test, from table 43) to determine pyrethroid resistance or susceptibility. Occasionally, two or even three pupae were found surviving the collectively reared treatment. However, these were discarded as the ensuing moths were too small to screen reliably with the previously described discriminating dose which had been calibrated for normal sized (200-240 mg) moths. This resulted in 51 usable samples for the collectively reared (competition) treatment while 312 larvae were reared successfully in the control (no competition) treatment (table 20). The percentage resistance of moths reared for each treatment (competition or no competition) were compared by a chi-squared test.

Larval competition: field study

This study aimed to determine the impact of natural selection on pyrethroid resistant and susceptible *H. armigera* larvae. Mixed (resistant/susceptible) field populations of *H. armigera* larvae were allowed to compete for the site at the tip of corn cobs. This is probably the most intense competition that *H. armigera* larvae are exposed to in nature as large numbers of eggs are laid on the attractive maize silks but only one larva (occasionally two) survives to occupy the site at the tip of the cob. Five commercial unsprayed maize crops were chosen (two at Emerald, November 1987 and three in the Namoi/Gwydir area, December 1987). A random sample of eggs was taken from the silks at each site, reared through individually on artificial diet to adults and screened on emergence with the previously described fenvalerate discriminating dose, to determine pyrethroid resistance or susceptibility. The same cohort was sampled later in the life cycle as large, fully fed larvae from the tips of cobs and again as pupae excavated from pupal chambers under the crop. These samples were also reared to adults and screened on emergence with the fenvalerate discriminating dose. The natural selection in this study acted on both the inherent differences between pyrethroid and susceptible larvae (as in the previously described laboratory competition study) as well as their interaction with external factors such as

Table 17. Time taken for field collected eggs of *Helicoverpa armigera*, reared on artificial diet at 25 ± 2°C, to develop to 30–40 mg larvae. Larvae then screened with the fenvalerate discriminating dose (0.2 µg/larva) to determine pyrethroid resistance or susceptibility. Numbers in brackets refer to the number of larvae assessed. Difference either signiificant (*, $P < 0.05$), highly significant (**, $P < 0.01$) or non significant (ns, $P < 0.05$), (t test).

Survey area	Year	Stage	Average number of days to reach 30–40 mg				
			Susceptible		Resistant		Difference (Res minus sus)
Namoi/Gwydir	1983/84	1	10.38	(891)	10.83	(72)	+0.45**
		2	9.13	(593)	9.40	(48)	+0.27ns
		3	8.82	(278)	9.64	(42)	+0.82**
	1984/85	1	9.05	(701)	9.20	(55)	+0.15ns
		2	8.48	(1,839)	8.64	(280)	+0.16*
		3	8.34	(2,058)	8.45	(801)	+0.11*
	1985/86	1	9.25	(1,604)	9.56	(131)	+0.31**
		2	9.51	(3,516)	9.66	(539)	+0.15*
		3	8.99	(2,864)	9.18	(2,356)	+0.19**
	1986/87	1	9.74	(1,117)	10.09	(542)	+0.35**
		2	9.50	(1,892)	9.73	(1,088)	+0.23**
		3	9.22	(2,465)	9.53	(1,842)	+0.31**
	1987/88	1	9.15	(721)	9.72	(173)	+0.57**
		2	9.16	(1,188)	9.47	(522)	+0.31**
		3	8.70	(1,246)	8.74	(782)	+0.04ns
	1988/89	1	9.12	(353)	9.17	(89)	+0.05ns
		2	10.37	(236)	11.46	(172)	+1.09**
		3	10.26	(416)	10.52	(638)	+0.26**
	1989/90	1					
		2	10.40	(171)	10.76	(177)	+0.36*
		3	10.57	(256)	11.06	(432)	+0.49**
Emerald	1985/86	1	11.59	(6,728)	12.16	(475)	+0.57**
		2	11.60	(2,224)	12.24	(467)	+0.64**
		3	13.06	(5,014)	13.58	(831)	+0.52**
	1986/87	1	12.42	(2,564)	12.74	(247)	+0.32**
		2	11.17	(1,193)	11.49	(444)	+0.32**
		3	12.17	(2,370)	12.20	(1,039)	+0.03ns
	1987/88	1	12.24	(1,687)	12.57	(340)	+0.33*
		2	10.79	(611)	11.10	(227)	+0.31*
		3	11.93	(1,437)	11.74	(535)	–0.19ns
	1988/89	1	12.59	(1,013)	13.04	(241)	+0.45**
		2	13.25	(197)	14.31	(123)	+1.06**
		3	14.12	(762)	14.20	(591)	+0.08ns
	1989/90	1					
		2	13.45	(42)	13.32	(34)	–0.13ns
		3	12.30	(225)	12.24	(278)	–0.06ns
Inverell	1987/88	1	9.34	(363)	9.93	(40)	+0.59**
		2	9.36	(537)	9.56	(133)	+0.20*
		3	10.08	(389)	10.38	(89)	+0.30ns
	1988/89	1	9.11	(227)	9.45	(60)	+0.34*
		2	10.77	(319)	11.24	(148)	+0.47*
		3	11.04	(410)	11.14	(308)	+0.10ns
	1989/90	1					
		2	11.10	(334)	11.17	(141)	+0.07ns
		3	11.58	(55)	11.62	(37)	+0.04ns

natural enemies, disease, weather, etc. The number of samples reared through from each life stage (eggs, large larvae or pupae) are given in table 20. The percentage resistance of moths reared from each life stage for each site, were compared by chi-squared tests.

Results

Early larval development

Both pyrethroid and endosulfan resistant *H. armigera* were slightly slower (up to one day) to develop to 30-40 mg larvae (tables 17 and 18, figures 28 and 29). Approximately 70% of the 42 pyrethroid Stage comparisons indicated significantly longer development times for resistant larvae, the remainder being non-significant. However, for endosulfan, only about 40% of the 30 Stage comparisons indicated significantly longer development times for resistant larvae, the remainder being either non-significant or, in one case, indicating a slightly shorter development time for resistant larvae. As expected, the initial cooling and subsequent transport at ambient temperatures of the samples from the remote

Table 18. Time taken for field collected eggs of *Helicoverpa armigera*, reared on artificial diet at 25 ± 2°C, to develop to 30–40 mg larvae. Larvae then screened with the endosulfan discriminating dose (10 µg/larva) to determine endosulfan resistance or susceptibility. Numbers in brackets refer to the number of larvae assessed. Difference either significant (*, P <0.05), highly significant (**, P <0.01) or non significant (ns, P <0.05), (t test).

Survey area	Year	Stage	Average number of days to reach 30–40 mg				
			Susceptible		Resistant		Difference (Res minus sus)
Namoi/Gwydir	1986/87	1	9.68	(817)	10.66	(58)	+0.98**
		2	9.75	(714)	9.86	(147)	+0.11ns
		3	9.21	(2,071)	9.46	(539)	+0.25**
	1987/88	1	9.04	(212)	9.29	(17)	+0.25ns
		2	9.22	(410)	9.38	(96)	+0.16ns
		3	8.67	(845)	8.63	(262)	−0.04ns
	1988/89	1	9.44	(195)	9.05	(19)	−0.39ns
		2	10.54	(125)	10.88	(17)	+0.34ns
		3	10.91	(595)	10.90	(70)	−0.01ns
	1989/90	1	10.42	(437)	10.20	(40)	−0.22ns
		2	10.34	(226)	10.84	(44)	+0.50**
		3	10.78	(493)	11.59	(93)	+0.81**
Emerald	1986/87	1	12.66	(1,024)	12.68	(84)	+0.02ns
		2	11.46	(862)	11.81	(221)	+0.35*
		3	12.18	(2,385)	12.15	(499)	−0.03ns
	1987/88	1	12.51	(940)	12.77	(96)	+0.26ns
		2	10.98	(409)	11.66	(67)	+0.68**
		3	12.03	(1,377)	11.68	(207	−0.35*
	1988/89	1	12.76	(932)	13.23	(80)	+0.47ns
		2	13.87	(216)	14.91	(35)	+1.04**
		3	14.39	(782)	14.86	(65)	+0.47ns
	1989/90	1					
		2	13.44	(32)	14.33	(9)	+0.89ns
		3	12.42	(369)	12.98	(100)	+0.56*
Inverell	1987/88	1	9.34	(200)	9.90	(29)	+0.56*
		2	9.37	(494)	9.80	(64)	+0.43**
		3	10.23	(273)	11.20	(19)	+0.97**
	1988/89	1	9.23	(142)	9.40	(15)	+0.17ns
		2	10.90	(287)	11.50	(20)	+0.60ns
		3	11.22	(578)	11.72	(32)	+0.50ns
	1989/90	1					
		2	11.26	(328)	11.50	(18)	+0.24ns
		3					

Emerald site, resulted in slightly longer development times (generally two to three days) for both susceptible and resistant Emerald larvae, in comparison to the other two study areas which were reared immediately at 25°C.

There were no differences between study areas nor was there any clear trend towards selection of fitness modifiers to overcome the slower development of the resistant larvae for either pyrethroids or endosulfan (i.e. no change over time).

Larval and pupal development: laboratory studies using field material

This study also clearly indicated slightly longer development times for resistant larvae (about half a day longer over a total larval period of approximately 20 days). These differences were detected in both early and later instars (table 19). Resistant larvae also had a greater proportion of slower developers with many larvae requiring an extra moult to complete development (table 19). Despite being slower to develop and therefore weighing less than susceptibles at equivalent times during the larval stage (table 19), resistant larvae did ultimately pupate to the same size pupa as susceptibles (table 19), the only difference being that they took half a day longer to do so. There were no differences in male or female pupal development times (table 19).

Larval competition studies

The laboratory study clearly showed that pyrethroid resistant larvae competed equally well with susceptible larvae under an intense artificially high competition density of 10 larvae per 20 ml (table 20).

The field study also showed that natural selection acting on *H. armigera* infesting maize, did not selectively favour either resistant or susceptible larvae or prepupae. In four out of the five crops, resistance levels did not significantly change throughout the entire cob inhabiting larval or soil burrowing prepupal stages (table 20). A loss of resistance was detected during the larval stage (53.3% down to 43.9%) in one of the five crops but the subsequent pupal sample cast some doubt on the significance of this result (table 20). Similar results were obtained for sites with either low (30-40%) or high (50-65%) pyrethroid resistance levels.

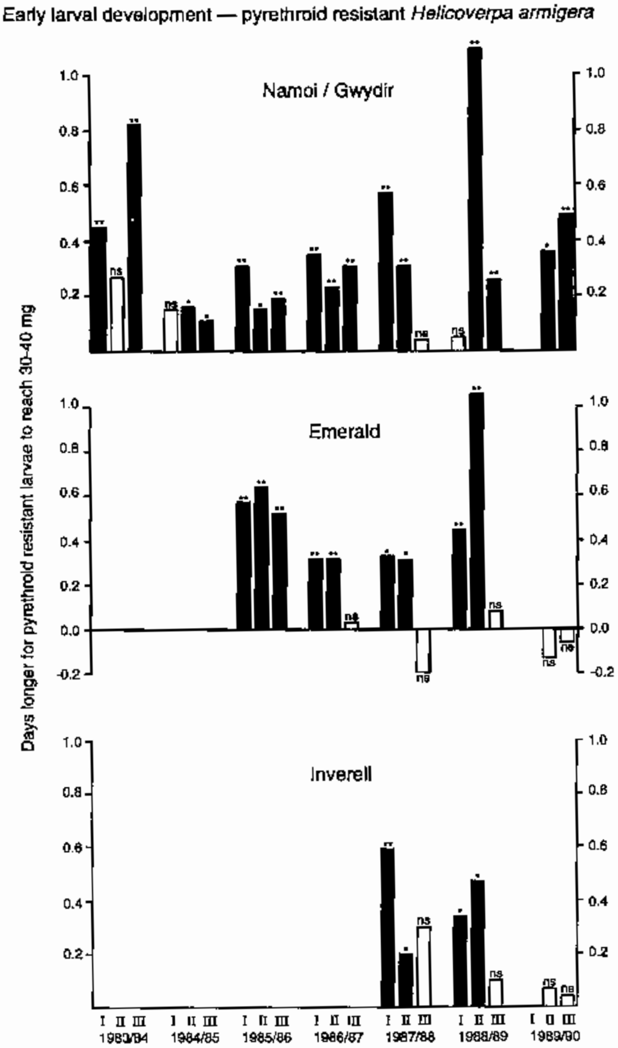


Fig. 28. Average number of days longer for pyrethroid resistant *Helicoverpa armigera* larvae to reach 30–40 mg (reared from field collected eggs on artificial diet at 25 ± 2°C). Solid columns indicate significantly longer development times for resistant larvae at $P < 0.05$ (*) or $P < 0.01$ (**). Open columns indicate development times not significantly different (ns, $P > 0.05$), (t test). Data from table 17.

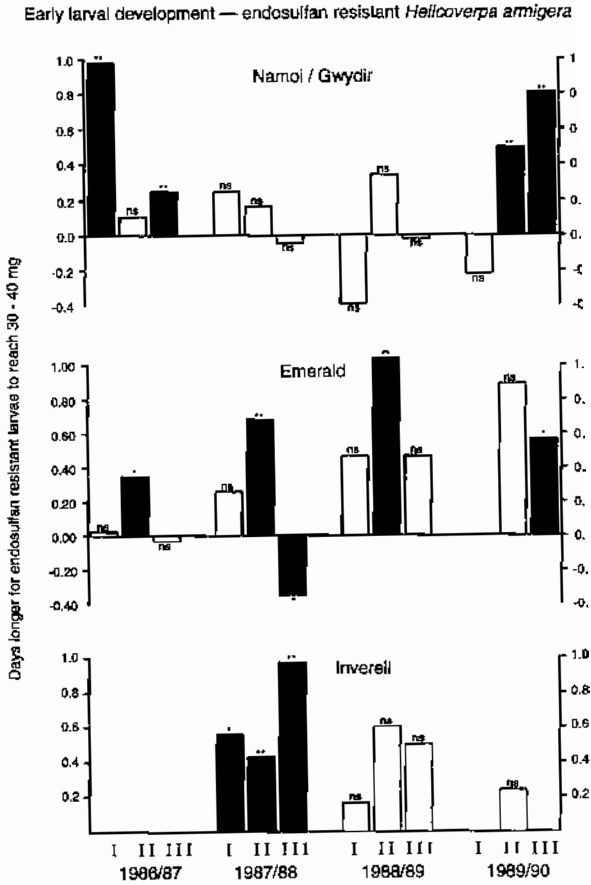


Fig. 29. Average number of days longer for endosulfan resista
Helicoverpa armigera larvae to reach 30–40 mg (reared from fie
collected eggs on artificial diet at 25 ± 2°C). Solid colum
indicate significantly longer development times for resista
larvae at $P < 0.05$ (*) or $P < 0.01$ (**). Open columns indica
development times not significantly different (ns, $P > 0.05$
(t test). Data from table 18.

Discussion

Both pyrethroid and endosulfan resistant larvae were shown to have marginally slower development times. This has also been shown for a wide range of variously resistant insects e.g. cyclodiene resistant anopheline mosquitoes (Emeka-Ejiofor *et al.*, 1983), DDT resistant houseflies (Pimentel *et al.*, 1951; McKenzie & Hoskins, 1954; Kerr, 1970), cyromazine resistant houseflies (Bloomcamp *et al.*, 1987), malathion resistant *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) (Singh & Morton, 1981) and pyrethroid resistant Colorado potato beetles (*Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)) (Argentine *et al.*, 1989b). However, occasionally, resistant insects have also been shown to be slightly faster e.g. cyclodiene resistant onion maggot (*Delia antiqua* (Meigen) (Dipter
Anthomyiidae)) (Missonier & Brunel, 1972) or to be n
different to susceptibles e.g. pyrethroid resistan
Liriomyza trifolii (Burgess) (Diptera: Agromyzidae) an
Heliothis virescens (Keil & Parrella, 1990 and Payne *et al*
1988, respectively). Development parameters wer
unchanged in larvae of *Helicoverpa zea* (Boddie) express
ing increased levels of monooxygenases (Neal, 1987
From an examination of the factors affecting the intrinsi
growth rate, Roush & Croft (1986) suggested that eve
small delays in development could affect populatio
growth rates more than similar differences in fecundit
However, it would seem that in this study, slight
longer development times do not manifest as significan
biological deficits. A similar situation has been found i
pyrethroid resistant *Leptinotarsa decemlineata* which ha
also been shown to have a marginally longer larva
development time but which does not result in any significant change in the intrinsic rate of increas
(Argentine *et al.*, 1989b). Thus, there would appear to b
no significant biological disadvantage to pyrethroi

Table 19. Various larval and pupal development parameters of susceptible and pyrethroid resistant *Helicoverpa armigera* reared on artificial diet (at 25°C) from field collected eggs in the Namoi/Gwydir, December 1986. n = number of larvae or pupae reared. *, **, ns = difference (top 2 tables) or column means (bottom 2 tables) statistically significant at (P <0.05), (P <0.01) or not significant (P <0.05), respectively (t test).

	Development time (days) from brown egg to				
	3rd instar	4th instar	5th instar	6th instar	pupa
susceptible n = 97	6.14	8.86	11.90	14.19	19.76
resistant n = 82	6.30	9.30	12.09	14.66	20.33
difference (res-sus)	$+0.16^{ns}$	$+0.44^{*}$	$+0.19^{ns}$	$+0.47^{ns}$	$+0.57^{*}$

	Larval weight (mg) at			Pupal weight (mg)
	10 days from brown egg	12 days from brown egg	14 days from brown egg	
susceptible n = 97	91.6	223.3	442.9	435.9
resistant n = 82	77.6	186.2	368.0	447.5
difference (sus-res)	$+14.0^{ns}$	$+37.1^{ns}$	$+74.9^{**}$	-11.6^{ns}

	% of larvae having a 6th instar	% of 'slow' larvae (i.e. weighing <40mg at 10 days from brown egg)	% of 'fast' larvae (i.e. weighing >150mg at 10 days from brown egg)
susceptible n = 97	33.0^{**}	26.8^{*}	22.7^{ns}
resistant n = 82	53.7^{**}	42.7^{*}	17.1^{ns}

	Pupal duration (days)			
	female		male	
susceptible	12.2^{ns}	n = 50	13.7^{ns}	n = 47
resistant	12.2^{ns}	n = 42	13.8^{ns}	n = 40

resistant *H. armigera* in the larval stage. If this is the case, then the unsprayed refugia would be expected to become increasingly contaminated and consequently less effective as sources for dilution. Under these circumstances, a rotation strategy would only be effective in slowing up the development of resistance, (i.e. in 'buying time'). This is precisely the situation described for the Australian rotation strategy in Section 2. Thus the complementary biology studies in this Section corroborate the resistance trends documented during evaluation of the Australian IRM strategy in Section 2.

However, one must qualify the above finding by recognizing that the resistant larvae used in this study were necessarily mixed heterozygotes and homozygotes, but presumably, principally the former. Thus, one cannot discount the possibility of significant disadvantages occurring in homozygotes. Also, the work in this study concentrated solely on larvae, prepupae and pupae. No work was done on the fecundity, fertility or mating competitiveness of resistant and susceptible moths. This could also be a potential source of biological disadvantage, and has been suggested for pyrethroid resistant *Heliothis virescens* (Campanhola & Plapp, 1989c; McCutchen *et al.*, 1989; Plapp *et al.*, 1990b). In fact, Plapp *et al.* (1990b) claim that the early season decline in pyrethroid resistance noted in their monitoring studies is due to reproductive disadvantages allowing rapid replacement of resistant insects (in the absence of insecticides) by their more reproductively successful susceptible compatriots. McCutchen *et al.* (1989) go on to suggest that these reproductive deficits will allow resistance to be managed and that without them, resistance could only get worse. This optimism for management of pyrethroid resistance in *H. virescens* in the USA, contrasts vividly with the reality of long term pyrethroid resistance management in Australia. As stated previously, the presence of a biological deficit is critical for the long-term success of an IRM rotation strategy. The optimism for the USA IRM strategy hinges on the claim for a reproductive deficit in pyrethroid resistant *H. virescens* moths. However, the data presented to support these claims (McCutchen *et al.*, 1989; Plapp *et al.*, 1990b) has a number of flaws or possible alternative explanations. For example, the authors suggest resistant females are less fertile, less fecund, attract less males and produce less pheromone. However, they compared unrelated strains of dissimilar genetic background which could also explain the differences noted (Roush & Croft, 1986).

Table 20. Percentage survival of *Helicoverpa armigera* moths (male/female combined) screened with a discriminating dose of fenvalerate (2.0 µg/newly emerged moth, topical eye test). Figures in brackets are the number of moths screened. Percentages in the same row, followed by the same letter, are not significantly different (chi-squared test, $P < 0.05$).

Field study — Moths reared from various life stages of a population cohort sampled from five maize crops, two at Emerald and three in the Namoi/Gwydir, either from eggs laid on silks (larvae fed on artificial diet), from large instar larvae taken from the tips of cobs or from pupae excavated from pupal chambers under the crop.

Lab study — Moths reared from either neonate larvae reared individually on artificial diet in 28 ml containers or from the sole surviving larva of ten neonates confined to a single container and fed on artificial diet *ad libitum*. Neonates obtained from eggs collected off maize silks in the field.

FIELD STUDY

	% Resistance of moths reared from field collected:-					
Trial site	eggs on silks		large larvae in cobs		pupae in soil	
Emerald, Qld. Nov 1987	36.5^{a}	(96)	28.7^{a}	(94)		
Emerald, Qld. Nov 1987	33.0^{a}	(115)	42.2^{a}	(90)		
Narrabri, NSW. Dec 1987	37.7^{a}	(114)	46.7^{a}	(276)	50.8^{a}	(126)
Narrabri, NSW. Dec 1987	53.3^{a}	(214)	43.9^{b}	(303)	38.7^{ab}	(62)
Myall Vale, NSW. Dec 1987	65.8^{a}	(161)	64.9^{a}	(245)	61.9^{a}	(155)

LAB STUDY

% Resistance of moths reared in lab from field collected neonate larvae:-	
reared singly	reared collectively (initial cohort size 10)
34.9^{a} (312)	35.3^{a} (51)

They also claimed that resistant females from the field had mated less than susceptibles and that resistant males from the field responded less to pheromone traps. The interpretation of their data did not take into account the fact that phenotypic resistance (as determined by survival at a discriminating dose) is not expressed in older moths as strongly as it is in younger moths (see Section 7). Thus their data on resistant females mating less could be equally well explained by genotypically resistant older (more mated) females being phenotypically scored as susceptibles. Their data on resistant males responding less to pheromone traps could also be equally well explained by the traps preferentially attracting older males (resistant or susceptible) which would be scored phenotypically as susceptibles. Thus the early season decline in pyrethroid resistance noted by Plapp *et al.* (1990b) in their monitoring studies may not be due to reproductive disadvantage at all but could be equally well explained by simple dilution from the refugia (as seems to be the case for Australia). The unbridled optimism for the long-term success of the USA IRM strategy should be restrained until more convincing evidence of a significant biological disadvantage to resistant moths or larvae is forthcoming.

There was no evidence in this study for the selection of fitness modifiers (co-adaptation) to overcome the slower development of either pyrethroid or endosulfan resistant larvae. This is an interesting theoretical challenge as the design of the Australian IRM strategy aims for continued access to pyrethroids over a long period, despite moderate levels of resistance. These are the conditions that should, at least theoretically, favour selection of fitness modifiers (Keiding, 1967; Georghiou & Taylor, 1976, 1986; McKenzie, 1986; Uyenoyama, 19 Thus, currently designed IRM strategies will most pr ably inadvertently select for fitness modifiers, occurred with diazinon resistance in *Lucilia cup* (Wiedemann) (Diptera: Calliphoridae) (McKenzie *et* 1982; McKenzie & Purvis, 1984). This is not of any sig icance in this particular study, as there appears to be significant biological disadvantage associated with slightly longer larval development periods. Howeve could be important in cases where significant biolog disadvantages do occur and where it is desirable to serve these for long-term successful IRM. This pote problem seems to elicit conflicting opinions in the literature. Recent reviews tend to discredit the genera of co-adaptation of fitness and resistance unless re tance is at a high frequency (Roush & Croft, 1986; Ro & McKenzie, 1987). However, other authors (e.g. Tay 1986) argue that the co-adaptation theory 'should be ter understood before abandoning the concept'. The in this study suggest that no co-adaptation has occu over a seven year period for pyrethroids (four years endosulfan). However, the general utility of this fin is unknown as each resistance episode and IRM cou strategy will no doubt present a unique situation.

Most of the work described in this Section refer pyrethroid resistance. Although it was shown that e sulfan resistant larvae were slightly slower to deve no work was done in this study to determine whe this was actually manifested as a significant biolog disadvantage. Thus the presence of a fitness deficit not be ruled out as a possible factor contributing to much more successful management of endosulfan re tance (see Section 2).

Helicoverpa armigera eggs, neonate and first instar larva on flowering sorghum.
Queensland Department of Primary Industries.

Section 6

Pyrethroid resistance: field selection in sorghum

Summary

Pyrethroids applied to flowering sorghum, at either the low midge rate or the higher *Helicoverpa* rate (20 g and 100 g fenvalerate a.i./ha, respectively), caused selective mortality of *Helicoverpa armigera* larvae and resulted in differential selection for resistance. This finding vindicated the original strategy decision to apply the same temporal restrictions on pyrethroid use for sorghum midge, *Contarinia sorghicola*, as those for *H. armigera*.

Introduction

Helicoverpa armigera has a wide range of crop hosts in Australia (Zalucki *et al.*, 1986), including maize, sorghum, wheat, triticale, sunflower, safflower, rapeseed, chickpea, lupins, pigeonpea, cowpea, mung bean, soyabean, tomato, tobacco and cotton. The graminaceous crops are particularly important *H. armigera* hosts as they are sown over large areas, are specific for *H. armigera* (*H. punctigera* is rarely found on monocots) and are invariably infested with large numbers of larvae (particularly the summer cereals, maize and sorghum). These last two crops (along with sunflowers) are the main alternative summer host crops to cotton and of these, sorghum is the most important because of the relatively large rain-grown area sown each summer.

Pyrethroids have been registered on sorghum for midge (*Contarinia sorghicola*) and *H. armigera* control for ten years or so. The registered rates used for sorghum midge control are significantly lower than those used for *H. armigera* (e.g. 20 g and 100 g fenvalerate a.i./ha, respectively). Midge is sprayed at flowering while *H. armigera* is usually controlled later on as medium to large larvae at the soft dough stage. However, *H. armigera* lays its eggs on flowering sorghum heads and early instar larvae can be present in the sorghum head at the same time that pyrethroids are being applied for midge control. Therefore, there exists the potential for pyrethroids, applied for midge control, to select inadvertently for pyrethroid resistance in contemporaneous *H. armigera* larvae. This potential was recognized during the initial stages of the design of the Australian IRM strategy and as mentioned previously in Section 1, resulted in the recommendation to apply the same restrictions on pyrethroid use for sorghum midge as those for *H. armigera*. This necessarily extempore recommendation was accepted by sorghum growers, despite possible increased costs if more expensive alternative chemicals needed to be used outside of the pyrethroid window. (N.B. This problem was minimized by careful timing of the pyrethroid window to suit both cotton and sorghum grower needs). However the recommendation was still only sustained by reasoned supposition and it was considered necessary to research the issue in order to validate or retract the strategy recommendation. The aim of this study was therefore to investigate the potential for low rate sorghum midge pyrethroid sprays, applied at flowering, to select for pyrethroid resistance in co-incident *H. armigera* populations. A positive link would vindicate the original strategy recommendation while the lack of any such correlation would necessitate its rescission.

Methods and materials

Effect on larval numbers

A 20 ha commercial block of rain-grown flowering sorghum near Narrabri, NSW, was divided into three equal areas and aerially sprayed (February 1987) with two rates of fenvalerate (20 and 100 g a.i./ha). The third area (upwind portion) was left as an unsprayed control. As mentioned previously, these represent the most commonly used registered rates for midge and *H. armigera* control, respectively, in sorghum. Sorghum heads were sampled pre-spray and each day from days 3-6 and days 10-13 after spraying. Fifty heads were sampled randomly from throughout each treatment block on each day. Eggs and larvae were extracted from the heads for counting by spinning the heads into a bucket (stalk below head rolled vigorously between the palms of the hands). The initial population sampled comprised mostly eggs. The same population cohort was sampled later as first to second instars at 3-6 days post-spray and again as third to sixth instars at 10-13 days post-spray. The four samples (3, 4, 5 and 6 days post-spray) were considered as four replicates for the first to second instar samples. Similarly, the four samples (10,11,12 and 13 days post-spray) were considered as four replicates for the third to sixth instar samples. The data for each instar group were analysed as a randomized complete block design with three treatments (unsprayed control, 20 g & 100 g a.i. fenvalerate/ha) and four replicates in time. Data were left untransformed and treatment means were compared by Duncan's new multiple range test.

Effect on resistance levels

Two commercial blocks of rain-grown flowering sorghum were used for this study: the Narrabri block described previously and a 15 ha block at Gatton in south-east Queensland. The Narrabri block received three treatments as described previously while the Gatton block received two (aerial application of fenvalerate at 20 g a.i./ha on one half and an unsprayed control on the other upwind portion). Larvae (as well as eggs in the case of the pre-spray sample) were sampled either pre-spray or at 7-14 days post-spray, reared through to moths on artificial diet and screened on emergence with the fenvalerate discriminating dose (2.0 µg/moth, topical eye test, from table 43) to determine pyrethroid resistance or susceptibility. There was some difficulty in collecting sufficient numbers of larvae from the sprayed treatments but also from the Gatton post-spray control treatment because of the significant impact of disease and natural enemies at this site. The number of samples successfully reared through for each treatment at each site are given in figure 30B. The percentage resistance of moths reared for the different treatments at each site were compared by chi-squared tests.

Results

Effect on larval numbers

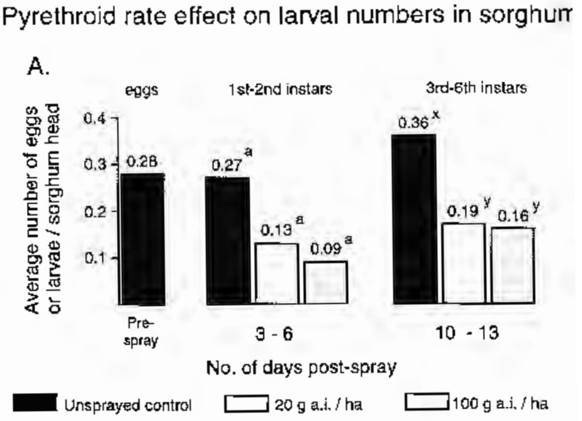
Both the low (20 g a.i./ha) and high (100 g a.i./ha) fenvalerate rates reduced larval numbers compared to the unsprayed control (fig. 30A). However, only the 10-13 day sample indicated statistically significant differences with the 3-6 day sample just falling short of significance (5% level). There was no difference between the rates, with both reducing the larval population by about one-half at 10-13 days post-spray.

Effect on resistance levels

Resistance levels increased significantly post-spray, compared to the unsprayed control, at both sites (fig. 30B), despite the relatively low numbers tested. There was no difference between the rates (Narrabri site) with both increasing resistance by about 1.7x. The low midge rate increased resistance 6x at the Gatton site.

Discussion

It was shown that pyrethroids, applied to flowering sorghum, removed a proportion of the *H. armigera* population (presumably the susceptibles) which resulted in a differential selection for resistance. The same result appears to prevail whether the low midge rate or high *Helicoverpa* rate is used. This is not altogether surprising as only early instars of *H. armigera* are present on sorghum, at the time when midge are being sprayed (i.e. flowering). Teakle & Byrne (1988) found that these early instars feed on the exposed anthers in flowering sorghum which present a large catching surface area for



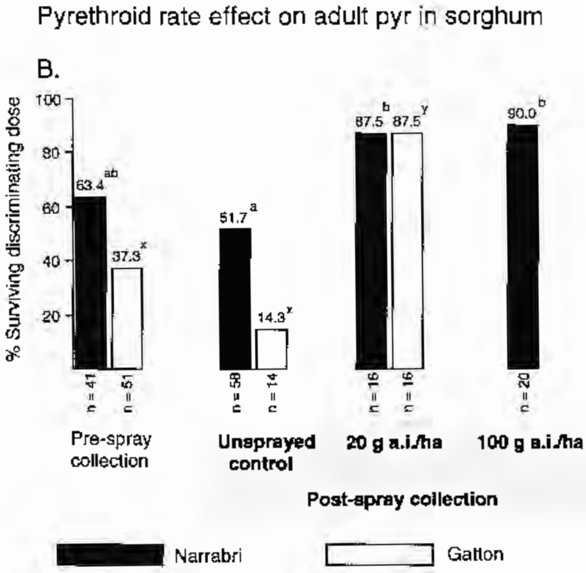


Fig. 30. The effect of fenvalerate application rate (20 g a.i./h normal low rate for sorghum midge (*Contarinia sorghicola*) con trol and 100 g a.i./ha normal high rate for *Helicoverpa armiger* control in sorghum) on: (A) selection for resistance in a coho of *H. armigera* sprayed as eggs on flowering sorghum i February 1987. Population sampled 3–6 and 10–13 days later a first–second and third–sixth instars, respectively. Means fo each sampling period, followed by the same letter, are not sig nificantly different (P <0.05) (Duncan's new multiple rang test); (B) the resistance levels of *H. armigera* moths bred fro larvae taken from sorghum heads before or after (7–14 days spraying at two sites (Narrabri, north-western NSW an Gatton, south-east Queensland) in February 1987. Resistanc expressed as the percentage of moths (male and female com bined) surviving a discriminating dose (adjusted for any con trol mortality) of fenvalerate (2.0 µg/moth, topical eye test). n total number of moths screened. Percentages (for each site followed by the same letter, are not significantly differen (P <0.05, chi-squared test).

Helicoverpa armigera larva in maize cob. *New South Wales Agriculture.*

Helicoverpa armigera larva hatching from egg. *New South Wales Agriculture.*

spray droplets. This renders these early instars particularly vulnerable to even low rates of highly active insecticides such as the pyrethroids. Consequently, the low midge rates for pyrethroids could quite conceivably kill early instar susceptible *H. armigera* on sorghum and even possibly some heterozygotes, if contacted early enough (Daly *et al.*, 1988a).

Clearly, pyrethroids applied at low rates for midge control in sorghum, can select inadvertently for pyrethroid resistance in co-incident *H. armigera* larvae. Thus, the original decision to apply the same restrictions on pyrethroid use for sorghum midge as those for *H. armigera*, was vindicated.

This research also helps explain the reports of poor performance of pyrethroids against *H. armigera* in sorghum where they had been used previously for midge control. Obviously, the midge spray at flowering selects for pyrethroid resistant *H. armigera* which, as larger larvae later on at the soft dough stage, would be virtually uncontrollable with any registered pyrethroid rate. This finding led to the adoption of the supplementary guideline for sorghum growers mentioned in Section 1, 'If a pyrethroid is used to control sorghum midge, do not follow up with a pyrethroid for *Heliothis* control, as the midge spray will have already selected for pyrethroid resistant *H. armigera*.'

Section 7

Pyrethroid resistance: selection of adults

Summary

Adult *Helicoverpa armigera* were shown to be capable of expressing pyrethroid resistance to both direct and indirect exposure. Male and female moths were equally resistant. Pyrethroid field sprays selected for resistance in moths which were repelled onto neighbouring unsprayed cotton and possibly also, lower into the unsprayed portion of the canopy. Selection of moths prior to egg laying is suggested as the explanation for the immediate increase in pyrethroid resistance which occurs within the Stage II window.

The phenotypic expression of pyrethroid resistance in adult *H. armigera* declined significantly with age, beginning sometime between four and six days and continuing for the effective life span of the moth. This was the main reason for the poor correlation of adult resistance (determined from pheromone trapped males) and resistance in field collected eggs found in a detailed study comparing both techniques on two properties. On a young emerging moth population, pyrethroids remained selective for four to five days. On an ageing population, pheromone trapped males significantly underestimated resistance. Endosulfan and profenofos sprays were neutral in their impact on pyrethroid resistance in both eggs and adults. The resistance peaks in eggs lagged four to seven days behind the pyrethroid applications. The advantages and disadvantages of monitoring resistance using pheromone trapped males are discussed.

Introduction

Helicoverpa spp. control in cotton is directed principally against the egg and larval stages. However, the adult stage occupies a spatially associated ecological niche and insecticides applied against eggs and larvae, can also affect moths. This was noted by Ruscoe (1977) and Morton (1979) but Topper (1987a) was probably the first to recognize the importance of adult mortality as a consequence of sprays directed against eggs and larvae in cotton. Most studies on resistance in *Helicoverpa* spp. have focused on larvae and unconsciously overlook the possible contribution from selection of moths. No doubt this is a consequence of the generally held belief that nectar feeding adult Lepidoptera would not express metabolic resistance (Wilkinson & Brattsten, 1972; Dittrich *et al.*, 1980; Joyce, 1982; Wilkinson, 1983; Hodgson, 1985; Brattsten, 1987a; Plapp *et al.*, 1988; Campanhola & Plapp 1989a, 1989b; McCutchen *et al.*, 1989; Roush, 1989). In fact many of these authors, as well as Day *et al.* (1983), suggest that sprays should be targeted on these life stages which have the weakest metabolic defences and which should not express metabolic resistance.

The possibility of pyrethroids selecting for resistance in adult *H. armigera* was not considered during the initial design of the Australian IRM stragegy for the reasons discussed previously. However, the monitoring studies very quickly detected an immediate increase in pyrethroid resistance which occurred within the Stage II window and which appeared too quickly to be explained by larval selection (see Section 2). A number of possibilities for this were explored in Section 2 but the most plausible was selection of moths prior to egg laying. Therefore, this study aims to document whether *H. armigera* moths can express pyrethroid resistance and if so, to investigate the possibility of using pheromone trapped males to monitor the impact of the IRM strategy on pyrethroid resistance levels.

Methods and materials

Resistance in moths: laboratory study

A pyrethroid resistant colony was formed from the fenvalerate discrimating dose survivors from the Emerald study area in the 1985/86 season. The F2 generation was bioassayed with fenvalerate on larvae and adults (topical eye and tarsal plate tests), as described in Appendix 2. Larval and adult resistance factors (LD/LC$_{50}$ resistant colony ÷ LD/LC$_{50}$ susceptible strain) were calculated using susceptible data from tables 37 and 43, respectively.

Resistance in moths: field monitoring data

If pyrethroids do indeed select for pyrethroid resistance in egg laying moths, then it should be possible to detect higher resistance levels on properties where pyrethroids had been recently applied. This theory was tested by closely examining the Namoi/Gwydir 1983/84 and 1984/85 Stage II pyrethroid resistance monitoring data (from Section 2) and correlating this with each property's spray history. Properties were classed as either sprayed or unsprayed (i.e. pyrethroids either applied or not applied on the property in the two to eight nights prior to the collection of the egg sample). The minimum spray interval of two nights was chosen as a one night interval (i.e. sprayed the night before egg collection) could have included a significant component of eggs laid before the spray event. The maximum spray interval of eight nights was chosen as this allows for the bulk of the female moth's reproductive life of 8-10 days (Topper, 1987b; Fitt, 1989). It also allows for the diminishing selection potential of the pyrethroid spray deposit due to partial degradation, growth dilution through leaf expansion and production of new unprotected foliage at the top of the canopy. During this analysis, it was noticed that some properties, which had not yet, or had not recently used a pyrethroid, had unusually high resistance levels. Invariably, these properties were observed to adjoin neighbouring properties which had indeed recently applied a pyrethroid. The possibility of selection followed by repellency of moths onto close neighbouring unsprayed fields was then considered, particularly in light of the well known irritant and repellent properties of pyrethroids (Ruscoe, 1977; Elliott *et al.*, 1978). Consequently, a second analysis of the resistance data was run, this time considering sprayed properties to be those where pyrethroids had been applied on the actual property itself or on close neighbours (defined arbitrarily as within 2 km). Resistance levels for sprayed and unsprayed properties were compared by t tests for each analysis for each season. The number of larvae screened for each treatment (i.e. sprayed or unsprayed) are given in figure 31.

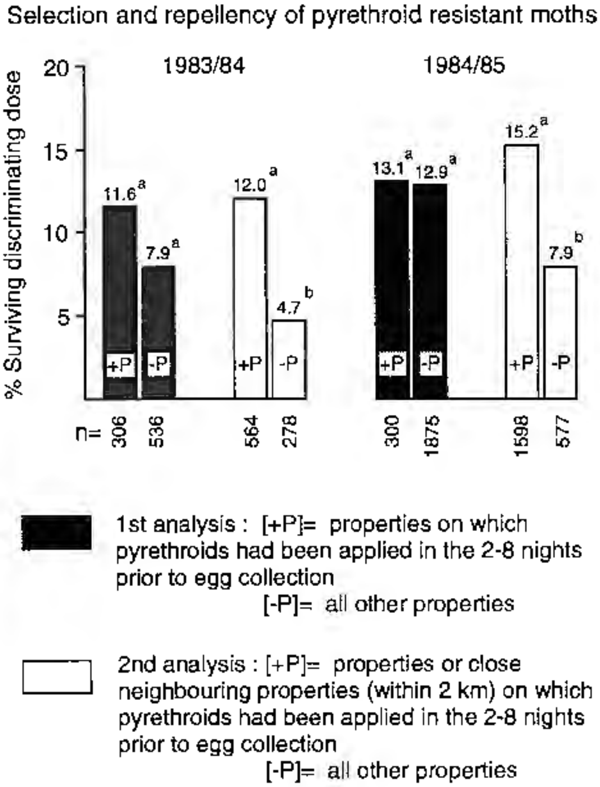


Fig. 31. Comparison of *Helicoverpa armigera* pyrethroid resistance levels (% of larvae, reared from field collected eggs, surviving the fenvalerate discriminating dose, 0.2 µg/30–40 mg larva) for properties in the Namoi/Gwydir study area for the 1983/84 and 1984/85 Stage II pyrethroid windows, where pyrethroids had [+P] or had not [–P] been applied in the 2–8 nights prior to egg collection. First analysis on whether sprays were applied on the collection property only. Second analysis on whether sprays were applied on the collection property or on close neighbouring farms (within 2 km). Means, for each paired comparison, followed by the same letter, are not significantly different (P <0.01) (t test). n = total number of larvae tested at each treatment.

Effect of moth age on expression of resistance

As part of the investigation of the possibility to use pheromone trapped males to monitor the impact of the IRM strategy, it was deemed important to research the possible influence of variable moth age (and hence physiological status) on phenotypic expression of pyrethroid resistance. Rather than measure this effect on a laboratory reared resistant colony, it was decided to use a wild mixed pyrethroid resistant/susceptible field strain. Consequently, advantage was taken of a large natural infestation of unsprayed *Helicoverpa armigera* larvae on adzuki beans (*Vigna angularis*) near Narrabri late in the 1986/87 season. The population was left to pupate and large numbers of pupae were excavated from their pupal chambers, brought back to the laboratory, and on emergence moths were split randomly into four treatments. For the first treatment, moths were screened immediately on the day of emergence with the fenvalerate discriminating dose (2.0 µg/moth, topical eye test, from table 43) and held (unfed) for 24 hours until assessment of mortality. For the other three treatments male and female moths were placed in 27 litre ventilated perspex cages (see Appendix 1) at up to 20 pairs per cage and reared on 10% honey solution at 25 ± 2°C and 60-80% r.h. for either 3-4, 6-7 or 10-11 days after emergence. They were then screened with the same discriminating dose as used for the newly emerged moths. Mating and egg laying were allowed to occur normally and cages were replaced every two to three days. There was no difference in the results for males or females, so the data for both sexes were combined. The number of moths

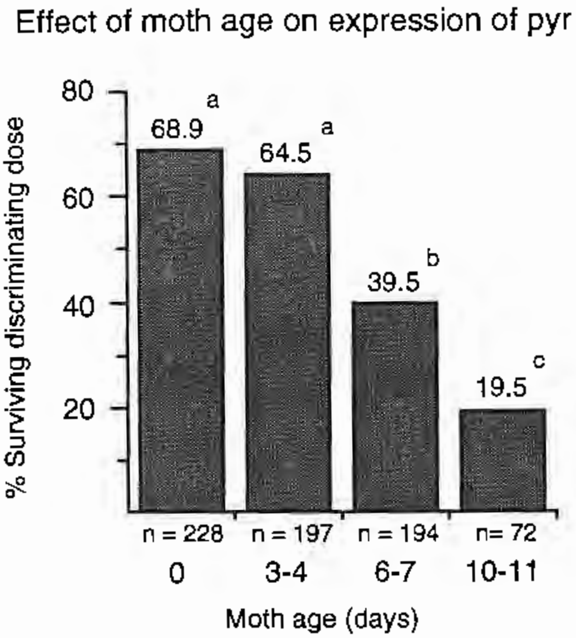


Fig. 32. Impact of ageing on the phenotypic expression of pyrethroid resistance in adults of *Helicoverpa armigera*, collected as pupae from a natural field infestation on adzuki beans, *Vigna angularis*, Narrabri Feb 1987. Resistance expressed as the number of reared moths surviving a discriminating dose (adjusted for any control mortality) of fenvalerate (2.0 μg/moth, topical eye test, from table 43) at 0, 3–4, 6–7 or 10–11 days after emergence. Moths (males and females combined) split equally among the four treatments on emergence and maintained on 10% honey in 27 litre laboratory rearing cages at 25 ± 2°C and 60–80% r.h. n = total number of moths screened. Percentages followed by the same letter are not significantly different (P <0.01, chi-squared test).

tested for each age group are given in figure 32 (note, the lower number of 10-11 days old moths tested was due to the increased mortality of moths reaching the end of their normal life expectancy range). Resistance estimates were corrected for any control mortality (acetone only) and compared using chi-squared tests.

Field resistance monitoring: moths versus eggs

The resistance monitoring programme described in Section 2 focused on the screening of larvae reared from field collected eggs. Other resistance monitoring programmes utilize pheromone trapped male moths for the same purpose. It was decided to critically evaluate these two resistance monitoring techniques by comparing them on two large blocks of commercial cotton near Narrabri during the 1986/87 season. The two blocks (390 and 340 ha) were actually contiguous cotton fields on two separately managed cotton properties (Auscott and Yarral, respectively). These two blocks were chosen as they were adjacent (Yarral) or close (Auscott) to two large blocks of unsprayed maize (total 106 ha) which were predicted to produce large numbers of *H. armigera* moths during the first half of the Stage II pyrethroid window (predicted from regular sampling of the larval population cohort). They were also chosen with the aim to explore the possibility of selection and repellency of pyrethroid resistant moths onto neighbouring unsprayed cotton. Male moths were trapped live in each cotton block using pairs of 'Texas' pheromone traps (Gregg & Wilson, 1991) placed 20, 50 and 100 m into each crop. Moths were cleared by 09.00h each day and then screened with the fenvalerate discriminating dose (2.0 μg/moth, topical eye test, from table 43) or acetone alone (to determine control mortality). Trapping commenced in the first week of the 1986/87 Stage II pyrethroid window and continued until the end of the first week of the following Stage III (a total of 49 days). At the same time, eggs were collected off each block every 2-3 days, reared through to 30-40 mg larvae and screened with the fenvalerate discriminating dose as detailed in Section 2. The numbers of larvae and moths screened are not given for each individual collection but are accounted for in the pooled binomial standard error estimates for each data point (fig. 33). As the two maize blocks were anticipated to be a major source of immigrants for the neighbouring Auscott and Yarral cotton blocks, the resistance level of this potential source population was determined by excavating pupae from under the maize crops and screening the newly emerged moths with the fenvalerate discriminating dose described previously. Spray decisions (timing and choice) were made independently by each property manager according to the pest pressure on each block. Auscott applied two pyrethroids (deltamethrin at 15 g a.i./ha), one at the beginning and the other at the end of the 42 day Stage II window. Yarral encountered higher pest pressure (probably because it was closer to the maize blocks) and applied three consecutive pyrethroids (lambdacyhalothrin at 18 g a.i./ha) during the two week period of moth emergence and subsequent heavy egg lays (fig. 33).

Results

Resistance in moths: laboratory study

Helicoverpa armigera clearly expressed resistance to pyrethroids in both the larval and adult stages, the latter whether the moths were exposed to the pyrethroid either directly (topical eye test) or indirectly (tarsal plate test) (table 21). The moths in the tarsal plate test were seen to be obviously irritated by the pyrethroid deposit and moved vigorously over and around the petri dish plate surface attempting to escape contact with the deposit. No such behaviour was observed in the control plates. Adult resistance factors were significantly higher than for larvae. Males and females were equally resistant (95% confidence intervals overlapped) for both testing techniques whether weights were adjusted to a standard 200 mg moth or not (adjusted data only, shown in table 21). Further evidence of the ability of *H. armigera* moths to express pyrethroid resistance is given for another eight colonies from both the Namoi/Gwydir and Emerald areas in 1987/88 season (Section 8, fig. 37), for the homozygous oxidative metabolic resistant strain (Section 10, table 35) and for a combined sample of all three study areas for the 1989/90 season (Section 10, fig. 50).

Resistance in moths: field monitoring data

When sprayed properties were defined strictly as those which had actually applied a recent pyrethroid themselves (first analysis in figure 31), resistance levels in the sprayed and unsprayed properties were not significantly different. This was unexpected as it was anticipated that if moths expressed pyrethroid resistance then selection of moths prior to egg laying would result in elevated resistance levels in the sprayed areas. However, it was then realized that this analysis ignored the possibility of these resistant moths being repelled onto close neighbouring blocks of unsprayed cotton which would then erroneously inflate the unsprayed resistance levels. When this possibility was allowed for (second analysis in figure 31), the two treatments clearly separated with the sprayed properties having obviously higher resistance levels in both seasons ($P < 0.1$).

Effect of moth age on expression of resistance

There was no significant drop in resistance level between newly emerged or three to four days old moths (fig. 32). However, by the time moths were six to seven days old, resistance levels had decreased (by just under one half) with a further decline (just over two-thirds) by 10-11 days.

Field resistance monitoring: moths

The resistance level of moths reared from pupae from the potential maize source population was 42% (fig. 33). These moths were predicted to emerge over a two to three week period early in the Stage II window and the virtually identical pre-spray level of adult resistance in both the Yarral and Auscott fields indicates strong circumstantial evidence that the maize blocks were indeed acting as an immigration source for the neighbouring cotton fields. Both properties then applied a pyrethroid and adult resistance responded rapidly increasing to about 70% on both properties but returned to their pre-spray levels within five days of the spray. The second pyrethroid spray was applied to the Yarral property only but adult resistance responded on both properties increasing to about 80% but then followed by a significant and rapid decline to very low levels of resistance (between 10 and 20%). This rapid decline began at about six to seven days after the peak emergence of moths from the adjacent maize blocks and reached its lowest point (on both properties) at 10 days after peak emergence. A third pyrethroid spray was then applied to the Yarral property while Auscott opted for the organophosphate profenofos. Once again adult resistance responded on both properties increasing to over 90% on Yarral and just under 70% on Auscott. Thereafter, adult resistance levels declined at a rate similar to the previous fall and remained low (10-30%) for the rest of the study. The endosulfan sprays applied during this period had no significant impact on adult pyrethroid resistance levels on either property nor did the deltamethrin spray applied at the end of the Stage II window on the Auscott property.

Field resistance monitoring: eggs

The resistance level in eggs (screened as larvae) from the Yarral property showed three distinct peaks which, after allowing for a six to seven day lag phase, corresponded well with the three pyrethroid applications on Yarral. However, resistance levels in eggs from the Auscott property remained relatively stable throughout this period but did respond to the late deltamethrin application (four day lag phase). The large and rapid declines in adult resistance noted after the second and third peaks for both Yarral (6-8x) and Auscott (3-4x) were not manifested in the egg samples with much smaller declines at Yarral (1.3-1.6x) and none at all on Auscott.

Discussion

Adult resistance

This study clearly showed that *H. armigera* adults are capable of expressing pyrethroid resistance in the laboratory (to both direct and indirect exposure) and that this can be manifested as selection of resistant moths in the field. Thus the selection of moths prior to egg laying is a plausible explanation for the immediate increase in pyrethroid resistance which occurs within the Stage II window (see Section 2). Pyrethroid resistance has also been shown to be expressed by adults of the closely related noctuids *Heliothis virescens* (McCaffery *et al.*, 1989b) and *Spodoptera exigua* Hübner (Brewer *et al.*, 1990). The expression of metabolic pyrethroid resistance in nectar feeding adult Lepidoptera has hitherto been considered minimal and that any adult resistance would be via other mechanisms e.g. nerve insensitivity (Brattsten, 1987a; Plapp *et al.*, 1988; Campanhola & Plapp, 1989a, 1989b; McCutchen *et al.*, 1989). The question of resistance mechanisms and expression of pyrethroid resistance in adult *Helicoverpa armigera* will be discussed further in Section 8.

Male and female moths were equally resistant whether allowance was made for the difference in weight between the sexes or not. Riordan & Gregory (1985) and Riedl *et al.* (1986) working on adult tsetse flies (*Glossina p. palpalis* (Robineau-Desvoidy) (Diptera: Glossinidae)) and codling moths (*Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae)) respectively, found the heavier females to be more tolerant but that this difference was removed when results were expressed per unit of body weight. This was probably because of the larger weight differential between the sexes in these two species (females 77-87% and 72% heavier, respectively) compared to the 5-10% difference for *H. armigera* in this study. This is consistent with Daly & Fitt's (1990) finding that small differences in body weight did not significantly affect phenotypic expression of pyrethroid resistance in *H. armigera*.

Repellency of resistant moths

The demonstration of selection of resistant moths in the field was dependent on making allowance for the possible dispersal of resistant moths onto nearby unsprayed cotton. Pyrethroids have been shown to be

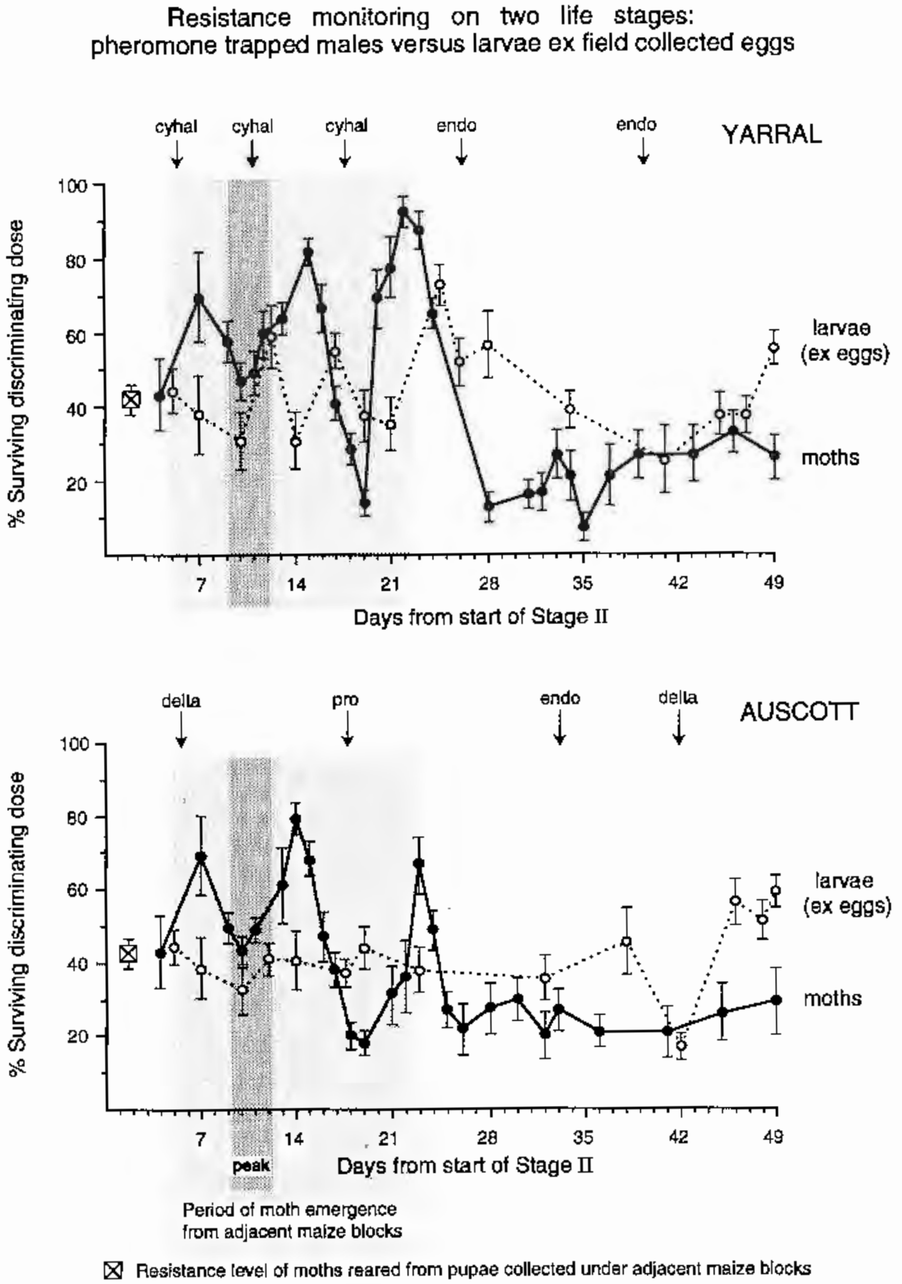


Fig. 33. Comparison of resistance monitoring on two life stages of *Helicoverpa armigera* (pheromone trapped male moths and larvae reared from field collected eggs) in contiguous cotton fields on two cotton properties (Auscott and Yarral) near Narrabri, NSW during the 1986/87 Stage II pyrethroid window (42 days from 10th Jan to 20th Feb) and one week into the following Stage III period. Resistance expressed as the percentage of each life stage surviving a discriminating dose of fenvalerate (0.2 µg/30–40 mg larva or 2.0 µg/moth, topical eye test, data from tables 37 and 43, respectively), adjusted for any control mortality ± pooled binomial standard error. Vertical arrows indicate insecticides applied to each field (lambdacyhalothrin 18, deltamethrin 15, endosulfan 720 or profenofos 750 g a.i./ha). Shaded areas indicate the duration and peak emergence of *H. armigera* moths from two large adjacent fields of unsprayed maize.

Table 21. Fenvalerate bioassay of adults and larvae of a strain of *Helicoverpa armigera* bred from fenvalerate discriminating dose snrvivors from the Emerald study area in 1985/86 season. Moths dosed either directly on the eye (topical eye test) or indirectly, by enforced contact with a treated glass plate (tarsal plate test). Moth LD$_{50}$ and LC$_{50}$s adjusted to standard 200 mg moth. Resistance factors (RF) are LD/LC$_{50}$ current strain ÷ LD/LC$_{50}$ of susceptible strain [larvae (0.03μg/30–40 mg larva); moths, topical eye test (0.30 and 0.40 μg per ♀ and ♂ moth, respectively) and moths, tarsal plate test (0.21 and 0.26 μg/cm^2 per ♀ and ♂ moth, respectively)]. Larval and moth susceptibility data from tables 37 & 43, respectively. * indicates non overlap of resistant and susceptible 95% confidence limits.

Moths: topical eye test

Sex	Av. weight of 1 day old, fed, unmated moths (mg) ± s.e.	LD$_{50}$ (μg/moth)	95% Conf. interval Lower	Upper	Slope	RF
♀	225 ± 2.9	31.4	23.97	41.45	2.1	105*
♂	200 ± 2.9	22.9	17.42	29.31	2.8	57*

Moths: tarsal plate test

Sex	Av. weight of 1 day old, fed, unmated moths (mg) ± s.e.	LC$_{50}$ (μg/cm^2)	95% Conf. interval Lower	Upper	Slope	RF
♀	248 ± 2.3	45.2	35.48	58.19	1.6	215*
♂	226 ± 2.0	66.1	51.07	88.59	1.6	254*

Larvae	LD$_{50}$ (μg/30–40 mg/larva)	95% Conf. interval Lower	Upper	Slope	RF
	0.52	0.38	0.74	1.8	17.3*

strong repellents to a wide range of susceptible pests including German cockroaches (*Blattella germanica* (Linnaeus) (Blattodea: Blattellidae)) (Rust & Reierson, 1978), twospotted mites (*Tetranychus urticae* Koch (Acarina: Tetranychidae)) (Penman & Chapman, 1983; Iftner *et al.*, 1986), boll weevil (*Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae)) (Moore, 1980), blackflies (*Simulium* spp. (Diptera: Simuliidae)) (Shemanchuk, 1981), mosquitoes (Diptera: Culicidae) (Taylor *et al.*, 1981) and lepidopterous larvae and moths e.g. *Helicoverpa* spp. adults (Gould, 1984; Moore, 1988) and *Plutella xylostella* (Linnaeus) (Lepidoptera: Yponomeutidae) (Kumar & Chapman, 1984). They have also been shown to be equally repellent to pyrethroid resistant insects such as hornfly (*Haematobia irritans* (Linnaeus) (Diptera: Muscidae) (Byford *et al.*, 1987a), housefly (*Musca domestica* Linnaeus (Diptera: Muscidae)) (Virgona *et al.*, 1983), green peach aphids (*Myzus persicae* (Sulzer) (Hemiptera: Aphididae)) (Sawicki & Rice, 1978) and rust-red flour beetles (*Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)) (Prickett & Ratcliffe, 1977). Originally it was thought that there was an inverse relationship between resistance and irritability (Georghiou, 1972) so that resistant insects were less irritated and could remain on treated deposits (i.e. repellency of resistant insects would not be a significant factor). However, in the early 1980s, the concept of independent expression of physiological resistance and irritancy response was put forward by several authors (e.g. Gould, 1984; Lockwood *et al.*, 1984; Pluthero & Singh, 1984). Evidence supporting this concept has been given for mosquitoes (Gerold & Laarman, 1964), spotted root maggot (*Euxesta notata* (Wiedemann) (Diptera: Otitidae)) (Hooper & Brown, 1965), twospotted mites (Penman *et al.*, 1988) and *Plutella xylostella* (Hoy *et al.*, 1990). The resistant strain of *H. armigera* tested in the laboratory in this study, although shown to be strongly pyrethroid resistant, was at the same time, still intensely irritated by the pyrethroid deposits in the tarsal plate test. Thus the pyrethroid resistant *H. armigera* in this study seem to express physiological resistance without hypo-irritability. This is consistent with the results presented here which suggest the selection and dispersal of resistant moths from sprayed to nearby unsprayed cotton. This was clearly indicated at a local level in the Auscott/Yarral study and also at a regional level for the 1983/84 and 1984/85 Namoi/Gwydir Stage II data. It is also possible that some surviving resistant moths may have remained in the sprayed crops and avoided the irritating pyrethroid deposits by sheltering lower in the canopy, where the pyrethroid had not penetrated, until the deposits had degraded sufficiently. This could help explain the field observations of many cotton growers and consultants who have commented that pyrethroid resistant *H. armigera* tend to lay on the lower flowers. Clearly both avoidance behaviours were probably utilized as some resistant moths remained behind to bolster the resistance levels on the sprayed properties. Otherwise the sprayed properties in the first analysis (sprayed neighbours excluded) would most likely have had lower resistance levels than the properties deemed to have been unsprayed.

Effect of moth age on phenotypic expression of resistance

The phenotypic expression of pyrethroid resistance in adult *H. armigera* dropped off dramatically with age in this study, beginning sometime between four and six days old and then continuing to further decline for the effective life span of the moth. A similar situation has been found for many other resistant and susceptible adult insects including pyrethroid resistant *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (Brewer & Trumble, 1989) and *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) (Sanderson *et al.*, 1989), malathion resistant *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) (Wool & Kamin-Belsky, 1983) and *Anopheles stephensi* Liston (Diptera: Culicidae) (Rowland & Hemingway, 1987), carbamate resistant houseflies (El-Aziz *et al.*, 1969) and susceptible codling moth (Riedl *et al.*, 1985), honeybees (*Apis mellifera* Linnaeus (Hymenoptera: Apidae)) (Delabie *et al.*, 1985) and the grasshopper *Melanoplus sanguinipes* (Fabricius) (Orthoptera: Acrididae) (Hinks & Ewen, 1986). In fact an examination of the literature could only reveal two cases to suggest that adult insecticide sensitivity does not increase with age. These are the special case of the larviporous blood feeding tsetse fly adult (*Glossina*) which is more tolerant after feeding (Riordan & Gregory, 1985) and the wind tunnel study on the pink bollworm (*Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)) (Haynes *et al.*, 1987) which showed no difference in adult sensitivity up to seven to eight days. However, the latter example, as the authors themselves suggest, can be criticized as the wind tunnel technique may have filtered out the less vigorous moths before bioassay (in fact only 50% of released moths were captured on the traps). Thus there is overwhelming evidence in the literature to support the general concept of a declining ability of ageing adult insects to express phenotypic resistance. A number of suggestions have been put forward to explain this phenomenon including declining monooxygenase activity (Hodgson 1983, 1985; Waters *et al.*, 1984; Agosin, 1985) or depletion of energy reserves (Willers *et al.*, 1987). The latter reference is particularly apt for this study as it refers to the closely related noctuid *Heliothis virescens* and indicates a particularly rapid deterioration at three to six nights after eclosion. This fits in neatly with the timing of the observed decline in this study but it would be inappropriate to dismiss the possibility of declining enzyme activity also contributing.

Resistance monitoring: moths versus eggs

The decline in the expression of phenotypic resistance with age raises the question of monitoring resistance by testing adults of unknown mixed age (such as found in pheromone trap catches). Many authors have recommended caution in doing so and suggest that this will underestimate resistance frequencies in the field (Wool & Kamin-Belsky, 1983; Riedl *et al.*, 1985; Georghiou *et al.*, 1987; Rowland & Hemingway, 1987; Sanderson *et al.*, 1989). Georghiou *et al.* (1987) suggested overcoming this problem by collecting larvae or pupae and rearing them through to adults which are then tested at a defined age. (N.B. This was the technique used for resistance estimates in Sections 5 & 6). However, this is a labour intensive approach which contrasts with the simplicity, ease and convenience which make the testing of pheromone trapped males so attractive.

The testing of pheromone trapped males in the Auscott/Yarral study yielded some very interesting findings. The first pyrethroid was applied on both fields on a young emerging population of moths and remained selective for a four to five day period. By that time, the pyrethroid deposits had obviously been diluted or degraded sufficiently to allow survival of the young susceptible moths. Wilson *et al.* (1983, 1986) found that fenvalerate applied at the normal registered field rate of 80-100 g a.i./ha resulted in leaf deposits of 0.2-0.4 $\mu g/cm^2$ which are of the order of the fenvalerate tarsal plate test discriminating dose for susceptible moths (1.6 $\mu g/cm^2$, from table 43) but which are well below the resistant strain LD$_{50}$s (45 and 66 $\mu g/cm^2$ for females and males, respectively, from table 21). Thus, a recently applied pyrethroid is obviously selective for resistant moths, but this selective advantage can be effectively negated after a few days by the combined effects of growth dilution and photodegradation. The second and third pyrethroid sprays applied to the Yarral property quickly selected for high adult resistance on both Yarral and the adjacent unsprayed Auscott property (indicating repellency of resistant moths as discussed previously). Both of these peaks (on both properties) were quickly followed by significant and rapid declines in phenotypic adult resistance. However, these declines were not equally manifested in the egg samples indicating poor correlation between genotype and its phenotypic expression in adult *H. armigera*. The declines in adult resistance after the second and third pyrethroids were shown to commence at five to seven days and reach their lowest point at about 10 days after spraying. This period correlates well with the period of increased sensitivity of *H. armigera* moths (6-11 days old) demonstrated earlier and indicates the expected response of an ageing moth population exposed to pyrethroids. A similar situation occurred with the last pyrethroid application on Auscott which significantly increased resistance in eggs but which had no effect on adult resistance in the relatively mature moth population. As expected, the endosulfan and profenofos applications were neutral in their impact on resistance in both eggs and adults. The resistance peaks in eggs were shown to lag four to seven days behind the pyrethroid applications. This delay can be attributed to the two to three day interval between egg collection periods (daily for moths) coupled with a moth pre-reproductive period of two to three days with peak egg lays at four to seven days (Topper, 1987b; Fitt, 1989).

The experience with resistance monitoring of pheromone trapped males in this study indicates poor correlation with resistance in field collected eggs, particularly during periods when older moths are the dominant age group. As mentioned earlier, many authors have drawn attention to this problem and recommend caution in testing adults of unknown mixed age. In addition to this problem, there are also a number of other potential pitfalls with the pheromone trapped male technique. These include the possible complication of sub-lethal doses of insecticides in sprayed crops (Riedl *et al.*, 1985), the possible impact of adult or previous larval diet (Robertson *et al.*, 1990), variation in temperature (particularly for within field assessment), the possible inability to detect all resistance mechanisms equally and the possibly altered behavioural response to pheromone traps of resistant insects (McCutchen *et al.*, 1989) or those affected by sub lethal doses (Moore, 1988). However, as mentioned earlier, there is no denying the attraction of the pheromone trapped male technique particularly for economic lepidopterous pests where pheromones are often commercially available and the other life stages are difficult to collect and/or expensive to rear. In fact the five studies published so far on resistance monitoring of pheromone trapped males, all focus on economic Lepidoptera including pink bollworm (Haynes *et al.*, 1987), lightbrown apple moth (*Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae)) (Suckling *et al.*, 1985), codling moth (Riedl *et al.*, 1985), *Heliothis virescens* (Plapp *et al.*, 1990b) and *Spodoptera exigua* (Brewer & Trumble, 1989). Despite all the problems and shortcomings of this technique, there is no doubt that it will continue to be used to monitor resistance, more because of its convenience than any scientific merit. Roush & Luttrell's (1989) pragmatic advice that the pheromone trapped male technique is better suited to monitoring resistance over large areas rather than on specific fields, is probably the best compromise.

The research outlined in this study resulted in the adoption of the following supplementary strategy guidelines mentioned in Section 1 :

- 'Avoid growing early maize or sunflowers near cotton, as they serve as early season nursery crops for resistant *H. armigera*'.
- 'Avoid consecutive sprays of pyrethroids where *H. armigera* are emerging from neighbouring early season alternative host crops, as resistance levels will be exacerbated by selection of moths before mating' (analogous to recommendations to avoid pre-mating selection given in Georghiou & Taylor (1976, 1986), Wood & Bishop (1981), Wood & Mani (1981), Mani & Wood (1984).

Section 8

Pyrethroid resistance: field resistance mechanisms

Summary

The dual insecticide ± synergist discriminating dose technique proved highly successful in determining the relative importance of pyrethroid resistance mechanisms present in field populations of *Helicoverpa armigera*, without the problems of alternative techniques. Synergist pre-treatment proved unnecessary therefore allowing use of the more convenient and labour efficient discriminating dose pre-mix.

Oxidative metabolic detoxification, probably via a polysubstrate monooxygenase system, was the major pyrethroid resistance mechanism in both larval and adult *H. armigera*. Residual piperonyl butoxide insensitive resistance (presumably nerve insensitivity, possibly acting in combination with the penetration resistance factor) was also present but at a low level. This latter resistance mechanism was expressed in larvae but moths appeared to express only weak phenotypic resistance. The predominant pyrethroid resistance mechanism employed by insects is discussed in relation to their feeding habit. The tenet that nectar feeding adult Lepidoptera are unable to express metabolic pyrethroid resistance is challenged.

Unrestrained pre-strategy pyrethroid selection pressure on sequential generations resulted in selection for elevated levels of *kdr* type nerve insensitivity and possibly even *super kdr*. Restriction of pyrethroid selection pressure to one generation per season favoured selection of the oxidative over the nerve insensitivity resistance mechanism. Two possibly complementary explanations are put forward for this; differential genetic dominance (semi-dominant oxidative mechanism versus recessive nerve insensitivity) and/or selection in more than one life stage (moths and larvae for the oxidative mechanism versus predominantly larvae only for the nerve insensitivity mechanism). It is suggested that insecticide resistance management strategies should be designed to avoid selection of elevated levels of the intractable nerve insensitivity resistance mechanism whereas low levels of this mechanism (normal *kdr*) are not considered difficult to manage. The demonstration that the strategy has favoured selection of the more amenable oxidative resistance mechanism invites the opportunity to develop possible chemical countermeasures.

Introduction

Pyrethroid resistance can be due to a number of mechanisms including reduced penetration, increased metabolism (both oxidative and esteratic) or nerve insensitivity. In the field, a selective advantage would be conferred on an individual that could delay the toxication process (e.g. through reduced penetration and/or target site sensitivity) which would then allow more time for metabolic detoxification by monooxygenases or esterases (Rossignol, 1988). Thus, the co-evolution of multiple resistance mechanisms should not be unexpected (Lockwood *et al.*, 1984; Via, 1986; Brattsten, 1990). However, the disruptive selection pressure from field use of insecticides channels this variation to yield a genetic system with discrete phenotypic classes (McKenzie, 1986) and field resistance is almost always controlled by one or two important mechanisms (Roush & Croft, 1986; Roush & McKenzie, 1987). Views on the relative importance of the mechanisms for pyrethroid resistance have been strongly influenced by the focus of many of the early studies on public health pests such as houseflies and mosquitoes, in which nerve insensitivity has been invariably demonstrated to be the major pyrethroid resistance mechanism (Brattsten *et al.*, 1986). This has led to the widely held belief that nerve insensitivity, and not metabolic detoxification, is the principal mechanism of pyrethroid resistance in a number of insect taxa (Plapp, 1976a; Sparks, 1981; Soderlund *et al.*, 1989). This belief has become so entrenched in the literature that some authors choose to gloss over their own data indicating the dominance of oxidative metabolic detoxification to conclude that 'there is no reason to doubt that target site insensitivity is the major factor in pyrethroid resistance' in their species of interest (e.g. Bull *et al.*, 1988).

The studies on pyrethroid resistant Australian *Helicoverpa armigera* in Section 3 indicated the dominance of the oxidative metabolic resistance mechanism, at least from the 1984/85 season onwards. However, the Via tolerance curve technique used in this analysis was somewhat subjective, very labour intensive and its cost effectiveness was being increasingly questioned. An alternative technique was sought which could generate the same information but more economically and more precisely. This study aims to explore the possibility of using a dual insecticide ± synergist discriminating dose technique to demonstrate the relative importance of the various pyrethroid resistance mechanisms present in field populations of *H. armigera* in Australia (a similar technique to that used by Farnham *et al.* (1984) and suggested by Raffa & Priester (1985)). It also aims to compare the mechanisms of pyrethroid resistance in adult and larval *H. armigera* and to discuss this in relation to the preferential selection of the more amenable oxidative resistance mechanism over the highly intractable nerve insensitivity mechanism, noted in Section 3.

Methods and materials

Monitoring study

Pyrethroid resistance levels were already being monitored in three separate areas using the discriminating dose technique on larvae reared from field collected eggs (see Section 2). In order to document the impact of the oxidative pyrethroid resistance mechanism in *H. armigera*, a third discriminating dose screen (in addition to the fenvalerate and endosulfan screens) was added to the monitoring programme from the 1987/88 season onwards. This involved the addition of the oxidative synergist piperonyl butoxide (Pbo) to the usual fenvalerate discriminating dose of 0.2 µg/30-40 mg larva (table 37). The maximum sub-lethal dose of Pbo (50 µg/30-40 mg larva, table 22) was added as recommended by Cochran (1987) and Scott (1990). For the first season (1987/88) the synergist was added one hour prior to the fenvalerate discriminating dose as recommended by Brindley (1977) and Scott (1990). However, this proved too tedious and inconvenient for the large number of samples involved so simultaneous application of the insecticide and synergist (in a pre-mix) was evaluated (see below and figure 34) and introduced for the following seasons. Thus, starting in the 1987/8 season, *H. armigera* larvae from each sample were split equally and randomly into three subsamples and tested with the fenvalerate or endosulfan discriminating doses (as previously) or the fenvalerate discriminating dose/Pbo combination. As there was no fenvalerate/Pbo discriminating dose determined for the 40-60 mg weight range, larvae from the fenvalerate/Pbo subsamples which grew through the 30-40 mg testing weight range, were transferred to the fenvalerate subsample and tested with the higher fenvalerate discriminating dose.

Sample analysis was similar to that described in Section 2. The samples were either pooled into collecting weeks and pyrethroid resistance (± synergist) graphed using 95% confidence intervals based on the between site binomial standard error (fig. 35), or into collecting Stages (I, II or III) and graphed as the amount of Pbo suppressible and residual resistance (- and + the standard error of the mean, respectively, in the top graph of figure 36) or the percentage suppressible (± standard error of the mean, in the bottom graph of figure 36). Numbers of larvae tested in each Stage are given in figure 36. Differences between the survival with fenvalerate alone versus the Pbo/fenvalerate mix within each Stage of the resistance management strategy were tested using paired t tests (top graph of figure 36).

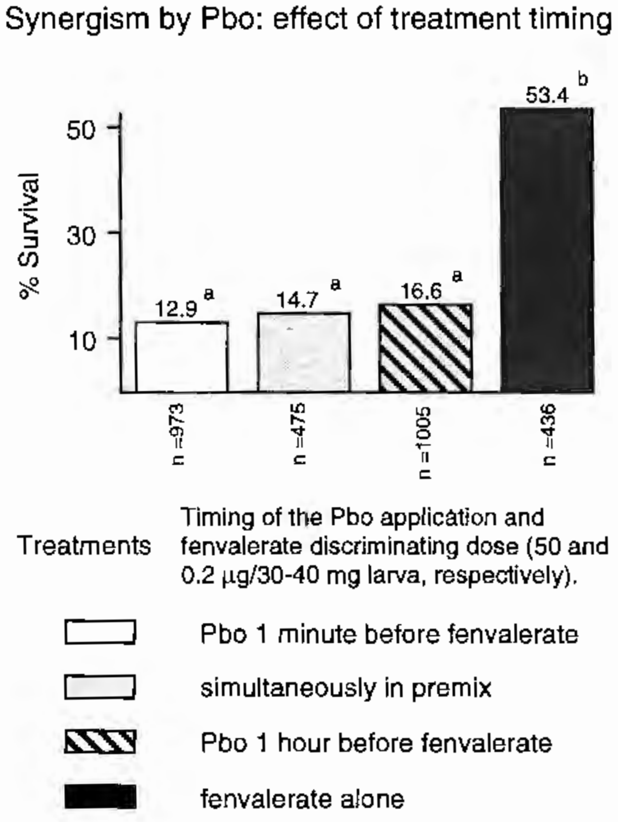


Fig. 34. Effect of synergist treatment timing on the suppression of pyrethroid resistance by Pbo (piperonyl butoxide) in a multigenic pyrethroid resistant field strain of *Helicoverpa armigera* collected in 1986/87 season. n = total number of larvae tested at each treatment. Column values, followed by the same letter, are not significantly different (P <0.01, chi-squared test).

Pbo timing study

As mentioned previously, a study was initiated to determine the necessity for pretreatment of the Pbo synergist. A multigenic pyrethroid resistant field strain of *H. armigera* was formed from the pooled fenvalerate discriminating dose survivors from the three monitoring areas in 1986/87 season. The larvae of the F1 generation

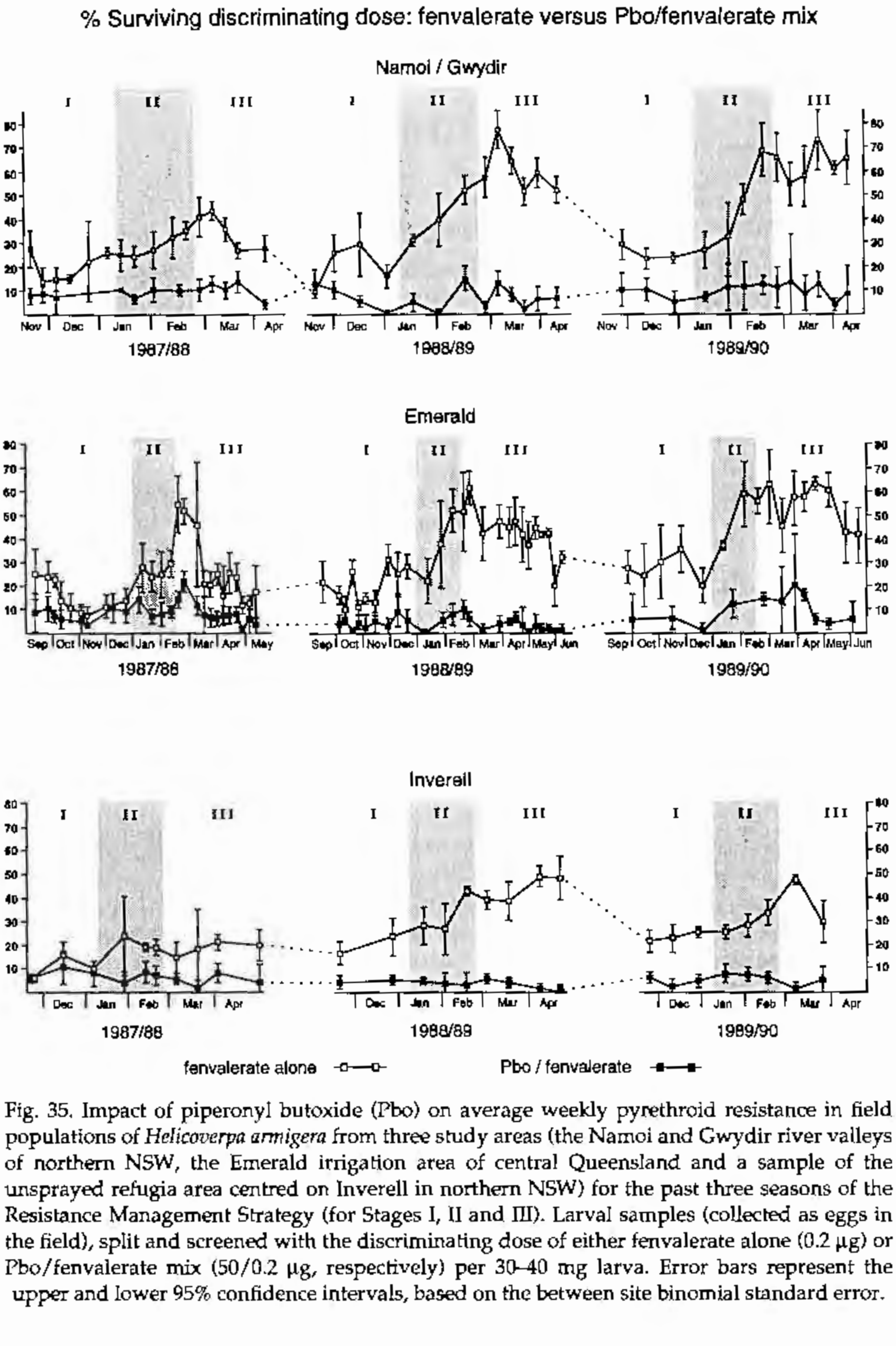


Fig. 35. Impact of piperonyl butoxide (Pbo) on average weekly pyrethroid resistance in field populations of *Helicoverpa armigera* from three study areas (the Namoi and Gwydir river valleys of northern NSW, the Emerald irrigation area of central Queensland and a sample of the unsprayed refugia area centred on Inverell in northern NSW) for the past three seasons of the Resistance Management Strategy (for Stages I, II and III). Larval samples (collected as eggs in the field), split and screened with the discriminating dose of either fenvalerate alone (0.2 µg) or Pbo/fenvalerate mix (50/0.2 µg, respectively) per 30–40 mg larva. Error bars represent the upper and lower 95% confidence intervals, based on the between site binomial standard error.

were split randomly into four subsamples and tested with Pbo and fenvalerate (50 & 0.2 µg/30-40 mg larva, respectively) in the following application sequences: 1. Pbo applied one hour before the fenvalerate dose. 2. Pbo applied one minute before the fenvalerate dose. 3. Pbo and fenvalerate applied simultaneously in a pre-mix. 4. Control treatment (fenvalerate alone).

The numbers of larvae tested in each treatment are given in figure 34. Treatments were compared using chi-squared tests.

Resistance mechanisms in moths and larvae

Adult *H. armigera* were shown to be capable of expressing pyrethroid resistance (see Section 7) however the mechanism of resistance was not studied. This study was designed to compare the resistance mechanisms in adults and larvae of eight multigenic pyrethroid resistant strains of *H. armigera*, bred from the pooled fenvalerate discriminating dose survivors from the Namoi/Gwydir and Emerald study areas in the 1987/88 season. These colonies were further selected in the labo-

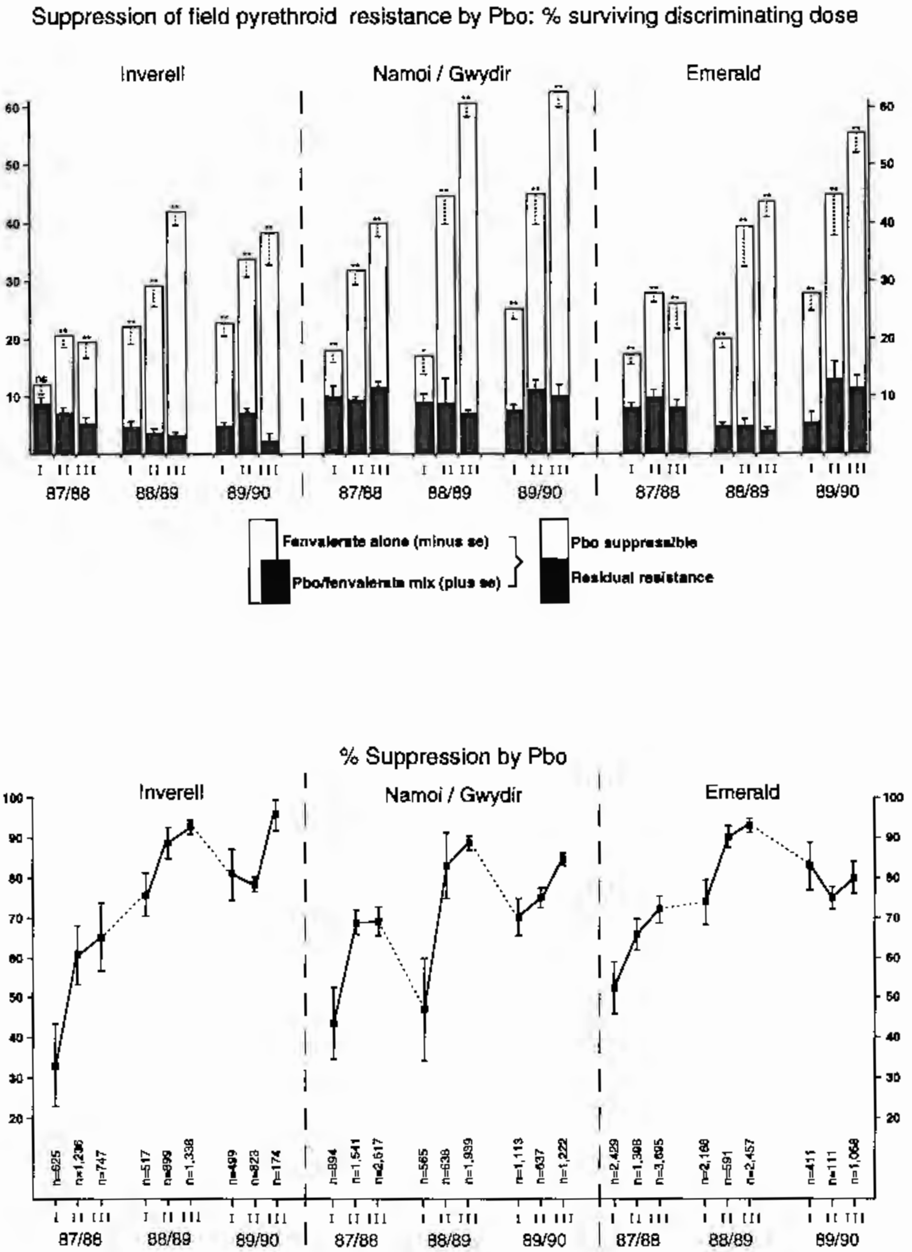
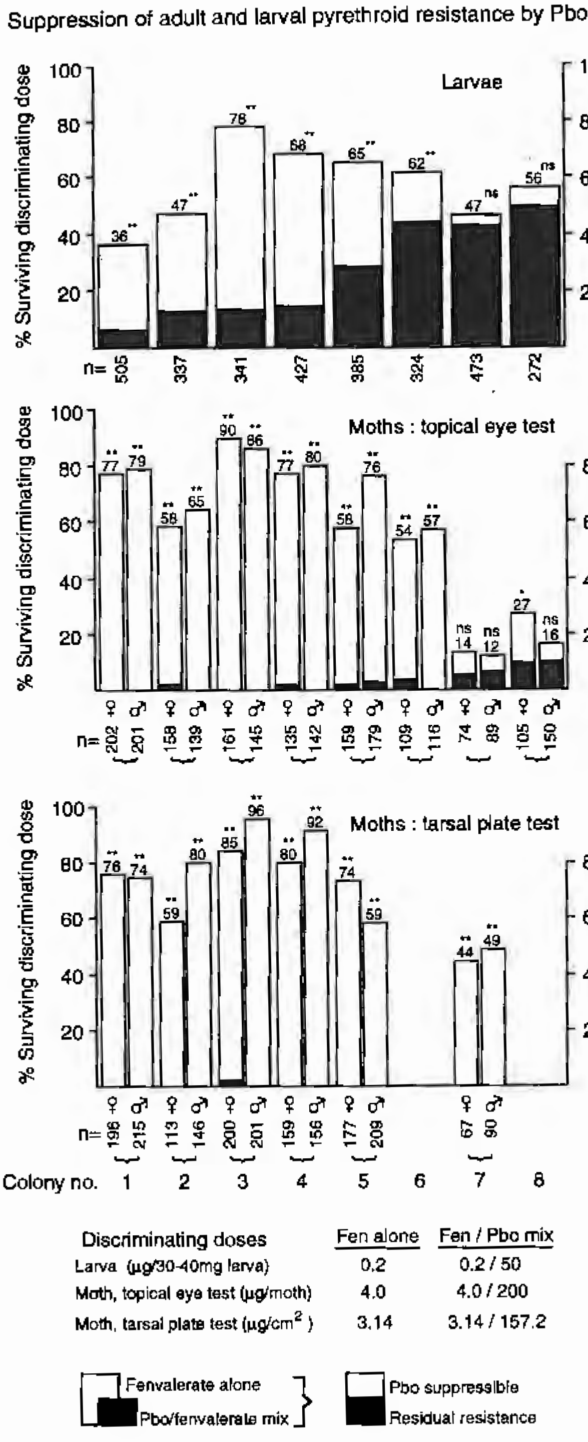


Fig. 36. Impact of piperonyl butoxide (Fbo) on pyrethroid resistance in field populations of *Helicoverpa armigera* from three study areas (the Namoi and Gwydir river valleys of northern NSW, the Emerald irrigation area of central Queensland and a sample of the unsprayed refugia area centred on Inverell in northern NSW). Larval samples (collected as eggs in the field), split and screened with the discriminating dose of either fenvalerate alone (0.2 µg) or Pbo/fenvalerate mix (50/0.2 µg, respectively) per 30–40 mg larva. The difference between the two screens is graphed as the amount of Pbo suppressible and residual resistance (– and + standard error, respectively) in the top graph and % snppressible in the bottom graph (± standard error). * and ** indicate significant differences between the survival with fenvalerate alone versus the Pbo/fenvalerate mix within each Stage (I, II and III) of the Resistance Management Strategy for the past three seasons, at $P < 0.05$ and 0.01 respectively; ns = not significantly different (paired t test). n = total number of larvae tested within each Stage.



Discriminating doses	Fen alone	Fen / Pbo mix
Larva (µg/30-40mg larva)	0.2	0.2 / 50
Moth, topical eye test (µg/moth)	4.0	4.0 / 200
Moth, tarsal plate test (µg/cm²)	3.14	3.14 / 157.2

Fig. 37. Impact of piperonyl butoxide (Pbo) on the suppression of pyrethroid resistance in adults and larvae of eight multigenic pyrethroid resistant strains of *Helicoverpa armigera*, bred from the fenvalerate discriminating dose survivors from the Namoi/Gwydir and Emerald study areas in 1987/88 season and selected in the laboratory with fenvalerate ± Pbo for two generations, to further increase resistance levels. Each colony split at F3 and larvae and moths screened with the discriminating dose (moths double) of either fenvalerate alone or fenvalerate/Pbo mix (data from tables 37 and 43, respectively). Moths dosed either directly on the eye (topical eye test) or indirectly, by enforced contact with treated glass plates (tarsal plate test). The difference between the survival ± Pbo is graphed as the amount of Pbo suppressible and residual resistance. * and ** indicate significant differences between the survival with fenvalerate alone versus the Pbo/fenvalerate mix (adjusted for any control mortality) at $P < 0.05$ & 0.01, respectively; ns = not significantly different, $P < 0.05$ (chi-squared test). n = total number of larvae or moths tested at each comparison.

ratory with fenvalerate (four with and four without Pbo) for another two generations in order to produce a variable mix of both the Pbo suppressible and Pbo insensitive pyrethroid resistance mechanisms. Each colony was split three ways at the F3 and one sub-sample was screened as larvae (numbers tested for each colony given in figure 37) with the discriminating dose of either fenvalerate alone or fenvalerate/Pbo mix (0.2 µg or 0.2/50 µg/30-40 mg larva, respectively, data from table 37). The other two subsamples were left unscreened and reared through to adults which were screened on emergence by both direct (topical eye test) and indirect (tarsal plate test) exposure, with twice the normal discriminating dose (data from table 43) of either fenvalerate alone (i.e. 4.0 µg/moth and 3.14 µg/cm², respectively) or fenvalerate/Pbo mix (i.e. 4.0/200 µg/moth and 3.14/157.2 µg/cm², respectively). The Pbo dose used on the adults was the maximum sub-lethal dose. The topical eye Pbo dose was split evenly between both eyes (100 µg/eye). Use ratios (Pbo : fenvalerate) were 250 : 1 and 50 : 1 for larvae and adults, respectively. The numbers of adults tested (male and female data presented separately) for each colony are given in figure 37. Survival with or without the Pbo synergist (adjusted for any control mortality) was compared for each colony using chi-squared tests.

Results

Pbo timing study

The Pbo significantly reduced pyrethroid resistance to the same level, whether the synergist was applied before or with the fenvalerate (fig. 34).

Monitoring study

The average weekly residual Pbo insensitive pyrethroid resistance levels were nearly always significantly lower than the resistance levels to the straight pyrethroid, for all three study areas in all seasons (fig. 35). The level of this residual Pbo insensitive resistance generally averaged below 10% for each Stage of the resistance management strategy (fig. 36). There was no concomitant increase in this residual Pbo insensitive resistance in response to the significant increases in pyrethroid resistance recorded within either the Stage II or III windows (due to selection of adults and larvae, respectively) except for the early Stage III period in the 1987/88 season at Emerald (fig. 35). During this period, the residual Pbo insensitive resistance clearly peaked (at just over 20%) at the same time as the sharp increase in resistance due to larval selection in the previous generation. The percentage suppression by Pbo was generally lower in the earlier years for all three areas and usually lowest at the beginning of the season (i.e. Stage I) (fig. 36). As there was little change in the residual Pbo insensitive resistance levels over the years, these changes in percentage suppression by Pbo reflect more the steadily increasing overall resistance over this three year period and the lower Stage I resistance levels, respectively. By the 1989/90 season, Pbo suppressed 70-95% of pyrethroid resistance in all three study areas. During the critical Stage II pyrethroid use period, the levels of suppression by Pbo have averaged 80-90% and 75-80% for the 1988/89 and 1989/90 seasons, respectively.

Resistance mechanisms in moths and larvae

Pyrethroid resistance levels in larvae ranged from approximately 40 to 80% with residual Pbo insensitive pyrethroid resistance ranging from 5 to 50% (fig. 37). Larvae from colonies 1-4 and 6-8 had predominantly Pbo sensitive and Pbo insensitive pyrethroid resistance, respectively, while colony 5 had similar levels of each. For colonies 1-4 resistance levels in adults were significantly higher than the corresponding larval resistance levels (for both exposure tests). For the other four colonies adult resistance levels were either similar to those of larvae or significantly lower (fig. 37). The level of residual Pbo insensitive resistance in adults was very significantly lower than the corresponding resistance level in larvae in all colonies, but especially for the indirect exposure method (tarsal plate test).

Discussion

Pbo timing

Contrary to expectations, the timing of the synergist application had no significant impact on its efficacy. Various authors had suggested that the degree of synergism would most likely be reduced if the synergist was applied simultaneously with the insecticide (Brindley, 1977; Scott, 1990). This finding then vindicates the use of the more convenient and labour efficient Pbo/fenvalerate discriminating dose pre-mix in the subsequent 1988/89 and 1989/90 seasons.

Monitoring study and field resistance mechanisms

The dual insecticide ± synergist discriminating dose technique was very successful in demonstrating highly significant and sustained suppression of pyrethroid resistance by Pbo for all three study areas. The strong suppression by Pbo in Stages II and III indicates metabolic detoxification, probably via a polysubstrate monooxygenase (= mixed function oxidase) system, as the major pyrethroid resistance mechanism in field populations of both adult and larval *H. armigera*, respectively. However, as Hodgson (1985) suggests, *in vivo* studies of synergists should not be used uncritically as these synergists are not necessarily specific for monooxygenases but may have general membrane effects, or perhaps inhibit other metabolizing enzymes as well. Hence, the claim of predominant oxidative metabolic detoxification should remain provisional until substantiated by specific *in vitro* studies. Nevertheless, this Pbo sensitive mechanism is the major pyrethroid resistance mechanism in the field and is expressed in both larvae and adults. McCaffery *et al.* (1989b) found a similar high level of Pbo suppressible pyrethroid resistance (>90%) in larvae and adults of *Heliothis virescens* from the USA, confirmed later *in vitro* to be clearly monooxygenase mediated (Clarke *et al.*, 1990). As mentioned previously in Section 7, the expression of metabolic pyrethroid resistance in nectar feeding adult Lepidoptera has hitherto been considered minimal and that any adult resistance would be via other resistance mechanisms e.g. nerve insensitivity (Brattsten, 1987a; Plapp *et al.*, 1988; Campanhola & Plapp, 1989a, 1989b; McCutchen *et al.*, 1989). This study, and that of McCaffery *et al.* (1989b), indicate otherwise and persistence with this tenet invites timely reappraisal, especially so in light of the possible poor phenotypic expression of nerve insensitivity pyrethroid resistance found in moths in this study (see later discussion).

In addition to adults, larvae also had a predominantly Pbo sensitive resistance mechanism indicating oxidative metabolic detoxification as the major field resistance mechanism. However, larvae also possessed a Pbo insensitive resistance mechanism which responded to selection pressure and could be expressed strongly. It is presumed that this residual Pbo insensitive resistance is the nerve insensitivity resistance mechanism, possibly acting in combination with the penetration resistance factor. Both of these resistance mechanisms have been recorded in field populations of pyrethroid resistant *H. armigera* in Australia (Gunning, 1988). This residual Pbo insensitive resistance was, however, apparently only weakly expressed in moths, especially in the indirect tarsal plate exposure test. A similar situation was found for the DDT nerve insensitivity resistance mechanism in cockroaches where there was no cross resistance to Type II pyrethroids in the surface contact tests (Scott *et al.*, 1986). The reason for the poor expression of the nerve insensitivity resistance mechanism in moths was not researched in this study. However, it is suggested that it may be due to either its inherently weak phenotypic expression in adults or possibly the poor phenotypic expression of the accompanying potentially synergistic penetration resistance factor in adults. Alternatively, the fenvalerate/Pbo discriminating doses used for the two adult testing techniques in this study may be too high relative to that used for the larval contact test (moths double and larvae the same discriminating dose as for fenvalerate alone), thus masking expression of phenotypic nerve insensitivity resistance in adults. However, this is unlikely as there was no concomitant increase in the field Pbo insensitive residual resistance recorded during the rapid increase in pyrethroid resistance within the Stage II window (see previous discussion). This immediate increase in pyrethroid resistance within the pyrethroid window, can be attributed to the selection of moths prior to egg laying (see Section 7). So clearly, pyrethroid selection pressure in the field results, virtually exclusively, in monooygenase mediated metabolic resistance in moths. Nevertheless, the possibility of adult nerve insensitivity resistance being masked by too high a discriminating dose should be investigated. Research on the relative expression of the two main pyrethroid resistance mechanisms in moths and larvae (monooxygenases and nerve insensitivity) is currently under way, using lower fenvalerate/Pbo discriminating doses for moths and results will be reported elsewhere.

The finding of a predominantly oxidative metabolic detoxification pyrethroid resistance mechanism for *H. armigera* was not altogether surprising. Phytophagous insects encounter various lipophilic plant compounds and are endowed with an array of microsomal monooxygenases with a predilection for metabolizing fat soluble xenobiotics to polar, hydrophilic materials that can be easily removed by excretion (Wilkinson, 1983; Hung *et al.*, 1990; Sun, 1990). In the 1980s, there were many studies on phytophagous agricultural pests which indicated a similar predominantly oxidative metabolic resistance mechanism, e.g. in diamondback moth (*Plutella xylostella* (Linnaeus) (Lepidoptera: Yponomeutidae)) (Ho *et al.*, 1983; Cheng *et al.*, 1986; Cheng, 1988; Yao *et al.*, 1988), in Colorado potato beetle (*Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)) (Soderlund *et al.*, 1983; Silcox *et al.*, 1985; Harris & Turnbull, 1986; Ahammad-Sahib *et al.*, 1990), in *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) (Broadbent, 1984; Keil & Parrella, 1990), in *Heliothis virescens* (McCaffery *et al.*, 1989b), in citrus thrips (*Scirtothrips citri* (Moulton) (Thysanoptera: Thripidae)) (Immaraju *et al.*, 1990b), in *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (Prabhaker *et al.*, 1988), in *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Collins, 1990) and in mushroom sciarid (*Lycoriella mali* (Fitch) (Diptera: Sciaridae)) (Brewer & Keil, 1989). Prior to this, most work on pyrethroid resistance mechanisms had been done on public health pests such as houseflies and mosquitoes largely because of the urgent problems they cause and their suitability for genetic and biochemical studies (Brattsten *et al.*, 1986). Invariably these were shown to possess predominantly nerve insensitivity pyrethroid resistance with only a minor contribution from monooxygenases, e.g. in houseflies (Farnham 1973, 1985; Elliott *et al.*, 1978; Farnham *et al.*, 1984; Ahn *et al.*, 1988), in mosquitoes (Halliday & Georghiou, 1985; Hemingway *et al.*, 1989), in cattle tick (*Boophilus microplus* (Canestrini) (Acarina: Ixodidae)) (Nolan *et al.*, 1977), in hornfly (Byford *et al.*, 1985; Roush *et al.*, 1986) and in German cockroaches (Scott *et al.*, 1990). The housefly is particularly interesting as Keiding (1986) found DDT resistant houseflies throughout the world but the nerve insensitivity resistance mechanism was only common in some areas in northern Europe. In fact, oxidative pyrethroid resistance mechanisms predominated in housefly populations from North America (Macdonald *et al.*, 1983b, 1985; Golenda & Forgash, 1985; Scott & Georghiou, 1986; Meyer *et al.*, 1987; Meyer & Georghiou, 1988a) and Japan (Golenda & Forgash, 1989). However there were exceptions with oxidative resistance in England (Nicholson & Sawicki, 1982) and nerve insensitivity in the USA (De Vries & Georghiou, 1980, 1981a, 1981b). The situation with houseflies then illustrates the complex potential interaction of the pyrethroid resistance mechanisms with selection and/or ecological factors and the need to research each resistance episode on its own merits. Nevertheless, the nerve insensitivity resistance mechanism is generally more important in the blood feeding (e.g. mosquitoes, ticks, hornfly) and omnivorous (e.g. cockroaches, houseflies), public health, and livestock pests than in phytophagous agricultural pests (Brattsten *et al.*, 1986). This potential bias was probably first recognized by Casida (1970) who remarked that 'it is tempting to continue emphasising houseflies in delving further into resistance mechanisms, but it is important to deal with other economic species as well, particularly agricultural pests'. Brattsten *et al.* (1986) also recognized the problem and suggested that extrapolating from public health pests may be dangerous 'because of differences in the physiology and ecology of blood and plant feeding species'. This latter point was brought out well by a deceptively titled but most fascinating study by Halliday & Feyereisen (1987). They noted that DDT and pyrethroid resistance increased after a blood meal in mosquitoes. They suggested that 'changes in nerve membranes caused by the nerve insensitivity factor, may have a normal physiological counterpart as found in mosquitoes in response to a blood meal'. Brown & Pal (1971) noted a similar situation in houseflies and the blood sucking reduviid disease vector *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) where insecticide susceptibility was dependent on adult diet and feeding patterns. It is intriguing to speculate whether blood feeding and omnivorous public health and livestock pests were forearmed with a nerve insensitivity mechanism on which natural selection for resistance could act and whether this may account for the greater importance of nerve insensitivity resistance mechanisms in these pests.

Preferential selection for oxidative resistance

Pyrethroid selection pressure in the field was clearly channelled to the oxidative metabolic pyrethroid resistance mechanism with a minor contribution from the nerve insensitivity/penetration resistance mechanisms. Penetration resistance on its own or in combination with non-metabolic resistance mechanisms, has generally been considered to be of only minor importance (Sawicki & Lord, 1970; Matsumura, 1983). The simplest explanation for the greater response of the oxidative over the nerve insensitivity resistance mechanism could be differential genetic dominance. The oxidative pyrethroid resistance mechanism in Australian *H. armigera* has been shown to be semi-dominant (Gunning & Easton, 1987; Daly, 1988; Daly & Fisk, 1992). While the nerve insensitivity gene has not yet been unequivocally shown to be recessive in Australian *H. armigera*, provisional data (R. Gunning & J. Daly, pers. comm.) indicate consistency with the general finding of recessive nerve insensitivity in a range of insect species (Farnham, 1977; Halliday & Georghiou, 1985; Brattsten *et al.*, 1986; Plapp, 1986; Roush *et al.*, 1986). Thus, the effectively greater dominance of the oxidative resistance mechanism could well be the simple explanation for its higher status. Conversely, the recessive nerve insensitivity pyrethroid resistance mechanism should be more easily managed (Curtis *et al.*, 1978; Wood & Bishop, 1981; Georghiou, 1983; Curtis, 1985; Croft & van de Baan, 1988) which is consistent with the data in this study.

However, an alternative explanation for the greater response of the oxidative resistance mechanism is possible. This mechanism was shown to be expressed strongly in both moths and larvae while the nerve insensitivity mechanism was clearly expressed only in larvae with possible weak phenotypic expression in moths. Thus in an IRM strategy which restricts pyrethroid use to one generation only per season, the oxidative resistance mechanism would be strongly selected for twice (in moths and larvae) but nerve insensitivity possibly only strongly once (in larvae). Thus, in such a system, selection pressure would be channelled to the oxidative resistance mechanism. This then raises the interesting question of the relative importance of the two resistance mechanisms in a system of intense and unrestrained selection pressure applied over sequential generations (as found in the pre-strategy era). Metabolic resistance is usually selected first as it involves changes in a system that is already primarily designed for defence against toxic chemicals (Brattsten, 1990). Target site resistance is more difficult to acquire because it involves changes in a crucially important process that is presumably, already optimized through evolution (Brattsten, 1990). Thus altered target site resistance, such as nerve insensitivity, should evolve later and probably only after prolonged and intense selection pressure. This has been found to be the case for chlorpyrifos resistant *Culex pipiens* Linnaeus (Diptera: Culicidae) in Italy which was initially due to an esterase but on increased selection pressure, insensitive acetylcholinesterase was found to predominate (Villani & Hemingway, 1987). As discussed in Section 3, the unrestrained and intense pre-strategy pyrethroid selection pressure over multiple generations of *H. armigera*, selected for strong *kdr* type nerve insensitivity resistance and possibly even elevated variants of this, such as *super kdr*. However, after the introduction of the strategy, which restricted pyrethroid use to one generation only per season, this appears to have been lost in favour of the oxidative resistance mechanism. This result is quite significant as *super kdr* (Gibson *et al.*, 1990) is a highly intractable resistance mechanism which gives much higher resistance than normal *kdr* (Farnham *et al.*, 1987) and which can be highly synergistic with normally minor metabolic resistance mechanisms (e.g. Factor 161 in houseflies, Sawicki *et al.*, 1986). The *kdr* type resistance mechanism alone seems to be relatively easy to manage as indicated in this study but also by Pree *et al.* (1990) evaluating their IRM strategy for the spotted tentiform leafminer *Phyllonorycter blancardella* (Fabricius) (Lepidoptera: Gracillariidae). This species has been shown to possess *kdr* type nerve insensitivity pyrethroid resistance only (Marshall & Pree, 1986; Pree *et al.*, 1986) and they found that the restriction of pyrethroid use to only one of the three generations per year, was successful in maintaining pyrethroid resistance at low levels over a three year period. Thus the *super kdr* type nerve insensitivity pyrethroid resistance mechanism should be avoided and future IRM strategies should be designed with this aim in mind.

The fact that the strategy has favoured the selection of the oxidative resistance mechanism over the nerve insensitivity mechanism is very important at the practical level. The more amenable oxidative resistance mechanism can be challenged by synergists and metabolically refractory altered pyrethroid structures. However, there are no known means to overcome the intractable nerve insensitivity mechanism. A similar approach (using enzyme blocking agents and structurally altered resistant molecules) has been successfully adopted by medical microbiologists aiming to counteract enzyme mediated antibiotic resistance (Ayliffe, 1987; Pearson, 1987). This study then leads into the final two sections dealing with the development of synergists (Section 9) and resistance breaking pyrethroids (Section 10) as possible chemical countermeasures for the predominant oxidative pyrethroid resistance mechanism.

Section 9

Pyrethroid resistance: synergists

Summary

Sixty-five synergists and seven solvents were tested on larvae of a pure breeding pyrethroid resistant strain of *Helicoverpa armigera* (homozygous for a metabolic detoxification mechanism fully suppressible by piperonyl butoxide (Pbo), presumably via a microsomal monooxygenase system). The methylenedioxyphenyl and acetylenic compounds were the most effective synergists with moderate activity from some organophosphate compounds, particularly the N-alkyl phthalimidomethyl phosphorodithioate, phosmet. All the other compounds tested were either ineffective or only marginally effective including the majority of organophosphates (12 out of 18 tested), pyrethroid analogues, N-alkyls, esterase and glutathione transferase inhibitors, various nitrogen heterocycles, juvenile hormone and analogues, formamidines, organochlorines, anti-oxidants and kojic acid. The most promising synergists indicated for further evaluation were synthetic analogues of Pbo, phosmet, propargite and possibly also fenthion, phosalone, azinphos-ethyl, pyrazophos and kinoprene. All solvents (except dodecane) had no impact on improving kill, indicating that the mode of action of Pbo and the other synergists in this study is principally true biochemical inhibition and not quasi-synergism (improved penetration). Dodecane marginally improved kill in the resistant but not the susceptible strain, indicating some preferential penetration synergism in the resistant strain.

A number of trials with Pbo were designed to facilitate the development of this synergist for commercial field use within the Australian IRM strategy. From these, it was found that: 1. A set rate of Pbo should be used, irrespective of the activity of the accompanying pyrethroid. 2. Residual activity of Pbo is poor (less than 50% of initial activity by two days post spray) but that this could be partially overcome by increasing the rate. 3. Straight Pbo applied onto a weathered pyrethroid deposit could restore control but only temporarily and would probably be of little practical field use. 4. No difference in residual activity could be found between the four formulations of Pbo tested. The synthetic analogue PBX was slightly less intrinsically active than the natural product (Pbo) but when tested in the field against Pbo at the same rate, was equivalent in efficacy to both the standard commercial and research Pbo formulations.

In order to preserve the long-term effectiveness of Pbo as a pyrethroid synergist within the Australian IRM strategy, an optimal use strategy (based on synergist rotation within the present insecticide rotation scheme) is proposed.

Introduction

In the previous chapter, it was shown that the strategy has favoured the selection of the oxidative pyrethroid resistance mechanism over the intractable nerve insensitivity mechanism. It was suggested that this development would then allow the opportunity to use synergists and resistance breaking pyrethroids to overcome the more amenable oxidative resistance mechanism. This study addresses the first option by exploring the range of available synergists against monooxygenase mediated pyrethroid resistance in *Helicoverpa armigera* in Australia and selecting the most promising for further study. Particular attention was paid to identifying synergists having the attributes necessary for a successful field synergist (that is photostability, residual contact activity, safety to mammals and the environment, no phytotoxicity, and good cost effectiveness). Because of the difficulty envisaged in convincing agrochemical companies to develop new synergists (discussed more fully for resistance breaking pyrethroids in Section 10), it was decided to concentrate on screening potential synergists which had already been commercialized for other

Table 22. Control mortality of various synergists and solvents tested alone on 30–40 mg fourth instar *Helicoverpa armigera* (topical application or mixed in diet). Where abbreviations are used, full chemical names are given in the captions of figs. 38–45.

Synergist/solvent	Rate/s	No. larvae (No. colonies) tested		% Mortality
Kojic acid	0.2% in diet	120	(2)	0
Olive oil	50 µg/larva	215	(2)	0
Corn oil	50 µg/larva	208	(2)	0
Sunflower oil	50 µg/larva	106	(2)	0
Kerosene	50µg/larva	291	(3)	0
PBO	50 µg/larva	1,948	(10)	0
Prochloraz	50 µg/larva	60	(1)	0
NK III	50 µg/larva	59	(1)	0
NK IV	50 µg/larva	59	(1)	0
Pix	50 µg/larva	60	(1)	0
Iprodione	50 µg/larva	269	(2)	0
Warf antiresistant	50 µg/larva	242	(2)	0
Codacide oil	50 µg/larva	225	(2)	0
Fenoxycarb	50 µg/larva	60	(1)	0
Juvenile hormone III	50 µg/larva	60	(1)	0
MDT	50 µg/larva	60	(1)	0
MDA	50 µg/larva	60	(1)	0
Propargloxyphthalimide	25 µg/larva	60	(1)	0
Triadimefon	50 µg/larva	60	(1)	0
Piperonyl alcohol	50 µg/larva	60	(1)	0
Hydroquinone	50 µg/larva	60	(1)	0
Catechol	50 µg/larva	60	(1)	0
Safrole	50 µg/larva	60	(1)	0
Methoprene	50 µg/larva	120	(2)	0
Hydropene	50 µg/larva	60	(1)	0
Pyriproxyfen	50 µg/larva	60	(1)	0
Pyrogallol	50 µg/larva	60	(1)	0
Propiconazole	50 µg/larva	60	(1)	0
KBR 1417	50 µg/larva	60	(1)	0
MGK 264	50 µg/larva	434	(3)	0.2
TPP	50 µg/larva	348	(4)	0.3
PBX	50 µg/larva	240	(4)	0.4
Thanite	50 µg/larva	243	(2)	0.4
DEM	50 µg/larva	275	(3)	0.4
Metyrapone	50 µg/larva	240	(2)	0.4
Sesamex	50 µg/larva	242	(2)	0.4
TCPB	20 & 50 µg/larva	200 & 201	(3 & 3)	0.5 & 5.5
Procymidone	50 µg/larva	267	(2)	1.1
Diclobutrazol	50 µg/larva	240	(2)	1.3
DEF	50 µg/larva	166	(3)	1.6
Resorcinol	50 µg/larva	60	(1)	1.7
CGA & 84708	5 & 10 µg/larva	243 & 250	(2 & 2)	3.7 & 29.6
Dodecane	0.5 & 1.0 µl/larva	232 & 165	(2 & 1)	3.9 & 15.8
Kinoprene	5 µg/larva	60	(1)	5.0
Chlordimeform	25 & 50 µg/larva	245 & 239	(2 & 2)	5.3 & 6.7
Cyromazine	10 µg/larva	60	(1)	8.3
Amitraz	10 & 25 µg/larva	245 & 240	(2 & 2)	9.0 & 14.6
Propargite	50 µg/larva	260	(2)	11.2
Propargyl alcohol	50 µg/larva	59	(1)	15.3

purposes. The alternative option of increasing the residual activity of effective but photolabile synergists, such as piperonyl butoxide (Pbo), was also explored.

Historically, most commercial use of synergists has been with pyrethrins where use rates of Pbo have been traditionally stated in terms of ratios of Pbo:toxicant (usually around 10:1) (Chadwick, 1963; Glynne Jones, 1983). However, with the newer pyrethroids, which can vary widely in activity (e.g. deltamethrin 15 g a.i./ha to fluvalinate 105 g a.i./ha, table 45), the retention of this concept would have resulted in possibly unnecessarily excessively high rates of Pbo with some pyrethroids. This had the potential to create disharmony among the pyrethroid manufacturers as those with the more active pyrethroids would have obviously had a significant commercial advantage. An alternative theory is that a set amount of synergist is needed to overcome the metabolic detoxification capability of the insect. If this proves to be the case, then the potential threat to the stability of the agrochemical industry support for the Australian IRM strategy will have been avoided. In addition, it would also result in conserving a precious limited resource (Pbo) and in significant cost savings to the grower. This study aims to resolve the question of

Table 23. Consistency of bioassays over 18 generations of laboratory culture in a pyrethroid resistant stain of *Helicoverpa armigera* homozygous for a metabolic detoxification mechanism fully suppressible by piperonyl butoxide, presumably via a monooxygenase system. Colony tested at each generation with the discriminating dose of fenvalerate (0.2 µg/30–40 mg larva, table 37), number of larvae tested in brackets. Colony bioassayed with fenvalerate at irregular intervals and LD_{50} expressed in µg/30–40 mg fourth instar larva. Resistance factors (RF) expressed as LD_{50} resistant strain ÷ susceptible strain (0.03 µg/larva, table 37).

Laboratory generation	% Surviving discriminaing dose		Fenvalerate LD_{50} (95% confidence intervals)		Slope	RF
Parental	90.1	(101)	1.51	(1.282, 1.768)	3.0	50.3
F_1	93.7	(48)	0.71	(0.604, 0.825)	3.4	23.7
F_2	91.3	(127)	0.91	(0.755, 1.094)	2.0	30.3
F_3	95.1	(205)				
F_4	92.7	(192)	1.65	(1.378, 2.005)	1.7	55.0
F_5	87.7	(65)				
F_6	81.2	(100)				
F_7	86.6	(112)				
F_8	93.7	(48)	0.90	(0.748, 1.108)	2.5	30.0
F_9	90.1	(72)				
F_{10}	87.5	(48)	0.98	(0.798, 1.201)	2.1	32.7
F_{11}	87.2	(262)				
F_{12}	88.4	(173)				
F_{13}	87.8	(49)	0.72	(0.612, 0.850)	2.9	24.0
F_{14}	91.2	(240)				
F_{15}	91.5	(47)	0.62	(0.527, 0.733)	3.1	20.7
F_{16}	88.3	(60)				
F_{17}	91.7	(60)				
F_{18}	90.0	(60)	0.67	(0.571, 0.782)	2.6	22.3

whether a synergist such as Pbo, should be used in a ratio to the toxicant or at a set rate irrespective of the activity of the insecticidal partner.

Synergists such as Pbo are well known as additives for susceptible insects in household insecticides (Glynne Jones, 1983). However, their use against resistant insects in the field has been far from successful due to the development of alternative nerve insensitivity resistance mechanisms (DDT synergists, Metcalf, 1989), overuse leading to resistance to Pbo itself (Pbo on pyrethroid resistant Colorado potato beetle (*Leptinotarsa decemlineata*), Forgash (1984), Silcox *et al.* (1985)) or photo-instability (Pbo on nuisance flies in Californian dairies, (Meyer & Georghiou, 1988a, 1988b)). This study aims to address these problems by identifying stable, cost-effective synergists and developing an optimal use strategy which exploits their utility but which preserves their effectiveness for the long term.

Methods and materials

Laboratory evaluation of synergists

Sixty-five synergists and seven solvents were tested on larvae of a pure breeding pyrethroid resistant strain of *Helicoverpa armigera* (homozygous for a metabolic detoxification mechanism fully suppressible by piperonyl butoxide, presumably via a microsomal monooxygenase system). Fourth instar 30-40 mg larvae were treated topically with the synergists, 5-15 min prior to the fenvalerate discriminating dose (0.2 µg/larva, table 37). Six of the seven solvents (Thanite, kerosene, sunflower, corn, olive and Codacide oils) were made up as 5% solutions in acetone with the fenvalerate discriminating dose and applied simultaneously in a premix. Two of the solvents (Thanite and Codacide oil) were also pretreated as described previously for the other synergists. The other solvent (dodecane) was used undiluted. The maximum sub lethal (or marginally lethal) dose of synergist was applied in 1.0 µl of acetone per larva (or distilled water if insoluble in acetone, e.g. cyromazine, chlordimeform hydrochloride, mepiquat chloride). Control mortalities for the synergists tested alone are given in table 22. Because of the high control mortality with 1.0 µl of dodecane (15.8%, table 22), this solvent was used at the lower volume of 0.5 µl per larva (only 3.9% control mortality, table 22). Also, because this solvent increased mortality slightly in the susceptible strain, the fenvalerate discriminating dose needed to be

Table 24. Impact of solvent on the toxicity of fenvalerate to a susceptible and pyrethroid resistant strain of *Helicoverpa armigera* (resistant strain homozygous for a metabolic detoxification mechanism fully suppressible by piperonyl butoxide, presumably via a microsomal monooxygenase system). LD_{50} expressed in µg/30–40 mg third-fourth instar larva. Data for dodecane adjusted for control mortality (4.8%) with solvent alone. Resistance factors (RF) expressed as LD_{50} resistant strain ÷ LD_{50} susceptible strain. * indicates no overlap of susceptible and resistant 95% confidence intervals. Discriminating doses for acetone from table 37. n = total number of larvae screened at each discriminating dose for dodecane.

Solvent	Susceptible						Resistant			
	LD_{50}	(95% C.i.'s)	Slope	% Mortality at 0.05	% Mortality at 0.1	% Mortality at 0.2	LD_{50}	(95% C.i.'s)	Slope	RF
1 µl acetone	0.030	(0.022, 0.033)	3.4		91.0	99.1	0.976	(0.798, 1.201)	2.1	32.5*
0.5 µl dodecane	0.021	(0.018, 0.025)	2.9	81.1 n = 60	100 n = 60		0.446	(0.364, 0.548)	1.6	21.2*

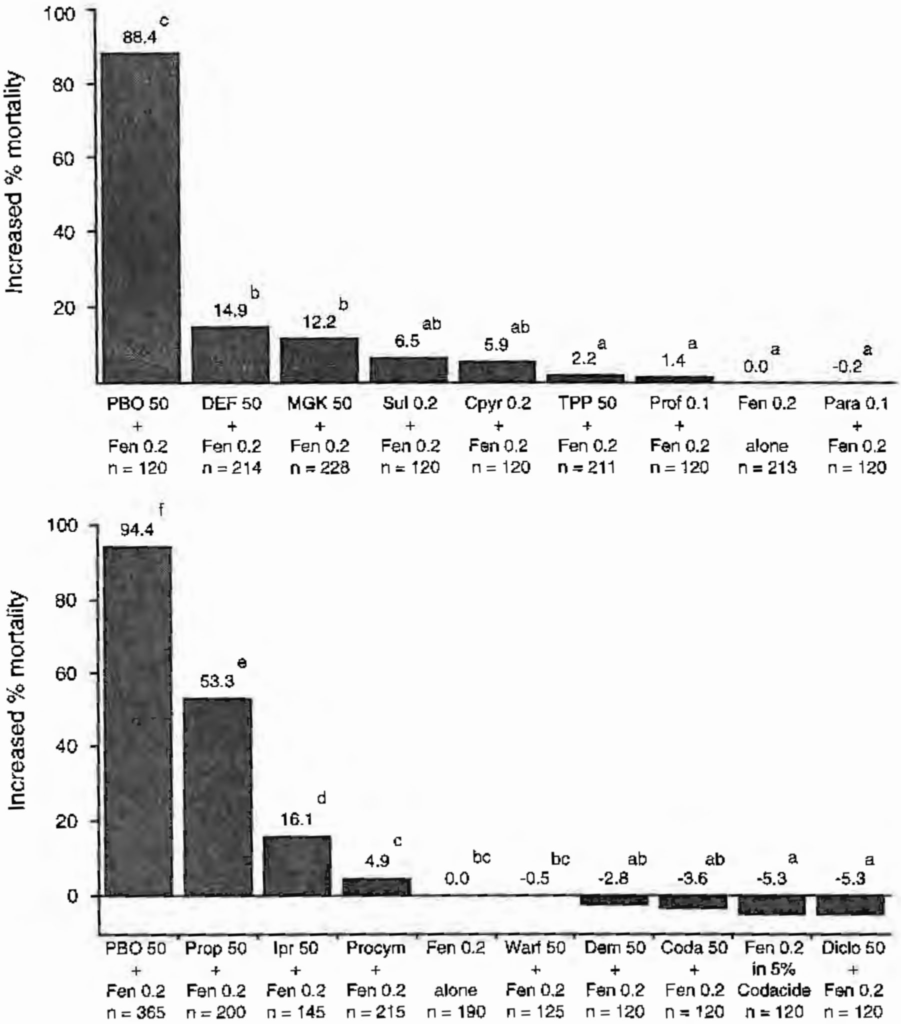


Fig. 38. Impact of various synergists and a solvent on mortality at the fenvalerate discriminating dose (0.2 µg/larva) on 30–40 mg fourth instar mixed function oxidase pyrethroid resistant *Helicoverpa armigera*. Results expressed as the increased mortality (adjusted for control mortality) over the base mortality with fenvalerate alone (10.8% in top graph, 5.3% in bottom graph). Synergists (µg/larva) applied topically in acetone, 5–15 minutes prior to fenvalerate (premix applied simultaneously). Within each graph, treatments with the same letter are not significantly different at the 5% level (chi-squared test). n = number of larvae tested. PBO – piperonyl butoxide, DEF - s,s,s,-tributylphosphoro-trithioate, MGK264 - N-(2-ethylhexy)bicyclo-(2,2,1)-5-heptene-2,3,-dicarboximide, Sul - sulprofos, Cpyr - chlorpyrifos, TPP - riphenyl phosphate, Prof - profenofos, Para - parathion, Prop - propargite, Ipr - iprodione, Procym - procymidone, Warf Antiresistant - N,N,-di-n-butyl-p-chlorobenzenesulphonamide, Dem - diethyl maleate, Coda - Codacide oil ® (95% rape seed oil and 5% emulsifiers, Microcide Ltd, UK), Diclo - diclobutrazol.

recalibrated to a lower level (0.1 µg/larva, table 24) when used with this solvent. Piperonyl butoxide/fenvalerate (50 & 0.2 µg/larva, respectively) and fenvalerate alone (0.2 µg/larva) were used as standards in each of the 10 tests (figs. 38-45). Results were expressed as the increased kill (adjusted for any control mortality, Abbott's formula) over the base kill with fenvalerate alone (variable for each test, noted in captions of figures 38-45, average 8.8% over the 10 tests). Numbers of larvae tested for each synergist or solvent varied usually between 100 to 200 (range 60 to 500) and are given in figures 38-45. Within each test, treatments were compared by a chi-squared test ($P < 0.05$). Some compounds were tested twice in separate tests (kerosene, MGK 264, propargite, DEF, DEM and PBX) and one thrice (TPP) while some other compounds were tested at a range of doses (chlordimeform, amitraz, dieldrin, endosulfan, DDT, CGA 84708, silafluofen, cycloprothrin and

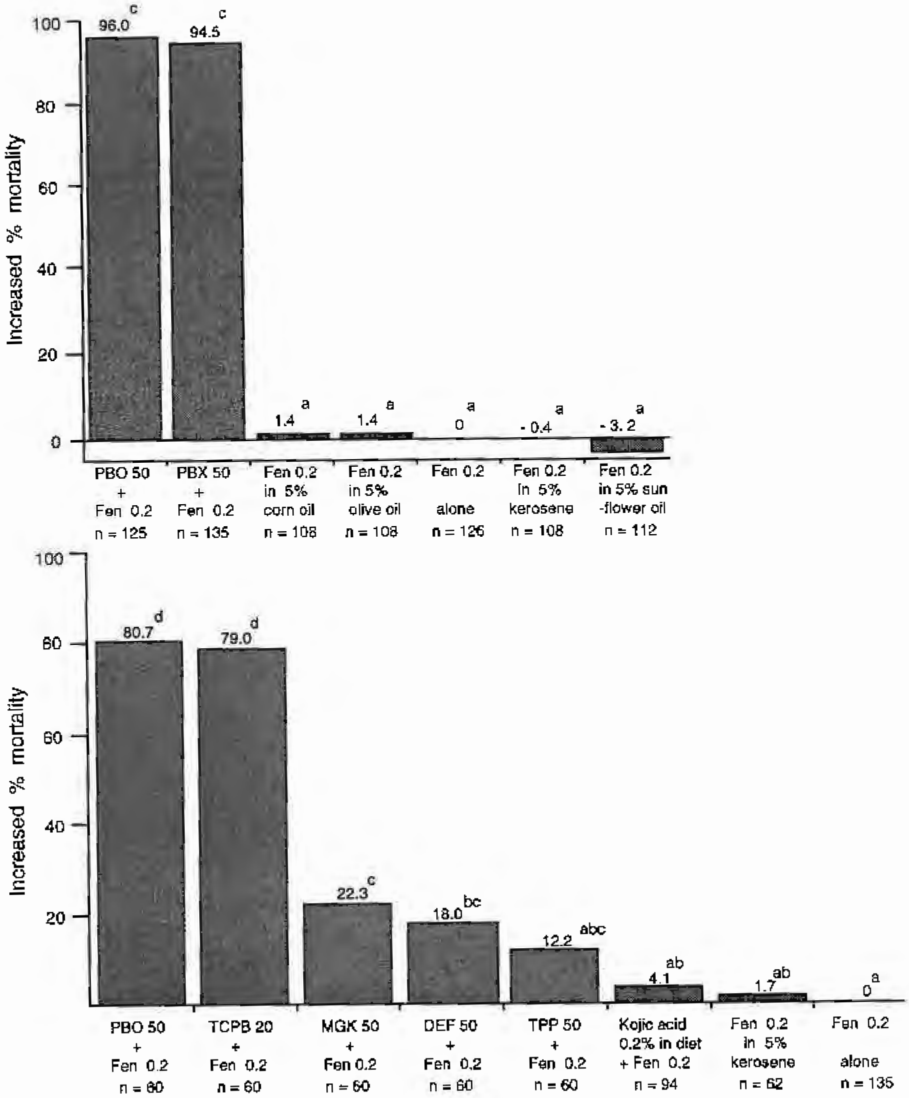


Fig. 39. Impact of various synergists and solvents on mortality at the fenvalerate discriminating dose (0.2 µg/larva) on 30–40 mg fourth instar mixed function oxidase pyrethroid resistant *Helicoverpa armigera*. Results expressed as the increased mortality (adjusted for any control mortality) over the base mortality with fenvalerate alone (3.2% in top graph, 19.3% in bottom graph). Synergists (µg/larva) applied topically in acetone, (except kojic acid mixed in diet), 5–15 minutes prior to fenvalerate (premixes applied simultaneously). Within each graph, treatments with the same letter are not significantly different at the 5% level (chi-squared test). n = number of larvae tested. PBO – piperonyl butoxide, PBX - synthetic homologue of PBO (ex Endura Spa, Italy), TCPB - 1,2,4-trichloro-3-(2-propynyloxy)benzene, MGK264-N - (2-ethylhexy)bicyclo-(2,2,1)-5-heptene-2,3-dicarboximide, DEF - S,S,S-tributylphosphoro-trithioate, TPP - triphenyl phosphate, Kojic acid - 2 hydroxymethyl-5-hydroxy-pyrone.

flucythrinate) (see below for details of full chemical names).

Because of the large number of synergists tested, it took nearly two years (approximately 15 laboratory generations) to complete the study. As mentioned previously, the study was carried out on a pure breeding homozygous resistant colony so variation in bioassay response over time should not have been a problem. However, in order to confirm this, the colony was bioassayed with fenvalerate at regular intervals and the results are given in table 23.

All the synergists (and solvents) tested in this study were applied topically, except kojic acid which was mixed in with the diet at 0.2%. Obviously, a topical test such as this will overlook synergists with poor cuticular penetration (Wilkinson, 1979; Motoyama *et al.*, 1990). However,

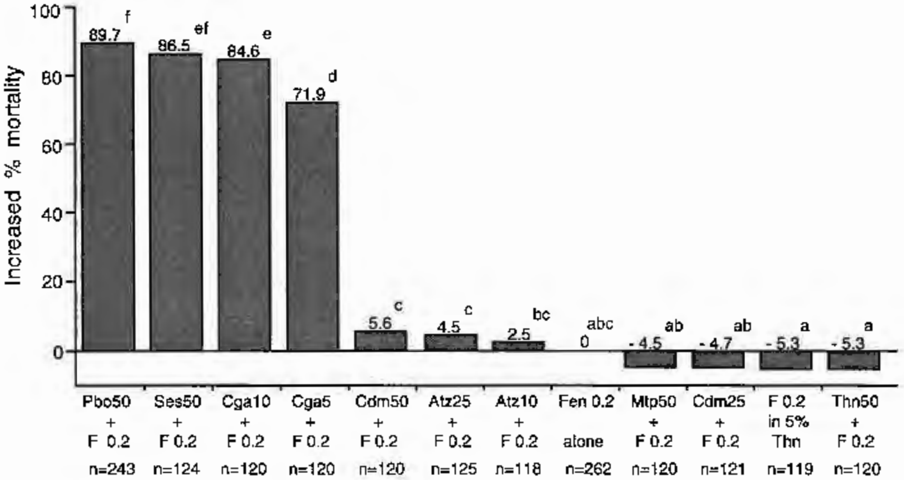


Fig. 40. Impact of various synergists on mortality at the fenvalerate discriminating dose (0.2 µg/larva) on 30–40 mg fourth instar mixed function oxidase pyrethroid resistant *Helicoverpa armigera*. Results expressed as the increased mortality (adjusted for any control mortality) over the base mortality with fenvalerate alone (9.5%). Synergists (µg/larva) applied topically in acetone, 5–15 minutes prior to fenvalerate (premix applied simultaneously). Treatments with the same letter are not significantly different at the 5% level (chi-squared test). n= number of larvae tested. Pbo - piperonyl butoxide, Ses - sesamex, Cga - N-2-nitrophenyl carbamicacid propynylester (CGA 84708; ex Ciba Geigy), Cdm - chlordimeform, Atz - amitraz, Mtp - metyrapone, Thn -Thanite (isobornyl thiocyanoacetate).

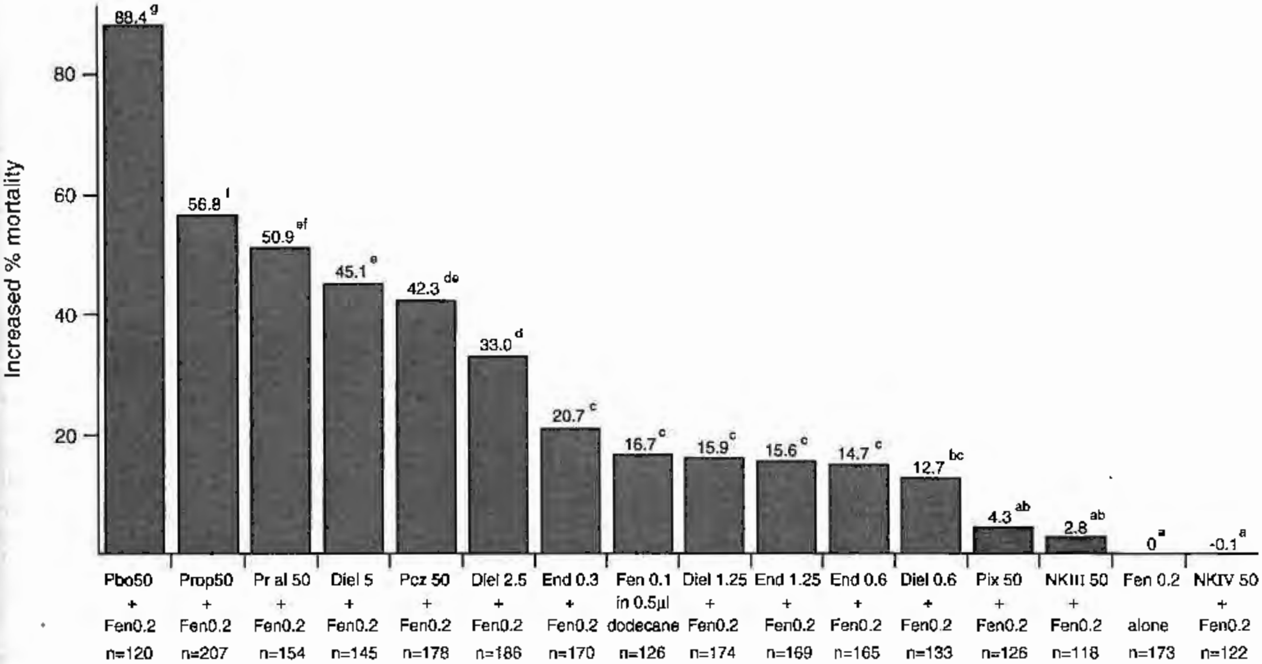


Fig. 41. Impact of various synergists and a solvent on mortality at the fenvalerate discriminating dose (0.2 µg/larva) on 30–40 mg fourth instar mixed function oxidase pyrethroid resistant *Helicoverpa armigera*. Results expressed as the increased mortality (adjusted for any control mortality) over the base mortality with fenvalerate alone (11.6%). Synergists (µg/larva) applied topically in acetone, 5–15 minutes prior to fenvalerate (premix applied simultaneously). Treatments with the same letter are not significantly different at the 5% level (chi-squared test). n= number of larvae tested. Pbo - piperonyl butoxide, Prop - propargite, Pr al - propargyl alcohol, Diel - dieldrin, Pcz - prochloraz, End - endosulfan, Pix - mepiquat chloride, NKIII - NKI 9978 (Bélai *et al.* 1988), NKIV - NKI 9988 (Bélai *et al.* 1988).

Table 25. The efficacy of various synergists and solvents [relative to piperonyl butoxide (Pbo)] in suppressing pyrethroid resistance in a strain of *Helicoverpa armigera* homozygous for a metabolic detoxification mechanism, fully suppressible by Pbo, presumably via a microsomal monooxygenase system. Fully effective and ineffective compounds not significantly different in activity from Pbo and fenvalerate alone, respectively, in figs. 38–45. Partially effective compounds significantly more active than fenvalerate alone but not as effective as Pbo in figs. 38–45. Full chemical names given in the captions of figs. 38–45.

Chemical group	Fully effective	Partially effective (% of Pbo activity)				Ineffective
		≥90	90 — 80	80 — 50	<50	
Methylenedioxyphenyls	Pbo, PBX, sesamex, MD toluene	PBX	MD aniline, safrole		piperonyl alcohol	
Organophosphates		phosmet	KBR 1417	fenthion, phosalone, azinphos-ethyl, pyrazophos	fenitrothion, triazophos	azamethiphos, monocrotophos, parathion, trichlorfon, profenofos, cholrpyrifos, sulprofos, diazinon quinalphos, methidathion
Pyrethroid analogues					silafluofen	flucythrinate, cycloprothrin
Acetylenic compounds	propargyloxyphthalimide, TCPB (propynyl ether)	CGA (propynyl ester), kinoprene		propargyl alcohol, propargite		
N-Alkyl compounds					MGK264, iprodione	procymidone, Warf antiresistant
Esterase, Glutathione transferase inhibitors					DEF, TPP	DEM, TPP
Nitrogen heterocycles					prochloraz, triadimefon	diclobutrazol, propiconazole, metyrapone, NK III & IV, cyromazine, mepiquat chloride
Juvenile hormone & analogues		kinoprene			fenoxycarb	JH III, methoprene, hydroprene, pyriproxyfen
Formamidines						chlordimeform, amitraz
Organochlorines				DDT, dieldrin	endosulfan	
Anti-oxidants						hydroquinone, catechol, pyrogallol, resorcinol
Solvents					dodecane	Codacide, corn, sunflower & olive oil, kerosene, Thanite
Other						kojic acid

this was done intentionally in order to screen for good contact activity rather than for stomach poisons which have limited utility in rapidly growing crops with protected feeding sites, such as cotton (Forrester 1991, in press).

Eighteen organophosphate compounds from a variety of structural groupings (table 26) were evaluated as synergists using the maximum sublethal (or marginally lethal) dose technique described previously. In addition, seventeen of these were also tested in full bioassays (with and without Pbo pre-treatment, 50 µg/larva) on both a susceptible and pyrethroid resistant strain of *H. armigera* (same strain as described previously). These compounds are listed in order of decreasing activity against the susceptible strain (without Pbo pre-treatment) in table 26. Resistance factors (LD_{50} resistant strain ÷ LD_{50} susceptible strain, both without Pbo) indicated any possible negative cross resistance which could be exploited. Susceptible and resistant synergistic ratios (LD_{50} susceptible or resistant strain (without Pbo) ÷ LD_{50} susceptible or resistant strain (with Pbo), respectively), indicated any possible bioactivation or detoxification by monooxygenases which could help explain the possible mechanism of any synergistic activity detected.

Bioassay techniques and analysis were as detailed in Appendix 2 except that only fourth instar 30-40 mg larvae were tested. Third instar larvae were excluded from these assays to minimize the possible variability due to fluctuating metabolic detoxification capacity during the moulting cycle (Wilkinson & Brattsten, 1972; Wilkinson, 1983; Collins & Hooper, 1984b; Hodgson, 1985). Sources of the various synergists and solvents for each of the structually related groups indicated in table 25 are given below.

Methylenedioxyphenyl compounds (see table 27 for structures)

Piperonyl butoxide (Pbo) 90.0%, 3,4-methylenedioxy toluene 97.0%, 3,4-methylenedioxy aniline 97.0%, safrole 97.0%, piperonyl alcohol 98.0% (all ex Aldrich Chem., Milwaukee, Wi, USA); PBX 90.0% (synthetic homologue of Pbo ex Endura Spa, Bologna, Italy); sesamex 90.0% ex P. Hughes, Biological & Chemical Research Institute, Sydney.

Organophosphates

Azamethiphos 95.4%, diazinon 90.0%, methidathion 97.6%, profenofos 91.3% (all ex Ciba Geigy, Sydney); azinphos-ethyl 96.3%, fenthion 99.5%, methyl parathion 96.8%, sulprofos 91.0%, trichlorfon 97.2% (all ex Bayer, Sydney); KBR 1417 97.0% (Behrenz *et al.*, 1990) ex Bayer, Germany; pyrazophos 99.0%, triazophos 42.8% (both ex Hoechst, Germany); monocrotophos 70.7% ex Shell, Melbourne; quinalphos 96.2% ex Sandoz, Sydney; chlorpyrifos 94.0% ex Dow, Sydney; phosalone 93.0% ex Rhone-Poulenc, Sydney; phosmet 93.5% ex ICI, Melbourne; fenitrothion 96.8% ex Sumitomo, Japan.

Pyrethroid analogues

Silafluofen, flucythrinate, cycloprothrin (see Section 10)

Acetylenic compounds

Propargyloxyphthalimide 97.0%, propargyl alcohol 99.0% (both ex Aldrich Chem., Milwaukee, Wi, USA); TCPB (1,2,4-trichloro-3-(2-propynyloxy) benzene (Brown, 1990)) 100% ex T. Brown, Clemson, USA; propar-

Table 26. Bioassay of 17 organophosphates with and without piperonyl butoxide (Pbo) pre-treatment (50 µg/larva) on a susceptible and pyrethroid resistant strain of *Helicoverpa armigera* (resistant strain homozygous for a metabolic detoxification mechanism fully suppressible by piperonyl butoxide, presumably via a microsomal monooxygenase system). LD$_{50}$s (expressed in µg/30–40 mg fourth instar larva), arranged in order of decreasing activity against the susceptible strain (without Pbo pre-treatment[3]). Resistance factors (RF) expressed as LD$_{50}$ resistant strain÷LD$_{50}$ susceptible strain. Synergistic ratios expressed as LD$_{50}$ susceptible or resistant strain (without Pbo) ÷ LD$_{50}$ susceptible or resistant strain (with Pbo), respectively. *, ^{ns} indicate non-overlap and overlap of 95% confidence intervals, respectively.

Organophosphate (No. of sulphur atoms in molecule)	Alone							Pbo pre-treatment							
	Susceptible strain			Resistant strain				Susceptible strain				Resistant strain			
	LD$_{50}$	(95% Confidence limits)	Slope	LD$_{50}$	(95% Confidence limits)	Slope	RF	LD$_{50}$	(95% Confidence limits)	Slope	Syn. ratio	LD$_{50}$	(95% Confidence limits)	Slope	Syn. ratio
parathion (1)	0.088	(0.076, 0.102)	3.6	0.197	(0.171, 0.228)	4.2	2.2*	0.485	(0.416, 0.565)	3.6	0.2*	0.522	(0.445, 0.610)	3.4	0.4*
quinalphos (1)	0.122	(0.102, 0.147)	2.6	0.133	(0.117, 0.152)	4.7	1.1^{ns}	0.784	(0.639, 0.974)	1.6	0.2*	1.417	(1.184, 1.693)	2.1	0.1*
profenofos (1)	0.144	(0.123, 0.169)	3.1	0.127	(0.115, 0.140)	6.7	0.9^{ns}	0.680	(0.581, 0.793)	3.4	0.2*	0.207	(0.179, 0.242)	3.6	0.6*
chlorpyrifos (1)	0.538	(0.459, 0.626)	3.4	0.422	(0.355, 0.500)	2.9	0.8^{ns}	1.932	(1.663, 2.243)	3.4	0.3*	1.006	(0.841, 1.195)	2.6	0.4*
sulprofos (3)	0.591	(0.508, 0.681)	3.9	0.412	(0.358, 0.472)	4.6	0.7*	1.868	(1.573, 2.214)	2.7	0.3*	1.019	(0.875, 1.181)	3.4	0.4*
monocrotophos (0)	0.696	(0.534, 0.878)	2.1	0.588	(0.452, 0.748)	1.5	0.9^{ns}	0.448	(0.355, 0.573)	1.9	1.6^{ns}	0.408	(0.328, 0.512)	1.9	1.4^{ns}
azamethiphos (1)	0.771	(0.636, 0.935)	2.2	0.747	(0.609, 0.902)	1.8	1.0^{ns}	0.654	(0.534, 0.800)	2.0	1.2^{ns}	0.506	(0.431, 0.591)	3.3	1.5*
fenitrothion (1)	1.042	(0.879, 1.231)	3.7	1.759	(1.502, 2.056)	3.1	1.7*	2.576	(2.148, 3.107)	3.1	0.4*	2.665	(2.243, 3.194)	2.6	0.7*
diazinon (1)	1.142	(0.989, 1.313)	3.9	0.936	(0.797, 1.094)	3.2	0.8^{ns}	2.253	(1.919, 2.654)	2.5	0.5*	1.808	(1.569, 2.080)	3.0	0.5*
methidathion(3)	1.238	(1.004, 1.517)	2.0	0.767	(0.638, 0.913)	2.6	0.6*	3.269	(2.739, 3.916)	2.0	0.4*	2.245	(1.936, 2.608)	2.8	0.3*
azinphos-ethyl (2)	1.393	(1.170, 1.651)	2.6	3.006	(2.568, 3.509)	2.5	2.2*	1.503	(1.261, 1.782)	2.1	0.9^{ns}	3.987	(3.289, 4.839)	1.7	0.8^{ns}
fenthion (2)	3.248	(2.624, 4.009)	2.3	5.139	(4.378, 5.987)	3.2	1.6*	4.521	(3.737, 5.454)	2.8	0.7^{ns}	4.367	(3.663, 5.206)	2.6	1.2^{ns}
triazophos (1)	3.631	(2.905, 4.526)	1.7	2.776	(2.293, 3.364)	2.2	0.8^{ns}	3.458	(2.822, 4.224)	1.9	1.1^{ns}	1.780	(1.533, 2.066)	3.4	1.6*
phosalone (2)	3.705	(2.910, 4.714)	1.8	8.534	(6.903, 10.59)	1.7	2.3*	9.445	(7.843, 11.38)	2.9	0.4*	13.17	(11.11, 15.55)	2.8	0.6*
trichlorphon (0)	10.19	(8.276, 12.56)	1.7	20.07	(16.95, 23.90)	2.7	2.0*	16.52	(13.78, 19.81)	2.3	0.6*	22.31	(18.72, 26.82)	2.5	0.9^{ns}
phosmet (2)	36.89	(28.65, 47.82)	1.4	55.87	(43.89, 73.04)	1.5	1.5^{ns}	8.918	(7.891, 10.05)	4.2	4.1*	20.34	(15.83, 26.26)	1.3	2.7*
pyrazophos (1)	106.7	(78.36, 151.9)	1.1	15.36	(12.43, 19.14)	1.7	0.1*	60.99	(44.02, 89.95)	1.0	1.7^{ns}	12.56	(10.53, 15.01)	2.4	1.2^{ns}

Organophosphate structure groupings

A] Nitrogen heterocycle phosphorothioates	(chlorpyrifos, azamethiphos, pyrazophos, triazophos, diazinon, quinalphos)
& dithioates	(phosalone, phosmet, azinphos-ethyl, methidathion)
B] Substituted phenyl phosphorothioates	(parathion, fenitrothion, profenofos, fenthion)
& dithioates	(sulprofos)
C] Phosphate	(monocrotophos)
D] Phosphonate	(trichlorfon)
E] Thiophosphonate	(KBR 1417) synergist only, not bioassayed

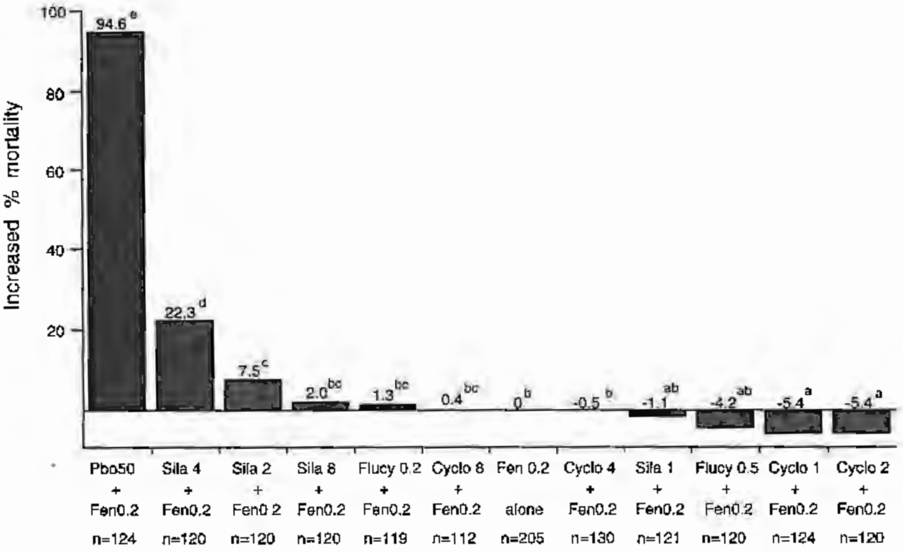


Fig. 42. Impact of three analogue synergists and piperonyl butoxide on mortality at the fenvalerate discriminating dose (0.2 µg/larva) on 30–40 mg fourth instar mixed function oxidase pyrethroid resistant *Helicoverpa armigera*. Results expressed as the increased mortality (adjusted for any control mortality) over the base mortality with fenvalerate alone (5.4%). Synergists (µg/larva) applied topically in acetone, 5–15 minutes prior to fenvalerate. Treatments with the same letter are not significantly different at the 5% level (chi-squared test). n = number of larvae tested. Pbo - piperonyl butoxide, Sila - silafluofen, Flucy - flucythrinate, Cyclo - cycloprothrin.

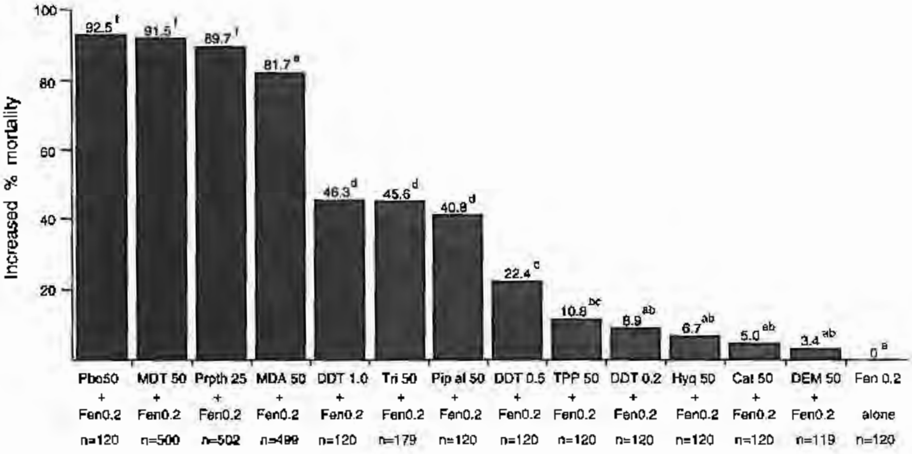


Fig. 43. Impact of various synergists on mortality at the fenvalerate discriminating dose (0.2 µg/larva) on 30-40 mg fourth instar mixed function oxidase pyrethroid resistant *Helicoverpa armigera*. Results expressed as the increased mortality (adjusted for any control mortality) over the base mortality with fenvalerate alone (7.5%). Synergists (µg/larva) applied topically in acetone, 5–15 minutes prior to fenvalerate. Treatments with the same letter are not significantly different at the 5% level (chi-squared test). n = number of larvae tested. Pbo - piperonyl butoxide, MDT - 3,4,-methylenedioxy toluene, Prpth - propargyloxyphthalimide, MDA-3,4-methylenedioxy aniline, DDT - dichlorodiphenyltrichloroethane, Tri - triadimefon, Pip al - piperonyl alcohol, TPP - triphenyl phosphate, Hyq - hydroquinone, Cat - catechol, DEM - diethyl maleate.

Table 27. Structure of methylenedioxyphenyl synergists screened in this study.

Compound

	R1	R2	R3	R4
piperonyl butoxide (Pbo)		$C_9H_{19}O_3$	C_3H_7	
sesamex		$C_8H_{16}O_4$		
safrole		C_3H_5		
3, 4– methylenedioxy toluene (MDT)		CH_3		
3, 4– methylenedioxy aniline (MDA)		NH_2		
piperonyl alcohol		CH_2OH		
PBX [a mixture of mono (30 ± 5%)				$C_9H_{19}O_3$
bis (60 ± 5%)	$C_9H_{19}O_3$			$C_9H_{19}O_3$
& tris (3 ± 1%)	$C_9H_{19}O_3$	$C_9H_{19}O_3$	$C_9H_{19}O_3$	
substitutions]				

gite 82.8% ex ICI, Melbourne; CGA 84708 (N-2-nitrophenyl carbamicacid propynylester, Dittrich *et al.*, 1981) unknown purity ex P. Hughes, Biological & Chemical Research Institute, Sydney; kinoprene 89.0% ex Sandoz, Sydney.

N-alkyl compounds

MGK264 (N-(2-ethylhexy)bicyclo-(2,2,1)-5-heptene-2,3-dicarboximide) 97.8% ex Chem Service, West Chester, Pa, USA; iprodione 97.1% ex Rhone-Poulenc, Sydney; procymidone 99.3% ex Sumitomo, Japan; Warf antiresistant (N,N,-di-n-butyl-p-chlorobenzenesulphonamide, Casida 1970) unknown purity ex R. Gunning, NSW Agricultural Research Centre, Tamworth.

Esterase, glutathione transferase inhibitors

DEF (s,s,s-tributylphosphoro-trithioate) 86.0% ex Chem Service, West Chester, Pa, USA; DEM (diethyl maleate) 98.0% ex Sigma Chem., St Louis, Mo, USA; TPP (triphenyl phosphate) 98.0% ex Aldrich Chem., Milwaukee, Wi, USA.

Nitrogen heterocycles

Prochloraz 98.3% ex Schering, Sydney; triadimefon 95.4% ex Bayer, Sydney; diclobutrazol 73.0% ex ICI, Melbourne; Pix® (mepiquat chloride) 99.0% ex Hoechst, Melbourne; propiconazole 94.0% and cyromazine 96.0% ex Ciba Geigy, Sydney; metyrapone 100% ex Sigma Chem., St Louis, Mo, USA; NK III & IV unknown purity (Bélai *et al.*, 1988) ex I. Bélai, Budapest, Hungary.

Juvenile hormone and analogues

Fenoxycarb 96.2%, pyriproxyfen 99.0%, methoprene 89.4% (all ex Wellcome, Sydney); hydroprene 97.0% & kinoprene 89.0% (both ex Sandoz, Sydney); juvenile hormone III 75.0% ex Sigma Chem., St Louis, Mo, USA.

Table 28. The efficacy of the synergist piperonyl butoxide (Pbo) in suppressing pyrethroid resistance in a strain of *Helicoverpa armigera* homozygous for a metabolic detoxification mechanism, fully suppressible by Pbo, presumably via a microsomal monooxygenase system. Larvae pretreated with Pbo doses 5–15 minutes prior to dosing with the pyrethroid discriminating dose (defined as the minimum pyrethroid dose needed to kill 100% of larvae after pretreatment with a *satis superque* dose of Pbo (50 µg/30–40 mg fourth instar larva). Data adjusted for any control mortality with the discriminating doses tested alone (all <12%)

Pyrethroid		Pbo				Ratio (Pbo ED_{50} : pyrethroid disc. dose)
Compound	Disc. dose µg/30–40 mg larva	ED_{50} µg/30–40 mg larva	95% Conf. interval		Slope	
			Lower	Upper		
deltamethrin	0.0125	2.02	1.648	2.478	1.4	162:1
lambdacyhalothrin	0.025	0.99	0.802	1.205	1.8	40:1
esfenvalerate	0.05	0.30	0.231	0.372	1.4	6:1
cypermethrin	0.2	0.59	0.475	0.716	1.5	3:1
fenvalerate	0.2	0.54	0.438	0.663	1.6	3:1
fluvalinate	0.5	0.55	0.439	0.552	1.7	1:1
cycloprothrin	1.0	1.23	1.049	1.438	2.4	1:1

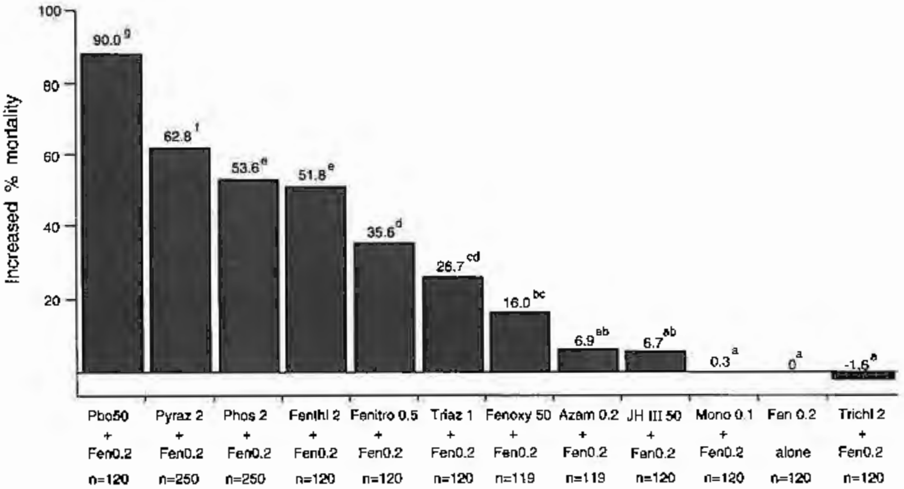


Fig. 44. Impact of various synergists on mortality at the fenvalerate discriminating dose (0.2 μg/larva) on 30–40 mg fourth instar mixed function oxidase pyrethroid resistant *Helicoverpa armigera.* Results expressed as the increased mortality (adjusted for any control mortality) over the base mortality with fenvalerate alone (10%). Synergists (μg/larva) applied topically in acetone, 5–15 minutes prior to fenvalerate. Treatments with the same letter are not significantly different at the 5% level (chi-squared test). n = number of larvae tested. Pbo - piperonyl butoxide, Pyraz - pyrazophos, Phos - phosalone, Fenthi - fenthion, Fenitro - fenitrothion, Triaz - triazophos, Fenoxy - fenoxycarb, Azam - azamethiphos, JHIII - juvenile hormone III, Mono - monocrotophos, Trichl - trichlorfon..

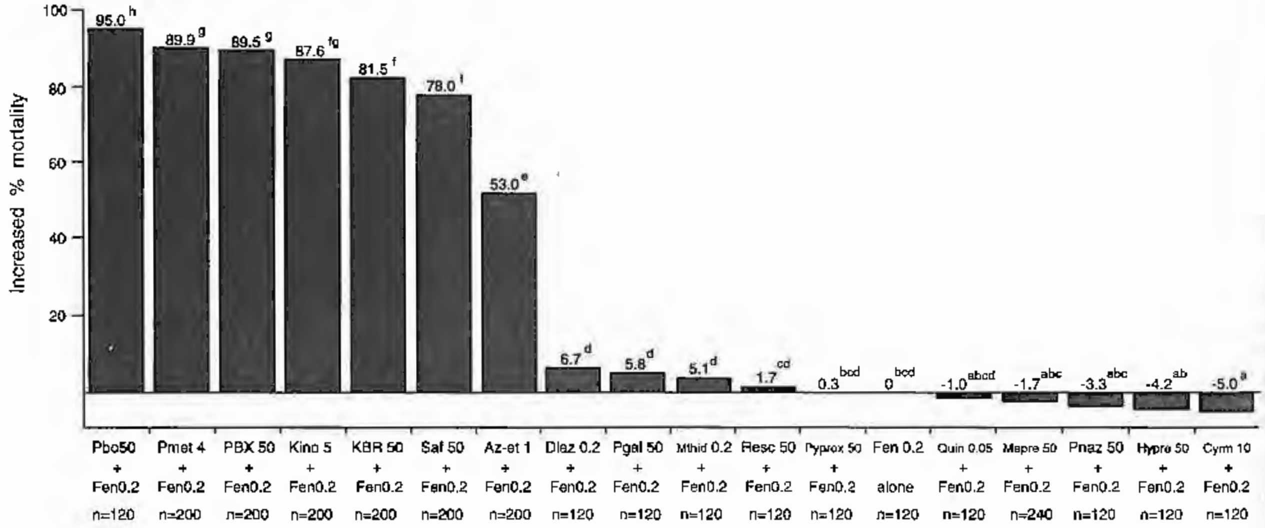


Fig. 45. Impact of various synergists on mortality at the fenvalerate discriminating dose (0.2 μg/larva) on 30–40 mg fourth instar mixed function oxidase pyrethroid resistant *Helicoverpa armigera.* Results expressed as the increased mortality (adjusted for any control mortality) over the base mortality with fenvalerate alone (5.0%). Synergists (μg/larva) applied topically in acetone, 5–15 minutes prior to fenvalerate. Treatments with the same letter are not significantly different at the 5% level (chi-squared test). n = number of larvae tested. Pbo - piperonyl butoxide, Pmet - phosmet, PBX - synthetic homologue of Pbo (ex Endura Spa, Italy), Kino - kinoprene, KBR - KBR 1417 thiophosphonate (Behrenz *et al.*, 1990), Saf - safrole, Az-et - azinphos-ethyl, Diaz - diazinon, Pgal - pyrogallol, Mthid - methidathion, Resc - resorcinol, Pyprox - pyriproxyfen, Quin - quinalphos, Mepre - methoprene, Pnaz - propiconazole, Hypre - hydroprene, Cyrm - cyromazine.

Table 29. The relative efficacy of two methylenedioxyphenyl synergists [piperonyl butoxide (Pbo) and a synthetic homologue (PBX, *ex* Endura Spa, Italy; refer to table 27 for structure)] in suppressing pyrethroid resistance in a strain of *Helicoverpa armigera* homozygous for a metabolic detoxification mechanism, fully suppressible by Pbo, presumably via a microsomal monooxygenase system. Larvae pretreated with Pbo or PBX doses 5–15 minutes prior to dosing with the fenvalerate discriminating dose (defined as the minimum fenvalerate dose needed to kill 100% of larvae after pretreatment with a *satis superque* dose of Pbo (50 µg/30–40 mg larva). Relative efficacy expressed as ED_{50} PBX ÷ ED_{50} Pbo. * indicates no overlap of Pbo and PBX 95% confidence intervals. Data adjusted for control mortality with the discriminating dose tested alone (8.3%).

Pyrethroid	Disc. dose µg/30–40 mg larva	ED_{50} µg/30–40 mg larva	95% Confidence interval		Slope	Relative efficacy PBX: Pbo
			Lower	Upper		
Pbo fenvalerate	0.2	0.68	0.561	0.831	1.7	
PBX fenvalerate	0.2	3.28	2.807	3.844	2.2	4.8* x less effective

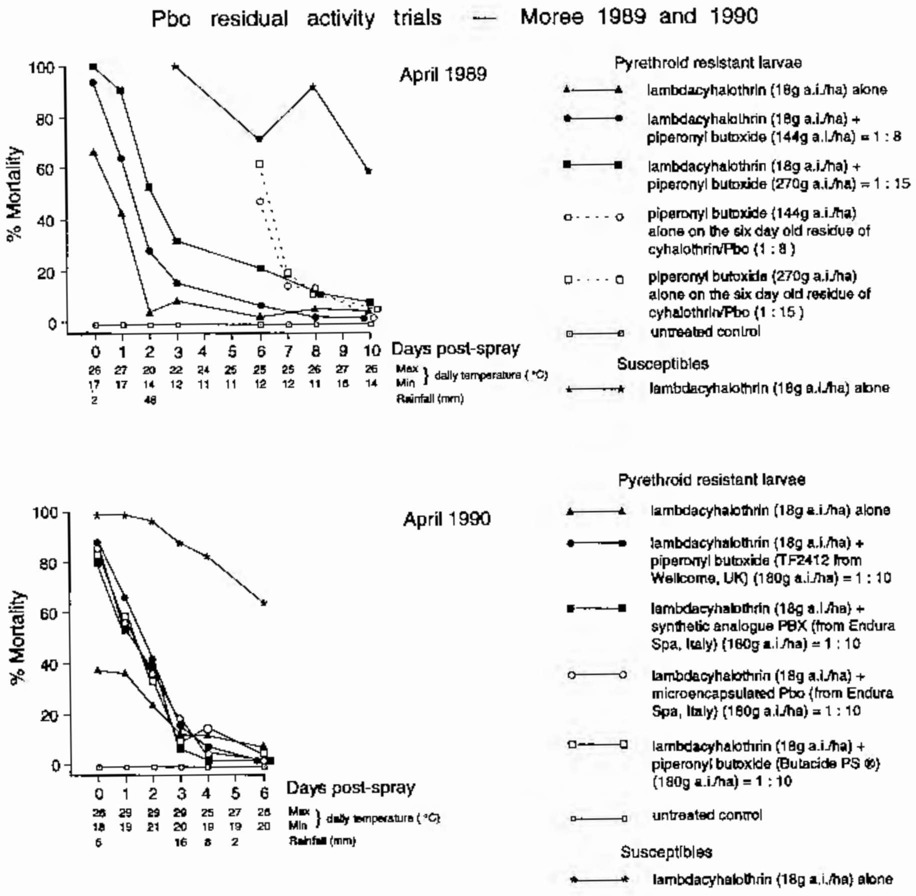


Fig. 46. Bioassay of early third instar (6.3 ± 0.3 mg) pyrethroid resistant {a strain homozygous for a resistance mechanism fully suppressible by piperonyl butoxide (Pbo)} and susceptible *Helicoverpa armigera* on fully expanded cotton leaves sprayed (and weathered) in the field with various lambdacyhalothrin and Pbo combinations. 1989 Pbo sprays all Waxalene Black formulation from ICI, UK. Larvae placed on 2.5 cm diameter leaf discs for 24 hours, then discs and larvae placed on artificial diet for a further 48 hours before assessment.

Formamidines

Chlordimeform hydrochloride 99.0% ex R. Gunning, NSW Agricultural Research Centre, Tamworth; amitraz 100% ex Schering, Sydney.

Organochlorines

Endosulfan, dieldrin (see Appendix 2); DDT 99.0% ex Shell, Melbourne.

Anti-oxidants

Hydroquinone 99.0%, catechol 99.0%, pyrogallol 99.0%, resorcinol 98.0% (all ex Aldrich Chem., Milwaukee, Wi, USA).

Solvents

Dodecane 99.0% ex Aldrich Chem., Milwaukee, Wi, USA; Codacide® oil (95% rape seed oil and 5% emulsifiers) ex Microcide Ltd, UK; corn, sunflower and olive oil (all 100% pure, purchased locally); kerosene, purity unknown, purchased locally; Thanite (isobornyl thiocyanoacetate) 90.0% ex Chem. Service, West Chester, Pa, USA.

Other

Kojic acid (2-hydroxymethyl-5-hydroxy-pyrone) 100% ex Sigma Chem., St Louis, Mo, USA.

Pbo: set rate versus ratio

The ability of Pbo to suppress pyrethroid resistance in a strain of *H. armigera* homozygous for a monooxygenase mediated metabolic detoxification mechanism, was tested for seven pyrethroids of variable potency (80-fold variation, with deltamethrin the most toxic to cycloprothrin the least, table 28). The ranking of activity in table 28 is the same as that for field use rates of these insecticides in cotton (table 45) (N.B. cycloprothrin is not shown in table 45 as it is not used in cotton). Cycloprothrin was included as it was desired to maximize the range in activity of compounds for the study and cycloprothrin was considered a relatively inactive pyrethroid with a high resistance factor (table 31). Larvae were pre-treated with varying Pbo doses 5-15 min prior to dosing with the relevant pyrethroid discriminating dose (table 28). This was defined as the minimum pyrethroid dose needed to kill 100% of larvae after pre-treatment with a *satis superque* dose of Pbo (50 μg/30-40 mg fourth instar larva). All the larvae within each pyrethroid test were treated with the same pyrethroid dose, only the Pbo dose was varied. Data were adjusted for any control mortality with the discriminating doses tested alone (all less than 12%). Bioassay techniques and analysis were as detailed in Appendix 2 except that only fourth instar 30-40 mg larvae were tested (same as for previous section). Because Pbo is not toxic in its own right (table 22), the data are expressed in ED_{50}s (effective dose) which is the dose of Pbo needed to suppress 50% of the metabolic pyrethroid detoxification capability in the monooxygenase mediated pyrethroid resistant *H. armigera* strain tested.

Relative synergistic activity of Pbo and a synthetic analogue

Laboratory screening of various synergists indicated significant activity of a synthetic analogue of Pbo. As the initial screening had tested only for presence/absence of synergistic activity and this compound (PBX from Endura Spa, Italy) was considered a possible replacement for the potentially limited natural product (Pbo), further tests were undertaken on the relative efficacy of these two compounds, using the same technique described in the previous section. Larvae were pre-treated with varying doses of Pbo or PBX 5-15 min prior to dosing with the fenvalerate discriminating dose (0.2 μg/30-40 mg fourth instar larva, table 29). Data were adjusted for control mortality with the discriminating dose alone (8.3%). Relative efficacy was expressed as the ED_{50} PBX ÷ ED_{50} Pbo.

Residual activity of Pbo

The residual activity of various Pbo formulations and rates was tested in two field trials at Moree, NSW, in the autumns of 1989 and 1990. Plots of a broadleaf cotton variety (Deltapine 90), sown on 1m row spacings, were sprayed with various insecticide/Pbo formulations in the field with a portable pressurized knapsack sprayer calibrated to deliver a spray volume of 50 l/ha. Each treatment area was 8 rows x 50 m long. Leaves were sampled randomly within each treatment area immediately after spraying and at daily intervals for 10 days (1989 trial) and six days (1990 trial). Care was taken to sample only leaves that were fully expanded at the time of spraying in order to avoid any growth dilution effects. The timing of the trials (early autumn) assisted greatly in this regard as the crops had 'cut out' (i.e. stopped rapid vegetative growth) by this time. Leaf deposits were weathered naturally in the field (post-spray temperature and rainfall given in figure 46). Sampled leaves were brought back immediately to the laboratory and leaf discs removed with a 2.5 cm diameter leaf punch. These discs were then positioned with forceps (adaxial surface upwards) flush at the bottom of the 7.5 ml (2.4 cm diameter) wells of the 12 well larval rearing trays described in Appendix 1. Early third instar larvae (average weight 6.3 ± 0.3 mg) of a pyrethroid resistant or susceptible strain of *H. armigera* were then placed on the leaf discs, one larva per disc (resistant strain homozygous for a resistance mechanims fully suppressible by Pbo). Whole leaves were then placed over the top of the 12 well rearing trays (adaxial surface downwards). The close fitting lid then held these top leaves in position exposing each larva to two treated leaf surfaces. Larvae remained exposed to the treated leaves for 24 h. Any live (or moribund) larvae were then removed from the treated leaves and placed singly on fresh artifical diet in 12 well rearing trays for a further 48 h before assessment. At the same time, a treated disc was placed about 5-10 mm above the diet (adaxial surface downwards) so that the larva had the opportunity to choose either feeding on the treated leaf or on untreated food. This was done to simulate the field conditions in cotton where larvae can avoid contacting spray deposits by remaining in protected feeding sites for a few days. Discs were rotated among larvae at 24 h so that larvae which had survived by then, were

Table 30. Statistical analysis (Generalised linear model regression, logit kill on log (days post spray +1)) of data from figure 46. Treatments compared by treatment contrasts from a generalised least square programme from NSW Agriculture, Biological and Chemical Research Institute. F-ratios very highly significant, *** (P <0.001) or non significant, ^{ns} (P <0.05).

Components of regression equations	Intercept (standard error)		Slope (standard error)	
1989 Trial treatments				
cyhalothrin alone	0.70	(0.251)	−4.85	(0.571)
cyhalothrin + Pbo (1:8)	2.57	(0.340)	−6.95	(0.706)
cyhalothrin + Pbo (1:15)	3.41	(0.342)	−6.14	(0.547)
Pbo respray (1:8)	−0.15	(0.245)	−4.74	(0.783)
Pbo respray (1:15)	0.43	(0.248)	−5.64	(0.782)
1990 Trial treatments				
cyhalothrin alone	−0.22	(0.228)	−2.45	(0.478)
cyhalothrin + Pbo (TF2412) (1:10)	2.42	(0.337)	−6.59	(0.696)
cyhalothrin + PBX (1:10)	1.80	(0.294)	−5.96	(0.630)
cyhalothrin + microencapsulated Pbo (1:10)	1.95	(0.304)	−6.02	(0.639)
cyhalothrin + Butacide (1:10)	1.77	(0.286)	−5.40	(0.599)

Treatment contrasts	F-ratios	
	Intercepts	Slopes
1989 Treatment comparisons		
cyhalothrin alone versus av. cyhalothrin + Pbo (1:8) & (1:15)	34.95***	1.26^{ns}
cyhalothrin + Pbo (1:8) versus cyhalothrin + Pbo (1:15)	17.06***	0.35^{ns}
Pbo respray (1:8) versus Pbo respray (1:15)	0.99^{ns}	0.21^{ns}
1990 Treatment comparisons		
cyhalothrin alone versus av. of all cyhalothrin + Pbo treatments	20.71***	23.65***
Between all cyhalothrin + Pbo treatments	0.68^{ns}	0.39^{ns}
1989 versus 1990 Treatment comparisons		
cyhalothrin + Pbo (1:8), 1989 versus av. of all cyhalothrin + Pbo treatments, 1990	0.46^{ns}	0.72^{ns}

exposed to discs which were known to have killed other larvae. This helped account for any possible variation in spray deposits.

There were five treatments in the 1989 trial:

1 & 2. Lamdacyhalothrin alone at the normal field rate (18 g a.i./ha, table 45) on both pyrethroid resistant and susceptible *H. armigera*.

3 & 4. Lambdacyhalothrin (18 g a.i./ha) + piperonyl butoxide, Waxalene Black formulation ex ICI, UK (144 or 270 g a.i./ha) on resistant larvae. (N.B. These treatments were resprayed on day six after the initial spray with the Pbo component only, figure 46).

5. An untreated control.

There were seven treatments in the 1990 trial:

1 & 2. Lambdacyhalothrin alone at the normal field rate (18 g a.i./ha, table 45) on both pyrethroid resistant and susceptible *H. armigera*.

3, 4 & 5. Lambdacyhalothrin (18 g a.i./ha) + piperonyl butoxide (180 g a.i./ha) on resistant larvae. Three separate Pbo formulations were tested:

a. Butacide PS®	-	the commercial standard (reportedly photostable) from ICI, Australia.
b. TF2412 formulation	-	a research formulation from Wellcome, UK.
c. Microencapsulated Pbo	-	a research formulation from Endura Spa, Italy.

6. Lambdacyhalothrin (18 g a.i./ha) + PBX (180 g a.i./ha) on resistant larvae.
7. An untreated control.

Four to six replications (each replicate being one tray of 12 larvae) were set up for each treatment for each sampling occasion. The number of replicates used depended on the day to day availability of suitably sized larvae and was usually five but never less than four. The percentage kill for each treatment over the sampling period for each year is shown in figure 46. The data were analysed on a Generalised Linear Model Least Square Program from the NSW Agriculture Biological & Chemical Research Institute, Sydney. Logit kill was regressed on log (days post spray +1). Components of the regression equations and F-ratios of selected treatment contrasts are given in table 30.

Results

Laboratory evaluation of synergists (summarized in table 25)

The pure breeding homozygous pyrethroid resistant colony was relatively stable in its phenotypic expression of resistance over time. Over 18 generations, there was only a 2.7-fold variation in LD_{50} while percentage survival at the discriminating dose ranged from a low of 81.2% in the F_6 to a high of 95.1% in the F_3 (table 23).

Methylenedioxyphenyl compounds

As expected, this group of compounds was the most effective, with three compounds equivalent in efficacy to Pbo. These were the synthetic analogue of Pbo from Endura Spa (PBX, fig. 39), the long chain sesamex (fig. 40) and the short chain methylenedioxy toluene (fig. 43). The polysubstituted PBX analogue was slightly less effective in a second test (fig. 45). The short chain methylenedioxy aniline (fig. 43) and safrole (fig. 45) were also moderately effective but the short chain piperonyl alcohol (fig. 43) was only marginally effective.

Organophosphates

Pbo was antagonistic to 10 of the 17 organophosphates tested in table 26 (parathion, quinalphos, profenofos, chlorpyrifos, sulprofos, fenitrothion, diazinon, methidathion, phosalone, trichlorphon) indicating that these compounds are bioactivated to more toxic oxon analogues by monooxygenases. Six compounds were unaffected by Pbo (monocrotophos, azamethiphos, azinphos-ethyl, fenthion, triazophos, pyrazophos) and one (phosmet) was strongly synergized indicating significant oxidative detoxification of this compound in both susceptible and resistant larvae (table 26). One of the hopes in this study was to find an organophosphate compound which was bioactivated by the monooxygenase/s in the resistant *H. armigera* strain, so that the organophosphate would be more toxic on the resistant strain. However, only two of the 10 bioactivated organophosphates in this study had negative cross resistance (sulprofos and methidathion) and then only at low levels (0.7 & 0.6 resistance factors, respectively, table 26). Pyrazophos, which showed no evidence of bioactivation, was the only other compound which had negative cross resistance and this was quite pronounced (0.1 resistance factor, table 26). Six organophosphate compounds (parathion, fenitrothion, azinphos-ethyl, fenthion, phosalone, trichlorphon) had low but significant resistance factors (range 1.6 to 2.3) indicating a low incipient level of organophosphate resistance in this strain.

In the synergist screens, most organophosphates tested (12 out of 18) were either ineffective (parathion, profenofos, chlorpyrifos, sulprofos (all fig. 38), trichlorphon, azamethiphos, monocrotophos (all fig. 44), quinalphos, diazinon, methidathion (all fig. 45)) or only marginally effective (fenitrothion, triazophos (both fig. 44)). Five compounds had intermediate to moderate activity (pyrazophos, phosalone, fenthion (all fig. 44) and azinphos-ethyl and the thiophosphonate synergist KBR1417 (both fig. 45)). Interestingly, the N-alkyl phthalimidomethyl organophosphate, phosmet gave very high synergism, falling just short of significance for full effectiveness (fig. 45).

Pyrethroid analogues

Two of the analogues tested (flucythrinate, cycloprothrin) were ineffective while the third (silafluofen) had only marginal activity at some rates (fig. 42).

Acetylenic compounds

As expected, these compounds were generally very effective synergists with two compounds equivalent in efficacy to Pbo. These were the N-alkyl/acetylenic hybrid propargyloxyphthalimide (fig. 43) and the propynyl ether TCPB (fig. 39). The propynyl ester CGA 84708 and the juvenoid with a terminal acetylenic bond (kinoprene) also had very high activity (figs 40 & 45, respectively). The miticidal propynyl sulphite propargite had similar intermediate activity in two separate tests (figs 38 & 41) while its metabolic breakdown product propargyl alcohol (Knowles & Hamed, 1990) was equally effective (fig. 41).

N-alkyl compounds

Warf antiresistant and the dicarboximide fungicide procymidone were both ineffective (fig. 38) while the closely related iprodione carboxamide fungicide had only marginal activity (fig. 38). The best known N-alkyl synergist MGK 264 had only marginal activity in two separate tests (figs 38 & 39).

Esterase, glutathione transferase inhibitors

DEM (a glutathione s-transferase inhibitor) was ineffective in two separate tests (figs 38 & 43). TPP (a carboxylesterase inhibitor) was ineffective in two tests (figs 38 & 39) and only marginally effective in a third (fig. 43). DEF (an inhibitor of esterases and the glutathione s-transferase system) was marginally effective in two separate tests (figs 38 & 39).

Nitrogen heterocycles

The imidazole (prochloraz) and triazole (triadimefon) fungicides were only marginally effective (figs 41 & 43, respectively). All other nitrogen heterocycles tested were ineffective, including the triazole fungicides (diclobutrazol and propiconazole, figures 38 & 45, respectively), metyrapone (fig. 40) and its imidazole and triazole analogues (NK III and NK IV, respectively, figure 41), the insect growth regulator cyromazine (fig. 45) and the plant growth regulator mepiquat chloride (fig. 41).

Juvenile hormone and analogues

The natural juvenile hormone III was ineffective (fig. 44) as were the synthetic analogues (juvenoids) methoprene, hydroprene and pyriproxyfen (all fig. 45). The juvenoid fenoxycarb was only marginally effective (fig. 44). The only juvenoid shown to have any significant synergistic activity was kinoprene and, as suggested previously, this is most likely due to the presence of a terminal acetylenic moiety.

Formamidines

Both chlordimeform and amitraz were ineffective at the two doses tested (fig. 40).

Organochlorines

Endosulfan showed a marginal synergistic activity but with no clear rate respnose (fig. 41). Surprisingly, both dieldrin and DDT displayed intermediate synergistic activity with a clear rate response (figs 41 & 43, respectively).

Anti-oxidants

All anti-oxidants tested were ineffective including hydroquinone and catechol (fig. 43) and pyrogallol and resorcinol (fig. 45).

Solvents

Dodecane was the only solvent which improved the kill compared to the normal acetone solvent and then only marginally (fig. 41). This was confirmed in the full bioassay study on pyrethroid resistant larvae (table 24) where the use of dodecane as a solvent in place of acetone lowered resistance factors, slightly but significantly, from 32.5x to 21.2x However, at the same time, there was no significant difference between acetone and dodecane in the susceptible strain.

All the other solvents proved no different from acetone including: Codacide oil (fig. 38) and Thanite (fig. 40) when used both as pre-mixes or when applied separately 5-15 min before the fenvalerate/acetone mix; corn, sunflower and olive oil as pre-mixes (fig. 39) and kerosene as a pre-mix in two separate tests (fig. 39).

Other

The fungal metabolite kojic acid was an ineffective synergist (fig. 39).

Pbo: set rate versus ratio

The Pbo ED50s varied between 0.3 to 2.02 µg/larva (6.7-fold variation) with no indication of any relationship between Pbo efficacy and pyrethroid potency, which varied 80-fold (0.0125 to 1.0 µg/larva) (table 28). When expressed as a ratio of Pbo : pyrethroid, there was a clear trend from a maximum ratio of 162:1 for the most active pyrethroid (deltamethrin) to a minimum of 1:1 for the least active pyrethroids (fluvalinate and cycloprothrin). Clearly, a set amount of Pbo was necessary to overcome the metabolic pyrethroid detoxification capacity in larvae and this was independent of pyrethroid activity.

Relative synergistic activity of Pbo and a synthetic analogue

The Pbo ED50 in this test (0.68 µg/larva, table 29) was not significantly different from that obtained in the previous test (0.54 µg/larva, table 28) done some 10 months earlier. This confirms the consistency of bioassay results over time noted previously for the pure breeding homozygous pyrethroid resistant colony (table 23). Based on comparison of ED50s, the synthetic analogue (PBX) was 4.8-fold less effective than the natural product (Pbo) in suppressing monooxygenase mediated pyrethroid resistance in this strain of *H. armigera.*

Residual activity of Pbo

The pyrethroid alone (lambdacyhalothrin 18 g a.i./ha) performed as expected on the susceptible strain giving total control initially and then declining only slowly to 65-70% kill at six days post-spray in both seasons with still good control (60% kill) at 10 days post-spray in the 1989 trial (fig. 46). There was no mortality in the untreated control in either year, indicating no problems with spray drift or over spraying. The pyrethroid alone killed some pyrethroid resistant larvae while the deposits were still fresh (66% and 38% kill immediately after spraying in 1989 and 1990, respectively) but this dropped off as the deposits weathered, particularly in the 1989 season. The addition of Pbo to the pyrethroid significantly improved kill over the pyrethroid alone in both seasons with the higher rate of Pbo (270 g a.i./ha) giving significantly longer residual than the lower rate (144 g a.i./ha) (table 30). For example, by two days post-spray, the pyrethroid alone was giving only 5% kill, while the addition of the low and high rates of Pbo improved kill to 28% and 52% kill, respectively (1989 data, fig. 46). By six days post-spray, the low rate of Pbo had also become ineffective but the high rate was still giving some, albeit reduced, measure of control (approx 20% kill, fig. 46). At the same time, the pyrethroid alone was still giving 70% kill on the susceptible strain, so it was suspected that the problem lay with rapid breakdown of the Pbo residues. To confirm this, at six days post-spray, the high and low Pbo treatments were resprayed with the same rates of Pbo without the pyrethroid. Control was immediately restored by both rates of Pbo to levels similar to the pyrethroid alone on susceptibles (no significant difference between rates, table 30). However, the impact was only temporary as any benefit from the extra Pbo had quickly dissipated by one to two days post-respray (fig. 46).

The first year's trial indicated a problem with rapid breakdown of Pbo residual activity but that this could be overcome partially by increasing the rate of Pbo. However, other options would be to extend the Pbo residual activity by either improving formulations or developing more stable synthetic analogues. These options were studied in the second year where two research formulations of Pbo and a synthetic analogue were compared to the standard, purportedly photostable, commercial formulation (Butacide PS®). All formulations of Pbo (and the synthetic analogue PBX) were used at the set rate of 180 g a.i./ha. The results indicated no difference between any of the treatments and their residual activity was no different to the Waxalene Black Pbo formulation (144 g a.i./ha) used in the 1989 trial (table 30) despite the slightly higher post-spray temperatures for the 1990 trial (on average 5°C hotter for the three days immediately after spraying, figure 46). The 48 mm of rain which fell on the second day of the 1989 trial had no significant impact on residual activity of either the pyrethroid alone (on susceptibles) or the Pbo (fig. 46), indicating a useful degree of rainfastness.

Discussion

Laboratory evaluation of synergists

The stability of the phenotypic expression of resistance in the pure breeding, homozygous, monooxygenase mediated pyrethroid resistant colony was considered satisfactory to allow comparison of the activity of synergists tested over a number of generations. The small changes in bioassay response over time recorded for the resistant strain in this study (2.7-fold variation in LD_{50} over 18 generations) are considered satisfactory given the potential problems of induction of monooxygenases by even small changes in diet (Perry *et al.*, 1971; Conney & Burns, 1972; Collins, 1985; Forrester, unpublished data) and suppression by sub-lethal infections of common laboratory rearing contaminants such as cytoplasmic polyhedrosis viruses (Brattsten, 1987b).

Methylenedioxyphenyl compounds

Methylenedioxyphenyl (MDP) compounds have been known as oxidative synergists since the work of H. L. Haller and associates in 1942 when they isolated sesamin from sesame oil and demonstrated its activity as a pyrethrum synergist (Beroza & Barthel, 1957). Since then, many reviews and studies have confirmed this activity (e.g. Casida, 1970; Wilkinson & Brattsten, 1972; Wilkinson *et al.*, 1972; Wilkinson, 1979, 1983; Hodgson, 1983; Agosin, 1985). In this study, the long chain natural and synthetic MDP compounds were all effective synergists while the short chain MDPs (except 3,4-methylenedioxytoluene) were generally less effective. This was also found by Casida (1970) who suggested: 'It appears that a long, polyether or oxygen containing side chain is ideal for pyrethroid synergism'. However, in a recent study on selective MDP monooxygenase inhibitors, Motoyama *et al.* (1990) found a number of selective inhibitors, with somewhat simpler structure than Pbo, that were quite effective pyrethroid synergists. This last study has probably been the most significant advance in research on monooxygenase synergists since the discovery by Haller and his associates of MDP synergist activity in 1942 (Haller *et al.*, 1942). Motoyama *et al.* (1990) have identified very effective *in vivo* resistance breaking MDP synergists with desirable selectivity between insects and mammals and which are effective with a number of chemical groups on a broad range of insect species. They have also gone on to give super-computer predictions of MDP compounds that could have even greater activity than Pbo and hopefully, perhaps, even photostability.

Organophosphates

It is well known that many organophosphate insecticides are bioactivated by monooxygenases through desulphuration to more toxic oxon analogues (Dittrich *et al.*, 1979; Kono *et al.*, 1983; Feng, 1984; Kulkarni & Hodgson, 1984; Sun *et al.*, 1985; Levi *et al.*, 1988; Campanhola & Plapp, 1989b; Brown, 1990; Chen, 1990). Seeking to exploit this, some workers have suggested searching for negatively correlated cross resistance where the oxidatively resistant strain metabolizes another insecticide (usually an organophosphate) to a more active toxicant at a greater rate than the susceptible strain (Casida, 1970; Wilkinson, 1983; Brattsten *et al.*, 1986). While excellent in theory, only a very few cases of negative cross resistance between pyrethroids and organophosphates have ever been documented (e.g. Kassai & Ozaki, 1984; Campanhola & Plapp, 1989a). This situation was also found in this study where only three out of the 17 organophosphates tested (sulprofos, methidathion and pyrazophos) were found to have negative cross resistance. The results with sulprofos and methidathion were not surprising as these compounds both contain three sulphur atoms (table 26) and the multiple sulphur containing organophosphates are known as better candidates for bioactivation (Schuphan & Casida, 1983). However, the result for pyrazophos is not consistent with this explanation as it contains only one sulphur atom and is not bioactivated. When used as synergists, only one of these compounds (pyrazophos) indicated any significant activity. Other organophosphates which displayed useful synergistic activity included fenthion, azinphos-ethyl, phosalone, KBR 1417 and particularly, phosmet. KBR 1417 is a known oxidase inhibitor (Behrenz *et al.*, 1990) and the result with phosmet is not altogether surprising, as it has a N-alkyl phthalimidomethyl grouping which has been shown to have oxidase inhibiting ability in other compounds (see later discussion). However, no explanation can be offered for the encouraging results for fenthion, azinphos-ethyl and phosalone except perhaps for phosalone where the active sulphur released during bioactivation, could bind to and inhibit the cytochrome P-450 detoxification enzyme/s (Kulkarni & Hodgson, 1984; Levi *et al.*, 1988).

Of the 18 organophosphates screened for oxidative synergistic activity in this study, only five compounds (phosmet, pyrazophos, fenthion, azinphos-ethyl and phosalone) warrant further investigation. These compounds are already commercially developed for other purposes and could find useful utility in combination with pyrethroids for management of pyrethroid resistance and perhaps even control of co-incident secondary pests. On the other hand, KBR 1417, although a reasonably effective oxidase inhibitor, is only an experimental compound and probably will not warrant the cost of commercial development.

Pyrethroid analogues

Analogue synergism is the use of mixtures of an insecticide and a structurally similar analogue, so that the analogue acts as a preferential suicide substrate for the detoxifying enzyme, thus protecting the more active partner.

This concept was used extensively to counter DDT dehydrochlorinase mediated DDT resistance (e.g. DMC and F-DMC, Moorefield & Kearns, 1955) and has been also suggested for other groups (Casida, 1970; Wilkinson, 1979). Three relatively highly resisted pyrethroids were chosen as possible analogue synergists in this study; cycloprothrin, flucythrinate and silafluofen (resistance factors 534, 78 and 34, respectively, figure 48). Surprisingly, none showed any significant activity, even the very highly resisted cycloprothrin and there seems little incentive to pursue this line of research further.

Acetylenic compounds

Propynyl ethers and esters have long been known as effective oxidase synergists with a similar spectrum of activity as MDP synergists (Sacher *et al.*, 1968; Casida, 1970; Wilkinson & Brattsten, 1972; Wilkinson *et al.*, 1972; Wilkinson, 1979, 1983; Golenda & Forgash, 1985; Brown & Payne, 1986; Cheng *et al.*, 1986; Cheng, 1988). These were also effective in this study (TCPB and CGA 84708) as well as the propynyl phthalimide and a juvenoid with a terminal acetylenic moiety (kinoprene). Given the lack of synergistic activity of the other juvenoids (and the natural juvenile hormone JH III) (see later discussion), it is clear that the active synergistic component of the kinoprene molecule is the terminal acetylenic moiety. The moderate synergistic activity of the propynyl sulphite propargite is very interesting as it is registered as a miticide in cotton and is being increasingly used in the Stage II pyrethroid window, often in combination with a pyrethroid. It is used at a relatively high rate (1500 g a.i./ha) and has some residual activity. Although not as active as the other propynyl compounds, the moderate synergistic activity of propargite should be studied further as the lack of full activity could be compensated for by the high dose rate and increased residual. The similar activity of propargite and its metabolic breakdown product (propargyl alcohol, Knowles & Hamed, 1990) confirms that the active synergistic component of these compounds is indeed the acetylenic moiety. This is particularly relevant to the discussion on the impact of inserting the acetylenic moiety into pyrethroids for potential oxidative resistance breaking activity (see Section 10).

It is interesting to note that Bohlmann (1988) recorded approximately 700 naturally occurring acetylenic compounds in plants, particularly in the Compositae. Su (1985) also noted that anacyclin (a relatively non-toxic lipid amide with two acetylenic moieties) co-occurs with the quite toxic lipid amide pellitorine in *Anacyclus pyrethrum*. Perhaps some of these naturally occurring acetylenes are attendant oxidase inhibitors for metabolically labile botanical insecticides as is the case for the methylenedioxyphenyl synergist sesamin, which co-occurs with natural pyrethrins/cinerins in *Chrysanthemum cinerariaefolium* (Casida, 1970; Krieger *et al.*, 1971). If this is the case, then these naturally occurring acetylenes may be useful models for designing oxidase synergists or for even indicating the presence of possible accompanying hitherto unknown insecticides.

N-alkyl compounds

N-alkyl compounds are known to be effective oxidase synergists in many insect species (Casida, 1970; Wilkinson & Brattsten, 1972; Wilkinson *et al.*, 1972; Wilkinson, 1979; Raffa & Priester, 1985; Cochran, 1987). The best known N-alkyl synergist is probably MGK 264 which appears to give only partial suppression of monooxygenase mediated pyrethroid and benzoylphenylurea resistance in *Plutella xylostella* where, at the same time, Pbo gives full suppression (Cheng *et al.*, 1986 and Perng *et al.*, 1988, respectively). The same situation (full suppression by Pbo and only marginal suppression by MG 264) seems to be the case also for this study on monooxygenase mediated pyrethroid resistance in the related lepidopteran *Helicoverpa armigera*. None of the other N-alkyl compounds tested (Warf antiresistant, procymidone or iprodione) worked any better than MGK 264.

Organochlorines

Surprisingly, DDT and the two cyclodiene insecticides dieldrin and endosulfan proved to be oxidase synergists of intermediate activity. This was unexpected as there is no obvious biochemical basis to explain this synergistic action. However, there have been two cases in the literature where cyclodienes have synergized pyrethroids in susceptible insects (toxaphene on *Heliothis virescens* and *Spodoptera frugiperda* (Brown *et al.*, 1982) and endrin on *S. littoralis* (El-Guindy *et al.*, 1981)). There has also been one reported case of a cyclodiene synergizing a pyrethroid in a resistant insect (endosulfan on pyrethroid resistant *S. littoralis* (Kern *et al.*, 1990)). This latter report particularly, prompted the assay of endosulfan as a potential synergist in this study and when activity was confirmed, a search of the literature revealed a possible explanation. Cyclodienes have been recorded as strong inducers of monooxygenases (dieldrin (Conney & Burns, 1972; Wilkinson & Brattsten, 1972) and endosulfan (Egaas *et al.*, 1988)) and it is known from work on other biphasic inducing agents that these stimulatory effects are often only transient and that these inducers can also act later as partial inhibitors (Rivière, 1983; Rivière *et al.*, 1984; Mougin *et al.*, 1991). If this indeed was the explanation for the intermediate synergistic activity of endosulfan in Kern *et al.*'s (1990) study, then other strong monooxygenase inducers (e.g. dieldrin) should also behave similarly. A search of the literature revealed DDT also as a strong inducer of monooxygenases (Conney & Burns, 1972; Rivière, 1983) as well as some other compounds, e.g. prochloraz, TPP and DEF (see later discussion). The intermediate synergistic activity of the strong monooxygenase inducers DDT and dieldrin recorded in this study, confirmed the biphasic induction/inhibition theory as a possible explanation for their activity.

The practical significance of these findings is probably limited as DDT and dieldrin are of course no longer options for use and endosulfan, having the lowest synergistic activity of the organochlorines tested, may not give satisfactory results in the field. Also, the use of pyrethroid/endosulfan mixtures in Stage II is not a desirable option as it would put extra selection pressure on endosulfan (see Section 2). For these reasons, oxidative synergism by organochlorines is seen more as a theoretical curiosity than as a practical solution to counter oxidative pyrethroid resistance.

Esterase, glutathione transferase inhibitors

Of the three inhibitors tested, only DEF (and TPP in one out of three tests) indicated some marginal synergist activity. McCaffery *et al.* (1989b) found no effect of DEF on pyrethroid resistance in *Heliothis virescens* but other workers have found DEF to give minor synergism of predominantly oxidative pyrethroid resistance (*Plutella xylostella* (Chen & Sun, 1986), *Leptinotarsa decemlineata* (Argentine *et al.*, 1989a), *Chrysoperla carnea* (Pree *et al.*, 1989), *Tribolium castaneum* (Collins, 1990)). Minor synergism by DEF in such situations is usually interpreted as meaning mixed resistance mechanisms, predominantly oxidative but with a small contribution from esterases. However, the same situation (high Pbo activity, minor DEF activity) was also encountered in this study using a pure breeding monooxygenase mediated pyrethroid resistant strain with no evidence of esterases as a resistance mechanism (Pbo fully effective and most organophosphates ineffective as synergists). Three possibilities are put forward as alternative explanations for the minor synergistic activity of the esterase inhibitor (DEF) on a pure breeding oxidative resistant strain of *Helicoverpa armigera*:

1. DEF is not completely specific for esterases as it is known to also block oxidases at high concentrations (Attia *et al.*, 1980; Scott, 1990).
2. DEF is a receptor agonist which has a high affinity for the receptor protein which recognizes xenobiotics (Plapp, 1986).
3. DEF is an inducer of monooxygenases (Perry *et al.*, 1971) and therefore is also a potential partial inhibitor (see previous discussion).

This latter point may also help explain the partial synergistic activity of TPP in this study, as TPP has also been shown to induce monooxygenases (Perry *et al.*, 1971; Conney & Burns, 1972).

Nitrogen heterocycles

Various nitrogen heterocycles (especially imidazoles and thiadiazoles) have been shown to bind directly to the heme moiety of cytochrome P-450 and have been recorded as oxidase synergists (Casida, 1970; Wilkinson & Brattsten, 1972; Wilkinson *et al.*, 1972; Wilkinson, 1979, 1983; Hodgson, 1983). In this study, a number of imidazole and triazole ergosterol biosynthesis (monooxygenase) inhibiting fungicides (prochloraz, triadimefon, diclobutrazol and propiconazole) were tested for synergistic activity in *H. armigera*. However, they were ineffective with only prochloraz and triadimefon having some minor activity. The result for prochloraz is not surprising as this compound has been documented as a potent inducer of cytochrome P-450 monooxygenases in plants and animals (Rivière, 1983; Rivière *et al.*, 1984; Johnston *et al.*, 1989; Mougin *et al.*, 1991) and therefore is also a potential partial inhibitor (see previous discussion). However, the partial activity of triadimefon was not expected as it was not recorded as a monooxygenase inducer in Japanese quail *Coturnix japonica* Temminck & Schlegel (Phasionidae) (Rivière *et al.*, 1984) whereas propiconazole (an ineffective synergist in this study) was an inducer along with prochloraz. This indicates the difficulty in extrapolating induction spectra between species.

The nitrogen heterocycle metyrapone is a well known vertebrate monooxygenase inhibitor which also has good *in vitro* inhibitory action in some insects (Bélai *et al.*, 1988). The same authors suggested that B-pyridine ring imidazole and triazole analogues of A-phenyl metyrapone (NK III & NK IV, respectively) were good *in vitro* inhibitors of microsomal cytochrome P-450 and may be useful insecticide synergists. On the basis of this prediction, these compounds were tested as oxidase synergists on *H. armigera* but were found to be ineffective. This negative result could well reflect the *in vivo* topical application method used in this study (*in vitro* in Bélai *et al.*, 1988) which highlights any problems with cuticular penetration, metabolic stability or distribution of potential synergists. However, as mentioned previously, the choice of the topical application over an alternative contact/ingestion test method was intentional in order to screen for good contact activity rather than for stomach poisons which have limited utility in rapidly growing crops with protected feeding sites, such as cotton (see methods and materials this Section).

The nitrogen heterocycle plant growth regulator (mepiquat chloride) was tested for synergistic activity, as it is being increasingly used in cotton. The nitrogen heterocycle insect growth regulator (cyromazine) was also tested because of its success in controlling insecticide resistant sheep blowfly (*Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae)). However, neither compound exhibited any contact synergistic activity.

Juvenile hormone and analogues

As well as detoxifying xenobiotics, monooxygenases are responsible for crucial steps in the biosynthesis of a number of insect hormones, e.g. juvenile hormone, precocenes and ecdysone (Hodgson, 1985). Juvenile hormone and its analogues have been shown to form a stable complex with cytochrome P-450, the same as for Pbo (Agosin, 1985). Juvenoids have also been shown to be suitable substrates for monooxygenases selected for by exposure to other insecticides (Plapp & Vinson, 1973; Hammock *et al.*, 1977). These findings suggested the use of these compounds as possible monooxygenase inhibitors. Consequently, the natural juvenile hormone (JH III) and five synthetic analogues (methoprene, hydroprene, pyriproxyfen, fenoxycarb and kinoprene) were tested for synergistic activity in *H. armigera*. However, only kinoprene expressed significant activity and, as discussed earlier, probably because of the presence of a terminal acetylenic moiety rather than any intrinsic juvenoid activity.

Formamidines

Chlordimeform (less so amitraz) has been shown to be a monooxygenase inhibitor (Plapp, 1976b, 1979; Dittrich *et al.*, 1981; Kamel *et al.*, 1987). It is also known to act as a substrate for monooxygenases and is bioactivated to more potent octopamine mimics (Knowles &

Schuntner, 1974; Brown, 1990) although Davenport & Wright (1985) suggest this only happens in ticks (Acarina: Ixodidae) and fireflies (Coleoptera: Lampyridae) with no such activity detected in Lepidoptera. Chlordimeform has been shown to be a pyrethroid synergist in pyrethroid resistant *Heliothis virescens* (no synergism found with amitraz) (Brown & Payne, 1986), susceptible and pyrethroid resistant *H. virescens* (Jensen, 1983; Campanhola & Plapp, 1989b) and in susceptible and pyrethroid resistant *Musca domestica* (amitraz also) (Liu & Plapp, 1990). However, chlordimeform did not synergize fenvalerate *in vivo* in the mouse (*Mus domesticus* Rutty (Muridae)) (Gaughan *et al.*, 1980).

Formamidines also have distinct behavioural modifying properties and have been shown to significantly enhance locomotory activity in *H. virescens* larvae (Benedict *et al.*, 1990). This increased locomotion can significantly increase the pick up of toxicant in self dosing residual assays and could be a confounding factor in claims of synergistic activity of formamidines using this assay technique (e.g. Campanhola & Plapp, 1989b). In this study, it was aimed to avoid this problem by administering both the formamidines and fenvalerate topically so that the toxicant dose was fixed and independent of any possible behavioural effects. As expected, both formamidines elicited a typical hyperactivity response in larvae in these tests, indicating satisfactory intoxication. However, neither formamidine indicated any sign of synergistic activity, suggesting that previous claims of pyrethroid synergism by formamidines were possibly more through a behavioural effect than any real biochemical effect.

Anti-oxidants

Methylenedioxyphenyl (MDP) synergists are said to act by binding at the methylenedioxy moiety and cleaving to yield an active carbene which links to the heme of the cytochrome P-450 to form a highly stable complex (Casida, 1970; Wilkinson *et al.*, 1983; Marcus *et al.*, 1987). However, an alternative explanation for MDP synergistic action is that the catechol degradation products, released on reaction of the MDP compound with the cytochrome P-450 dependent monooxygenase system, compete with the insecticide at some limiting stage of metabolism (Casida, 1970). Catechol (and the closely related anti-oxidants resorcinol, hydroquinone and pyrogallol) have also been shown to slow the breakdown (photolysis) of pyrethrum films (Glynne Jones, 1960). However, in this study, none of these four anti-oxidants indicated any sign of synergist activity. This tends to support the former explanation for the mode of action of MDP synergists, also suggested in Casida (1970).

Solvents

Most well known synergists, including Pbo, are fat soluble and therefore favour penetration through the insect cuticle (Gast, 1961; Casida, 1970). For example, Pbo has been recorded as a penetration synergist in susceptible *Spodoptera frugiperda* (Gist & Pless, 1985), in susceptible *S. littoralis* (Christie & Wright, 1990), in susceptible and resistant *Musca domestica* (De Vries & Georghiou, 1981b; Ahn *et al.*, 1988) and in susceptible *S. eridania* (Cramer), but not *H. virescens* (Anderson *et al.*, 1986). On the other hand, Pbo was not found to be a penetration synergist in pyrethroid resistant *Musca domestica* (Golenda & Forgash, 1985; Macdonald *et al.*, 1985; Bull & Pryor, 1990). Because of this possible dual mode of action of synergists, one should be careful to account for the possibility of purely physical penetration synergism when screening for biochemical synergist activity. This problem was probably first noted by Sun & Johnston (1972) who coined the term 'quasi-synergism' to describe the increased cuticular penetration component of total synergism. They suggested using two alternative solvent systems (a 5% kerosene mix in acetone and Thanite®) to negate the possible improved cuticular penetration of lipophilic synergists such as Pbo. Both these solvents were tested in this study along with Codacide (rapeseed), corn, sunflower and olive oil and the organic solvent, dodecane. None of the solvents (except dodecane) was effective in improving the kill of the fenvalerate discriminating dose indicating that the mode of action of Pbo and the other synergists in this study is principally true biochemical inhibition and not improved penetration (quasi-synergism). The result with dodecane is interesting as this solvent has been documented as a penetration synergist in both susceptible and resistant *Musca domestica*, but with a 2.5-fold greater effect in the resistant strain (Sawicki & Lord, 1970). A similar situation was found in this study with some marginal improvement in the kill in the resistant strain (both in the discriminating dose test and the full bioassay) but with no significant effect in the susceptible strain. Thus, dodecane may be a preferential penetration synergist in the resistant strain and the possibility of Pbo acting in a similar manner (as well as classical monooxygenase inhibition) is discussed in Section 10.

Other

Kojic acid is a secondary fungal metabolite that is commonly produced by many species of *Aspergillus* and *Penicillium*. It has been shown to inhibit monooxygenases and has been suggested as an inexpensive alternative for methylenedioxyphenyl synergists (Dowd, 1988a, 1988b) but was ineffective in this study.

Pbo: rate, synthetic analogues, residual activity

The synergist screens indicated some potential alternative oxidase synergists for further study. However, in the short term, Pbo will be developed as the synergist for field use. The first question that needed to be answered was, what rate should be used? and then, should this vary in ratio to the activity of the pyrethroid? This study quite clearly indicated that a set amount of Pbo should be used, irrespective of the activity of the accompanying pyrethroid (also suggested by Silcox *et al.*, 1985). The residual activity trial indicated poor residual activity of Pbo but that this could be partially overcome by increasing the rate. The top rate of Pbo used in this study was 270 g a.i./ha which was only 50% effective at 2 days. It was shown that a straight Pbo spray applied onto a weathered pyrethroid deposit could restore control temporarily but the quick drop off in activity and growth dilution in the field would probably jeopardize the utility of this concept for field use. Pbo is currently registered in Australia for use as a pyrethroid synergist at a set rate of 250-350 g a.i./ha. It is recommended to be sprayed early evening or at night to maximize its impact on nocturnal moths and to delay breakdown in sunlight (Shaw, 1991). The attempts to increase the residual activity of Pbo by modifying the formulation were unsuccessful in this study. However, this area of research is considered potentially fertile and further research on more residual formulations should be strongly encouraged.

The synthetic analogue PBX was slightly less intrinsically active than the natural product (Pbo) but when tested in the field against Pbo at the same rate, was equivalent in efficacy and residual to both the standard commercial and research Pbo formulations. This finding is quite important as the natural product is in limited supply, so the development of a cost-effective and hopefully, photostable synthetic analogue is considered critical if oxidative synergists are to be used in significant quantities in large scale field crop IRM strategies.

Pbo use strategy

As mentioned previously, the use of synergists against resistant insects in the field has been far from successful due to several reasons, but principally due to *de novo* resistance to the synergist mixture. Most examples of resistance to synergistic mixtures reported in the literature have been with DDT (e.g. DDT/chlorfenethol on houseflies and mosquitoes (March *et al.* (1952), Brown (1967), respectively); DDT/Warf antiresistant on houseflies and mosquitoes (Pillai *et al.* (1963), Forgash (1964), respectively) and DDT/toxaphene on *Helicoverpa armigera* (fig. 1 and Wolfenbarger *et al.*, 1981)). However, there have also been three reports of resistance to synergistic mixtures involving Pbo (with carbaryl on houseflies (Georghiou, 1962); with pyrethroids on Colorado potato beetle (Forgash, 1984) and with pyrethroids on diamondback moth (Chen & Sun, 1986)). The resistance to synergistic mixtures can be through the development of alternative nerve insensitivity mechanisms (Ranasinghe & Georghiou, 1979; Metcalf, 1989) but mostly through resistance to the synergist itself (e.g. metabolic degradation) or to its mode of action (Moorefield & Kearns, 1955; Nicholson & Sawicki, 1982; Silcox *et al.*, 1985; Chen & Sun, 1986; Hung & Sun, 1989).

Historical problems with resistance to synergistic mixtures clearly indicated the need for careful use of Pbo within the Australian IRM strategy. The recommendation to use Pbo as a pyrethroid synergist was first incorporated into the Australian strategy in 1990/91 season. Right from the introduction of Pbo, precautions were taken to avoid its overuse. It was strongly recommended that Pbo be used only once per season on one only of the three pyrethroids recommended during the Stage II window, preferably with the last or second last pyrethroid spray (Shaw, 1991). The impact of this use is being closely followed and will be reported elsewhere. However, because of the severity of the pyrethroid resistance problem in *H. armigera* in Australia, there is a real need to develop a second synergist to rotate with Pbo. The first pyrethroid applied during Stage II usually works well enough alone but the second and third pyrethroids are increasingly requiring supplementation. This study of course, indicated some potential alternative oxidase synergists to fulfil this role. However, in the meantime, *Bacillus thuringiensis* (Bt) has been developed as a *de facto* second pyrethroid synergist to provide a 'safety net' for the pyrethroids by killing resistant larvae which may survive the pyrethroid component of the mixture (Forrester, in press). However, one of the disadvantages with using Bt in this way, is that it has no impact on moths and some of the potential synergists identified in this study may prove useful alternatives in this regard. Thus, the Australian IRM strategy is developing a synergist rotation strategy within an insecticide rotation strategy (already outlined in Section 1). This concept of synergist rotation has not been adopted in the field previously but its potential has been quite nicely demonstrated in two elegant laboratory studies on pyrethroid and synergist resistant *Plutella xylostella* (Chen & Sun, 1986; Yao *et al.*, 1988).

The past field use of synergists has been mostly unsuccessful but this should not deter future efforts. There have been some successes and probably the most famous example is the use of mixtures of organophosphates and Kitazin/KitazinP (a fungicidal organophosphate carboxylesterase inhibitor) for control of organophosphate resistant green rice leathopper (*Nephotettix cincticeps* (Uhler) (Hemiptera: Cicadellidae)) (Sawicki, 1981; Georghiou, 1983; Miyata & Saito, 1984). In fact, since 1977 these synergistic mixtures have been so successful in continuing to control *N. cincticeps* in China, that this insect is now regarded as a secondary pest (Tang *et al.*, 1988).

So there is hope for the long-term successful management of synergists in field resistance situations and on this positive note it is probably pertinent to revive Wilkinson's (1979) prophetic comment, 'Research on synergists is valuable from a theoretical point of view and for the day when we really may have to make the most of the insecticidal compounds already available'. Perhaps we are already there.

Section 10

Pyrethroid resistance: resistance breaking pyrethroids

Summary

The structural requirements for designing a resistance breaking pyrethroid to overcome oxidative metabolic pyrethroid resistance in *Helicoverpa armigera* were studied. A range of pyrethroid structures were tested on a well defined pure breeding pyrethroid resistant strain of *H. armigera* (homozygous for a metabolic detoxification mechanism fully suppressible by piperonyl butoxide, presumably via a microsomal monooxygenase system). Highest resistance factors were to the ester bonded phenoxybenzyl alcohol pyrethroids, particularly to those with an aromatic acid moiety. Changes to the alcohol moiety alone could overcome most, if not all, resistance. Simple benzyl alcohols were the most effective followed by cyclopentenolones and a methylated biphenyl alcohol. However, the benzylfurylmethyl alcohol (bioresmethrin) was not effective. The incorporation of a synergophore grouping into the alcohol moiety was fully effective for Scott's Py III (methylenedioxyphenyl) and prallethrin (propynyl) but only partially effective for tetramethrin (N-alkyl). Changes to the acid moiety had little effect except for the incorporation of a synergophore methylenedioxyphenyl grouping (Cheminova I) which was just as effective as for the same insertion in the alcohol moiety. The change to a central ether bond from the conventional ester bond lowered resistance. Reversion to an unsubstituted alpha carbon analogue from the conventional alpha cyano group also lowered resistance.

Piperonyl butoxide (Pbo) had little effect on pyrethroid toxicity in the susceptible strain except for the single isomers deltamethrin and esfenvalerate. However, it was more than fully effective in overcoming resistance and actually reduced resistance factors to significantly below one in the resistant strain. This indicated the possibility that Pbo could be acting both as a classical monooxygenase inhibitor and a preferential penetration synergist in resistant larvae.

Partial or full resolution of racemic mixtures had minimal impact on increasing toxicity in the susceptible strain. However, partially or fully resolved isomers were clearly much more toxic on resistant strains, indicating a possible blocking effect of the inactive isomers during the toxication process with the higher pyrethroid doses applied to resistant larvae. Cis isomers had only slightly higher resistance factors than trans isomers.

Seven fully effective resistance breaking pyrethroids were identified in this study and one of these (the simple benzyl alcohol, Series Two) was shown to be equally effective on both adults and larvae of *H. armigera*. It was also shown to work equally well on laboratory or field material and gave results similar to a pyrethroid/Pbo combination. However, none of the resistance breakers identified so far are able to satisfy all of the requirements necessary for an ideal resistance breaking pyrethroid (i.e. good resistance breaking activity at low rates, photostability, residual activity similar to current pyrethroids and safety to mammals). Factors acting against the possible commercialization of successful resistance breaking compounds are discussed.

Introduction

The previous chapter dealt with the possibility of developing synergists to counter the predominant oxidative pyrethroid resistance present in field populations of *Helicoverpa armigera* in Australia. A second possibility is to alter the structure of the pyrethroid molecule itself so that it is no longer a suitable substrate for the insect's detoxifying enzyme/s. This concept of a resistance breaking compound is not new and has been suggested by numerous authors (e.g. Lipke & Kearns, 1959; Hammock *et al.*, 1977; Casida *et al.*, 1983; Wilkinson, 1983; Scott & Georghiou, 1986; Farnham *et al.*, 1987). However, for various reasons, the realization of the concept has met with only limited success and only in the DDT, carbamate and organophosphate groups (Fukuto & Mallipudi, 1983; Metcalf, 1989).

A related approach is the incorporation of a synergistically active grouping (synergophore) into a molecule containing an insecticidally active grouping (toxophore) in order that it might serve as its own synergist. This concept of self synergism is also not new, having been first attempted with pyrethroids (piperonyl

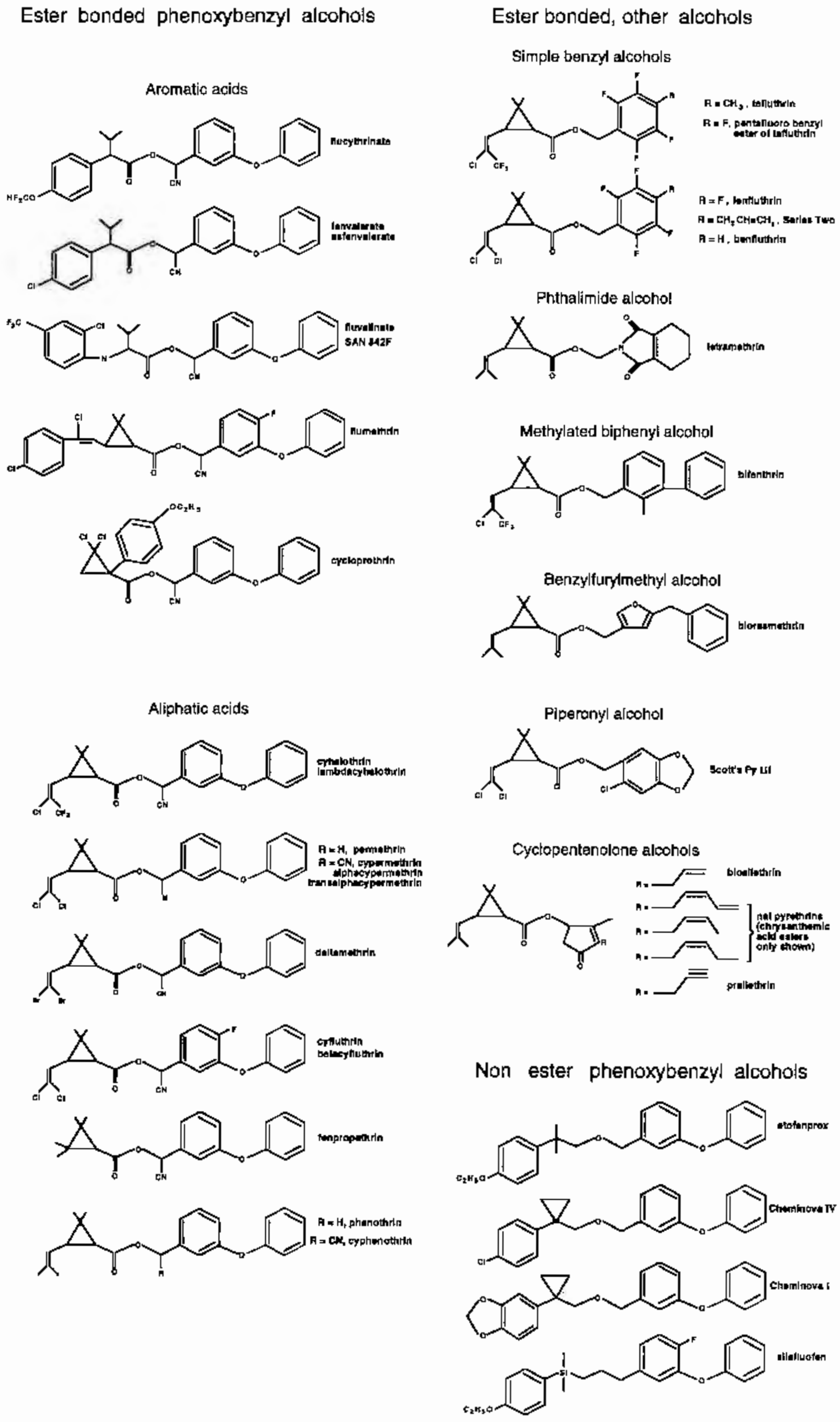


Fig. 47. Structures of pyrethroids tested in this study.

chrysanthemates) by H. Staudinger and L. Ruzicka in 1924 and patented 25 years later by M. E. Synerholm (Barthel, 1961). Further refinements of these compounds with a synergophore group on the alcohol moiety were made by Barthel (1961) and Scott & Georghiou (1986) while similar compounds with a synergophore on the acid moiety were reviewed by Casida (1970). Successful self synergism has also been demonstrated for various synergophores (methylenedioxyphenyls and propynyl ethers) in other chemical groups, especially with carbamates (Casida, 1970; Wilkinson, 1979; Wallace & Zerba, 1989a, 1989b). However, once again, these compounds have met with only limited commercial acceptance.

This study aims to explore the requirements for the design of a successful resistance breaking or self synergizing pyrethroid by comparing the relative degree of synergism across closely related compounds, as suggested by Raffa & Priester (1985). The use of the well defined pure breeding pyrethroid resistant strain of *H. armigera* (homozygous for a metabolic detoxification mechanism fully suppressible by piperonyl butoxide, presumably via a microsomal monooxygenase system), rather than an undefined polygenic resistant strain, allows precise observations on the impact of structural changes on metabolic resistance, a technique recommended strongly by Elliott *et al.* (1987). The study also aims to identify and suggest ways to counteract the factors militating against the adoption of these compounds into commercial practice. The ability of these resistance breaking pyrethroids to overcome metabolic resistance in both larval and adult life stages is also assessed. The incorporation of a candidate resistance breaking compound into the Namoi/Gwydir resistance monitoring programme for one season allowed the assessment of its performance on field collected pyrethroid resistant *H. armigera*, relative to the pyrethroid Pbo/screen outlined in Section 8.

Methods and materials

Effect of pyrethroid structure on metabolic resistance in larvae

Thirty-five diverse pyrethroid structures were tested on larvae of both a laboratory susceptible and a pure breeding pyrethroid resistant strain of *H. armigera* (homozygous for a metabolic detoxification mechanism fully suppressible by piperonyl butoxide, presumably via a microsomal monooxygenase system). Bioassay technique and analysis were as detailed in Appendix 2 except that only fourth instar 30-40 mg larvae were tested. Third instar larvae were excluded from these bioassays to minimize the possible variability due to fluctuating metabolic detoxification capacity during the moulting cycle (Wilkinson & Brattsten, 1972; Wilkinson, 1983; Collins & Hooper, 1984b; Hodgson, 1985). Sources of the various pyrethroids for each of the structurally related groups indicated in figure 47 are given below.

Ester bonded phenoxybenzyl alcohols with aromatic acids

Flucythrinate 80.0% ex American Cyanamid; fenvalerate 94.3% ex Shell, Melbourne; esfenvalerate 83.8% ex Shell, Melbourne; fluvalinate 91.1% (IR cis R, IR cis S) ex Sandoz, Sydney; SAN 842 F (fluvalinate, IR cis S) 80.1% ex Sandoz, Sydney; flumethrin 60.0% ex Bayer, Sydney; cycloprothrin 65.0% ex Rhone-Poulenc, Sydney.

Ester bonded phenoxybenzyl alcohols with aliphatic acids

Cyhalothrin 92.3% ex ICI, Melbourne; lambdacyhalothrin 96.5% ex ICI, Melbourne; permethrin 94.6% ex FMC, Brisbane; cypermethrin 93.7% ex FMC, Brisbane; alphacypermethrin 96.2% (IR cis S, IS cis R) ex FMC, Brisbane; transalphacypermethrin 89.8% (IR trans S, IS trans R) ex FMC, Brisbane; deltamethrin 99.4% ex Hoechst, Melbourne; cyfluthrin 93.4% ex Bayer, Sydney; betacyfluthrin 97.0% ex Bayer, Sydney; fenpropathrin 91.9% ex Sumitomo, Japan; d-phenothrin 92.1% ex Sumitomo, Japan; cyphenothrin 95.8% ex Sumitomo, Japan.

Ester bonded, simple benzyl alcohols

Tefluthrin 90.7% ex Incitec, Brisbane; pentafluorobenzyl ester of tefluthrin (McDonald & Punja, 1986) 95.0% ex ICI, UK; fenfluthrin, purity unknown, ex J. Scott, Cornell, USA; Series Two (R 152611) 86.0% ex ICI, UK; benfluthrin 97.5% ex Bayer, Sydney.

Ester bonded, phthalimide alcohol

Tetramethrin 94.7% ex Wellcome, Sydney.

Ester bonded, methylated biphenyl alcohol

Bifenthrin 90.4% ex FMC, Brisbane.

Ester bonded, benzylfurylmethyl alcohol

Bioresmethrin 95.7% ex Wellcome, Sydney.

Ester bonded, piperonyl alcohol

Scott's Py III (Scott & Georghiou, 1986) unknown purity, ex J. Scott, Cornell, USA.

Ester bonded, cyclopentenolone alcohols

Bioallethrin 93.1% ex Wellcome, Sydney; natural pyrethrins 50.0% ex Wellcome, Sydney; prallethrin 94.2% ex Sumitomo, Japan.

Non ester phenoxybenzyl alcohols

Etofenprox 96.3% ex Mitsui Toatsu, Japan; Cheminova I and IV (Pedersen, 1986) 96.0 & 97.0% respectively, ex Cheminova, Denmark; silafluofen (Hoe 498, Stübler *et al.*, 1989) 98.2% ex Hoechst, Melbourne.

The various pyrethroids are listed in order of decreasing activity against the susceptible strain in table 31 and graphed in order of decreasing resistance factors in figure 48.

Effect of Pbo on metabolic resistance in larvae

The impact of pretreatment with piperonyl butoxide (Pbo) (50 µg/30-40 mg fourth instar larva) was tested on larvae of both the laboratory susceptible and pyrethroid resistant *H. armigera* strain, as detailed above. Resistance factors (LD_{50} resistant strain with Pbo ÷ LD_{50} susceptible strain with Pbo) are given for 20 pyrethroid structures in table 32 and compared to the resistance factors for the same pyrethroids without Pbo pretreatment in figure 49.

Effect of isomer resolution on pyrethroid toxicity

The bioassay data for five sets of racemates and their fully or partially resolved isomers were extracted from table 31 and the increased toxicity of the resolved isomers compared for the susceptible and pyrethroid resistant strain (table 33). The relative toxicity of fenvalerate and its fully resolved isomer esfenvalerate was tested in 12 strains of susceptible *H. armigera*, four strains of susceptible *H. punctigera* and in eight pyrethroid resistant *H. armigera* strains with varying levels of polygenic resistance (table 34). The 24 strains were founded from larvae collected from the field in the 1986/87 season and bioassayed in the $F_{1\text{-}2}$, as detailed previously.

Effect of pyrethroid structure on metabolic resistance in moths

Three pyrethroids were tested on moths (topical eye test) of both a laboratory susceptible and a pure breeding pyrethroid resistant strain of *H. armigera* (homozygous for a metabolic detoxification mechanism fully suppressible by Pbo, presumably via a microsomal monooxygenase system). Bioassay technique and analysis were as detailed in Appendix 2. The three pyrethroids chosen included two conventional phenoxybenzyl synthetic pyrethroids, one with an aromatic acid (fenvalerate) and the other with an aliphatic acid (cypermethrin), as well as the resistance breaking simple benzyl alcohol pyrethroid, Series Two (table 35).

The ability of Pbo and the Series Two pyrethroid to overcome metabolic resistance in moths and larvae was compared on material collected from the field in late 1989/90 season. The Stage III fenvalerate screen survivors from all three study areas (Namoi/Gwydir, Emerald and Inverell) were combined and reared through to moths (for the adult topical eye tests) or to F_1 larvae (for the larval tests). The moths and larvae were screened with the discriminating doses (detailed in fig. 50) of either fenvalerate alone, fenvalerate/Pbo or Series Two alone. The percentage surviving the discriminating dose for the three screens within each life stage, were compared using chi-squared tests. The total number of moths (male/female combined) and larvae tested are given in figure 50.

Effect of pyrethroid structure on field pyrethroid resistance

The results on the pure breeding pyrethroid resistant strain of *H. armigera* indicated that the Series Two pyrethroid was an effective resistance breaker at least in this laboratory strain. The next step was to test it against field genetic material, so the compound was incorporated into the Namoi/Gwydir resistance monitoring programme for one season (1989/90) as a fourth discriminating dose screen. The *H. armigera* larvae from each sample for this season were split equally and randomly into four subsamples and tested with the discriminating dose of either fenvalerate, fenvalerate/Pbo, endosulfan (as previously described) or Series Two (0.1 µg/30-40 mg larva, from table 41). As there was no Series Two discriminating dose determined for the 40-60 mg weight range, larvae from the Series Two subsamples which grew through the 30-40 mg testing weight range, were transferred to the fenvalerate subsample and tested with the higher fenvalerate discriminating dose (see Section 2). Sample analysis was similar to that described in Section 2. The samples were either pooled into collecting weeks and percentage survival at the discriminating dose (for fenvalerate alone, fenvalerate/Pbo mix and Series Two alone) graphed using 95% confidence intervals based on the between site binomial standard error (right hand graph in figure 51) or into collecting Stages (I, II or III) (left hand graph in figure 51) and separated by paired t tests. Numbers of larvae tested for each screen in each Stage, are given in figure 51.

Results

Effect of pyrethroid structure on metabolic resistance in larvae

The highest resistance factors were to the ester bonded phenoxybenzyl alcohol pyrethroids, particularly to those with an aromatic acid moiety (fig. 48). Within the aliphatic acid ester bonded phenoxybenzyl pyrethroids, change from the conventional halogenated vinylcyclopropanecarboxylic acid moiety to a tetramethylcyclopropanecarboxylic acid (fenpropathrin) had no impact on the resistance factor. However, the reversion to the original unsubstituted chrysanthemic acid (phenothrin, cyphenothrin) did seem to lower the resistance factor. The reversion to an unsubstituted alpha carbon analogue from the normal alpha cyano group (e.g. cyphenothrin to phenothrin and cypermethrin to permethrin) also had a significant impact in lowering the resistance factor (6.9 down to 2.9 and 25.2 down to 6.8, respectively).

Any significant divergence from the conventional phenoxybenzyl alcohol moiety in ester bonded pyrethroids resulted in a significant decrease in resistance except for the benzylfurylmethyl alcohol pyrethroid bioresmethrin which had intermediate levels of resistance. The simple benzyl alcohol pyrethroids were all full resistance breakers except for tefluthrin (methyl substitution on position 4) which was only just slightly resisted. The synergophore (N-alkyl) phthalimide alcohol pyrethroid tetramethrin was only slightly resisted as was the methylated biphenyl alcohol pyrethroid bifenthrin. The original cyclopentenolone alcohol pyrethrins, bioallethrin and the natural pyrethrins were, interestingly, also only slightly resisted. The synergophore cyclopentenolone (propynyl) and piperonyl (methylenedioxyphenyl) alcohol pyrethroids (prallethrin and Scott's Py III, respectively) were also full resistance breakers. The non ester pyrethroids were quite divergent in their response, despite all four having a conventional phenoxybenzyl alcohol moiety. The methylene bonded silaneophane silafluofen was highly resisted, the aromatic acid ether bonded phenoxybenzyls (Cheminova IV and etofenprox) were only slightly to moderately resisted while the ether bonded phenoxybenzyl with the synergophore (methylenedioxyphenyl) piperonyl acid moiety (Cheminova I) was a full resistance breaker.

In all, seven compounds were identified as full resistance breakers (Series Two, Cheminova I, prallethrin, fenfluthrin, benfluthrin, Scott's Py III and the pentafluorobenzyl ester of tefluthrin) with a further five having only very low resistance factors (≤ 2.2) (tefluthrin, bioallethrin, natural pyrethrins, bifenthrin and tetramethrin).

Table 31. Bioassay of 35 pyrethroid structures on a susceptible and pyrethroid resistant strain of *Helicoverpa armigera* (resistant strain homozygous for a metabolic detoxification mechanism fully suppressible by piperonyl butoxide, presumably via a microsomal monooxygenase system). LD_{50}s (expressed in µg/30–40 mg fourth instar larva), arranged in order of decreasing activity against the susceptible strain. Resistance factors (RF) expressed as LD_{50} resistant strain ÷ LD_{50} susceptible strain. *, ^{ns} indicate non-overlap and overlap of susceptible and resistant 95% confidence intervals, respectively.

	Susceptible strain			Resistant strain			
Pyrethroid	LD_{50} (95% Confidence limits)		Slope	LD_{50} (95% Confidence limits)		Slope	RF
cyfluthrin	0.006	(0.005, 0.007)	2.4	0.081	(0.067, 0.098)	2.2	13.2*
betacyfluthrin	0.008	(0.007, 0.009)	3.5	0.033	(0.028, 0.039)	2.9	4.3*
lambdacyhalothrin	0.011	(0.009, 0.013)	3.0	0.132	(0.110, 0.162)	2.2	12.1*
deltamethrin	0.013	(0.011, 0.015)	2.3	0.154	(0.134, 0.176)	4.0	12.0*
esfenvalerate	0.015	(0.013, 0.017)	2.3	0.445	(0.372, 0.529)	2.0	30.3*
Series Two	0.015	(0.013, 0.017)	3.4	0.016	(0.014, 0.020)	2.6	1.1^{ns}
permethrin	0.016	(0.014, 0.019)	2.4	0.110	(0.093, 0.131)	2.7	6.8*
bioresmethrin	0.022	(0.019, 0.026)	3.4	0.236	(0.193, 0.292)	1.7	10.6*
cyhalothrin	0.023	(0.019, 0.026)	4.4	0.396	(0.319, 0.497)	1.8	17.5*
bifenthrin	0.026	(0.023, 0.030)	3.7	0.054	(0.046, 0.063)	2.4	2.1*
fenvalerate	0.034	(0.028, 0.040)	3.0	1.65	(1.378, 2.005)	1.7	49.2*
alphacypermethrin	0.034	(0.029, 0.040)	2.9	0.305	(0.254, 0.372)	2.4	9.0*
flucythrinate	0.037	(0.030, 0.045)	2.5	2.86	(2.34, 3.46)	1.8	78.0*
fenpropathrin	0.040	(0.034, 0.048)	2.7	1.38	(1.14, 1.69)	2.3	34.3*
cypermethrin	0.044	(0.039, 0.051)	4.0	1.11	(0.908, 1.343)	1.7	25.2*
transalphacypermethrin	0.045	(0.037, 0.055)	2.2	0.328	(0.271, 0.397)	2.6	7.2*
tefluthrin	0.068	(0.057, 0.081)	3.4	0.105	(0.091, 0.123)	3.3	1.6*
San 842F	0.089	(0.079, 0.102)	4.5	1.89	(1.58, 2.25)	2.5	21.1*
fluvalinate	0.112	(0.096, 0.132)	2.7	3.66	(3.01, 4.44)	1.5	32.7*
phenothrin	0.113	(0.093, 0.137)	2.2	0.325	(0.260, 0.408)	1.8	2.9*
flumethrin	0.125	(0.106, 0.149)	2.8	6.49	(4.67, 9.17)	0.8	51.9*
etofenprox	0.133	(0.111, 0.160)	2.4	0.510	(0.411, 0.633)	1.8	3.8*
Cheminova IV	0.168	(0.142, 0.198)	3.0	0.867	(0.718, 1.047)	2.3	5.2*
cyphenothrin	0.181	(0.148, 0.221)	2.1	1.25	(1.023, 1.519)	2.1	6.9*
benfluthrin	0.233	(0.194, 0.283)	2.4	0.160	(0.137, 0.188)	3.4	0.7$^{*}_{ns}$
penta-fluoro tefluthrin	0.236	(0.207, 0.270)	5.3	0.200	(0.172, 0.236)	3.4	0.8
cycloprothrin	0.243	(0.212, 0.279)	3.5	129.7	(70.5, 307.0)	0.5	534*
fenfluthrin	0.289	(0.248, 0.337)	3.6	0.130	(0.112, 0.153)	3.4	0.5*
silafluofen	0.365	(0.310, 0.428)	3.3	12.58	(10.24, 15.46)	1.7	34.4*
natural pyrethrins	0.371	(0.311, 0.440)	2.8	0.740	(0.626, 0.874)	2.8	2.0*
Cheminova I	0.403	(0.345, 0.472)	3.6	0.132	(0.109, 0.161)	2.2	0.3*
Scott's Py III	1.26	(1.05, 1.52)	2.3	0.62	(0.530, 0.736)	3.1	0.5$^{*}_{ns}$
prallethrin	1.60	(1.36, 1.89)	2.9	1.28	(1.12, 1.47)	3.9	0.8
tetramethrin	2.35	(1.96, 2.81)	2.4	5.15	(4.21, 6.24)	1.9	2.2*
bioallethrin	3.12	(2.68, 3.65)	3.3	5.37	(4.46, 6.57)	2.5	1.7*

Of these, only two compounds (Series Two and bifenthrin) had activity in the top third of the table against susceptibles (table 31), with the activity of Series Two approaching that of some of the most active currently commercially available pyrethroids such as esfenvalerate, deltamethrin and lambdacyhalothrin. In fact, on the resistant strain, Series Two was the most active pyrethroid (on the basis of LD_{50}s in table 31) followed by betacyfluthrin, bifenthrin and cyfluthrin.

Effect of Pbo on metabolic resistance in larvae

The Pbo pretreatment had little effect on pyrethroid toxicity in the susceptible strain with no effect in 11 out of the 20 pyrethroids tested and only a minor effect (< 2.0 synergistic ratio) in a further six (table 32). Interestingly, the highest synergistic ratios on the susceptible strain were found for the two single isomer pyrethroids tested esfenvalerate and deltamethrin (2.7 and 3.6, respectively). As expected, the synergistic ratios on the resistant strain were high to very high except for the full resistance breakers fenfluthrin, pentafluorobenzyl ester of tefluthrin, Series Two and Scott's Py III (3.4, 2.3, 2.2 and 0.9, respectively). The only non significant synergistic ratio was with Scott's Py III which was not surprising because of its synergophore methylenedioxyphenyl grouping on the alcohol moiety. The synergistic ratios on the resistant strain (table 32) were always higher than the corresponding resistance factors (table 31) and even after allowing for some slight synergistic effects of Pbo on the susceptible strain (mentioned previously), the resulting resistance factors for the pyrethroid/Pbo combination were, with few exceptions, significantly below one (table 32, fig. 49). Indeed, Pbo significantly increased the toxicity of pyrethroids in the resistant strain (17 out of 20 tested in table 32) by 1.4- to 3.3-fold compared to the susceptible (resistance factors 0.7 and 0.3, respectively). This was found for even highly resisted compounds such as cycloprothrin, flucythrinate, flumethrin, fenvalerate, fluvalinate, esfenvalerate

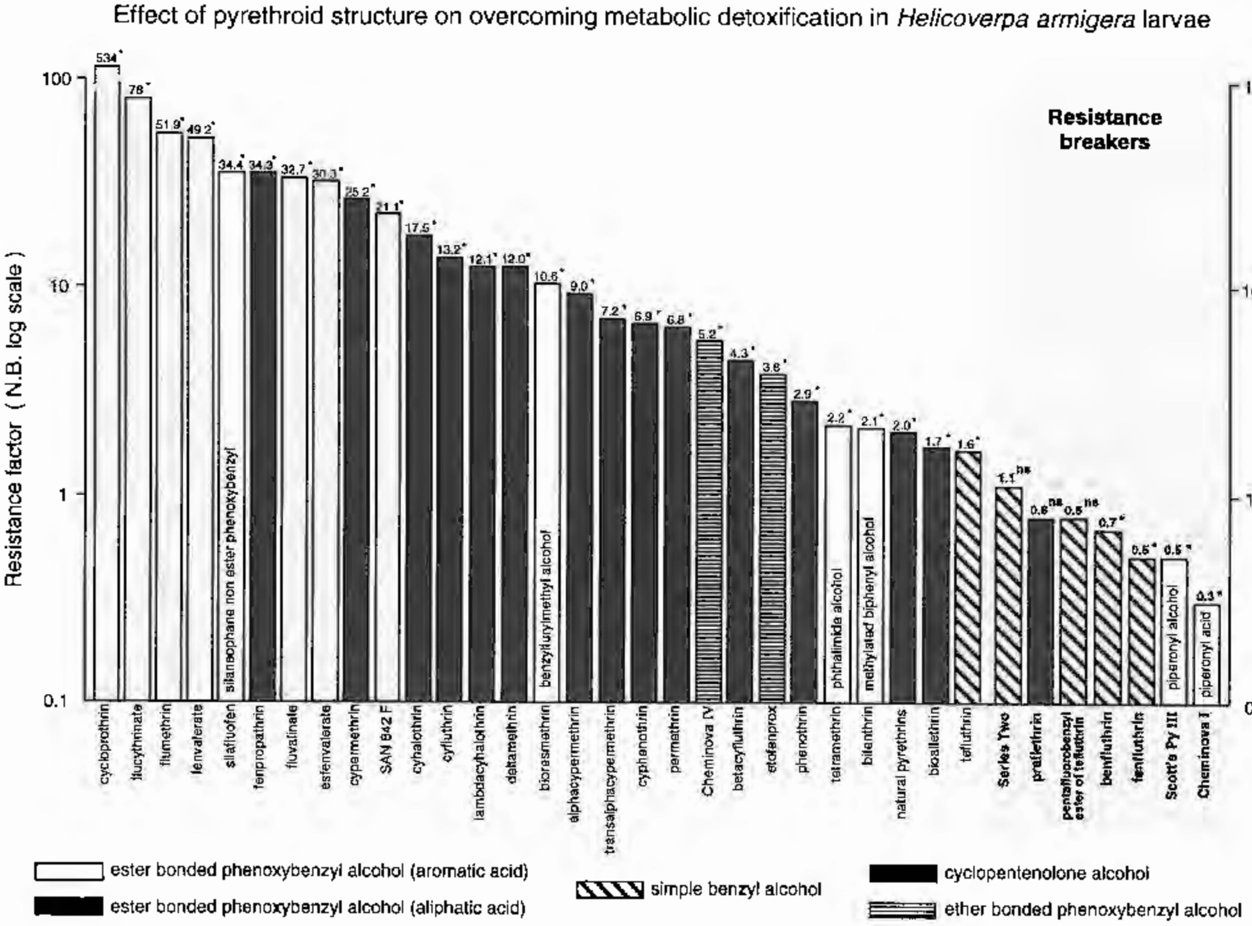


Fig. 48. Resistance factors (LD50 resistant strain ÷ LD50 susceptible strain) for 34 pyrethroid structures tested against a pyrethroid resistant strain of *Helicoverpa armigera* homozygous for a metabolic detoxification mechanism fully suppressible by piperonyl butoxide, presumably via a microsomal monooxygenase system. Resistance factors (above columns) indicate the degree of either non-overlap (*) or overlap (ns) of resistant and susceptible 95% confidence intervals. Data from table 31.

and cypermethrin (resistance factors 25-534x). Interestingly, this same phenomenon was found for the synergophore (methylenedioxyphenyl) pyrethroids, Scott's Py III and Cheminova I when tested alone on the resistant strain (resistance factors 0.5 & 0.3, respectively, figure 48).

Effect of isomer resolution on pyrethroid toxicity

The partial or full resolution of five pyrethroid racemate mixtures (fenvalerate, cyfluthrin, fluvalinate, cyhalothrin, cypermethrin) had little impact on increasing toxicity against the susceptible strain (significant only for esfenvalerate and lambdacyhalothrin, 2.3- and 2.1-fold, respectively, table 33). However, the fully or partially resolved isomers were much more toxic on the resistant strain (all six significantly more toxic, including the cis isomers (IR-cis S, IS-cis R) in alphacypermethrin and the trans isomers (IR-trans S, IS-trans R) in trans cypermethrin). This resulted in significantly lower resistance factors for all the resolved isomers with only a slight difference between cis and trans cypermethrin (9.0 and 7.2, respectively, table 33).

The same trend was found for the more broadscale testing of fenvalerate and its fully resolved isomer esfenvalerate on field material during the 1986/87 season (table 34). Esfenvalerate was found to be slightly more toxic than fenvalerate on susceptible *H. punctigera* and *H. armigera* (2.5 and 2.7x, respectively) but much more toxic on resistant *H. armigera* populations (up to 12.3x on the most resistant population). In fact, there was a good correlation between the level of pyrethroid resistance (as indicated by the resistance factor) and the increased toxicity of esfenvalerate (table 34).

Effect of pyrethroid structure on metabolic resistance in moths

Moths were similarly resistant to the conventional aromatic and aliphatic acid ester bonded phenoxybenzyl pyrethroids (fenvalerate and cypermethrin, respectively) (table 35). The simple benzyl pyrethroid Series Two,

Table 32. Bioassay of 20 pyrethroids on a susceptible and pyrethroid resistant strain of *Helicoverpa armigera*, after pretreatment with piperonyl butoxide (Pbo) (50 µg/30–40 mg fourth instar larva). Resistant strain homozygous for a metabolic detoxification mechanism, fully suppressible by piperonyl butoxide, presumably via a microsomal monooxygenase system. LD$_{50}$s (expressed in µg/30–40 mg fourth instar larva), arranged in order of decreasing activity against the susceptible strain. Resistance factors (RF) expressed as LD$_{50}$ resistant strain (with Pbo) ÷ LD$_{50}$ susceptible strain (with Pbo). Synergistic ratios expressed as LD$_{50}$ susceptible or resistant strain (without Pbo, data, from table 31) ÷ LD$_{50}$ susceptible or resistant strain (with Pbo), respectively. *, ^{ns} indicate non-overlap and overlap of 95% confidence intervals, respectively.

	Susceptible strain				Resistant strain				
Pbo+	LD$_{50}$ (95% Confidence limits)		Slope	Syn. ratio	LD$_{50}$ (95% confidence limits)		Slope	RF	Syn. ratio
deltamethrin	0.0036	(0.0032, 0.0041)	2.9	3.6*	0.0022	(0.0019, 0.0026)	2.6	0.6*	69.9*
betacyfluthrin	0.0043	(0.0036, 0.0052)	2.2	1.8*	0.0021	(0.0018, 0.0024)	3.2	0.5*	15.7*
cyfluthrin	0.0048	(0.0041, 0.0057)	2.8	1.3^{ns}	0.0051	(0.0043, 0.0061)	2.6	1.1^{ns}	15.8*
esfenvalerate	0.0055	(0.0048, 0.0063)	3.7	2.7*	0.0041	(0.0035, 0.0047)	3.8	0.7*	109*
lambdacyhalothrin	0.0093	(0.0079, 0.0109)	3.1	1.2^{ns}	0.0084	(0.0073, 0.0096)	3.9	0.9^{ns}	15.7*
Series Two	0.014	(0.012, 0.017)	3.6	1.0^{ns}	0.008	(0.007, 0.009)	3.6	0.5*	2.2*
cyhalothrin	0.015	(0.012, 0.018)	3.1	1.5*	0.004	(0.003, 0.005)	1.9	0.3*	102*
bifenthrin	0.017	(0.015, 0.020)	3.5	1.5*	0.007	(0.006, 0.008)	3.9	0.4*	7.7*
flucythrinate	0.027	(0.022, 0.033)	2.8	1.4^{ns}	0.007	(0.006, 0.008)	2.2	0.3*	415*
alphacypermethrin	0.029	(0.025, 0.034)	3.2	1.2^{ns}	0.012	(0.010, 0.015)	2.5	0.4*	25.0*
fenvalerate	0.030	(0.025, 0.035)	4.0	1.1^{ns}	0.016	(0.013, 0.019)	2.7	0.5*	106*
cypermethrin	0.040	(0.035, 0.047)	3.2	1.1^{ns}	0.015	(0.013, 0.018)	2.1	0.4*	73.5*
fluvalinate	0.055	(0.048, 0.065)	2.8	2.0*	0.029	(0.025, 0.034)	2.5	0.5*	126*
tefluthrin	0.056	(0.050, 0.064)	6.8	1.2^{ns}	0.023	(0.020, 0.027)	3.3	0.4*	4.5*
penta-fluoro tefluthrin	0.090	(0.078, 0.104)	3.5	2.6*	0.087	(0.075, 0.101)	3.3	1.0^{ns}	2.3*
cycloprothrin	0.130	(0.113, 0.149)	3.0	1.9*	0.093	(0.080, 0.108)	3.6	0.7*	1,397*
etofenprox	0.133	(0.116, 0.154)	4.0	1.0^{ns}	0.056	(0.049, 0.065)	3.7	0.4*	9.1*
fenfluthrin	0.143	(0.121, 0.170)	2.8	2.0*	0.039	(0.032, 0.046)	2.6	0.3*	3.4*
flumethrin	0.167	(0.139, 0.204)	2.6	0.7^{ns}	0.098	(0.080, 0.120)	2.0	0.6*	66.3*
Scott's Py III	1.65	(1.42, 1.91)	3.5	0.8^{ns}	0.725	(0.612, 0.850)	3.1	0.4*	0.9^{ns}

which had been earlier shown to be a full resistance breaker in larvae of the pure breeding oxidative pyrethroid strain of *H. armigera*, was also shown to be effective in breaking resistance in moths in this strain (except for a slight residual 1.8x resistance in females, table 35).

A similar result was found during the more broad-scale testing of the Series Two compound on field material from late 1989/90 season. Series Two was shown to be equivalent in activity to the pyrethroid/Pbo mix on both larvae and moths (fig. 50). The slightly less effective result on moths (5.4 versus 1.4% residual resistance for Series Two versus fenvalerate/Pbo mix, figure 50) may be due to a genuinely lower resistance breaking activity of Series Two on moths (some indication of this for female moths in table 35) or slight differences in kill rates of the Series Two and fenvalerate/Pbo moth discriminating doses.

Effect of pyrethroid structure on field pyrethroid resistance

The simple benzyl resistance breaking pyrethroid Series Two, was found to be equivalent in activity to the fenvalerate/Pbo mix throughout the whole season (except on one sampling occasion in late Stage III (fig. 51). Series Two and the fenvalerate/Pbo mix were significantly better than fenvalerate alone in both the Stage II adult selection period and the Stage III larval selection manifestation period. This is consistent with previous findings indicating equivalent metabolic resistance breaking activity of Series Two and Pbo in both moths and larvae (see previous comments). As anticipated, Series Two had no impact in overcoming the residual nerve insensitivity type resistance mechanism and was equivalent to the fenvalerate/Pbo mix in this regard.

Discussion

Effect of pyrethroid structure on metabolic resistance in larvae

The typical oxidation sites on pyrethroids have been well documented for many insect species. These sites are the 4′ (preferentially) but also the 2′ and 6 positions on the alcohol moiety and the geminal dimethyl group on the cyclopropane ring on the acid moiety (Casida *et al.*, 1983; Soderlund *et al.*, 1983; Leahey, 1985b; Ruigt, 1985). Working specifically on susceptible *H. armigera* and *Heliothis virescens*, Lee *et al.* (1989) found the 2′, 4′ and the geminal dimethyl group to be the most important oxidation sites with the geminal dimethyl group on the acid moiety being much less important for *Helicoverpa armigera* than *Heliothis virescens*. Working on pyrethroid resistant *H. virescens*, Little *et al.* (1989) found the main oxidation sites to be the 2′ and 4′ positions on the alcohol moiety with little evidence for any on the geminal dimethyl group. Bearing this in mind, one should suspect that altering the structure of the alcohol moiety would have the best chance in countering oxidatively based metabolic resistance in *Helicoverpa armigera* and indeed, this was found to be the case in this study.

Changes in the alcohol moiety

The phenoxybenzyl group has been the basis for the alcohol moiety of every pyrethroid ever used against

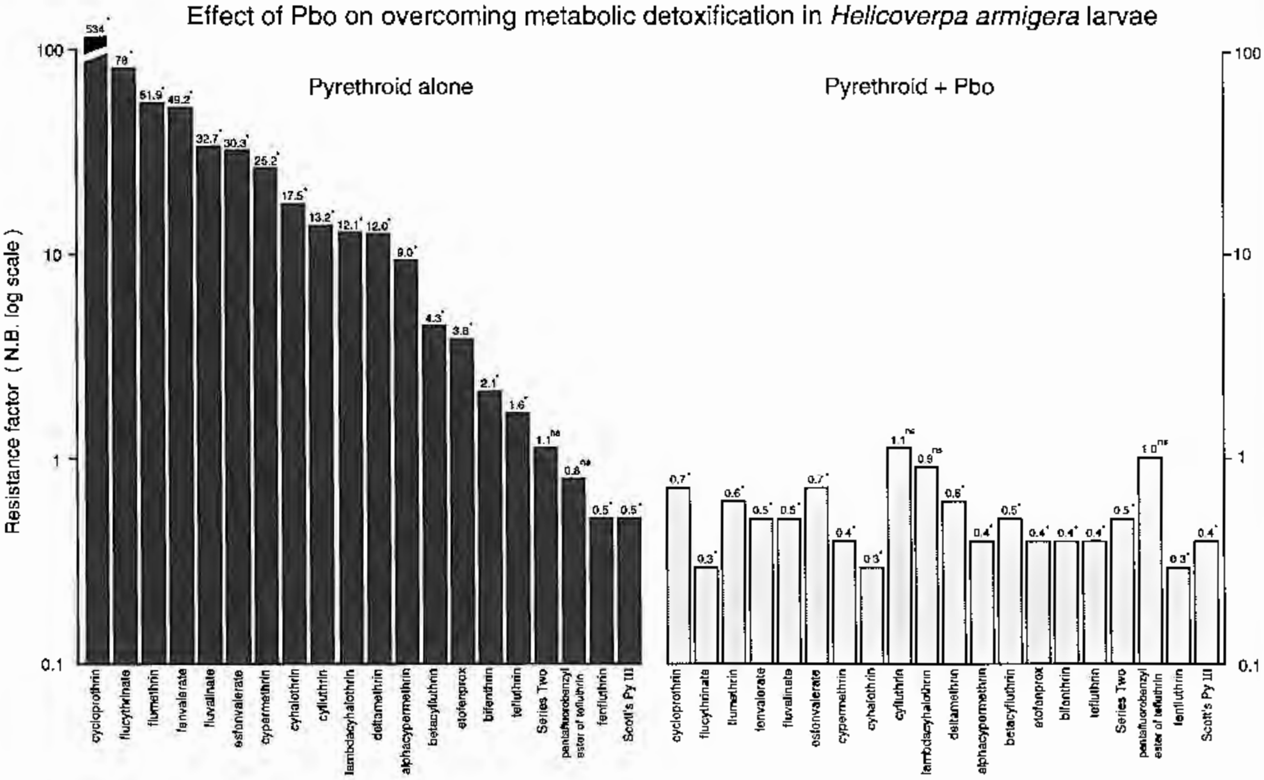


Fig. 49. Resistance factors (LD50 resistant strain ÷ LD50 susceptible strain) for 20 pyrethroids tested alone and after pretreatment with piperonyl butoxide (Pbo) (50 µg/30–40 mg fourth instar *Helicoverpa armigera* larva). Resistant strain homozygous for a metabolic detoxification mechanism, fully suppressible by Pbo, presumably via a microsomal monooxygenase system. Resistance factors (above columns) indicate the degree of either non-overlap (*) or overlap (ns) of resistant and susceptible LD50 95% confidence intervals. Data for pyrethroids alone from table 31 and for pyrethroids + Pbo from table 32.

Helicoverpa spp. in cotton in Australia (fenvalerate, esfenvalerate, deltamethrin, cypermethrin, alphacypermethrin and lamdacyhalothrin from figure 58 as well as more recently cyfluthrin and fluvalinate). Thus selection pressure on the metabolically susceptible phenoxybenzyl alcohol moiety has been intense. Changes to this group alone can overcome resistance. For example, reduction of the alcohol to a single benzyl ring breaks resistance fully in fenfluthrin, benfluthrin, Series Two and the pentafluorobenzyl ester of tefluthrin. Tefluthrin itself is still slightly (but significantly) resisted but this can be overcome by blocking the potential oxidation site at the 4 position (Heath & Leahey, 1989) with a fluorine atom replacing the methyl group to obtain the pentafluorobenzyl ester analogue of tefluthrin. However, this does not seem to be necessary for the other simple benzyl alcohol pyrethroid benfluthrin which is not protected at the 4 position. It is significant that the four resistance breaking simple benzyl alcohol pyrethroids mentioned above all have conventional halogenated vinylcyclopropanecarboxylic acid moieties with a potentially metabolically susceptible geminal dimethyl group. The fact that these four are still full resistance breakers indicates the unimportance of the geminal dimethyl group as an oxidation site which is consistent with the findings of Lee *et al.* (1989) and Little *et al.* (1989) mentioned earlier. Halliday & Georghiou (1985) and Scott & Georghiou (1986) also found lower resistance factors for the simple benzyl alcohol pyrethroid fenfluthrin in pyrethroid resistant *Musca domestica* and *Culex quinquefasciatus*, respectively.

The methylated biphenyl alcohol pyrethroid biphenthrin had a very much reduced resistance factor. This was unexpected as it is still susceptible to oxidation at the 2′ and 4′ positions. However, it does lack the ether linkage between the phenyl rings in the alcohol and biphenyl ether cleavage to desphenyl fenvalerate was shown to be a major degradation pathway for fenvalerate resistant Colorado potato beetle, *Leptinotarsa decemlineata* (Soderlund *et al.*, 1987). This may also be an important metabolic resistance pathway for *H. armigera* in Australia and should be studied further. Halliday & Georghiou (1985) and Scott & Georghiou (1986) also found lower resistance factors for bifenthrin, as for fenfluthrin above. The relevance of this to overcoming resistance in the field is unknown but it is interesting to note the results of Charoenying *et al.* (1989) working on pyrethroid resistant *H. armigera* in sunflowers in Thailand and Cheng *et al.* (1988) working on *Spodoptera exigua* on onions in Taiwan. Both groups found biphenthrin to work better than conventional phenoxybenzyl pyrethroids. Although Cheng *et al.* (1988) do not men-

Table 33. Bioassay of five pyrethroids and their fully or partially resolved isomers on a susceptible and pyrethroid resistant strain of *Helicoverpa armigera*. Resistant strain homozygous for a metabolic detoxification mechanism fully suppressible by piperonyl butoxide, presumably via a microsomal monooxygenase system. LD$_{50}$ and resistance factor (RF) data taken from table 31. Increased toxicity of resolved isomer expressed as the LD$_{50}$ racemate mixture ÷ LD$_{50}$ resolved isomer. *, ns indicate non-overlap and overlap of 95% confidence intervals, respectively (confidence intervals from table 31).

	Susceptible		Resistant		
Racemate and resolved pyrethroid isomer/s (No. active of total no. of isomers)	LD$_{50}$	Increased toxicity of resolved isomer	LD$_{50}$	RF	Increased toxicity of resolved isomer
fenvalerate (1/4)	0.0336		1.6546	49.2*	
esfenvalerate (1/1)	0.0147	2.3*	0.4449	30.3*	3.7*
cyfluthrin (2/4)	0.0061		0.0807	13.2*	
betacyfluthrin (2/2)	0.0076	0.8^{ns}	0.0329	4.3*	2.5*
fluvalinate (1/2)	0.1118		3.660	32.7*	
SAN 842 F (1/1)	0.0894	1.3^{ns}	1.886	21.1*	1.9*
cyhalothrin (2/4)	0.0226		0.3957	17.5*	
lambdacyhalothrin (2/2)	0.0109	2.1*	0.1319	12.1*	3.0*
cypermethrin (4/8)	0.0441		1.1103	25.2*	
alphacypermethrin (2/2)	0.0339	1.3^{ns}	0.3053	9.0*	3.6*
transalphacypermethrin (2/2)	0.0452	1.0^{ns}	0.3276	7.2*	3.4*

tion whether the *S. exigua* they were working with were resistant to pyrethroids or not, one would have to strongly suspect they were. The benzylbenzyl ester pyrethroids are an interesting group which warrant further study as they are potential resistance breakers, are relatively active on phytophagous mites and have good activity, being generally only slightly less toxic than corresponding phenoxybenzyl esters (Elliott, 1990).

The cyclopentenolone alcohol pyrethroids bioallethrin and natural pyrethrins also had significantly lower resistance factors. However, the benzylfurylmethyl alcohol pyrethroid bioresmethrin, which was one of the early compounds discovered en route from the natural pyrethrins to the synthetic pyrethroids (Davies, 1985) had an intermediate resistance factor. This general transition from highest resistance to phenoxybenzyl esters, less to furylmethyls and then less again to cyclopentenolones has also been noted for a number of predominantly oxidatively pyrethroid resistant insect species such as *Musca domestica* (Harris *et al.*, 1982; Scott & Georghiou, 1986; Solomon *et al.*, 1990), *Culex quinquefasciatus* (Priester & Georghiou, 1980) and *Tribolium castaneum* (Collins, 1990). Interestingly, it has also been noted for the nerve insensitivity pyrethroid resistance mechanism in *Musca domestica* (De Vries & Georghiou, 1980; Farnham *et al.*, 1987; Ahn *et al.*, 1988).

The incorporation of a synergophore grouping into the alcohol moiety was fully effective for Scott's Py III (methylenedioxyphenyl) and prallethrin (propynyl) but only partially effective for tetramethrin (N-alkyl). This correlates well with the relative activity of these synergist groups when used alone (table 30). It is interesting to note that Scotts Py III and prallethrin retain their full resistance breaking capability despite the presence of a potentially metabolically susceptible geminal dimethyl group in the acid moiety. This was also found for the full resistance breaking simple benzyl alcohol pyrethroids and confirms the unimportance of the geminal dimethyl group as an oxidation site.

Changes in the acid moiety

Resistance factors were significantly higher for phenoxybenzyl esters within aromatic acids. This has also been noted for pyrethroid resistant *Plutella xylostella* in Taiwan (Liu *et al.*, 1981, 1984; Cheng *et al.*, 1986). The reason for this is not clear as there is no mention in the literature of any primary oxidation sites on these acids other than the normal geminal dimethyl group (Leahey, 1985b). However, there is a possibility that the extra phenyl ring on the acid moiety in these compounds may provide another potential site/s for oxidation and this should be studied further.

The reversion to the original unsubstituted chrysanthemic acid (phenothrin, cyphenothrin) was successful in partially lowering the resistance factor. The halogenated vinylcyclopropanecarboxylic acid has been the basis for the acid moiety of most of the pyrethroids used against *Helicoverpa* spp. in cotton in Australia (deltamethrin, cypermethrin, alphacypermethrin and lambdacyhalothrin from figure 58, as well as more recently cyfluthrin). The only exception is the phenylacetic aromatic acid of the relatively lower use (fig. 58) pyrethroids fenvalerate and esfenvalerate. So the lower resistance factor for these chrysanthemic acid esters was not altogether surprising and is cousistent with similar findings for structural divergence from the standard commercial phenoxybenzyl alcohol moiety. However, the same logic was not consistent for the significantly altered tetramethylcyclopropane acid pyrethroid fenpropathrin which was highly resisted. The incorporation of a synergophore grouping (methylenedioxyphenyl) into the acid moiety (Cheminova I) was just as effective as for the same insertion in the alcohol moiety. It is interesting to note that Cheminova I was still a full resistance breaker despite

Table 34. Relative toxicity (LD$_{50}$ fen ÷ LD$_{50}$ esfen) of fenvalerate and its resolved isomer esfenvalerate on various field strains of susceptible *Helicoverpa punctigera* and pyrethroid resistant and susceptible *Helicoverpa armigera*, collected in the 1986/87 season. Resistant *H. armigera* strains with varying levels of mixed resistance mechanisms. Resistance factors (RF) expressed as the LD$_{50}$ of current strain ÷ LD$_{50}$ of a susceptible strain (0.03 µg fen, 0.012 µg esfen and 0.011 µg fen, 0.005 µg esfen/30–40 mg larva for *H. armigera* and *H. punctigera*, respectively). Susceptible data for fenvalerate from tables 37 (*H. armigera*) & 42 (*H. punctigera*); susceptible data for esfenvalerate average of current susceptible lines. * indicates no overlap of the 95% confidence intervals (data not shown) of the fenvalerate and esfenvalerate LD$_{50}$s.

Strain data		Fenvalerate		Esfenvalerate		Relative toxicity esfen: fen
Collection site	Host	LD$_{50}$	RF	LD$_{50}$	RF	
Susceptible *Helicoverpa punctigera*						
Emerald, Q.	cotton	0.0090	0.8	0.0033	0.7	2.7*
Narrabri, NSW.	cotton	0.0094	0.9	0.0036	0.7	2.6*
Bourke, NSW.	cotton	0.0130	1.2	0.0044	0.9	3.0*
Emerald, Q.	cotton	0.0149	1.4	0.0085	1.7	1.8*
		Average		0.005		2.5
		± standard error		± 0.0012		± 0.26
Susceptible *Helicoverpa armigera*						
Kerang, VIC.	maize	0.022	0.7	0.008	0.7	2.8*
Narrabri, NSW.	cotton	0.024	0.8	0.007	0.6	3.4*
Narrabri, NSW	maize	0.024	0.8	0.014	1.2	1.7*
Wee Waa, NSW.	sunflowers	0.024	0.8	0.009	0.8	2.7*
Melbourne, VIC.	maize	0.024	0.8	0.008	0.7	3.0*
Laboratory susceptible colony		0.029	1.0	0.008	0.7	3.6*
Wee Waa, NSW.	sunflowers	0.031	1.0	0.018	1.5	1.7*
Emerald, Q.	cotton	0.035	1.2	0.018	1.5	1.9*
Wee Waa, NSW.	maize	0.038	1.3	0.014	1.2	2.7*
Wee Waa, NSW.	maize	0.039	1.3	0.016	1.3	2.4*
Wee Waa, NSW	sunflowers	0.040	1.3	0.010	0.8	4.0*
Wee Waa, NSW.	sunflowers	0.040	1.3	0.017	1.4	2.4*
		Average		0.012		2.7
		± standard error		± 0.0013		± 0.21
Resistant *Helicoverpa armigera*						
Wee Waa, NSW.	maize	0.057	1.9	0.016	1.3	3.6*
Wee Waa, NSW.	maize	0.061	2.0	0.023	1.9	2.7*
Narrabri, NSW.	maize	0.071	2.4	0.023	1.9	3.1*
Moree, NSW.	cotton	0.181	6.0	0.056	4.7	3.2*
Emerald, Q.	cotton	0.242	8.1	0.050	4.2	4.8*
Narrabri, NSW.	cotton	0.597	19.9	0.090	7.5	6.6*
Emerald, Q.	cotton	2.090	70.0	0.346	28.8	6.0*
Emerald, Q.	cotton	9.127	304.2	0.744	62.0	12.3*

possessing a conventional metabolically susceptible phenoxybenzyl alcohol moiety.

Effect of central bond

The change to a central ether bond from the conventional ester bond resulted in lower resistance factors. Etofenprox and Cheminova IV had relatively low resistance factors (3.8 and 5.2, respectively) while fenvalerate (a conventional ester bonded pyrethroid structurally similar to Cheminova IV) had a high resistance factor (49.2). The reason for the lower resistance factor in the Cheminova IV ether analogue is unclear as Cheminova IV still has a conventional metabolically susceptible phenoxybenzyl alcohol moiety. It may be that Cheminova IV and etofenprox are quickly metabolized by monooxygenases to their generally less toxic 2′/4′ hydroxy analogues (Hutson, 1983; Reed *et al.*, 1983; Brattsten *et al.*, 1986) but that these partially toxic analogues can only be slowly removed by conjugation because of the inability of the normal esterases to cleave the non-ester central bond. This rate limiting step would slow down the detoxification process and result in only partially successful resistance breakers. However, the non ester/non ether methylene bonded silaneophane pyrethroid (silafluofen) did not follow this logic and was highly resisted.

Changes at the alpha carbon position

The reversion to an unsubstituted alpha carbon analogue from the conventional alpha cyano group significantly lowered resistance. This is not surprising as the alpha cyano group has been present on every pyrethroid ever used against *Helicoverpa* spp. in cotton in Australia (fenvalerate, esfenvalerate, deltamethrin, cypermethrin, alphacypermethrin and lambdacyhalothrin from figure 58, as well as more recently cyfluthrin and fluvalinate). The non-cyano pyrethroid permethrin was never commercially accepted for *Helicoverpa* spp. control in Australian cotton and has been restricted to a minor use

Table 35. Topical eye test bioassay of three pyrethroids on a pyrethroid resistant strain of *Helicoverpa armigera*, homozygous for a metabolic detoxification mechanism fully suppressible by piperonyl butoxide, presumably via a microsomal monooxygenase system. LD_{50}s adjusted to standard 200 mg moth. Resistance factors (RF) expressed as LD_{50}s resistant strain ÷ LD_{50} susceptible strain. Susceptible strain LD_{50}s 0.30/0.40, 0.10/0.19 and 0.04/0.04 for fenvalerate female/male, cypermethrin female/male and Series two female/male moths, respectively (data from tables 43 and 44). *, ^{ns} indicate non-overlap and overlap of susceptible and resistant 95% confidence intervals, respectively.

Chemical	Sex	Av. weight of 1 day old, fed, unmated moths (mg) ± s.e.	LD_{50} (µg/moth)	95% Conf. interval		Slope	RF
				Lower	Upper		
fenvalerate	♀	221 ± 1.7	10.51	7.81	13.83	1.3	35.0*
	♂	203 ± 1.6	12.21	9.76	15.17	1.7	30.5*
cypermethrin	♀	221 ± 1.7	3.51	2.58	4.77	1.1	35.1*
	♂	203 ± 1.6	2.96	2.43	3.58	2.1	15.6*
Series Two	♀	221 ± 1.7	0.072	0.064	0.081	5.2	1.8*
	♂	203 ± 1.6	0.053	0.046	0.063	2.8	1.3^{ns}

product mainly in vegetables. Hence, selection pressure was intense on alpha cyano pyrethroids and the lower resistance factors for compounds diverging from the conventional alpha cyano structure is consistent with similar findings for divergence from the conventional commercial halogenated vinylcyclopropanecarboxylic acid and phenoxybenzyl alcohol moieties mentioned previously. Other researchers have also found lower resistance factors for non-cyano pyrethroids in pyrethroid resistant *Plutella xylostella* (Liu *et al.*, 1981; Cheng *et al.*, 1986) and *Tribolium castaneum* (Collins, 1990).

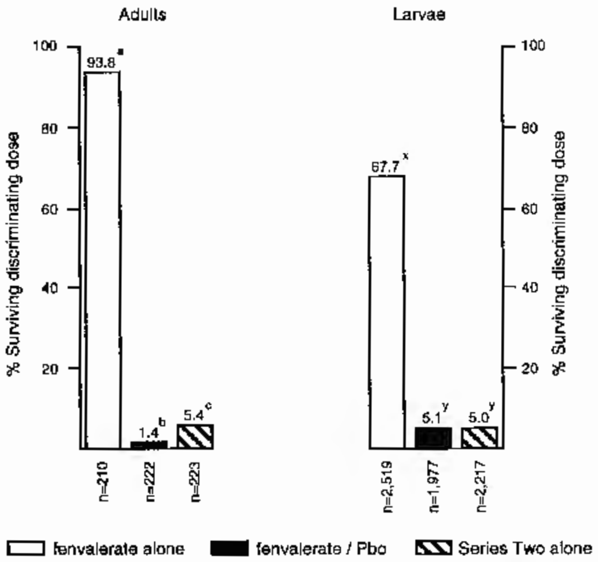
Synergophore substitutions at the alpha carbon position were not investigated in this study due to the unavailability of suitable compounds. However, they certainly warrant further investigation as the potentially synergophoric ethynyl substitution at the alpha carbon has been shown to be an effective alternative for the cyano group in both benzylbenzyl and phenoxybenzyl chrysanthemates (Matsuo *et al.*, 1976). Indeed, Scott & Georghiou (1986) found such a compound (CGA 112'567) to be partially effective in overcoming predominantly oxidative pyrethroid resistance in *Musca domestica*, but this particular result was confounded by a concomitant 4' chloro substitution on the metabolically susceptible phenoxybenzyl moiety of this compound. Thus, the value of the ethynyl substitution at the alpha carbon as a potential resistance breaker remains unclear. However, this is certainly a potentially fertile area for the design of resistance breaking pyrethroids considering the utility of the acetylenic group in overcoming oxidative degradation noted for many synergists (see Section 9) and prallethrin in this study.

Effect of Pbo on metabolic resistance in larvae

As anticipated, Pbo had little effect on pyrethroid toxicity in the susceptible strain. However, there were a few exceptions, notably the single isomer pyrethroids deltamethrin and esfenvalerate, where Pbo increased toxicity (slightly but significantly) in the susceptible strain. Apparently, there is some limited oxidative metabolism of these pyrethroids which occurs naturally in susceptibles and this could explain the greater variability and lower slopes noted for deltamethrin during the calibration of fenvalerate and deltamethrin susceptible bioassays in Appendix 2. This supports the decision (discussed in Appendix 2) not to adopt deltamethrin for the subsequent discriminating dose based monitoring study, despite its being the most popular pyrethroid in commercial use at the time. For the same reason (as well as historical continuity) it has been decided to retain fenvalerate as the pyrethroid discriminating dose, despite its being recently replaced commercially by its fully resolved isomer esfenvalerate. It is not clear from this study why deltamethrin and esfenvalerate should be selectively oxidatively metabolized in susceptibles, nor whether there is any significance in the fact that they are both fully resolved isomers.

Pbo was not only fully effective in suppressing resistance to even the most highly resisted pyrethroids but it actually lowered resistance factors significantly below one. In other words, Pbo was more effective on the resistant strain than the susceptible. This result was quite unexpected and can possibly be explained by allowing for the possible penetration synergistic action of Pbo in addition to its well known inhibition of monooxygenase mediated metabolic degradation. This additional possible mode of action of Pbo was discussed fully in Section 9 where some evidence was presented to suggest that at least one solvent (dodecane) partially improved kill in the resistant but not the susceptible strain, presumably through better cuticular penetration in the resistant strain (also noted for *Musca domestica* by Sawicki & Lord, 1970). Perhaps the data in this study suggest a similar phenomenon is occurring for Pbo which is acting both as a classical monooxygenase inhibitor and a preferential penetration synergist in resistant larvae. However, some non-methylenedioxyphenyl resistance breaking pyrethroids (e.g. fenfluthrin, benfluthrin and

Suppression of pyrethroid resistance by Pbo and Series Two pyrethroid — Namoi / Gwydir, Emerald, Inverell 1989/90 combined



	Discriminating dose	
	Larval (µg / 30-40 mg larva)	Adult (µg / newly emerged moth)
fenvalerate	0.2 (table 37)	2.0 (table 43)
fenvalerate / Pbo	0.2 / 50	2.0 / 100
Series Two	0.1 (table 41)	0.2 (table 44)

Fig. 50. Impact of piperonyl butoxide (Pbo) and a resistance breaking pyrethroid (Series Two) on pyrethroid resistance in adults and larvae of *Helicoverpa armigera* reared from field material collected from the Namoi and Gwydir river valleys of northern New South Wales, the Emerald irrigation area of central Queensland and a sample of the unsprayed refugia centred on Inverell in northern New South Wales. The 1989/90 Stage III fenvalerate screen survivors from all three areas combined, reared through to moths (for the adult topical eye tests) or to F1 larvae (for the larval tests), and then screened with the appropriate discriminating dose for susceptibles (data source in brackets). Adult and larval column means, followed by the same letter, are not significantly different (P <0.05) (chi-squared test). n = total number of moths (male/female combined) or larvae tested.

some others not shown here) can also lower resistance factors significantly below one when tested alone, so other factors are also no doubt operating.

Effect of isomer resolution on pyrethroid toxicity

As expected, partial or full resolution of racemic mixtures had minimal impact on increasing toxicity against the susceptible strain. The small increase in toxicity documented for the full resolution of fenvalerate to esfenvalerate in susceptible *Helicoverpa* spp. (approx 2.5-fold) is similar to that found by Yoshioka (1978) and Hirano (1989b). However, fully or partially resolved isomers were clearly much more toxic than racemic mixtures when tested on resistant strains. This has also been noted for pyrethroid resistant *Heliothis virescens* (Leonard *et al.*, 1988b) and *Haematobia irritans* (Sparks & Byford, 1988). However, no consistent differences were found for nerve insensitivity pyrethroid resistant *Musca domestica* (Farnham *et al.*, 1987). This suggests the possibility that the insecticidally inactive isomers may be blocking the active isomers at some point in the toxication process (penetration through to the site of action). Generally there has been no clear demonstration of competitive interaction of toxic and non-toxic isomers (Miller & Salgado, 1985). However, Davies (1985) gives a good example of inert isomers exerting a differential blocking effect in a range of insect and mite pests. A similar blocking effect could also be occurring with the higher pyrethroid doses applied to the resistant larvae in this study. However, the reason for the differential reaction of resistant and susceptible larvae remains unclear from this study and must await further research.

The cis isomers in cypermethrin were found to have a slightly higher resistance factor than the trans isomers. The same situation has been found for pyrethroid resistant *Helicoverpa armigera* from Thailand (Ahmad & McCaffery, 1988). This is not surprising as cis isomers are generally better substrates for hydroxylation by oxidases than trans isomers while the reverse is true for metabolism by ester cleavage (Jao & Casida, 1974; Soderlund & Casida, 1977; Soderlund *et al.*, 1983; Dauterman, 1985; Ruigt, 1985). However, the differences are not great and probably have no practical significance for field control. This contrasts with the situation for metabolic degradation by DDT dehydrochlorinase which showed strict structural and steric specificity for certain DDT stereoisomers (Sternburg *et al.*, 1954; Lipke & Kearns, 1959).

Design and commercial adoption of resistance breaking pyrethroids

This study has shown that it is indeed possible to identify pyrethroid structures capable of overcoming oxidative metabolic pyrethroid resistance in both adult and larval *Helicoverpa armigera* and that these work equally well on laboratory or field strains. However, all of the compounds identified so far fall short of the ideal requirements for a resistance breaking pyrethroid which are listed below:

- Good resistance breaking activity at low rates (less than 20 g a.i./ha).
- Stability in sunlight.
- Residual activity as good as current pyrethroids.
- Safety to mammals, etc.

Of the seven full resistance breaking pyrethroids identified in this study, only one (the simple benzyl Series Two) comes close to meeting these requirements. However, the lower molecular weight of the simple benzyl pyrethroids increases their volatility and thus reduces their utility as residual foliar sprays. All the other resistance breaking pyrethroids identified in this study are either not stable in sunlight, have short residual or are active only at uneconomically high rates. Some leads for designing future resistance breaking

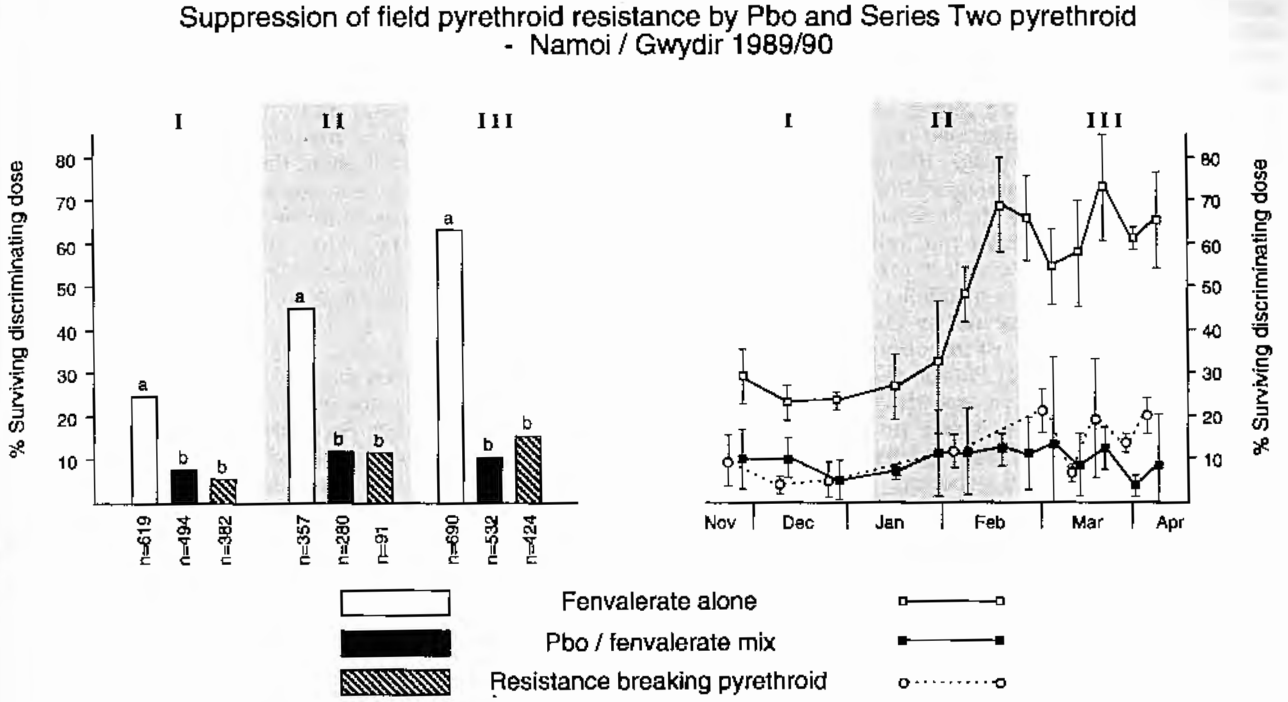


Fig. 51. Impact of piperonyl butoxide (Pbo) and a resistance breaking pyrethroid (Series Two) on pyrethroid resistance in field populations of *Helicoverpa armigera* from the Namoi and Gwydir river valleys of northern NSW in the 1989/90 season. Larval samples (collected as eggs in the field), split and screened with the discriminating dose of either fenvalerate alone (0.2 µg, from table 37) or Pbo/fenvalerate mix (50/0.2 µg, respectively) or Series Two (0.1 µg, from table 41) per 30–40 mg larva. Left hand graph shows data pooled for each Stage (I, II, III) of the Resistance Management Strategy. Columns, within each Stage, followed by the same letter, are not significantly different at $P < 0.05$ (paired t test). n = total number of larvae tested for each screen in each Stage. Similarly, right hand graph shows data graphed as a weekly average ± the 95% confidence interval, based on the between site binomial standard error.

pyrethroids have already been identified or suggested in this study and there seems to be considerable potential for modifying the alcohol moiety of pyrethroids without significantly impairing potency (Katagi & Kurita, 1989). Therefore it is probably only a matter of time before Hervé's (1982) prophecy is fulfilled, 'It will undoubtedly be possible to find some pyrethroids that are active against resistant strains by varying the acids and alcohols and changing the isomers'. Perhaps the greatest challenge will not be to discover the ideal resistance breaking pyrethroid but to convince agrochemical industry to commercialize it.

Elliott (1989) described the development of new pyrethroids as 'largely a question of economics'. His aphoristic comment is particularly appropriate for the development of resistance breaking pyrethroids which most agrochemical producers would consider possible, but extremely risky. They are concerned that any investment in developing a new resistance breaking pyrethroid would be quickly lost if use of the oxidative metabolic resistance breaker were not managed correctly. They are particularly concerned with the inability of these potential resistance breakers to overcome the nerve insensitivity type pyrethroid resistance. This fear is quite well founded as many of the world's pyrethroid resistance problems are characterized by elevated levels of *kdr* or even *super kdr* type nerve insensitivity gained through unrestrained and intense pyrethroid selection pressure over multiple pest generations. As mentioned in Section 8, it may be possible to design insecticide resistance management (IRM) strategies to avoid this scenario and there is clearly a movement in this direction within the international research community with the formation of the International Organisation for Pesticide Resistance Management (Smale, 1990). As the world-wide resistance problem continues to deteriorate, the influence of such organizations will increase and hopefully we will begin to see the implementation of successful IRM strategies throughout the world. This will provide an incentive to agrochemical industry to develop oxidative resistance breaking pyrethroids as they begin to perceive a long-term sustainable market for their investment. This problem is not new as noted by Metcalf (1989) in his review of the search for resistance proof DDT analogues and synergists for the resistance disrupted USA *Aedes aegypti* yellow fever vector eradication campaign in the 1960s. Deutero-DDT was identified as a resistance breaker for DDT dehydrochlorinase but was never commercialized because of its high cost relative to DDT ($20 and $0.45/kg, respectively). The cost problem was compounded by the encroachment of the *kdr* type nerve insensitivity resistance mechanism which obviated efforts to combat DDT resistance with resistance proof analogues and synergists (Metcalf, 1989). However, we should not be overly dismayed by this experience with *A. aegypti*. As mentioned previously in Section 8, blood feeding public health and livestock pests are probably predisposed to developing nerve insensitivity type pyrethroid resistance mechanisms while many phytophagous agricultural pests have predominantly oxidative metabolic resistance mechanisms. Furthermore, implementation of carefully designed IRM strategies may be able to select preferentially for the more amenable oxidative metabolic resistance mechanism. The oxidative resistance mechanisms developed by some of the world's most important pyrethroid resistant agricultural pests, e.g. *Plutella xylostella*, *Leptinotarsa decemlineata* and *Helicoverpa* spp. (discussed in Section 8) show remarkable parallels. This is probably not surprising as most of these resistance problems were selected with conventional phenoxybenzyl pyrethroid esters such as fenvalerate, deltamethrin, cypermethrin and permethrin which were used almost exclusively for phytophagous agricultural pests until the mid-1980s. For example, these four pyrethroids alone accounted for over 97% of world-wide pyrethroid use in the early 1980s (Hervé, 1985). Thus it is not unreasonable to assume that a resistance breaking pyrethroid designed to counter oxidative metabolic resistance in, say, pyrethroid resistant *Helicoverpa armigera* in Australia, may have more general utility in overcoming oxidative pyrethroid resistance in many other key phytophagous agricultural pests throughout the world. If this is so, and if IRM strategies can be successfully implemented, then resistance breaking pyrethroids are a potentially lucrative market and the price differential for resistance breaking and conventional compounds may not be so large as that documented in the *A. aegypti* example stated earlier.

We have come a long way in the understanding of resistance over the last three decades. We have also had some experience in successfully managing pyrethroid resistance (see earlier Sections). The incorporation of synergists and resistance breaking pyrethroids is a logical extension of this management programme. We are rapidly accumulating the knowledge required to develop a sustainable IRM strategy employing synergists and/or resistance breaking pyrethroids. Let us hope the agrochemcial industry has the foresight and courage to complement these developments.

Discussion

This study has shown that resistance is not necessarily a 'one-way street' (Keiding 1967) and that it can be managed to at least slow down the resistance treadmill, thereby extending the usefulness of available chemicals (Hammock & Soderlund, 1986; Sawicki & Denholm, 1987). The economic implications of successful insecticide resistance management (IRM) have been shown by Cox & Forrester (1992) who compared the impact of managed and unmanaged pyrethroid resistance in *Helicoverpa armigera* in the Australian and Thai cotton industries, respectively. They found that the Thai cotton industry declined dramatically despite a strong local demand for raw cotton for the booming textile industry, while the Australian cotton industry thrived and steadily expanded. Similar dramatic collapses due to uncontrolled resistance have been recorded previously (e.g. Hearn, 1975; Bottrell & Adkisson, 1977) and there is no doubt that those countries adopting successful IRM strategies, will enjoy significant economic advantage in highly competitive international markets. However, it must be stressed that successful IRM is not just a clever re-organization of chemical countermeasures into mixture and/or rotation schemes. IRM strategies must complement good integrated pest management (IPM) practices and only when IRM is properly incorporated into acceptable IPM programmes, will there be any hope of successful resistance management (Bull & Menn, 1990; Croft, 1990; Forrester, 1990a; Forrester & Fitt, 1992). As Riley (1990) suggests 'it is those states and countries that rely on these means of cotton production, that will manage the resistance problem'.

As mentioned in the Prologue, this study attempts to redress the imbalance between the large body of theoretical information on the genetics, physiology and biochemistry of resistance and the lack of application of these in practical IRM. It is hoped that this study has been successful in this regard. Many useful points for practical IRM have been highlighted throughout this study and some have already been incorporated into the 'Supplementary Guidelines' for the strategy (Forrester, 1990b; Shaw, 1991), for example:

- Grow early maturing crops to avoid dominant *H. armigera* late in the season, high resistance and expensive Stage III insecticides.
- Avoid growing certain alternative host crops (especially early maize and sunflowers) near cotton, as they serve as early season nursery crops for resistant *H. armigera.*
- Avoid consecutive sprays of pyrethroids where *H. armigera* are emerging from neighbouring early season alternative host crops, as resistance levels will be exacerbated by selection of moths before mating.
- Sample over-wintering pupae under cotton stubble and cultivate should they exceed threshold.
- If a pyrethroid is used to control sorghum midge, do not follow up with a pyrethroid for *Helicoverpa* control, as the midge spray will have already selected for pyrethroid resistant *H. armigera.*
- Add piperonyl butoxide (Pbo) to one only of the three recommended pyrethroid sprays (either the second or the third), preferably at night or late evening, to maximize its activity against moths.

The monitoring technique employed in this study (discriminating dose screening of larvae reared from field collected eggs) proved extremely successful and had a number of advantages over the conventional resistance monitoring technique (full bioassay of lab reared F1 progeny) or the alternative discriminating dose testing of pheromone trapped male moths of uncertain age or previous pesticide exposure. The development and refinement of this sensitive and cost-effective technique proved invaluable in maintaining user confidence in the strategy, combating complacency and allowing the fine tuning of strategy recommendations as and when necessary (Riley, 1990) (e.g. the reduction of the pyrethroid window from 42 to 35 days and the decision to commercialize Pbo).

However, as in most research, just as many questions seem to have been raised as resolved. Perhaps the most important is what has been the reason for the preferential selection of the oxidative metabolic