

Cotton Research and Development Corporation

FINAL REPORT

Project Title Ecological genetics of endosulfan
resistance in *Helicoverpa armigera*

Project No CSE18C

**Research
Organisation** CSIRO Division of Entomology

Principal Researcher: Dr Joanne Daly

 CSIRO Division of Entomology
 GPO Box 1700
 CANBERRA ACT 2601

 Tel: 06-246-4148
 Fax: 06-246-4000

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Introduction

The cotton industry is heavily dependent on the use of insecticides for pest management, particularly of *Helicoverpa armigera* and *H. punctigera*. Reduction in the use of these chemicals is a CRDC goal. This is achievable through the preservation of susceptibility to existing insecticides such as endosulfan. This insecticide is the most commonly used pesticide in spring and early summer (stage 1) in cotton because of its high efficacy, low cost and short-term impact on predators and parasites. Loss of this insecticide to resistance would reduce seriously the options for pest control in cotton in spring.

Resistance to endosulfan was widespread in New South Wales and Queensland during the 1970's and field failures were reported. Resistance was lost from field populations after the introduction of the synthetic pyrethroids in the late 1970's. It reappeared in the mid-1980's as the usage of endosulfan increased under the *Resistance Management Strategy*. Frequencies, however, have not raise rapidly but have averaged 10-30% and widespread problems with pest control have not been reported.

This project studied the factors responsible for frequency changes in endosulfan resistance in populations of *H. armigera* in cotton. From these results it should be possible to predict under what conditions resistance could reduce the efficacy of endosulfan for the control of *H. armigera* and lead to field failures.

Objectives and Summary of Results

(1) *To isolate a strain of H. armigera highly resistant to endosulfan and determine the inheritance of this resistance.* Resistance to endosulfan is sex-linked character, with full dosage compensation in the female. Expression of resistance is at low level in larvae and higher in adults.

(2) *To determine the factors important in the development of endosulfan resistance in the field and to predict under what conditions field failures will occur.* Commercial applications of endosulfan can discriminate only poorly between susceptible and resistant larvae. However, it is predicted that resistance can lead to field failures or poor control when insecticide applications are below recommended rates, where deposit residues are poorly distributed or reduced, or applications occur under adverse weather conditions.

(3) *To complete studies in the biology of pyrethroid resistance.* There is little evidence that pyrethroid resistant *H. armigera* are less able to survive diapause than susceptible ones. Expression of pyrethroid resistance appears to decline in adults after a period of pupal diapause.

Results and Discussion

A. The Genetic Basis of Resistance

An endosulfan resistant strain of *H. armigera* was developed from insects supplied by Dr Neil Forrester (NSW Department of Agriculture, Narrabri). The founding laboratory colony were survivors of Dr Forrester's monitoring program of the Namoi/Gwydir Valley in stage 2, 1989/90.

Resistance appeared to be conferred by a single major gene which was sex-linked. In lepidoptera the female is the heterogametic sex (XY) and the males are homogametic (XX). Possible genotypes for resistance are given below in Table 1.

Table 1. Possible genotypes for endosulfan resistance in *H. armigera*

Sex	Genotype	Phenotype
Female	R-	resistant
	r-	susceptible
Male	RR	resistant
	Rr	intermediate resistant
	rr	susceptible

where **R** is the resistant allele, **r** is the susceptible allele, and **-** is the absence of the allele on the Y chromosome in the female. Figure 1 illustrates the dose-response for adult *H. armigera* strains. Full dosage compensation was observed in the females, that is, resistant females (R-) with only one copy of the resistance allele had the same level of resistance as the homozygous resistant (RR) males that had two copies. The pattern of inheritance was confirmed in a series of backcrosses in which the expected pattern of segregation was observed.

In addition to the major gene effects, other genetic variation for endosulfan resistance was observed within the resistant strain. This additional resistance was of the order of 4-fold and did not appear to be due to a major resistance factor.

Resistance factors in the resistant and susceptible strains were determined using a variety of techniques, in a variety of stages. The results are given in Table 2. Resistance was expressed in both larvae and adults. Resistance varied from 2-166 fold depending on the life-stage and age of insects and also on the technique used to determine resistance. When larvae were exposed to treated leaves, as would be the case in the field, resistance factors were low, 2-14 fold and declined with the age of the larvae. So, although larger larvae are generally more tolerant of insecticide than younger ones, resistance appeared to confer less of an advantage in older larvae. In contrast to low resistance factors in leaf bioassays, estimates from topical dose bioassays of 7-day larvae were 66 fold. In adults, estimates varied from 10-166 fold, depending on the genetic background in which resistance was expressed. The highest resistance values may be an artefact of hybrid vigour - they were observed in the resistant daughters in F1 crosses between resistant and

susceptible lines. These high levels would not be expected under field conditions except under very intense selection pressure.

Table 2. Comparison of the LD50s of susceptible and resistant *H. armigera*.

Strain	Age in days	LD50	95% C.I.		Slope	Resistance Factors
a. Larvae exposed to treated leaves						
Susceptible	0	39	32	47	1.9	
Resistant		547	451	664		14
Susceptible	2	249	195	317	1.6	
Resistant		1,620	1,280	2,050		6.5
Susceptible	3	499	425	587	2.8	
Resistant		3,220	2,780	3,730		6.5
Susceptible	5	941	771	1,150	2.3	
Resistant		4,870	4,020	5,900		5.2
Susceptible	7	2,340	2,040	2,681	4.9	
Resistant		10,310	8,990	11,825		4.4
Susceptible	10	5,660	4,660	6,860	2.9	
Resistant		11,870	10,010	14,070		2.1
b. Larvae with topical dose						
Susceptible	7	960	896	1,020	2.5	
Resistant		63,800	59,400	68,400	2.9	66
c. Adults in treated vials						
Susceptible		9	8.5	9.5	3.7	
Heterozygous males, average		64	61	68	2.3	7
Resistant -						
Low		91	69	122	2.3	10
Average		234	220	249	1.8	26
High		560	504	623	2.0	62
Highest		1,490	1,210	1,830	3.9	166

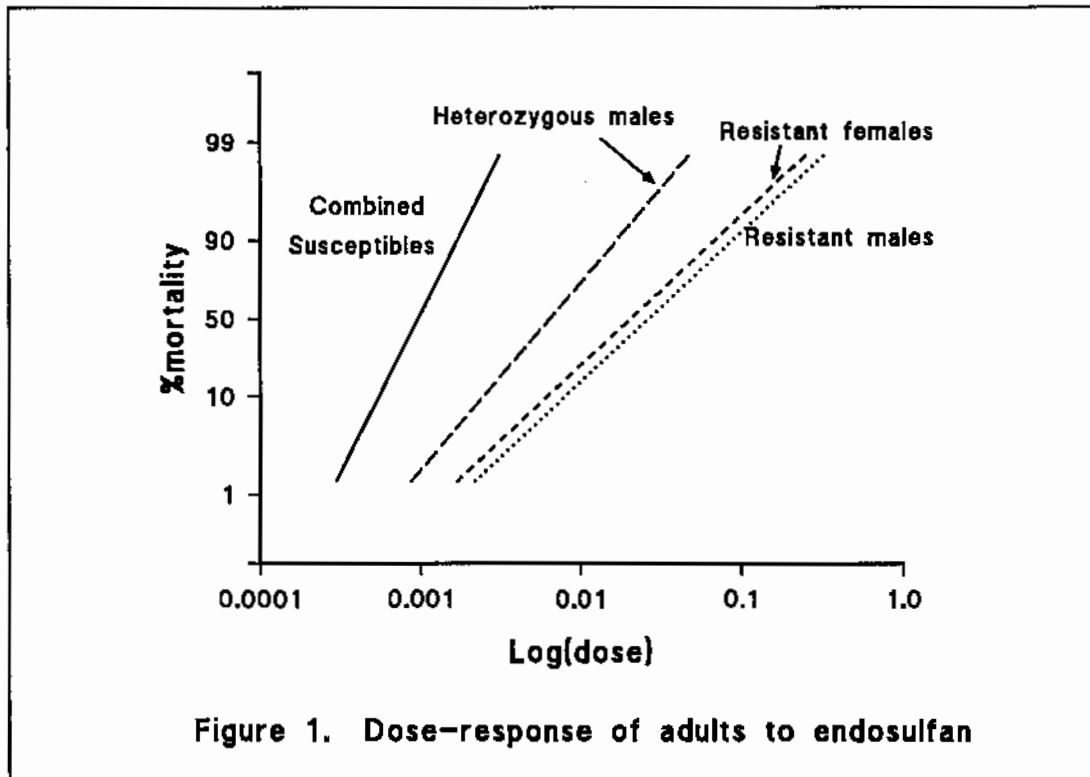
Notes: LD50 given as:

For leaves: % concentration $\times 10^{-4}$ (w/v) of 0.5 ml solution applied in Potter Tower. Endosulfan was dissolved in a solution of 1.5:1.5:3:9 parts of acetone:ethanol:oil:0.05% Triton X in water.

For Topical dose: % concentration $\times 10^{-4}$ (w/v) applied in 1 μ l of acetone to dorsal surface of 4th instar larvae, 28-40 mg in body mass.

For adults: % concentration $\times 10^{-4}$ (w/v) of 0.5 ml solution in acetone rolled onto the interior surface of a scintillation vial.

All endosulfan used was technical grade.

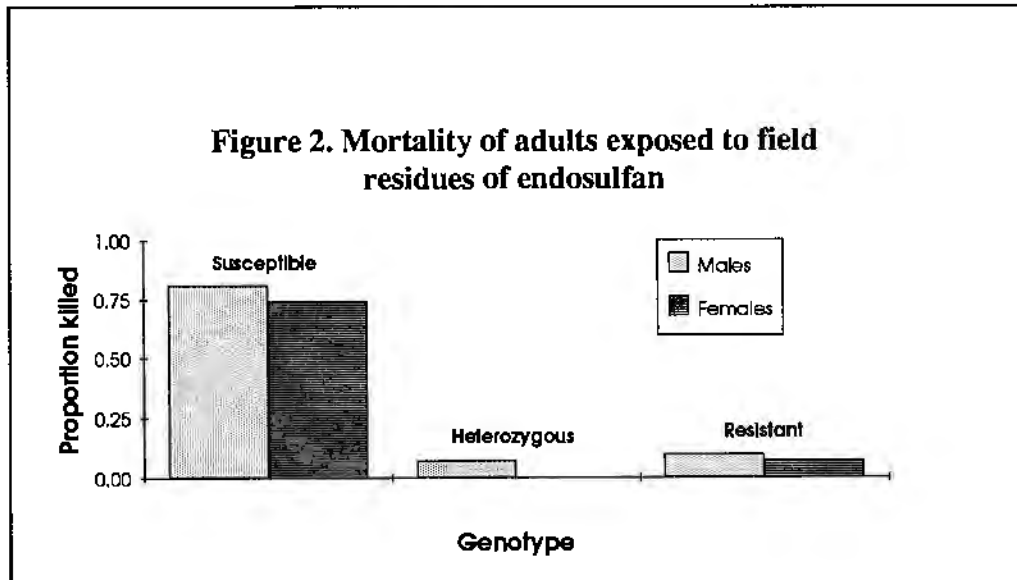


This form of resistance, as a sex-linked character with dosage compensation, can lead to a rapid increase in frequencies because even when rare, resistance is fully expressed in females. Compare this with the normal inheritance of resistance for autosomal (non sex-linked) genes in which full expression is rare until frequencies exceed 10% or more, when homozygous resistant individuals become common.

B. Field Studies of Endosulfan Resistance

Field studies were undertaken in January 1992 (1991/92 season) and in December 1992 (1992/93 season). These studies attempted to simulate exposure of *H. armigera* to endosulfan under field conditions. Larvae were placed onto leaves with deposits of endosulfan applied commercially to cotton crops at 3 litres per hectare. Adults were placed in the crop directly into the flight path of spray planes.

In adults there was good discrimination between genotypes (Figure 2). More than 75% of susceptible adults died after spraying, compared with less than 10% of either the heterozygous males or resistant females and males. Estimate of relative viability of susceptible adults was 0.26, a value that indicates strong selection is occurring in the adult life-stage under these conditions.



Larval mortality results differed between the two seasons, although the trend was the same. In both years, there was poor discrimination between the genotypes by exposure of larvae to treated leaves (Figures 3 & 4). In January 1992, most larvae 2-days old or less, died when exposed to fresh spray residues of endosulfan. There was very little difference in survival between resistant and susceptible individuals. However, when placed onto leaves sprayed two days before the experiment, very few larvae died. These results are consistent with short residual effect of endosulfan in mid-summer.

In December 1992, only minor differences were observed between genotypes. On fresh deposits most larvae died. However, in this season, survival of larvae on two day deposits was higher than in January 1992. Indeed, mortality was of the order of 40-60%.

Although the differences between the genotypes was not large in either experiment, estimates of relative viabilities for susceptibles were still low. In December 1992, these estimates were about 0.5, a moderate level of selection. An exception was in 2-day larvae on fresh deposits, 0.07, again indicating a high level of selection in this category.

Why is endosulfan resistance a problem in field conditions when it appears to confer little advantage? Figures 3 & 4 present the mortality of genotypes averaged over replicates. Figure 5 illustrates the variation in mortality between replicates for one case, neonate larvae on fresh residues. It is evident that there is considerably more variation in mortality between samples when resistant larvae are exposed than with susceptible or heterozygous individuals. One possibility is that resistance confers a greater advantage when the concentration of insecticide is reduced by either by poor application or adverse weather conditions.

Figure 3. Larval Mortality in January 1992

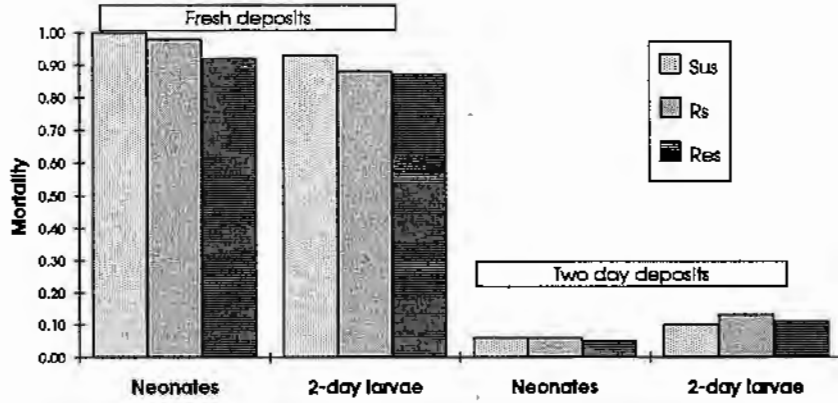


Figure 4. Larval mortality in December 1992.

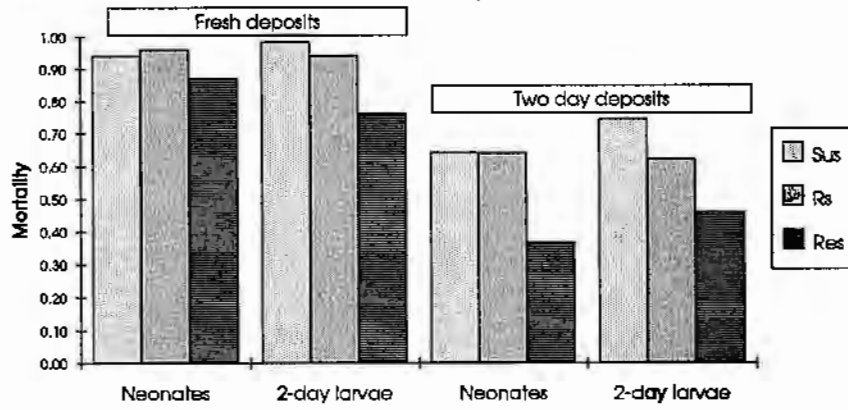
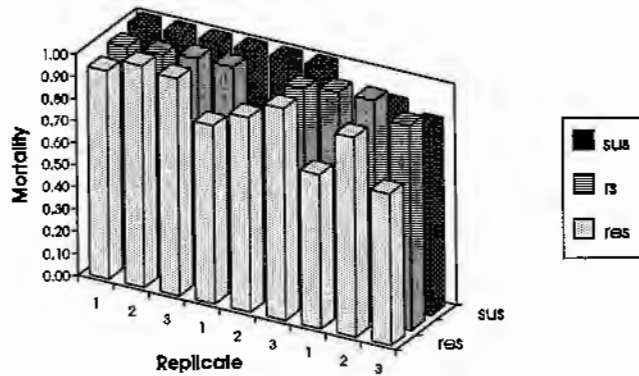


Figure 5. Mortality of neonate larvae on fresh deposits



C. Biology of Pyrethroid Resistance

Pyrethroid resistance frequencies decline each year from the high levels observed in autumn to more moderate levels in springtime. Although Daly & Fitt (1990) and Fitt & Daly (1990) indicate that this decline is due primarily to immigration from unsprayed areas, there is some evidence (Daly & Fitt 1990) that resistant insects were less able to survive overwintering diapause. In laboratory experiments, the frequency of resistance did decline in groups that had experienced diapause. However, selective mortality could not account for this and it appears that tolerance to pyrethroids declines either as a consequence of diapause or from the extended time of development associated with diapause. Thus, unlike *Heliothis virescens*, monitoring for resistance in adult *H. armigera* is not possible using treated vials because resistance frequencies would be underestimated in spring.

Conclusions and Recommendations

Endosulfan resistance is sex-linked in *H. armigera*. The particular mode of expression, in which females that carry the resistance allele are fully resistant, means that there is a potential for a rapid increase in resistance frequencies under field conditions. The resistance factors that could be observed using topical dose testing (50-160 fold) are similar to the levels reported during the 1970s. However, there is only poor discrimination between resistant and susceptible larvae when they are exposed to endosulfan under similar conditions to that in the field (resistance factors 2-14 fold). Thus, we predict from laboratory experiments that resistance will increase in frequency in the field but will not cause many problems with insect control.

The field experiments confirm that resistance to endosulfan is under moderate selection in both larvae and adults. Thus, continued field use of endosulfan will probably lead to an increase in resistance frequencies. We can speculate further that the rate of increase will depend on the number of applications that are used against *H. armigera* (versus *H. punctigera*) and with the proportion of those applications that are mixtures versus endosulfan alone. However, field failures with this insecticide should be sporadic and unpredictable. This effect, coupled with the apparent low residual life of endosulfan in mid-summer, would argue strongly in favour of mixtures of endosulfan with other insecticides/bioinsecticides if there is a risk of adverse weather conditions when *H. armigera* is present in large numbers.

Communication of Results

PUBLICATIONS 1991-1993

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- Kennaugh, L., D. Pearce, J.C. Daly & A.A. Hobbs. 1993. A piperonyl butoxide synergizable resistance to premethrin in *Helicoverpa armigera* which is not due to increased detoxification by cytochrome P450. *Pesticide Biochem. Physiol.* 45: 234-241
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- Daly, J.C., 1993. Ecology and genetics of insecticide resistance in *Helicoverpa armigera*: interactions between selection and gene flow. *Genetica.* in press.

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Conference Proceedings

- Daly, J.C. 1991. Future needs in resistance management for *Heliothis armigera*, pp 33-40. [Eds] P.H. Twine & M.P. Zalucki. *In 'A Review of Heliothis research in Australia. Conference & Workshop Series QC91006, Queensland Department of Primary Industries, Brisbane.*
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Daly, J.C. 1993. Insecticide resistance and its management, pp 25-29. Report of Proceedings, Australian Sheep Blowfly Workshop, Canberra, December 1992. CSIRO Division of Entomology, Canberra, April 1993.

Submitted for Internal Review

Daly, J.C. & J.H. Fisk. Decrease in tolerance to pyrethroids in resistant *Helicoverpa armigera* after pupal diapause.

VIDEOS

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The Cottco Report. August 1991. Cottco Ag Sales, produced by Evan Layt and Ian McClement, Qld.