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FINAL REPORT OF CSE 1L

(July 1987-June 1990)

Ecological Genetics of Pesticide Resistance in *Heliothis armigera*

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SUMMARY OF MAJOR FINDINGS

This study determined the genetic basis of pyrethroid resistance in *Heliothis armigera* and characterised those factors that lead to cyclical fluctuations in the frequency of resistance in field populations. The major findings were:

(1) A single major semi-dominant gene, associated with mixed function oxidases, is responsible for most pyrethroid resistance in field populations.

(2) The nerve insensitivity mechanism, which appeared to be important in field failures with pyrethroids in the early 1980's, no longer contributes significantly to the expression of resistance in field populations.

(3) Field application rates kill resistant larvae < 4-days old.

(4) Very small resistant larvae can survive field exposure to pyrethroids as the pesticide decays or is diluted by plant growth.

(5) Field application rates kill susceptible but not resistant adults.

(6) Pupae overwintering under cotton crops have high levels of resistance and high survival.

These findings have been integrated into the resistance management strategy for control of spring/summer pests in broadacre crops in eastern Australia. In particular, these results led to, or validated, recommendations about the commercial use of pyrethroids:

. use pyrethroids only on larvae < 5 mm,

. do not use pyrethroids at low rates against non-*Heliothis* pests,

. do cultivate cotton crop stubble to destroy overwintering populations of *Heliothis*,

. use a synergist, piperonyl butoxide, with pyrethroids to reduce selection pressure for resistance.

Ecological Genetics of Pesticide Resistance in *Heliothis armigera*

Field of Research	Crop and Environment Protection
Field Code	1.1

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INTRODUCTION

The use of insecticides for pest control in broadacre crops in Queensland and northern New South Wales is regulated by a resistance management strategy. The strategy was introduced in 1983 after resistance to the synthetic pyrethroids led to field failures in the control of the major pest species, *Heliothis armigera*, in the Emerald district in Queensland. Subsequently, pyrethroid resistance was observed to be widespread in *H. armigera* populations in eastern Australia. Endosulfan resistance in *H. armigera* had been a problem in the 1970's.

When the strategy was designed little was known about pyrethroid resistance in *H. armigera*, and vital information about the biology of the species was lacking. So the design was based on general notions about resistance rather than tailor-made to suit the cotton industry or the specific cases of resistance. The strategy aimed to moderate the frequencies of resistance to the pyrethroid and endosulfan insecticides in *H. armigera* by limiting selection pressure for resistant individuals. In cotton crops, the use of endosulfan was restricted to spring and early summer and that of synthetic pyrethroids to a 5-6 week period in January and February.

Since 1983, pyrethroids have continued to be used for *H. armigera* control and resistance frequencies have cycled annually. Resistance is at relatively low frequency in spring; there is a rise during, and immediately after, the pyrethroid use period in mid-summer; and then a decline by the beginning of spring. The extent to which these fluctuations are caused by the strategy cannot be tested directly because of the serious financial constraints of establishing a control region. Such a region would have been allowed the continued use of pyrethroids for *Heliothis* control, for comparison with regions which adopted the strategy. Yet assessment of the strategy, and its effects on resistance frequencies in *H. armigera*, is vital if the cotton industry is to avoid unnecessary costs incurred from restrictions in the choice of chemical pesticides.

This project (CSE 1L) has taken an alternative approach to validate the strategy: it has studied the genetics of resistance and has determined those factors which have influenced the evolution of the resistance genes. From this information inferences can be drawn about how the strategy has altered the relative importance of the factors.

An ability to categorise resistant individuals into different genotypes is essential before resistance can be studied in the field. Laboratory studies determined the number of major genes involved in pyrethroid resistance. Field studies then examined the causes of annual fluctuations in frequencies of these resistance genes. Experiments evaluated the selection pressure on both larvae and adults when pyrethroids were applied to commercial farms. Observations were made on the effect of overwintering, and of immigration on the winter/spring decline in frequencies (see final report of grant DAN 36L, 1987-90).

Collaboration was undertaken with Dr Gary Fitt, CSIRO Division of Entomology, Narrabri (field studies of winter and spring generations), Dr Robin Gunning and Mr Neil Forrester, New South Wales Agriculture and Fisheries, and Dr Andrew Hobbs, University of Western Australia (all collaborated on aspects of the mechanisms and genetics of resistance).

OBJECTIVES

The following objectives were proposed for the project:

1. to determine the number of genes involved in resistance to synthetic pyrethroids,
2. to determine those factors which affect the frequency of these genes in sprayed and unsprayed populations. Particular emphasis will be placed on the life-stage when selection for resistance occurs,
3. to establish reference strains of H. armigera, each homozygous (true-breeding) for each resistance gene isolated. This work will extend to resistance to other insecticides, and
4. to establish linkage relationships between these genes and other genetic (electrophoretic) markers.

Objectives 1-3 were achieved and preliminary studies were begun with objective 4 (see below). Major findings have been published (see attached list).

RESEARCH FINDINGS

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1. Genetic Studies

In *H. armigera,* pyrethroid resistance is expressed in all larval instars and in adults. Laboratory crosses indicate that the major component of pyrethroid resistance is inherited as a single, incompletely dominant gene, *mfo* (Fig. 1) [publication 15]; it is completely dominant in expression under field conditions (see Fig. 5 below). The gene confers 33-fold increase in tolerance to pyrethroids in 4th instar larvae (Fig. 1) [15] and 200-fold increase in freshly emerged adults (Fig 2) [4,9]. Expression of MFO resistance in adults declines with their age; it is almost absent in adults > 6-d old (Fig. 3) [9].

The resistance phenotype can be suppressed in both adults and larvae with the addition of the synergist, piperonyl butoxide, which is specific to the mixed function oxidase (MFO) enzymes (Fig. 2). Thus, it can be inferred that the mechanism of resistance is associated with this group of enzymes [15].

A true discriminating dose between heterozygous and susceptible individuals does not exist in 4th instar larvae. Between 15-50% of heterozygous individuals are killed at the diagnostic dose currently used in field monitoring programs. As a consequence, programs which monitor 4th instar larvae will underestimate pyrethroid resistance frequencies [15].

A second mechanism, nerve insensitivity (NIS), was shown to be a relatively minor component of resistance. By itself, it did not appear to be contributing to the resistance phenotype under bioassay conditions [4]. The genetics of this mechanism appear to be complex: the resistance phenotype within a strain containing on NIS mechanism was increased over a 3-5 generations of selection. Furthermore, Dr R.V. Gunning observed an increased nerve insensitivity in the selected strain. The limited effect of NIS on resistance phenotype in contemporary populations is in contrast to observations by Dr R.V. Gunning shortly after field failures with pyrethroids in 1983. At that time Dr Gunning found that nerve insensitivity was the main mechanism of resistance. However, our ability to increase the expression of NIS expression with selection indicates that NIS could again become a problem if pyrethroid use increased.

2. Field Studies

Field studies examined the expression of MFO resistance in *H. armigera* exposed to commercial applications of insecticide. Although resistant larvae, < 4-d old, are killed when exposed to fresh applications of synthetic pyrethroids (Fig. 4), selective mortality can occur in smaller larvae as the insecticide is degraded by weather (Fig. 5): 2 days after application with 2-d old larvae and 8 days after application with neonate larvae [8].

Another important life-stage in which selection can occur is in adults. Experiments gave direct evidence of adult selection in the field. Both susceptible and resistance adults were restrained on platforms in cotton fields and exposed to aerial applications of pyrethroids at commercial rates. Between 57-75% of resistant adults survived compared with 23% of susceptible ones [9].

The frequency of pyrethroid resistance is known to cycle annually. Of particular interest is the decline in frequency observed from the autumn generation of *H. armigera* to that in the first and second spring generations. Resistance frequencies were examined in overwintering and spring populations of *H. armigera* in the Namoi/Gwydir Valleys (in collaboration with Dr Gary Fitt; see also final report of DAN 36L). In 1987 and in 1988, a majority of the overwintering pupae were found in cotton fields. These populations had significantly higher levels of resistance, and lower levels of parasitism, than populations found under other crops. Little selective mortality was observed in these pupae so that populations of moths emerging in springtime would be expected also to have high resistance frequencies (Fig. 6).

In spring, larval populations with high frequencies of resistance (60-70%) were observed concurrently with the emergence of local overwintering populations. However, other populations were sampled before local emergence had significantly lower frequencies of resistance. From the results of this study, and those of the ecology of *Heliothis* (G.P. Fitt), it appears that the spring decline in resistance frequencies is due to immigration of *H. armigera* which are less resistant than the resident populations [2,3,5,9].

3. Reference Strains

Three pure-breeding reference strains for resistance studies have been established during the course of this project. These are:

F3.1:	Pyrethroid resistant; established from a single pair cross; contains metabolic resistance.
AN02:	Similar to F3.1 except that it is more outbred.
K:	Contains nerve insensitivity but no metabolic resistance.

More recent studies have established strains for endosulfan resistance.

4. Cytogenetics of *Heliothis* species

(J.H. Fisk)

Both *H. armigera* and its sibling species, *H. punctigera,* were observed to have 31 pairs of chromosomes which are in a graded series of sizes with none distinctive. *H. punctigera* had a consistently higher number of dumb-bell shaped bivalents at male meiotic first metaphase than did *H. armigera* [6].

Female meiosis was confirmed to be achiasmatic [6]. This observation will assist in the mapping of genes to specific chromosomes.

PUBLICATIONS 1987-1991

Ecological Genetics of Resistance

1. Daly, J.C. 1991. Future needs in resistance management for *Heliothis armigera,* pp 33-40. [Eds] P.H. Twine and M.P Zalucki. *In* 'A Review of Heliothis research in Australia. Conference and Workshop Series QC91006, Queensland Department of Primary Industries, Brisbane.
2. Daly, J.C. and G.P. Fitt. 1990. Resistance frequencies in overwintering pupae and the spring generation of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in northern New South Wales, Australia: selective mortality and gene flow. J. Econ. Entomol. 83: 1682-1688.
3. Daly, J.C. and G.P. Fitt. 1990. Monitoring for pyrethroid resistance in relation to body weight in adult *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). J. Econ. Entomol. 83: 705-709.
4. Daly, J., S. Trowell and A. Hobbs. 1990. Ecological genetics of *Heliothis.* pp 259-266. Australian Cotton Growers Research Conference, Broadbeach, QLD, August 1990.
5. Fitt, G.P. and J.C. Daly. 1990. Abundance of overwintering pupae and the spring generation of *Helicoverpa* spp. (Lepidoptera: Noctuidae) in northern New South Wales, Australia: implications for pest management. J. Econ. Entomol. 83:1827-1836.
6. Fisk, J.H. 1989. Karyotype and achiasmatic female meiosis in *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) (Lepidoptera: Noctuidae). Genome 32: 967-971.
7. Daly, J.C. 1988. Insecticide resistance in *Heliothis armigera* in Australia. Pesticide Science. 23: 165-176.
8. Daly, J.C., J.H. Fisk and N.W. Forrester. 1988. Selective mortality in field trials between strains of *Heliothis armigera* (Hübner) (Lepidoptera: Noctuidae) resistant and susceptible to synthetic pyrethroids: functional dominance of resistance and age-class. J. Econ. Entomol. 81: 1000-1007.
9. Daly, J.C., G.P. Fitt and J.H. Fisk. 1988. Pyrethroid resistance in pupae and adult *Heliothis armigera,* pp 73-78. Australian Cotton Growers Research Conference, Surfers Paradise, August 1988.

10. Daly, J.C. and D.A.H. Murray. 1988. Evolution of resistance to synthetic pyrethroids in *Heliothis armigera* (Hübner) (Lepidoptera: Noctuidae) in Australia. J. Econ. Entomol. 81: 984-988.

11. Fitt, G.P. and J.C. Daly. 1988. The overwintering foe, pp 13-24. Australian Cotton Growers Research Conference, Surfers Paradise, August 1988.

13. Daly, J.C. 1987. Ecological genetics of *Heliothis armigera* and pyrethroid resistance. Aust. Cotton Grower 8 (4): 38-41.

14. Fitt, G.P., J.C. Daly, A.G.L. Wilson and M. Terras. 1987. Identification of *Heliothis* eggs using electrophoresis. Aust. Cotton Grower 8(4): 54-56.

Submitted for Publication

15. Daly, J.C. and J.H. Fisk. Inheritance of metabolic resistance to the synthetic pyrethroids in the moth, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), from New South Wales, Australia. Submitted to Bull. ent. Res.

Other Publications (1987-91) on Heliothis, Insecticide Resistance Management Strategies or Population Genetics.

16. Daly, J.C. 1990. Methods for studying population genetics in *Heliothis*, pp 157-170. [Eds] M. Zalucki and P. Twine. *In* 'Handbook on Research on the Biology of *Heliothis*' Springer-Verlag.

17. Daly, J.C. 1990. Resistance management strategies - Theory and practice. pp 72-83. [Eds] J.A. McKenzie and P. Martin. Proceedings of 'Future Research Needs in Resistance Management in the Wool Industry', Australian Wool Corporation, Melbourne, March 1990.

18. Daly, J.C. and J.L. Patton. 1990. Dispersal, gene flow and allelic diversity between local populations of *Thomomys bottae* pocket gophers in the coastal ranges of California. Evolution 44: 1283-1294.

19. Roush, R.T and J.C. Daly. 1990. The role of population genetics in resistance research and management, pp 97-153. *In* R.T. Roush and B. Tabashnik, 'Pesticide Resistance in Arthropods', Chapman and Hall, New York.

20. Trowell, S. and J. Daly. 1990. A *Heliothis* identification kit. pp 267-275. Australian Cotton Growers Research Conference, Broadbeach, QLD, August 1990.

21. Daly, J.C. 1989. The use of electrophoretic data in a study of gene flow in the pest species *Heliothis armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae), pp.115-141. *In* H.D. Loxdale and J. Den Hollander [Eds], Electrophoretic Studies on Agricultural Pests, Systematics Special Volume No 39. Clarendon Press, Oxford.

22. Fisk, J.H. and J.C. Daly. 1989. Electrophoresis of *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) (Lepidoptera: Noctuidae): genotype expression in eggs and allozyme variations between life stages. J. Aust. ent. Soc. 28: 191-192.

23. Gregg, P.C. and J.C. Daly. 1989. The Australian species of *Heliothis*: identification, genetic variation and migration. Acta Phytopatholog et Entomol. Hung. 24: 85-91.

24. Farrow, R.A. and J.C. Daly. 1987. Long-range movements as an adaptive strategy in the genus *Heliothis* (Hübner) (Lepidoptera: Noctuidae): a review of its occurrence and detection in four pest species. Aust. J. Zool. 35: 1-24.

TRAVEL

(* indicates partly funded by the Cotton Research Council)

Overseas Trips

1. 11th International Congress of Plant Protection, Manila, Phillipines, October 1987. Presented an invited paper on 'Insecticide resistance in *Heliothis armigera* in Australia'.

* 2. United Kingdom, April 1989. Attended a symposium on 'Electrophoretic studies on agricultural pests', Rothamsted Experimental Research Station, England, April 1988. Presented a paper 'The use of electrophoretic data in a study of gene flow in the pest species *Heliothis armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae).

Visited four groups involved in studies on resistance. Presented a seminar on 'Pyrethroid resistance in *Heliothis armigera* in Australia'.

3. 18th International Congress of Entomology, Vancouver, Canada, July 1988. Presented two invited papers: 'Status of pyrethroid resistance in *Heliothis armigera* (Lepidoptera: Noctuidae) after four years of management: the Australian experience' and 'Ecological genetics of *Heliothis armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae)'.

Local Conferences/workshops

Papers were presented at all the meetings below except the 1988 ACGRA meeting.

* 1. Australian Cotton Growers Research Conference, Broadbeach, QLD, August 1988 and 1990.

* 2. Australian Entomological Society, Brisbane, May, 1988 and Sydney, 1989

3. Chairman of Organising Committee, Australian Entomological Society, Canberra, July 1990.

4. Workshop on 'Future Research for '*Helicoverpa*', Oilseeds Research Council, Brisbane, February 1990.

5. Workshop on 'Future Research Needs in Resistance Management in the Wool Industry', Australian Wool Corporation, Melbourne, March 1990.

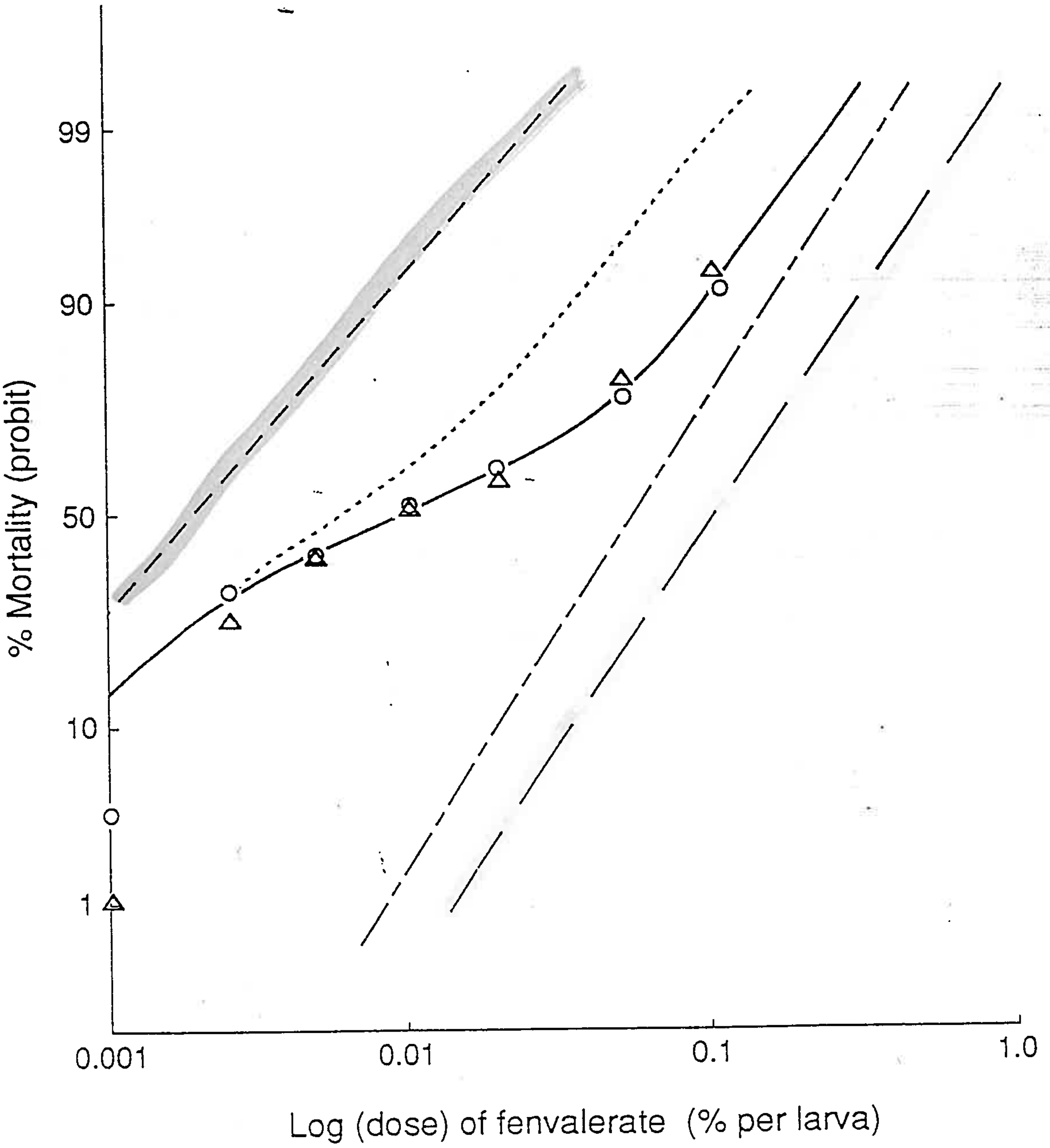

Fig 1. Dose response lines for (a) susceptible strain in 1987-88 (- - -); (b) resistant homozygote Mfo in 1987 (—— ——); (c) high F_1 line (—— - - ——); (d predicted backcross line between high F_1 and susceptible strains (———) and between low F_1 and susceptible strains (.....); (e) observed mortality for backcrosses from high F_1 (Δ) and from low F_1 (0) from all generations.

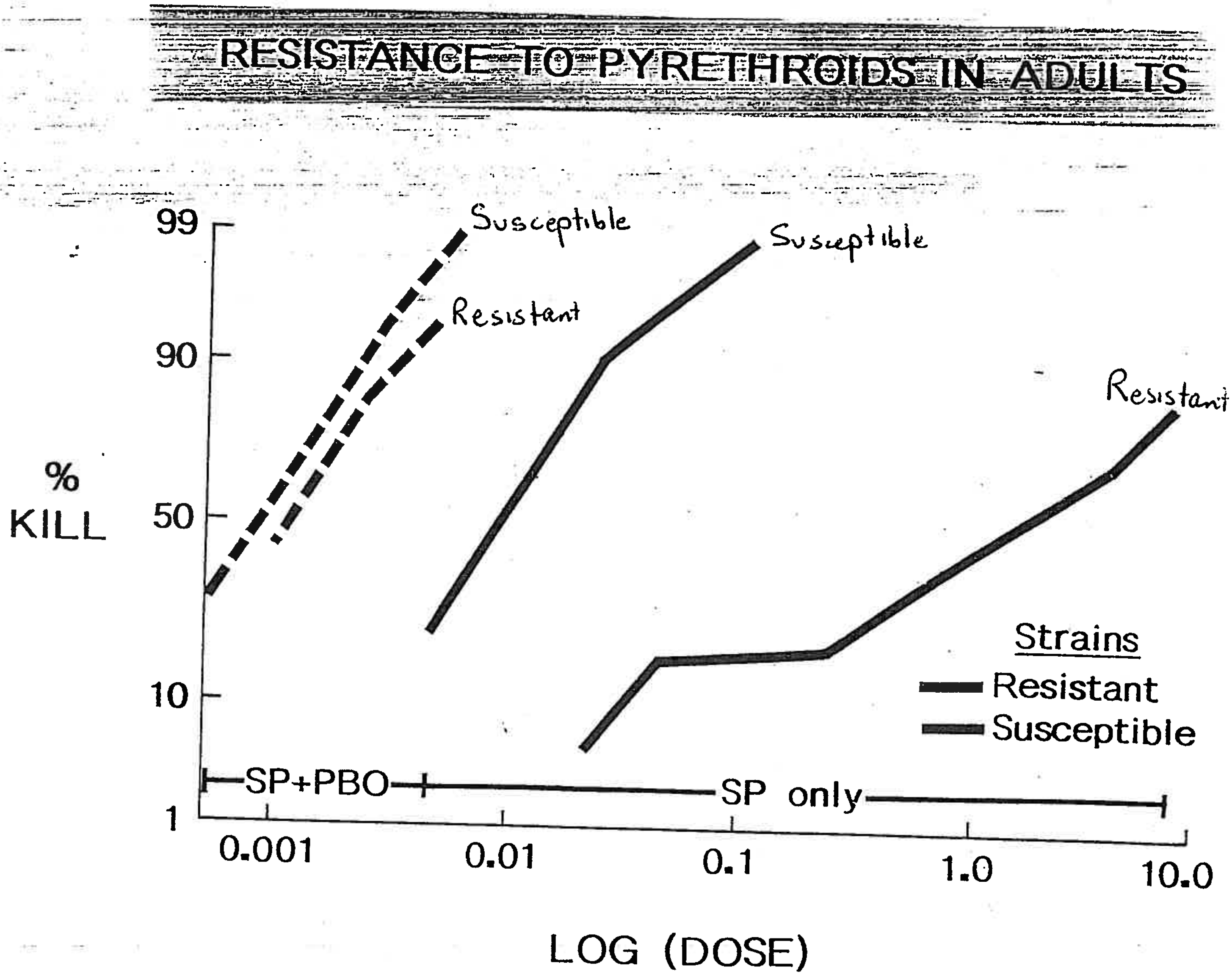

Figure 2. Bioassay response of pyrethroid resistant and susceptible *H. armigera* adults in the presence and absence of the synergist piperonyl butoxide.

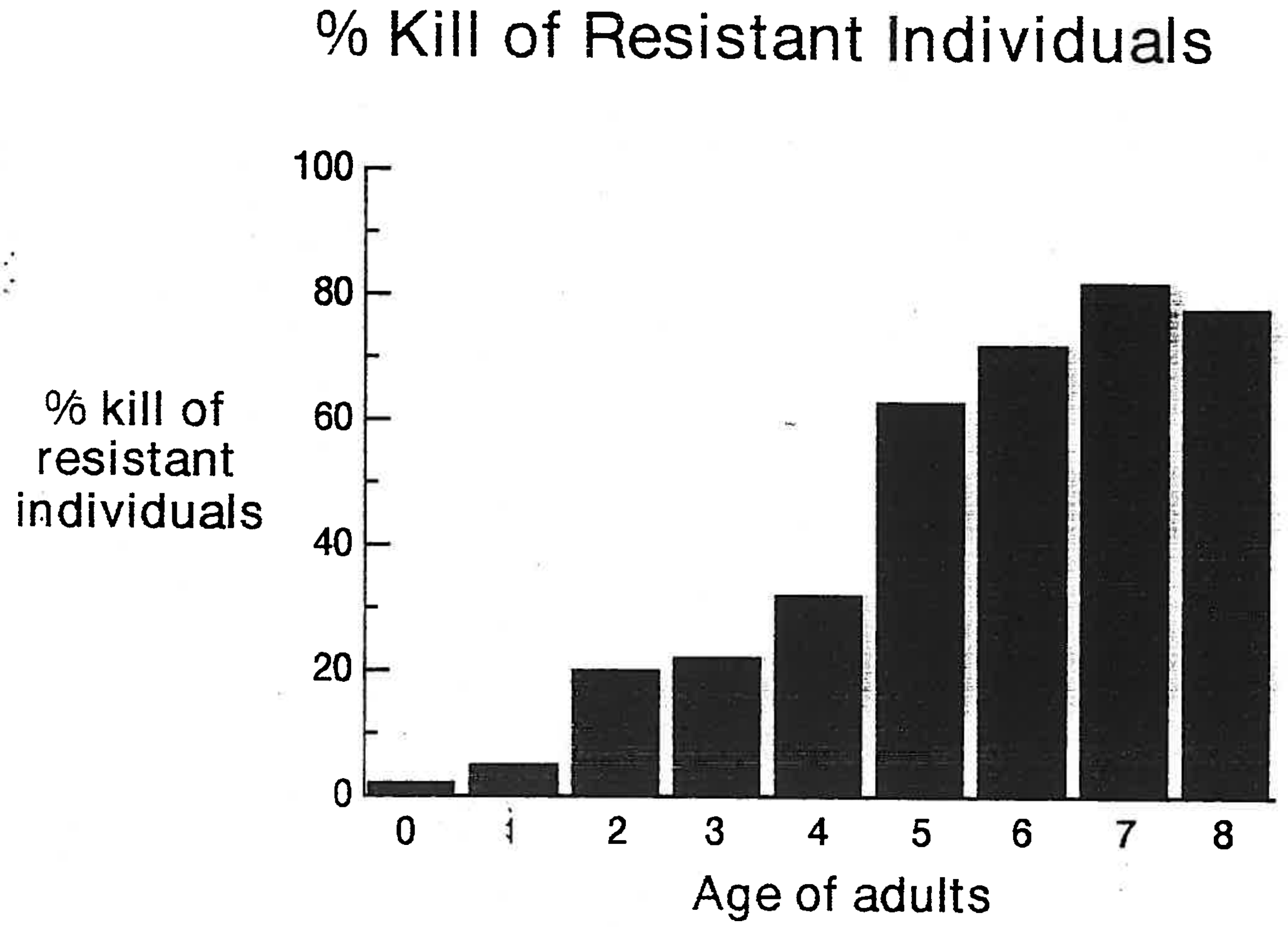

Figure 3. The percentage of resistant adults that are killed at the LD_{99} for susceptible individuals. Note that resistant adults become less tolerant as they get older.

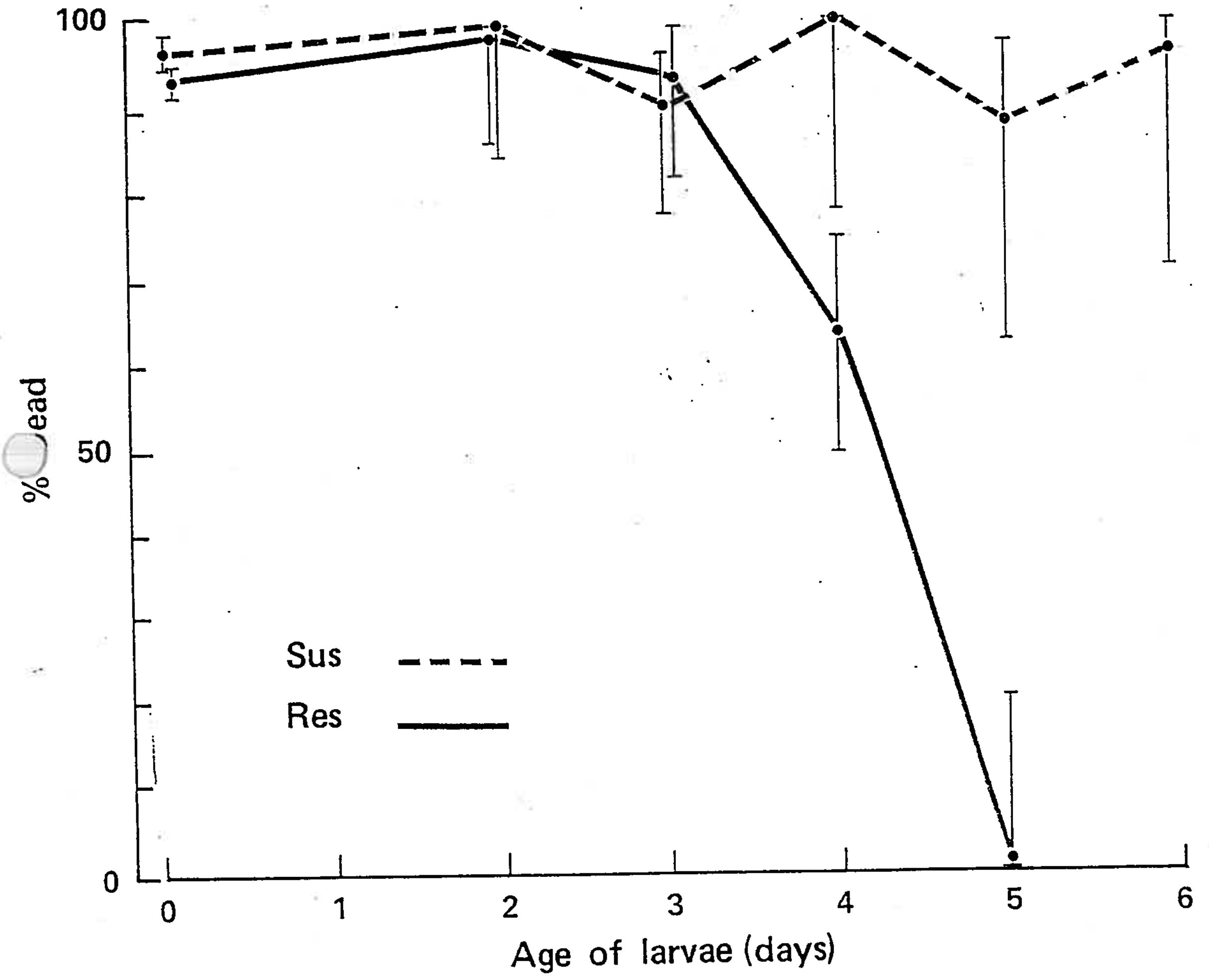

Figure 4. The percentage of larvae that are killed when exposed to field application rates of pyrethroids. Note that resistant larvae become more tolerant to pyrethroids as they get older.

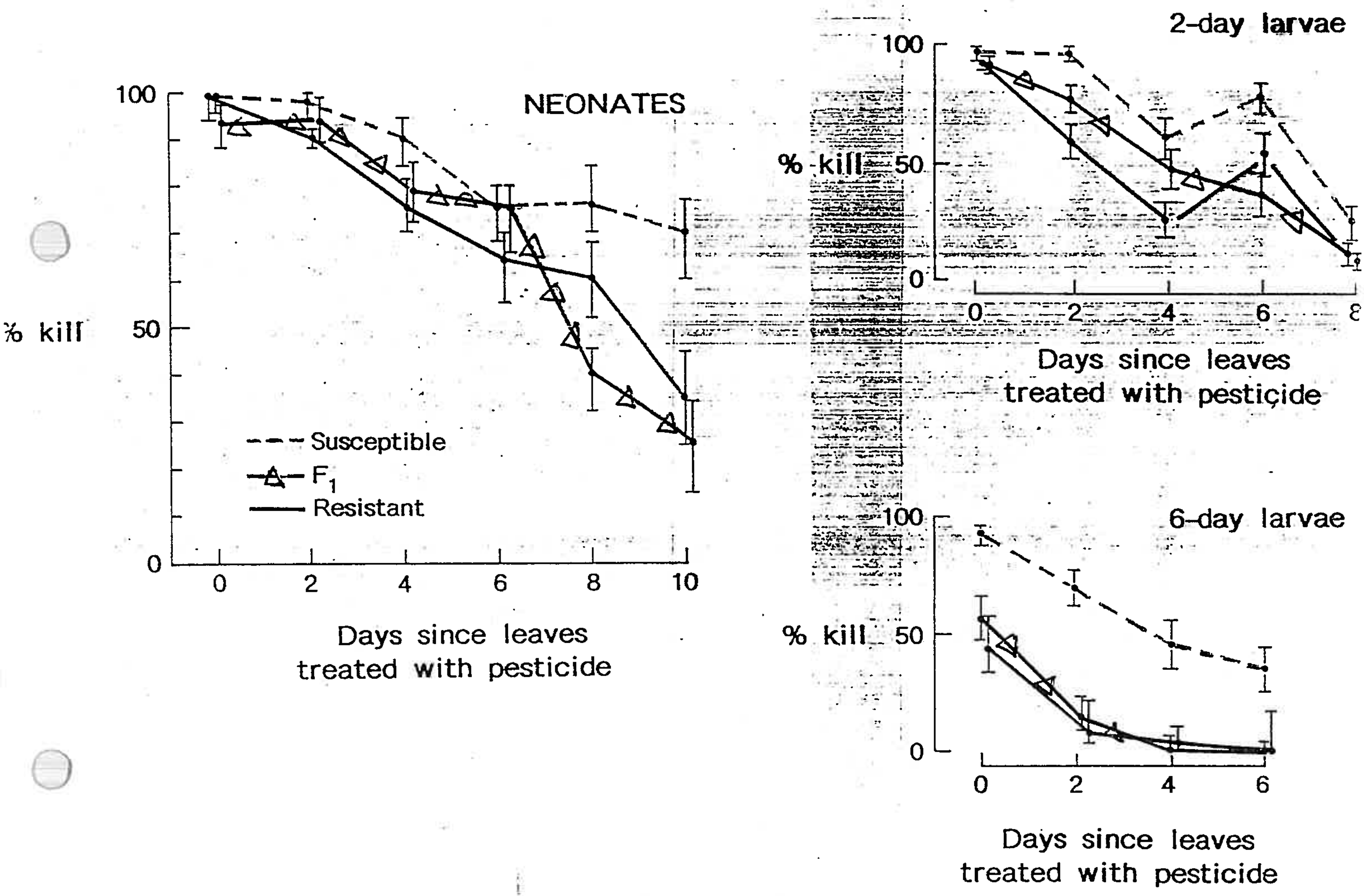

Figure 5. The response of larvae of different ages to decay of pyrethroids applied to cotton leaves in commercial field. Note that as the insecticide decays resistant larvae, < 4-d old can survive exposure.

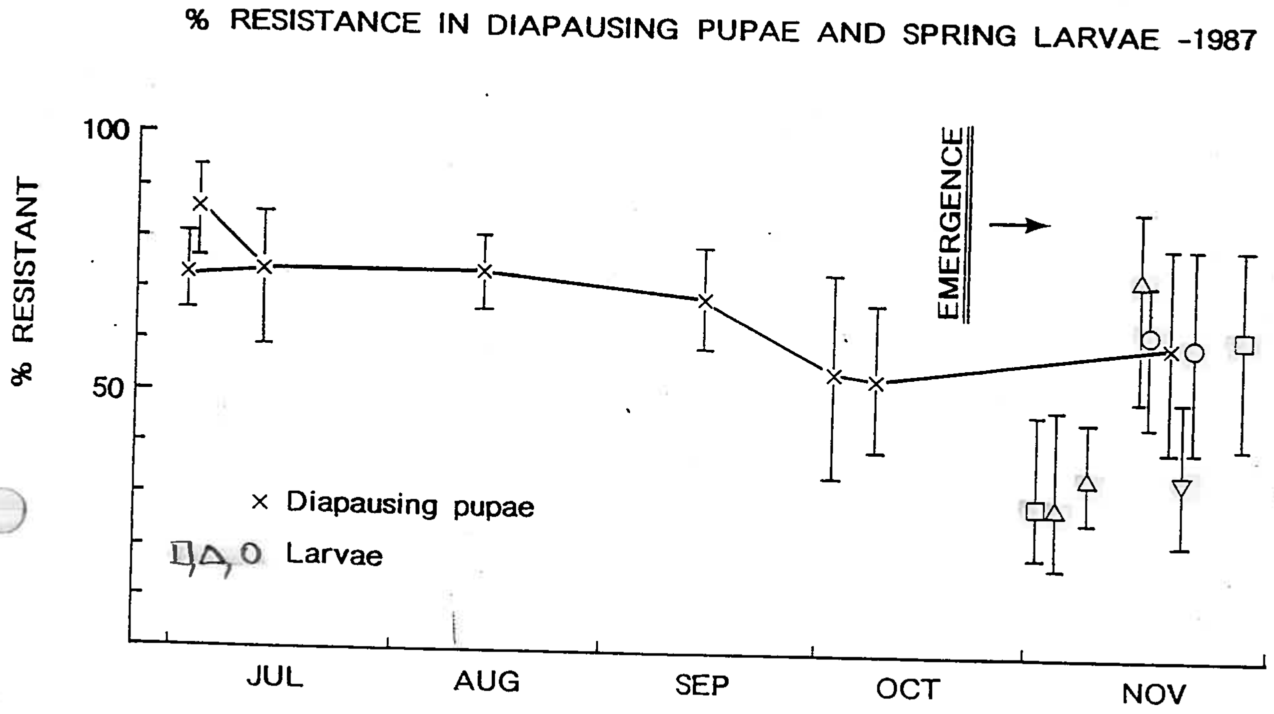

Figure 6. The frequency of resistance in pupae diapausing under cotton crop stubble and in larvae found in spring crops in the Namoi/Gwydir Valleys.