Ecology, evolution and the effective use of pesticides

John A. McKenzie, Zhenzhong Chen and Phil Batterham
Centre for Environmental Stress and Adaptation Research
Department of Genetics, University of Melbourne, Vic, 3010
Email: dean@science.unimelb.edu.au

Summary
Studies in L. cuprina *and* D. melanogaster *have demonstrated that it is possible to generate single gene resistant variants in the laboratory using a combination of mutagenesis, to increase the variation available, and selection, above the* LC_{100} *of susceptibles, to specifically screen that variation.*

The data from L. cuprina *are predictive of the results observed for naturally occurring field resistance to dieldrin and diazinon. This gives us confidence that the results gained for cyromazine are robust. The availability of data before an insecticide is used allows comparisons to be made between competing products. These data also inform decisions as to which concentrations and delivery systems should be used to minimise the evolution of resistance and to maximise the probability of effective control.*

In this context the timing of when insecticides are used can be considered not just as a reactive measure in response to a fly wave but as a potentially proactive measure to have significant impact on the population dynamics of the blowfly at a time when it is ecologically vulnerable.

Keywords
Predicting insecticide resistance, timing of application

Introduction

The management of agricultural ecosystems is still largely dependent on the judicious use of pesticides. This is certainly the case in the control of the Australian sheep blowfly, *Lucilia cuprina* where, for at the very least the intermediate future, the availability of efficacious insecticides is essential (Levot, 1993). Insecticide use has, however, almost universally resulted in the development of resistance in this and other pests (McKenzie, 1996; Denholm *et al.*, 1999).

A survey of the literature identifies general observations about the evolutionary processes and the resistance mechanisms involved (Table 1). The reasons for such outcomes are discussed elsewhere (McKenzie, 1996), but it should be noted that there is not necessarily uniform agreement about the conclusions drawn. For example, there is considerable debate concerning the reasons for preferential single gene responses to selection (McKenzie, 2000; Groeters and Tabashnik ,2000).

Table 1. Summary of the outcomes observed for various parameters in the evolution of insecticide resistance

Parameter	Observed Outcome
Genetic basis	Single gene
Biochemical mechanisms	Relatively restricted
Selection intensity	Concentration-dependent
Cost to fitness	Variable
Coadaptation	Very rare

Such debates aside, it remains true that once resistance develops in the Australian sheep blowfly, control requires sheep to be treated more frequently (McKenzie and Batterham, 1998) with the potential to exacerbate problems of occupational health and safety, residues and environmental

degradation. The resistance literature abounds with models to respond to these difficulties (Roush, 1989; Denholm *et al.,* 1999) but, typically, strategies are put in place after resistance has evolved (Roush, 1989; Forrester *et al.,* 1993; Denholm *et al.,* 1999). A more desirable approach would be to attempt to manage susceptibility (Daly and McKenzie, 1986; Tabashnik, 1990; McKenzie, 1996) by using appropriate treatment concentrations at a time when they have maximum impact on the population dynamics of the pest. Ideally the concentrations used, and the subsequent decay curves, should minimise the likelihood of resistance evolving.

Work by our group, using *L. cuprina* and *Drosophila melanogaster* (as a model organism), has been directed at these aims. We have investigated if novel timing of insecticide treatment significantly influences the population dynamics of *L. cuprina.* We have also used laboratory mutagenesis and selection regimes to test if it is possible to recreate previously naturally occurring resistances and to predict resistance mechanisms to new insecticides before they evolve in the field.

The ecological use of insecticide

In south-eastern Australia sheep are usually treated in November/December to protect them from blowfly strike. A treatment may also be necessary in autumn depending on weather conditions and blowfly activity (Foster *et al.,* 1975). Early treatment of sheep (September) aims to remove sheep as a resource when blowfly numbers are low after emergence post overwintering (Foster *et al.,* 1975; McKenzie, 1990; McKenzie, 1994). A control experiment of early treatment (McKenzie and Anderson, 1990) yielded promising results (Table 2). Both sheep blowfly numbers and strike rate showed significant decrease following early treatment. Importantly, the carrion species *Calliphora stygia* and *C. augur*, which are not obligate parasites of sheep (Waterhouse, 1947), show no significant difference between treatment regimes, providing an independent control for the treatment-related changes observed for *L. cuprina* populations.

Table 2. The effect of an early treatment strategy on the number of blowflies trapped and the strike-rate in sheep

Season	Ratio of number of flies trapped (ETA/NTA)		Strike-rate (ETA/NTA)
	Lucilia cuprina	*Calliphora* spp.	
Control	1.79	1.20	1.82
Experimental	0.70	0.95	0.36

In experimental seasons an area with early treatment of sheep in September (ETA) is compared with a normal treatment (late November) area (NTA). During the control seasons sheep from both areas were treated in late November (the data are averaged over seasons and derive from McKenzie and Anderson, 1990; after McKenzie, 1996).

Even more importantly, over the several years of the study, fewer treatments were requir ed to achieve satisfactory strike control in autumn in early treatment areas than in normal treatment areas. More effective control had the extra benefit of economic advantage (McKenzie and Anderson, 1990). Similar strategies have subsequently been suggested for the control of *L. sericata*, the primary cause of strike in sheep in the United Kingdom (Wall *et al.,* 1993).

While early treatment of sheep in the reported study resulted in less overall treatments, advocacy of early treatment strategies must be made with several caveats. Prophylatic treatment would only be considered in areas where a spring treatment was normal practice. It would be enhanced if treated populations were susceptible to the insecticide used. With respect to this latter criterion, management of a susceptible population would be enhanced if the likely resistance mechanisms were identified before they evolved in the field. This would inform the concentration of insecticide applied to sheep, provide data of possible selective pressures for resistance (McKenzie and Whitten, 1982), allow comparison of the potential of competing products to develop

resistance and to consider delivery strategies to minimise this (McKenzie, 1996). The question then becomes; is it possible to predict the genetic basis of resistance to a novel chemical before it evolves in natural populations? The answer may well be yes (McKenzie and Batterham, 1998).

Predicting resistance

Control experiments

Before a new chemical is introduced into the field for blowfly control, will a regime of laboratory mutagenesis and selection effectively screen for likely genetic variants that subsequently confer resistance? To have confidence in the process we must first demonstrate that such a regime can select for resistance mechanisms that have evolved to previously used chemicals.

Resistance to the cyclodiene dieldrin and to the organophosphorus insecticide diazinon evolved in *L. cuprina* in the 1950's and 1960's respectively (Hughes and McKenzie, 1987). In each case resistance is essentially controlled by allelic substitution at a single genetic locus. The dieldrin–resistance gene (*Rdl*) maps to chromosome V, resistance to diazinon is controlled by the *Rop-1* gene on chromosome IV (McKenzie and Batterham, 1998).

Following mutagenesis, selection at concentrations above the LC_{100} of susceptibles (McKenzie 2000) resulted in several laboratory-generated resistant variants for each insecticide. The genetic basis for each variant was identical to that observed in natural populations (Smyth *et al.,* 1992; McKenzie *et al.,* 1992). That is, laboratory-generated dieldrin-resistance mapped to *Rdl*, diazinon-resistance to *Rop-1*. Even more intriguingly, the molecular bases of the laboratory variants were also identical to those that had evolved in the field. Dieldrin-resistance is due to a single amino acid substitution serine^{302} $\rightarrow$ alanine (McKenzie and Batterham, 1998), a substitution that is also observed in *D. melanogaster* and several other species (ffrench-Constant, 1994).

Diazinon-resistance results from one or other of two amino acid changes (glycine^{137} – aspartic acid, tryptophan^{251} - leucine) (McKenzie and Batterham, 1998), both of which occur in natural populations (Newcomb *et al.,* 1997; Campbell *et al.,* 1998a).

Thus, there is an excellent capacity of mutagenesis and selection regimes to recreate field resistance in the laboratory. We can therefore approach prediction of resistance mechanisms for chemicals, to which resistance is yet to evolve in the field, with considerable confidence.

Resistance to cyromazine

Resistance to cyromazine has yet to evolve in natural populations of *L. cuprina*. Laboratory mutagenesis and selection has, however, been successful in screening four resistant variants. These map to two loci on chromosomes IV and V respectively (Yen *et al.,* 1996). The variants share characteristics with naturally occurring variants from natural populations of *Musca domestica* (Shen and Plapp, 1990; Keiding *et al.,* 1992) and with laboratory generated resistant variants in *D. melanogaster* (Adcock *et al.,* 1993; Daborn *et al.,* 2000).

In all cases resistance ratios are low. It would also seem that, unlike the situation for diazinon – and dieldrin-resistance, there are a number of genetic options available by which resistance to cyromazine can evolve. It is possible that there may be more than one target for this insecticide (Daborn *et al.,* 2000). Several different resistance loci have been identified in *L. cuprina* (Yen *et al.,* 1996) and *D. melanogaster* (Adcock *et al.,* 1993; Daborn *et al.,* 2000). For the *L. cuprina* variants there is generally a fitness cost associated with resistance to cyromazine (Yen *et al.,* 1996) but this is not reported in the other species.

Using the results; managing susceptibility

Choosing an insecticide

The rate at which resistance evolves initially depends on the relative fitness of homozygous susceptibles and resistant heterozygotes over a range of concentrations (McKenzie and Whitten, 1982; Scott *et al.*, 2000). Subtle interactions can occur between genotypes. These interactions may be concentration-dependent (McKenzie, 1996). However, given the availability of resistant variants, an initial estimate of the likely rate of resistance evolution may be gained by comparing the LC_{100} ratios of heterozygotes and susceptibles. On the basis of these ratios we would predict that dieldrin-resistance would evolve more quickly than diazinon-resistance, which in turn would evolve more rapidly than resistance to cyromazine. When data on the relative fitness of homozygotes are added (McKenzie and Whitten, 1982; Smyth *et al.*, 1992; McKenzie *et al.*, 1992; Yen *et al.*, 1996) these predictions are made more firmly.

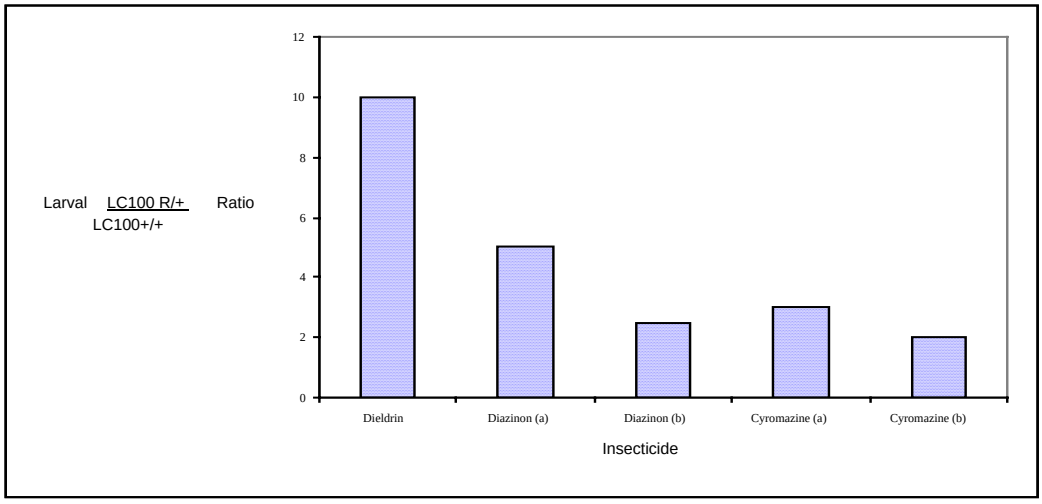

Figure 1. Larval ratio of LC_{100} R/+/ LC_{100}+/+ for strains of *L. cuprina* generated in the laboratory that are resistant to dieldrin, diazinon or cyromazine. Dieldrin-resistance is controlled by the *Rdl* gene. Diazinon-resistance is controlled by the *Rop-1* gene. Two variants (a) gly^{137}-asp and (b) trp^{251}- leu were found. The latter variant also provides resistance to the dimethyl OPs such as malathion (Campbell *et al.*, 1998a). Cyromazine-resistance is controlled by loci on chromosomes IV ((a), *Cyr(4))* or Chromosome V (b), *Cyr(5)*). (Data derived from McKenzie, 1987; Smyth *et al.*, 1992; McKenzie *et al.*, 1992; Yen *et al.*, 1996; Campbell *et al.*, 1998a; Campbell *et al.*,1988b; McKenzie and Batterham, 1998).

The predictions are supported by the field data. Resistance to dieldrin and diazinon evolved after two and ten years respectively (Hughes and McKenzie, 1987). Resistance to cyromazine is yet to evolve in natural populations of *L. cuprina* after over twenty years of usage of the chemical for blowfly control. It is clear, even without the benefit of hindsight, what chemical would have been viewed as the least likely to generate resistance had comparative mutagenesis, selection and fitness estimation experiments been conducted prior to release. Such comparisons should be done before the release of new chemicals for blowfly control.

Armed with the fore-knowledge of likely resistance mechanisms we are well placed to consider appropriate delivery/decay characteristic systems to minimise the evolution of resistance and help manage susceptibility.

Concentrations and decay curves

Heterozygotes will occur by random spontaneous mutation in a susceptible population irrespective of whether an insecticide is present. Mutation-selection balance theory predicts initial resistant allelic frequencies of between 10^{-3} and 10^{-13} depending on which assumptions are made (Whitten and McKenzie, 1982; McKenzie, 1996). If heterozygotes are exposed to concentrations of the insecticide above the LC_{100} of this phenotype, selection for resistance will not occur. Selection for single gene variants only results initially when the concentration to which individuals are exposed lies between the LC_{100} of susceptibles and that of resistant heterozygotes. Predictive knowledge of the type of resistance that is likely to evolve allows these concentrations to be defined (McKenzie, 1996).

Furthermore, if the insecticide can be delivered with a square, or rectangular, wave decay pattern, at an initial concentration above the LC_{100} of heterozygotes, the probability of resistance evolving is minimised (McKenzie, 1987; McKenzie, 1996). This is possible in the case of cyromazine. For instance, Anderson *et al.* (1989) have shown that square wave delivery is feasible and that blowfly control is effective when the insecticide is delivered from an intraruminal capsule.

Acknowledgements

We are grateful for the support from the Australian Research Council and the International Wool Secretariat at different stages of our studies.

References

Adcock, G.J., Batterham, P., Kelly, L.E., and McKenzie, J.A. (1993). Cyromazine resistance in *Drosophila melanogaster* (Diptera: Drosophilidae) generated by ethyl methanesulfonate mutagenesis. *Journal of Economic Entomology* **86**, 1001-1008.

Anderson, N., McKenzie, J.A., Laby, R.H., Strong, M.B., and Jarrett, R.G. (1989). Intraruminal controlled release of cyromazine for the prevention of *Lucilia cuprina* myiasis in sheep. *Research in Veterinary Science* **46**, 131-138.

Campbell, P.M., Newcomb, R.D., Russell, R.J., and Oakeshott, J.G. (1998a). Two different amino acid substitutions in the ali-esterase, E, confer alternative types of organophosophorus insecticide resistance in the sheep blowfly, *Lucilia cuprina*. *Insect Biochemistry and Molecular Biology* **28**, 139-150.

Campbell, P.M., Yen, J.L., Masoumi, A., Russell, R.J., Batterham, P., McKenzie, J.A., and Oakeshott, J.G. (1998b). Cross-resistance patterns among *Lucilia cuprina* (Diptera: Calliphoridae) resistant to organophosphorus insecticides. *Journal of Economic Entomology* **91**, 367-375.

Daborn, P.J., McKenzie, J.A., and Batterham, P. (2000). A genetic analysis of cyromazine resistance in *Drosophila melanogaster* (Diptera: Drosophilidae). *Journal of Economic Entomology* **93**, 911-919.

Daly, J.C., and McKenzie, J.A. (1986). Resistance management strategies in Australia. The *Heliothis* and 'Wormkill' programmes. *Proceedings of the Brighton Crop Protection Conference*, British Crop Protection Council, Croydon, England 951-959.

Denholm, I., Prickett, J.A., and Devonshire, A.L. (Eds) (1999). *'Insecticide Resistance': From Mechanisms to Management.* (CABI Publishing: Wallington).

ffrench-Constant, R.H. (1994). The molecular and population genetics of cyclodience insecticide resistance. *Insect Biochemistry and Molecular Biology* **24**, 335-345.

Forrester, N.W., Cahill, M., Bird, and L.J., Layland, J. (1993). Management of pyrethroid and endosulphan resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Australia. *Bulletin Entomological Research Supplement* **1**, 1-32.

Foster, G.G., Kitching, R.L., Vogt, W.G., and Whitten, M.J. (1975). Sheep blowfly and its control in the pastoral ecosystem of Australia. *Proceedings of the Ecological Society of Australia* **9**, 213-229.

Groeters, F.R., and Tabashnik, B.E. (2000). Roles of selection intensity, major genes, and minor genes in evolution of insecticide resistance. *Journal of Economic Entomology* **93**, 1580-1587.

Hughes, P.B., and McKenzie, J.A. (1987). Insecticide resistance in the Australian sheep blowfly, *Lucilia cuprina:* speculation, science and strategies. *In* 'Combating Resistance to Xenobiotics. Biological and Chemical Approaches'. (Eds. M.G. Ford, D.W. Holloman, B.P.S. Khambay, R.M. Sawicki). pp162-167. (Ellis Horwood: Chichester, England).

Keiding, J., El-Khodary, A.S., and Jespersen, J.B. (1992). Resistance risk assessment of two insect development inhibitors, diflubenzuron and cyromazine, for control of the house fly *Musca domestica* L. Part II: Effect of selection pressure in laboratory and field populations. *Pesticide Science* **35**, 27-37.

Levot, G.W. (1993). Insecticide resistance: new developments and future options for fly and lice control on sheep. *Wool Technology and Sheep Breeding* **41**, 108-119.

McKenzie, J.A. (1987). Insecticide resistance in the Australian sheep blowfly – messages for pesticide usage. *Chemistry and Industry* **8**, 266-269.

McKenzie, J.A. (1990). Selection at the dieldrin resistance locus in overwintering populations of *Lucilia cuprina* (Wiedemann). *Australian Journal of Zoology* **38**, 493-501.

McKenzie, J.A. (1994). Selection at the diazinon resistance locus in overwintering populations of *Lucilia cuprina* (the Australian sheep blowfly). *Heredity* **73**, 57-64.

McKenzie, J.A. (1996). *Ecological and Evolutionary Aspects of Insecticide Resistance.* (R.G. Landes/Academic Press: Austin, USA).

McKenzie, J.A. (2000). The character or the variation: the genetic analysis of the insecticide-resistance phenotype. *Bulletin of Entomological Research* **90**, 3-7.

McKenzie, J.A., and Anderson, N. (1990). Insecticidal control of *Lucilia cuprina:* strategic timing of treatment. *Australian Veterinary Journal* **67**, 385-386.

McKenzie, J.A., and Batterham, P. (1998). Predicting insecticide resistance: mutagenesis, selection and response. *Philosophical Transactions of the Royal Society of London: Biological Sciences* **353**, 1729-1734.

McKenzie, J.A. and Whitten, M.J. (1982). Selection for insecticide resistance in the Australian sheep blowfly *Lucilia cuprina. Experientia* **38**, 84-85.

McKenzie, J.A., Parker, A.G., and Yen, J.L. (1992). Polygenic and single gene responses to selection for resistance to diazinon in *Lucilia cuprina. Genetics* **130**, 613-620.

Newcomb, R.D., Campbell, P.M., Ollis, D.L., Cheah, E., Russell, R.J., and Oakeshott, J.G. (1997). A single amino acid substitution converts a carboxylesterase organophosophorus

hydrolase and confers insecticide resistance on a blowfly. *Proceedings of the National Academy of Science USA* **94**, 7464-7468.

Roush, R.T. (1989). Designing resistance management programs: how can you choose? *Pesticide Science* **26**, 423-441.

Scott, M., Diwell, K., and McKenzie, J.A. (2000). Dieldrin resistance in the Australian sheep blowfly, *Lucilia cuprina:* chance, selection and reponse. *Heredity* **84**, 599-604.

Shen, J., and Plapp, F.W. (1990). Cyromazine resistance in the house fly: genetics and cross resistance in diflubenzuron. *Journal of Economic Entomology* **83**, 1689-1697.

Smyth K.A., Parker, A.G., Yen, J.L., and McKenzie, J.A. (1992). Selection of dieldrin-resistant strains of *Lucilia cuprina* (Diptera: Calliphoridae) after ethyl methanesulphonate mutagenesis of a susceptible strain. *Journal of Economic Entomology* **85**, 352-358.

Tabashnik, B.E. (1990). Modeling and evaluation of resistance management tactics. *In* 'Pesticide Resistance in Arthropods' (Eds. R.T. Roush, B.E. Tabashnik). pp153-182. Chapman & Hall: New York, USA).

Wall, R., French, N. and Morgan, K.L. (1993). Sheep blowfly population control: development of a simulation model and analysis of management strategies. *Journal of Applied Ecology* **30**, 743-751.

Waterhouse, D.F. (1947). The relative importance of live sheep and of carrion as breeding grounds for the Australia sheep blowfly *Lucilia cuprina. CSIRO Australian Bulletin **217**.*

Whitten, M.J., and McKenzie, J.A. (1982). The genetic basis for pesticide resistance. *Proceedings of the 3rd Australasian Conference of Grassland Invertebrates* (Ed. K.E. Lee). 1-16 (South Australian Government Printer: Adelaide).

Yen, J.L., Batterham, P., Gelder, B., and McKenzie, J.A. (1996). Predicting resistance and managing susceptibility to cyromazine in the Australian sheep blowfly *Lucilia cuprina. Australian Journal of Experimental Agriculture* **36**, 413-420.