Aspects of insecticide resistance in New Zealand strains of the sheep blowflies, *Lucilia cuprina* and *Lucilia sericata*

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Thank God it's over or is it just a beginning?

LIST OF PUBLICATIONS FROM THESIS

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LIST OF ABBREVIATIONS

[^{14}C]	Carbon labelled insecticide
*	Significant at the 95% probability level
**	Significant at the 99% probability level
a.i.	Active ingredient
ACh	Acetylcholine
AChE	Acetylcholinesterase
aNA	Alpha-naphthyl acetate
ANOVA	Analysis of variance
BChE	Butyrylcholinesterase
BHC	Benzene hexachloride
bNA	Beta-naphthyl acetate
Bp	Base pair
BSA	Bovine serum albumin
BSP	Sulphobromophthalein
Bt	*Bacillus thuringiensis*
CDNB	1-chloro-2,4-dinitrobenzene
CNS	Central nervous system
CSF	Combined resistant field strain
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DCNB	1,2-dichloro-4-nitrobenzene
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DEF	S,S,S,-tributylphosphorotrithioate (TBTP)
dH_2O	Distilled water
DNA	Dioxyribonucleic acid
dNTP	Dexynucleoside triphosphate
DPM	Disintegration particles per minute
DTNB	5,5'-dithiobis(2-nitrobenzoic acid)
DTT	Dithiothreitol
E_3	Esterase 3 (*Lucilia cuprina*)
EDTA	Ethylenediaminetetra acetic acid
E_o	Total cholinesterase activity
EPN	*O*-ethyl *O*-*p*-nitrophenyl phosphonothionate
EPNO	*O*-ethyl-*O*-*p*-nitrophenyl phenylphosphonothioate
GABA	4-aminobutyric acid
GSH	Glutathione
GST	Glutathione *S*-transferase
HEPES	(N-[-hydroxyethyl]piperazine-N'-[2-ethanesulphonic acid])
IGR	Insect growth regulator
IPM	Integrated pest management
Kda	Kilodaltons
Kdr	Knock-down resistance
Ki	Pseudo-first-order rate constant governing the inactiviation of

	AChE by an organophospate insecticide
LC_{50}	Lethal concentration killing 50% of the population
LC_{99}	Lethal concentration killing 99% of the population
LcEcR	Ectysteriod receptor
LD_{50}	Lethal dose killing 50% of the population
MCE	Malathion carboxylesterase
µci	Microcurie
MFO	Mixed function oxidase
mg	Milligram
mg/l	Milligrams/litre
min	Minute
mm	Millimetres
8-MOP	8-methoxypsoralen
MtB	Methylthiobutyrate
NADPH	Nicotinamide-adenine dinucleotide phosphate, reduced
nm	Nanometres
NS	Not significant at the 95% probability level
NSW	New South Wales
NZ	New Zealand
OP	Organophosphorus insecticide
OPA(3, 4, 7)	Operon primer kit A variants
PAGE	Polyacrylamide gel electrophoresis
PBO	Piperonyl butoxide
PCA	Principal co-ordinates analysis
PMB	Pentamethylbenzene
PMSF	Phenylmethylsulfonyl fluoride
p-NBC	*p*-nitrobenzyl chloride
ppm	Parts per million
PTU	*1*-phenyl-2-thiourea
PVP	Polyvinylpyrrolidone
PVPP	Polyvinylpolypyrrolidone
RAPD PCR	Random amplified polymorphic DNA polymerase chain reaction
RF	Resistance factor = LC_{50} field strain/LC_{50} susceptible strain
RNA	Ribonucleic acid
SDS	Sodium dodecyl sulphate
SE	Standard error
SP	Synthetic pyrethroid insecticide
Stdev	Standard deviation
TBPT	S,S,S,-tributylphosphorotrithioate (DEF)
TEA	0.04 *M* Tris-acetate buffer containing 0.001 *M* EDTA
TPP	Triphenyl phosphate
Tris	Tris(hydroxymethyl)-amino-methane
µl	Microlitres
w/v	Weight per volume
w/w	Weight per weight

ABSTRACT

A treated surface bioassay was used to detect insecticide resistance to five organophosphorus insecticides (diazinon, chlorfevinphos, chlorpyrifos, dichlofenthion and propetamphos), the synthetic pyrethroid, deltamethrin, and the insect growth regulator, diflubenzuron, in larvae of field strains of *Lucilia cuprina*, and diazinon in *Lucilia sericata* from 1990 to 1997. Organophosphate resistance was widespread in both species of blowfly and *L. cuprina* exhibited side-resistance between diazinon, chlorpyrifos, dichlofenthion and propetamphos but no cross-resistance could be demonstrated with diazinon and deltamethrin, or between diazinon and diflubenzuron. Phenotypic expression of resistance appeared to be influenced by environmental factors such as season, region, sheep density and year.

The relative efficacies of four of the above OPs and the extent to which the OP resistance status of *L. cuprina* affected this, was assessed by comparing a susceptible strain of *L. cuprina*, and a strain known to exhibit OP side-resistance, in a series of *in vivo* trials. All four treatments provided 19 – 21 weeks protection against susceptible larvae but chlorfenvinphos provided the longest protection (16 – 17 weeks), followed by propetamphos (15 – 16 weeks), dichlofenthion (10 –13 weeks) and diazinon (9 – 13 weeks), against the resistant strain.

Penetration of insect cuticle, non-oxidative and oxidative mechanisms that might contribute to resistance to organophosphate insecticides were examined in larvae and adults from field isolates of *L. cuprina*. Only non-oxidative mechanisms were considered with respect to *L. sericata*. The basal mechanism of resistance appeared

to be an altered esterase (the E_3 esterase) in *L. cuprina* and was also suspected to contribute to OP-resistance in *L. sericata*. Adult and larval strains of *L. sericata* showed no relationship between level of GST conjugating activity and resistance to diazinon, suggesting that these enzymes do not constitute a mechanism of OP-resistance in this species. Contrasting this was the observation of a strong relationship between the level of GST conjugating activity, with respect to model substrate DCNB, and resistance to diazinon in both adult and larval strains of *L. cuprina*. This relationship was supported by a study of the OP-conjugating properties of partially purified glutathione S-transferases from resistant *L. cuprina* larvae.

Neither an increase in acetylcholinesterase content nor an insensitive AChE in the central nervous system of larvae of *L. cuprina* and larvae or adult *L. sericata* appear to have been selected for in the resistant field strains examined. There did however appear to be a positive correlation between resistance to diazinon and susceptibility of AChE inactivation by tetrachlorvinphos in *L. cuprina* adults. The significance of this factor was investigated further by multiple regression and it was concluded that this did not have a main effect on resistance in adults.

Samples of *L. cuprina* were further characterised by RAPD-PCR to determine intra- and inter-strain variation. Key features of this analysis were the calculation of phylogenetic similarities in an attempt to better define a 'field strain', the mode of dispersal of the species throughout New Zealand as well as to identify genetic markers of high level insecticide resistance. No markers of resistance were identified but this study emphasised the need to treat each sample as a separate strain as

sampling from the same area at different times showed that samples were not identical suggesting there is variable gene flow in and out of areas and populations.

Based on biochemical identification of resistance mechanisms, consideration was given to the use of potentiating pairs of insecticides as a method of prolonging the life of some existing OP insecticides. It was shown that prospects for using such pairs is limited due to the widespread OP-resistance observed in *L. cuprina.* Finally, rather than reiterate discussion of resistance mechanisms, the conclusion of this thesis looks towards applying knowledge of resistance mechanisms effectively within the framework of integrated pest management system (IPM). It discusses areas of weakness in knowledge that need to be addressed and emphasises the need for a susceptibility reservoir if IPM is to succeed at a national level.

INTRODUCTION

Sheep myiasis in New Zealand and approaches for its control

Blowfly strike (cutaneous myiasis) of sheep is a major problem in many sheep producing countries of the world including South Africa, Britain, Australia and New Zealand. Heath (1990) estimated losses to the New Zealand sheep industry at $30-40 million annually. Estimated losses to the Australian industry range from $50 million (Brideoake 1979) to $200 million (Beck *et al.* 1985) annually. The main costs are associated with prevention of strike including labour costs for implementing management practises such as shearing, crutching and docking. These are discussed in more detail later. The cost of chemical treatment is also significant, as is that of production losses due to deaths, loss of wool quality and reductions in live weight (Broadmeadows *et al.* 1983, Raadsma & Baker 1983).

Myiasis of sheep occurs when larvae of dipterous insects invade and feed on the living tissues and bodily fluids of the sheep (Zumpt 1965). This condition, known as blowfly strike, is an example of facultative parasitism whereby larvae can develop on both living and dead organic material (Zumpt 1965) although some species such as *Lucilia cuprina* (Wiedemann) show a greater preference for a live host (Norris 1959, Waterhouse 1947). Egg-laying by adult primary strike agents is triggered by certain predisposing factors such as faecal soiling, wounds (from docking and shearing), urine wetting or external sources of moisture and bacterial infections such as fleece rot and dermatophilosis (Graham 1979, Merritt 1979, Chin & Gogolewski 1991). Eggs hatch and larvae begin feeding on the live host and continue to do so

throughout their three larval instars. The length of life cycle is temperature dependent but at optimal lab conditions (25°C) it takes 7-12 days for larval development depending on species. Once larvae have completed development they leave the host and pupate in the soil. After emergence from puparia adults require a protein meal for sexual maturation (Norris 1959).

In Australia the most common primary myiasis fly is *Lucilia cuprina* (the Australian sheep blowfly). It is estimated to initiate over 90% of strikes (Watts *et al.* 1976). *Lucilia cuprina* is also the major cause of strike in South Africa (Waterhouse & Paramonov 1950). In England and Wales where *L. cuprina* has not been recorded, *Lucilia sericata* (Meigen) (the European or common green blowfly) is the predominant fly causing strike on sheep (French *et al.* 1992). *Lucilia sericata* is also present in Australia but is normally confined to urban areas (Hall & Wall 1995) although it can initiate strike (Beck *et al.* 1985).

Lucilia cuprina is a relatively recent arrival to New Zealand compared with Australia where it was reported as early as the 1880s (Graham 1979). In New Zealand, specimens of *L. cuprina* were first identified retrospectively from material collected in 1984 in a country-wide flystrike survey (Bishop 1993) although the species was only first recognised as occurring in New Zealand in 1988. It is estimated however, to have been introduced during the late 1970s (Heath 1991). Prior to the introduction of *L. cuprina*, *L. sericata* and *Calliphora stygia* (Fabricius) were the most important strike species. *Lucilia sericata* has been present in New Zealand for over 100 years although it was previously misidentified as *L. caesar* by Hutton (1901) (Miller 1939, Dear 1986). Numbers of single species strikes by *L. cuprina* are common but this

species is often found together with either *L. sericata* or *C. stygia* (brown blowfly), or both (D. Bishop personal communication). *Chrysomya rufifacies* (Macquart)(hairy maggot fly) is another important species in North Island strike samples. *Chrysomya rufifacies* is a secondary strike fly and is predacious on other Dipterans. Circumstantial evidence in New Zealand suggests it may also initiate many strikes (Monzu 1979, Norris 1959, Vogt & Woodburn 1979).

In the 1990/91 season (September - April) approximately 19% (n=389) of samples were single species *L. cuprina* strikes and 47% were *L. cuprina* in combination with other species. These proportions remained relatively unchanged in 1991/92 (September - April) (D. Bishop unpublished data). By 1993/94 however *L. cuprina* was present in 60% of 400 samples of larvae from field strike (D. Bishop unpublished data). Hall & Wall (1995) believe it may displace *L. sericata* as the most important strike species as it has in Australia (G. Levot personal communication) although *L. sericata* has shown no decline in its representation over several years of strike survey (Table 1).

Table 1. Percentage occurrence of *Lucilia cuprina* and *Lucilia sericata* in flystrike samples received by AgResearch, Wallaceville (extracted from AgResearch, Flystrike Newsletters produced by D. Bishop).

Flystrike Season (≈ **August – June**)	**% occurrence of flystrike species**	
	Lucilia cuprina	***Lucilia sericata***
1993 – 94	58.1	56.4
1994 – 95	69.8	58.1
1995 – 96	59.3	59.0
1996 – 97	66.1	46.5
1997 - 98	61.0	57.0

Each species of New Zealand's heterogeneous flystrike fauna has a distinct seasonal pattern, which differs between North and South Islands of New Zealand. In the North Island *C. stygia* is active from July to May and from October to April in the South. *Calliphoria stygia* tends to be adapted to lower temperatures (Norris 1959). The two green blowflies *L. sericata* and *L. cuprina* have a similar range, being active between September and May in the north, peaking between January and March. In the south *L. cuprina* is active between February and May whereas *L. sericata* is active for a longer period between November and May. *Chrysomya rufifacies*, which is only found in North Island strikes (D. Bishop personal communication), occurs between December and May (Heath & Bishop 1995). Flystrike is therefore a problem that can occur in up to ten months of a year in certain regions of New Zealand and in some years has occurred during the whole 12 months.

Control of flystrike is achieved primarily by insecticidal treatment of sheep. Insecticidal control can either be preventative or curative. Curative measures involve removal of wool from infected areas and the application of an insecticidal dressing of high concentration. Prior to the 1930s this was the only form of control available in Australia (Graham 1979). Preventative measures are the most popular form of control used today. Preventatives are applied before or at the onset of strike (Monzu *et al.* 1983) either as saturation applications or back-line applications such as pour-on or spray-on preparations. The latter two methods apply a high concentration of insecticide in a strip along the backline of the sheep that can subsequently spread throughout the epicuticle of the sheep's skin (Shanks 1975). Back-line applications

are increasing in popularity due to their ease of use and the reduction in the time of application.

The effectiveness of insecticidal treatment can be enhanced by a variety of other management practises that reduce the fly population and the susceptibility of sheep to strike. Shearing, crutching and docking are three important management strategies that reduce the attractiveness of the sheep. Timing of shearing to coincide with insecticide treatment can reduce the cost per unit of insecticide application especially where saturation strategies are employed. It also ensures that insecticide is more evenly distributed in the skin and fleece. Crutching (removal of soiled wool around the anus) and docking (removal of tails in lambs) reduce the build-up of faecal soiling of fleece. Faecal soiling can be due to a number of factors that can be alleviated. For instance, treatment of endoparasites can reduce faecal scouring (Baille 1979). Grazing management may also reduce scouring as it may result from a change in pasture type and will continue until the sheep's digestive system adjusts (Baille 1979). Aphid infested lucerne has also been shown to cause scouring (Baille 1979).

A surgical procedure known as 'mulesing' can reduce breech strike in merinos. A wrinkly breed of sheep known as the Vermont strain of merino was introduced into Australia during the 1880s (Graham 1979). These merinos have a particularly high wool yield but their excessive skin folds result in a build-up of moisture due to sweat, urine and faecal staining. This makes them particularly susceptible to breech strike. Mulesing surgically removes the wool bearing skin around the crutch area to reduce build-up of moisture (Moule 1948). Mulesing only occurs in Australia and

significantly reduces breech strike although it can be an attractive oviposition site for blowflies until healed (Cook & Steiner 1990). Morley & Johnstone (1983) present a comprehensive review of the history and development of the mules operation.

Bait-bin trapping and destruction of carrion can achieve reduction of the fly population. Bins are baited with offal to attract flies, and bin design prevents flies escaping. This method aims to attract flies away from their live host as well as reduce fly numbers. Destruction of carrion reduces alternative breeding sites and potential refugia from insecticides.

In Australia, even before 1920, attempts were made to reduce the fly population by the release of parasitoid wasps. This however failed to reduce flies to an economically manageable level (Graham 1979). The same was also attempted in New Zealand (Heath & Bishop 1989). Three wasp species of importance are *Alysia manducator* (Cameron), *Tachinaephagus zealandicus* (Ashmead) and the endemic parasitoid *Aphaereta aotea* (Hughes & Woodstock). These species have only occurred in 1.1% of strike samples during an eight-year period (1986 - 1994) (Bishop *et al.* 1996).

Development of a vaccine to protect sheep against *L. cuprina* is thought to be a promising route of protection currently under study in Australia. There is evidence to suggest that sheep acquire resistance to blowfly attack after 4-8 infestations through the development of antibody (Sanderman *et al.* 1986, Eisemann *et al.* 1990). IgG antibodies to the antigens of *L. cuprina* have been identified in the sera of struck sheep (O'Donnell *et al.* 1980, Seaton *et al.* 1992). Experimental vaccines have so far

been based on proteases secreted by larvae (Sanderman 1990), and membrane proteins of larvae (Johnson *et al* 1992).

Genetic control of insect pests may also be a useful development for biological control. For instance, the screwworm fly, *Cochliomyia hominivorax*, has been partly controlled in America by the release of radiation-sterilised males (Bushland 1975). Genetic control measures against *L. cuprina* have been trialled by Australian workers. Methods have involved sterile insect release as well as releases of flies with two chromosomal rearrangements. These were sex-limited translocations and compound chromosomes. Compound chromosomal strains were rendered partially fertile when mated with members of their own strain but completely sterile when mated with wild flies (Foster 1980). Sex-limited translocated females were constructed with an eye-colour mutation which rendered them blind and unable to survive and reproduce in the wild. Males of this strain carried the eye colour mutation that could be spread to female offspring (Foster 1980).

All three methods have been trialled in small-scale releases in New South Wales, Australia and a larger scale release of mutant males on Flinders Island off the Eyre Peninsula of South Australia (Davidson 1989). Some degree of success was achieved but the cost of mass rearing and application were considered high in comparison to the economic benefits, especially with the sterile release method (Whitten *et al.* 1977). Although New Zealand is a large land area in terms of the logistics of a mass release programme it might have a more successful outcome than in Australia. For example, the mountainous topography of New Zealand may be advantageous in that it provides barriers to fly migration thus isolating populations of

flies that could be controlled by localised extirpation through genetic modification. The observed rapid spread of *L. cuprina* throughout New Zealand may mean that such genetic control methods may only provide temporary relief however. Information on these genetically altered strains of *L. cuprina* is available if this type of programme is ever incorporated into an integrated pest management (IPM) system in New Zealand.

Insect control using bacterial pathogens such as *Bacillus thuringiensis* (Cooper & Pinnock 1983), iridoviruses (Longworth & Kalmakoff 1977) and rhabditid nematodes (Bedding 1983) are also considered to be potentially useful. *Bacillus thuringiensis* (Bt.) shows the most promise at present and is mainly used for the control of lepidopteran pests but certain strains have been shown to have larvicidal effects on *L. cuprina* (Cooper & Pinnock 1983). Evaluation of the toxicity of several Bt. strains is currently under evaluation in New Zealand (P. Wigley & C. Chilcott, personal communication).

With the exception of sterile male release and Bt. many of the methods discussed above are already operating to control flies. If used in an orchestrated manner these methods for the control of flystrike can be seen as an example of an IPM system. Insecticidal control is an essential part of this management scheme but the system has been undermined by the development of resistance to some insecticides by key fly species.

Until recently insecticide resistance had not proved to be a problem in New Zealand despite insecticides being the major method of fly prevention since the introduction

of arsenicals in 1919 (A. Heath personal communication). New Zealand and Australia have had a similar history of insecticide usage since treatments began. Arsenicals were replaced with a succession of compounds including DDT, BHC, cyclodienes (dieldrin and aldrin), carbamates and numerous organophosphorus compounds (OPs) including diazinon (Hughes 1977). In Australia *L. cuprina* has been documented as resistant to representatives of most groups including cyclodienes (dieldrin, aldrin; Shanahan 1959), carbamates (butacarb; Hughes & McKenzie 1987) and organophosphorus compounds (diazinon, Shanahan 1966). The emergence of resistance in *L. cuprina* in Australia to some of these insecticides has led to reduced periods of protection (Shanahan & Roxburgh 1974) in the case of the cyclodienes and carbamates. This has resulted in their withdrawal from the market.

Prior to the introduction of *L. cuprina* in New Zealand, there has only been one report of resistance developing in a blowfly species. Hart (1961) showed the flystrike-initiating *L. sericata* in the Marlborough region to be resistant to dieldrin. Withdrawal of this compound from the market reduced the risk of higher and presumably more widespread resistance developing. Monitoring of insecticide resistance continued for a number of years by S. Millar. Resistance monitoring later became a consistent feature of flystrike research when *L. cuprina* was found to be increasing its range southward (D. Bishop personal communication) and complaints of reduced effectiveness of insecticides became more numerous (A. Heath personal communication).

Despite growing worldwide concern over insecticide residues in the environment, insecticidal control is still of paramount importance for the management of pests and

will continue to be, at least in the near future. Strategies to minimise the development and increase of resistance are needed as new insecticides are difficult to discover, and costly to develop, register and market. Research on resistance mechanisms provides information that is integral to the implementation of IPM strategies. Beneficial knowledge includes identification of resistant populations and understanding of resistance mechanisms which could lead to the discovery of alternative insecticides or formulations. A biochemical approach can identify enzyme systems of importance and molecular biology can illuminate the basic changes that are expressed as resistance mechanisms. Many key enzyme systems are already known for resistant insect species.

This thesis aims firstly to identify the extent of OP-resistance in strains of *L. cuprina* and *L. sericata* in New Zealand and investigates how this affects the efficacy of OPs in the field (Section 1). Secondly, it attempts to characterise and compare biochemical mechanisms of OP-resistance in both species (Section 2). An attempt is made to determine whether geographic differences in the expression of resistance within and between populations of *L. cuprina* can be explained by genetic variation (Section 3). Lastly, a solution to reduce the expression of resistance is discussed and the application of possible potentiating pairs of OPs towards *L. cuprina* larvae based on this toxicological and biochemical information is investigated (Section 4).

SECTION 1

Assessing insecticide resistance in field populations of *Lucilia cuprina* and *Lucilia sericata*

Introduction

Prevention of flystrike of sheep in New Zealand is achieved mainly by dipping using three classes of insecticides; insect growth regulators (IGRs) including the benzoyl ureas and triazine compounds, synthetic pyrethroids (SPs) and organophosphorus compounds (OPs) either alone or as mixtures.

Insect Growth Regulators

The benzoyl urea, diflubenzuron (Zenith®, Novartis New Zealand Ltd; Fleececare®, Ancare Distributors Ltd), its analogue SIR-8514 (triflumuron) and the triazine, cyromazine (Vetrazin®, Backstop®; Novartis New Zealand Ltd) act as morphogenics or insect growth regulators that interfere with an insect's life cycle. These insecticides are ingestive poisons that inhibit the formation of cuticular proteins during moulting, resulting in retardation of larval growth and larval malformations (Binnington *et al.* 1987, Baille & Wright 1985, Mulder & Gijswijt 1973). Diflubenzuron causes a decrease in chitin production in *L. cuprina* due to its interference with a step in the chitin biosynthesis pathway (Turnball *et al.* 1980, Turnball & Howells 1982, 1983, Binnington 1985). Cyromazine causes epidermal cells to invade the insect cuticle resulting in necrotic lesions developing in intersegmental membranes (Reynolds & Blakey 1989). Cyromazine is thought to affect protein synthesis indirectly via the insect's hormonal system (Friedel *et al.*

1988). Cyromazine has also been shown to significantly reduce egg production but not hatch rate in laboratory evaluations of *L. cuprina* (Friedel & McDonell 1985). In contrast, diflubenzuron is an effective ovicide that inhibits eclosion (Grosscurt 1977, Levot & Shipp 1983, 1984). Triflumuron has however, been shown to be 6 – 8 times more effective as an ovicide as diflubenzuron in strains of *L. cuprina* in Australia (Levot & Shipp 1983, 1984).

Tolerance to cyromazine in field strains of *Musca domestica* has been identified (Bloomcamp *et al.* 1987, Shen & Plapp 1990). Resistance to cyromazine has also been induced in *Drosophila melanogaster* by mutagenesis with ethyl methanesulfonate followed by selection with the insecticide (Adcock *et al.* 1993). Similarly, four strains of *L. cuprina* have shown resistance as a result of mutagenesis (Yen *et al.*1996) but no natural resistance to cyromazine has been detected in Australian field strains of *L. cuprina* (G. Levot personal communication).

Synthetic Pyrethroids

Synthetic pyrethroids (SPs) have been used mostly to control the sheep body louse, *Bovicola ovis*, acting as disrupters of nerve impulse transmission by keeping open sodium channels in neuronal membranes (Laufer *et al.*1984). This causes an increase in neurotransmitters from nerve cells discharging repetitively and eventually leads to paralysis in the insect. Synthetic pyrethroids have also been found to be capable of suppressing oviposition behaviour in blowflies (Orton & Shipp 1983). It was thought that because the SPs disrupted oviposition behaviour it was less likely that resistance would develop in blowflies (Orton & Shipp 1983). Indeed Sales *et al.* (1989) found that no significant resistance had developed in field populations of *L. cuprina* in

Australia to the SP deltamethrin despite its use against *B. ovis* since 1981. They were however, able to induce resistance to deltamethrin in a laboratory-selected combined field strain (CFS) of *L. cuprina*, which stabilised resistance factors as high as 25 in larvae and 7 in female adults. Sales *et al.* (1996) also demonstrated a low-level cross-resistance between cypermethrin and diazinon. They pointed out however, that this cross-resistance did not affect the ability of cypermethrin to suppress oviposition by *L. cuprina*. This CFS also showed cross-resistance to OPs, carbamates and side-resistance to other SPs. Examples of synthetic pyrethroids used primarily or adventitiously for fly control in New Zealand include cypermethrin (cyperfly®, Ancare; Cypor, Novartis New Zealand Ltd); alphacypermethrin (Duracide® and Vanquish Long Wool®, SmithKline Beecham Animal Health); and cypermethrin in combination with the OP chlorpyrifos (Flypel®, Ancare) (Appendix 1.1.17).

Organophosphorus Compounds

The organophosphorus insecticides (OPs) are a class of insecticides that are all esters of variants of phosphoric acid. They can be classified into twelve types of OP i.e. phosphates, phosphonates, phosphorothionates, phosphorodithioates, phosphorodithiolates, phosphorothionates, phosphorodiamidates, phosphinates, phosphinothionates phosphoramidothionates, phosphoramidothiolates and phosphorothiolates (Chambers & Levi 1992). Aliphatic OPs have a linear carbon chain-like structure and include malathion, dicrotophos and dichlorvos. Phenyl OPs have a phenyl ring, and hydrogens may be displaced with Cl_2, NO_2, CH_3 or S. This group includes parathion, methyl parathion and isofenphos. The ring structure of heterocyclic OPs may include different atoms such as O, N or S. This group includes diazinon, azinphos methyl and chlorpyrifos (Chambers & Levi 1992).

Organophosphates act by inhibiting acetylcholinesterase in the insect's central nervous system. They are primarily used as flystrike larvicides but can also kill adult flies. They are well represented in the product line of available fly preventatives and are the primary focus of this toxicological study. Organophosphate insecticides currently available for fly control include coumaphos (Asuntol Powder®, Asuntol liquid®; Bayer NZ Ltd), diazinon (Top Clip 40®, Diazinon 40®, Novartis New Zealand Ltd, and Diazinon 40 sheep dip®, Nufarm), propetamphos (Seraphos® and Seraphos 500®, chlorfenvinphos (Supreme DFF®, Schering-Plough Animal Health), dichlofenthion (Trigon DFF®, Schering-Plough Animal Health) and chlorpyrifos (Xterminate 10®, Ancare) (Appendix 1.1.17). Trigon DFF® has since been withdrawn from the market.

With the exception of chlorfenvinphos, which is already in its active form, all of the above OPs need to be converted to their oxon form by bioactivation before they exert their toxic action. For example, diazinon is converted to diazoxon, which is a more potent cholinesterase inhibitor than diazinon (Yang *et al.*1971). This reaction is catalysed by the insect's microsomal mixed function oxidase system (El-bashir & Oppenoorth 1969). This is discussed in more detail in chapter 3.5.

The key characteristic of an effective flystrike preventative insecticide is the ability to kill the pest rapidly so that the host is not predisposed to further strike. Predisposition to strike is increased by the attractiveness of open wounds and subsequent bacterial infection. Added advantages of an insecticide are a long protective period and the ability of the insecticide to translocate throughout the wool

as well as providing activity against resistant strains of blowflies (Hodson *et al.* 1983, Harrison & Rundle 1983). Failure of insecticides to fulfil expectations can be due to a number of factors including poor insecticidal application, inappropriate timing of application and faulty equipment.

Resistance has been an overwhelming determinant of reduced protection periods of insecticides against *L. cuprina* in Australia. The OPs were first introduced there in the mid 1960s and by the 1970s resistance by *L. cuprina* in Australia had taken a firm hold (Hughes 1977). Moderate organophosphate side-resistance in *L. cuprina* has been identified between diazinon, chlorfenvinphos and coumaphos (Hughes 1981) and low-level side-resistance between diazinon and propetamphos (Levot 1990). As a result, resistance to diazinon has effectively reduced protection periods from over 16 weeks when initially available to less than 6 weeks subsequently (Levot & Boreham 1995).

Establishing resistance as a prime cause of reduced protection periods against flystrike in New Zealand has been used as a starting point for monitoring insecticide resistance. Ideally, resistance monitoring attempts to measure changes in the frequency and magnitude of resistance over time and space. Monitoring is therefore most useful in the early stages of the development of resistance. Monitoring can also be a useful tool for the evaluation of alternative control tactics and for assessing risk of the development of resistance to new insecticides.

The first chapter (1.1) in this section establishes the resistance status of *L. sericata* to diazinon and *L. cuprina* to five OPs (diazinon, chlorfenvinphos, chlorpyrifos,

dichlofenthion and propetamphos). It also assesses the consequences of side resistance to these OPs for *L. cuprina* control and explores the effects of environmental factors on levels of resistance in both species in New Zealand. The toxicological response of *L. cuprina* to deltamethrin and diflubenzuron is also briefly addressed.

The second chapter (1.2) deals with the relative efficacy of four OPs (diazinon, chlorfenvinphos, dichlofenthion and propetamphos) *in vivo*, towards susceptible and resistant strains of *L. cuprina.* The aim of this study is to determine the relative protective nature of OPs in a field simulation.

The focus of monitoring in this thesis has been directed mostly towards resistance to OPs due not only to their extensive use and wide range of marketed formulations but also because of lack of evidence for changed susceptibility on the part of blowflies to other insecticide groups.

CHAPTER 1.1

Insecticide resistance status of *Lucilia cuprina* and *Lucilia sericata* based on a treated-surface toxicological bioassay

Introduction

Initial assessment and continual resistance monitoring is fundamentally important to any resistance management program. The success of the program is therefore dependent on the availability of a simple, reliable method of testing.

Two simple *in vitro* methods are *(a)* a treated-surface bioassay and *(b)* topical application of a known dose of insecticide. Topical application methods have the advantage that a precise quantity of insecticide of a known concentration can be administered to individual insects. Such methods are arduous if employing a range of concentrations to determine a level of resistance. They can, however, be useful if considering a discriminating dose approach. A discriminating dose is a threshold concentration that will kill susceptible insects but not resistant individuals. The resistance status of a species needs to have been characterised previously before a discriminating dose approach can be used effectively (Roush & Miller 1986). Topical application methods have been used for characterising resistance in strains of adult *L. cuprina* in Australia (Hughes 1982, Gleeson *et al.* 1994). Topical application of adult *L. cuprina* is addressed in this chapter but only in using a discriminating dose of diazinon (0.02%). Unfortunately it was not practical to investigate large numbers of strains over a concentration range. The accuracy of this method is also shown to be questionable.

Levot (1990) has described an efficient, reproducible larval bioassay for monitoring resistance in field populations of *L. cuprina* in Australia by employing a treated surface method. Organophosphate insecticide treatment of sheep tends to target larvae almost exclusively, so this method is of more practical relevance than using adults in a bioassay. The advantages of this treated-surface bioassay are that it is simple, fast to prepare and relatively inexpensive. Laboratory conditioning of larvae can also be avoided by testing early generations of a strain. Furthermore, it permits many insecticides to be tested simultaneously and is also a useful technique for assessing the feasibility of new insecticides and insecticide mixtures without the expense of large field trials.

The purpose of this study was to establish the resistance of New Zealand strains of both *L. cuprina* and *L. sericata* using a treated-surface bioassay. The resistance response of *L. cuprina* to five OPs was assessed but only diazinon was considered with respect to *L. sericata* strains. The SP, deltamethrin, and the IGR, diflubenzuron, have also been considered with respect to *L. cuprina*. Where possible, the influence of region, season, density of sheep and year are considered as explanatory factors for the level of resistance in both species.

Materials and Methods

Insects

Lucilia cuprina susceptibles. a laboratory susceptible strain of *Lucilia cuprina*, with no history of organophosphorus insecticide exposure was obtained from the Biological and Chemical Research Institute, Rydalmere, Sydney, Australia.

Lucilia sericata susceptibles. a laboratory susceptible strain of *Lucilia sericata*, with no history of organophosphorus insecticide exposure was obtained from ICI (New Zealand) Ltd (now Coopers Division, Schering-Plough Animal Health Ltd) in mid-1985 and maintained in culture at the Wallaceville Animal Research Centre, Upper Hutt, for over 100 generations.

Field Strains. L. cuprina and *L. sericata* field strains were derived from instances of flystrike. Variable numbers of larvae from struck sheep were mailed to Wallaceville Animal Research Centre in 250 ml sample pottles containing a small amount of vermiculite. Larvae were reared through to adults and maintained as single species' strains. Toxicological testing was carried out on larval offspring of first generation field strains (F2). Flies were fed sugar and water *ad libitum*, with larvae being reared on a combined diet of processed pet food and minced ox liver.

Toxicology

Larval dose-response bioassays

Toxicological testing used a method described by Levot (1990). Briefly, 120 mm x 30 mm pieces of Whatmann chromatography paper (3MM) were impregnated with 1 ml of insecticide in acetone. Insecticide concentrations were chosen after a preliminary test to give a 0-100% larval mortality. Technical grade diazinon (91.5%) and propetamphos (90.1%) were obtained from Young's Animal Health (New Zealand) Ltd. (now Novartis New Zealand Ltd). Chlorfenvinphos (81.7%) and dichlofenthion (99.4%) were obtained from Pitman Moore (NZ) Ltd (now Schering-Plough Animal Health), chlorpyrifos (93%) and diflubenzuron (99.1 %) were from Ancare Distributors Ltd and deltamethrin (99.3%) was from Ciba Geigy New

Zealand Ltd (now Novartis New Zealand Ltd). Papers treated with acetone only were used as a control. Papers were rolled and placed into 50 mm x 10 mm glass vials. Between 50 and 150 first instar larvae, less than two hours old and hatched from eggs laid on ox liver were placed into the vials. One ml of fortified sheep serum was added and the vials plugged with non-absorbent cotton wool. The serum had been fortified with 2% yeast extract and 0.5% monobasic potassium orthophosphate. Larvae were incubated for 24 hours at a constant 25°C and with a 24-hour photophase. To record efficacy, treated papers were unrolled into warm water. Larval movement/immobility was detected against a dark background and used to calculate % mortality.

Larval dose-response bioassays were performed by myself until December 1993 after which I kindly acknowledge the technical assistance of Mr Neville Haack at AgResearch, Wallaceville Animal Research Centre, Upper Hutt.

Adult topical application bioassays

Individual female flies (3 – 6 days old, not gravid) from 17 strains were immobilised with CO_2 and then treated with a discriminating dose of 0.5 µl of 0.02 % diazinon in acetone (Shanahan 1966). Controls were treated with acetone only. The dose was applied to the upper thorax of each fly using a 0.5 µl microcapillary tube. Batches of 20 flies were placed into plastic containers (110 mm diameter x 60 mm depth) with mesh lids. Sample size ranged from 56 - 180 individuals depending on the availability of flies. A water-saturated cotton dental plug and a single sugar cube were placed on the mesh lid to provide flies with food for the duration of the experiment. Flies were kept at a constant 25°C under fluorescent lighting with a

16:8 light/dark regime. Percent mortality was recorded after 24 hours. Numbers of survivors of the discriminating dose were used as a phenotypic estimate of resistant individuals (Hughes 1981).

Individual flies from two strains (131, Takapau 21.04.91 and a laboratory OP-susceptible strain) were weighed and the means determined for both sexes. The mean weight of females only was determined for other strains.

Statistical Analysis

Dose response was analysed by probit regression (Finney 1952). A probit regression computer program was obtained from the Biological and Chemical Research Institute, Rydalmere, Sydney. Results are presented as LC_{50} and LC_{99} with 95% fiducial limits, slope and standard error of the log-dose probit line. Slopes indicate change in mortality per unit change in insecticide concentration (Robertson & Priesler 1992). Percent control mortalities were corrected using Abbott's transformation (1925). Resistance factors (R.F.'s) were calculated by dividing the LC_{50} of the field strain by that of the susceptible strain.

Probit data were analysed in three ways by analysis of variance (ANOVA) methods in S-Plus (Everitt 1994). First, the LC_{50} for each insecticide and species was used as the dependent variable. Region, season, year and sheep densities were used as predictors of the magnitude of resistance where the linear model allowed. Appendices 1.1 – 1.3 describe the designation of categories for region, season and sheep density. LC_{50} data for diazinon were also used to construct frequency distribution graphs for *L. cuprina* and *L. sericata.* These were constructed to

determine variation among field strains and as a comparison to organophosphate susceptible strains for both species.

Secondly, the slope of the log-dose probit line (ldp-line) for each insecticide was analysed using the corresponding LC_{50} as a predictor. Where applicable, region and season were included as possible predictors of slope in this model. This type of analysis was undertaken to provide an indication of factors affecting the phenotypic variation in response to insecticides within a population. It is often assumed that shallow slopes are indicative of heterogeneous populations with respect to resistance. Susceptible (SS) populations and homozygous resistant (RR) populations vary little genetically and therefore produce steep ldp-lines that equate to either very low or very high LC_{50}s. Heterozygous resistant individuals, on the other hand, typically show intermediate LC_{50}s as a result of large variation of response within the population, which is depicted by a shallow slope (Falconer 1989, Lande 1981). The OP susceptible strains for both *L. cuprina* and *L. sericata* were removed from analysis as frequency distribution histograms of LC_{50}s showed them to be quite distinct from field samples and therefore to be outliers (Appendix 1.1.13). The underlying hypothesis for this analysis can therefore be more accurately stated as: an increase in slope parallels an increase in resistance, which is a function of a decrease in genetic variation within a population (Rosenheim & Hoy 1986).

Log-dose probit lines were also used in plots of the response of individual strains of *L. cuprina* to many insecticides and also of many strains to one insecticide. These types of graph provide an indication of the factors affecting the phenotypic variation in response to insecticides within a population. Comparisons of such plots can

provide information regarding differences in quality and quantity of insecticide detoxification enzymes (Hardman *et al.* 1959, Kuperman *et al.* 1961, Robertson & Rappaport 1979), but need to be confirmed by biochemical assessment.

Lastly, ANOVA was used to determine the magnitude of side-resistance between pairs of OP insecticides as well as cross-resistance between diazinon and deltamethrin. As with previous analyses the influence of region as a predictor was considered. Data sets were not large enough to include season, year and sheep density as parameters in this analysis.

Regression diagnostics used for each analysis were standardised residuals, hat matrices and coefficient estimate graphs. A description of regression diagnostics can be found in the glossary.

Results

The LC_{50} for the susceptible strain, which is used in calculation of all resistance factors, was determined in March 1992 and has been monitored at irregular periods since this time. The mean LC_{50} of the susceptible strain over this time was 0.059 ± 0.011.

Dose response bioassays – (i) Summary statistics

See Appendix 1.1.4 for ANOVA tables showing the relationship between insecticides and environmental factors.

Lucilia cuprina

(a) Diazinon A total of 359 samples from 81 localities were tested over 8 years (1990 - 1997). LC_{50}s ranged from 0.39 mg/l to 2.76 mg/ml (RF 7.8 – 55.2 x's).

The highest resistance to diazinon was recorded from farm 004, Bulls (3 February 1992), the lowest by farm 014, Rotorua (7 December 1994)(Appendix 1.1.5). LC_{50}s of *L. cuprina* larvae were strongly affected by region and sheep density ($p<0.005$) but not season or year (Appendix 1.1.4). Data were F-tested (for two-sample variance) with either region 1, or sheep density 1, to determine the extent of their influence. The means for these are presented in tables 1.1.1and 1.1.2.

Table 1.1.1 Diazinon LC_{50}s for *Lucilia cuprina* larvae presented by region.

Region	Number of samples	Mean LC_{50} (mg/l) (min – max.)	
1	22	1.08 (0.63 – 1.90)	
2	38	1.09 (0.39 – 1.67)	NS
3	87	1.23 (0.48 – 2.19)	NS
4	110	1.27 (0.48 – 2.76)	NS
5	42	1.68 (0.71 – 2.74)	*
6	60	1.15 (0.41 – 2.74)	NS

* Significant at the 95% probability level
NS not significant at the 95% probability level

Table 1.1.2 Diazinon LC_{50}s for *Lucilia cuprina* larvae presented by sheep density.

Sheep Density (number of sheep)	Number of samples	Mean LC_{50} (mg/l) (min – max.)	
1. > 8 million	56	1.17 (0.41 – 2.74)	
2. 7 – 8 million	72	1.37 (0.66 – 2.76)	NS
3. 4 – 5 million	29	1.22 (0.59 – 2.13)	NS
4. 3 – 4 million	28	1.11 (0.52 – 1.67)	*
5. 2 – 3 million	87	1.15 (0.48 – 2.13)	*
6. < 2 million	87	1.40 (0.39 – 2.74)	NS

(b) Chlorfenvinphos A total of 30 samples from 22 localities were tested over 3 years (1992-1994). LC_{50}s ranged from 0.28 mg/l to 0.81 mg/ml (RF 2.5 – 7.4 x's). The highest resistance to chlorfenvinphos was recorded from farm 009, Blenheim (9 January1994), the lowest by farm 014, Blenheim (24 February 1993)(Appendix 1.1.6). Resistance to chlorfenvinphos could not be predicted by region, season or year.

(c) Chlorpyrifos A total of 53 samples from 29 localities were tested over 4 years (1991 - 1994). LC_{50}s ranged from 0.31 mg/l to 2.02 mg/ml (RF 2.1 – 13.5 x's). The highest resistance to chlorpyrifos was recorded from farm 012, Hastings (27 November 1991), the lowest by farm 035, Levin (11 April 1994)(Appendix 1.1.7). LC_{50}s of *L. cuprina* larvae were strongly affected by region, season and year ($p<0.05$). Data were F-tested (for two-sample variance) with region 1, season 1 or the year 1991, to determine the extent of their influence. The means for these are presented in tables 1.1.3 and 1.1.5. Figure 1.1.10 shows the yearly trend in response of *L. cuprina* and *L. sericata* to the organophosphorus compounds tested in this study and expresses them as percentage change in mean LC_{50} from those recorded in 1991. This figure (1.1.10) shows a decline in chlorpyrifos resistance over successive years.

Table 1.1.3 Chlorpyrifos LC_{50}s for *Lucilia cuprina* larvae presented by region.

Region	Number of samples	Mean LC_{50} (mg/l) (min – max.)	
1	4	0.63 (0.46 – 0.75)	
2	8	0.69 (0.44 – 0.92)	NS
3	15	1.01 (0.41 – 2.02)	*
4	14	0.81 (0.31 – 1.39)	NS
5	7	0.99 (0.74 – 1.22)	NS
6	5	0.77 (0.59 – 0.95)	NS

Table 1.1.4 Chlorpyrifos LC_{50}s for *Lucilia cuprina* larvae presented by season.

Season	Number of samples	Mean LC_{50} (mg/l) (min – max.)	
1	33	0.90 (0.41 – 1.56)	
2	17	0.70 (0.31 – 1.36)	NS
4	3	1.24 (0.67 – 2.02)	**

** Significant at the 99% probability level

Table 1.1.5 Chlorpyrifos LC_{50}s for *Lucilia cuprina* larvae presented by year.

Year	Number of samples	Mean LC_{50} (mg/l) (min – max.)	
1991	11	1.00 (0.56 – 2.02)	
1992	17	1.01 (0.67 – 1.56)	NS
1993	5	0.75 (0.41 – 0.91)	NS
1994	20	0.67 (0.31 – 0.95)	**

(d) Dichlofenthion A total of 57 samples from 31 localities were tested over 4 years (1991 - 1994). LC_{50}s ranged from 0.90 mg/l to 5.45 mg/ml (RF 2.9 – 17.6 x's). The highest resistance to dichlofenthion was recorded from farm 014, Blenheim (26 January 1992), the lowest by farm 036, Kaiwaka (14 March 1994)(Appendix 1.1.8). LC_{50}s of *L. cuprina* larvae were strongly affected by region, season and year ($p<0.005$). Data were F-tested (for two-sample variance) with region 1, season 1 or year 1991, to determine the extent of their influence. There appeared to be no significant differences between region or season with this analysis suggesting that the ANOVA model was able to detect interactive influence by these factors. The means for these are presented in tables 1.1.6 and 1.1.8. Figure 1.1.10 shows a decline in dichlofenthion resistance over successive years. Figure 1.1.11 compares regional trends in response of *Lucilia cuprina* larvae to dichlofenthion, as well as chlorpyrifos and dichlofenthion.

Table 1.1.6 Dichlofenthion LC_{50}s for *Lucilia cuprina* larvae presented *by region.*

Region	Number of samples	Mean LC_{50} (mg/l) (min – max.)	
1	4	1.52 (0.90 – 2.03)	
2	8	1.86 (1.04 – 2.79)	NS
3	15	2.65 (1.39 – 4.30)	NS
4	15	2.16 (0.93 – 3.69)	NS
5	8	3.53 (2.20 – 5.45)	NS
6	7	2.10 (0.98 – 2.88)	NS

Table 1.1.7 Dichlofenthion LC_{50}s for *Lucilia cuprina* larvae presented by season.

Season	Number of samples	Mean LC_{50} (mg/l) (min – max.)	
1	34	2.76 (1.24 – 5.45)	
2	18	1.69 (0.90 – 4.30)	NS
4	5	2.32 (1.39 – 3.85)	NS

Table 1.1.8 Dichlofenthion LC_{50}s for *Lucilia cuprina* larvae presented by year.

Year	Number of samples	Mean LC_{50} (mg/l) (min – max.)	
1991	13	2.60 (1.24 – 4.07)	
1992	19	3.06 (1.64 – 5.45)	*
1993	4	2.71 (2.54 – 2.88)	*
1994	21	1.59 (0.90 – 3.15)	*

(e) Propetamphos A total of 61 samples from 26 localities were tested over 4 years (1991 - 1994). LC_{50}s ranged from 0.33 mg/l to 0.89 mg/ml (RF 2.2 – 5.9 x's). The highest resistance to propetamphos was recorded from farm 023, Masterton (7 January 1992), the lowest by farm 010, Te Kuiti (15 January 1992) (Appendix 1.1.9). LC_{50}s were unaffected by region, season or year.

(f) Deltamethrin A total of 26 samples from 15 localities were tested over 3 years (1991 - 1993). LC_{50}s ranged from 0.33 mg/l to 2.75 mg/ml. No laboratory SP-susceptible strain was established so resistance factor could not be calculated. The highest LC_{50} for deltamethrin was recorded from farm 003, Wanganui (27 January 1993), the lowest by farm 111, Masterton (30 December 1991)(Appendix 1.1.10). LC_{50}s were unaffected by region which was the only predictor that the linear model could fit to the data set.

(g) Diflubenzuron A total of 16 samples from 12 localities were tested during 1997. LC_{50}s ranged from 0.15 mg/l to 3.98 mg/ml (RF 1.4 – 36.2x's). The

highest resistance to diflubenzuron was recorded from farm 091, Waipukurau (14 April 1997), the lowest was shared by farms 085 and 060, from Timaru and Waiau respectively (20 March 1997, 27 January 1997)(Appendix 1.1.11). The data set was too small to make reliable predictions on the effects of region, season and year, although there was a suggestion that LC_{50}s differed between regions ($p = 0.04$).

Lucilia sericata

(a) Diazinon A total of 99 samples from 61 localities were tested over 7 years (1991 - 1997). LC_{50}s ranged from 0.18 mg/l to 2.06 mg/ml (RF 3.0 – 34.3 x's). The highest resistance to diazinon was recorded from farm 009, Blenheim (5 March 1996), the lowest by farm 114, Ashburton (9 January 1996) (Appendix 1.1.12). LC_{50}s of *L. sericata* larvae were strongly affected by regional ($p<0.005$) and yearly ($p = 0.03$) parameters. Data were F-tested (for two-sample variance) with either region 1 or year 1991, to determine the extent of factor influence. The means for these are presented in tables 1.1.9 and 1.1.10. Figure 1.1.10 shows a decline in diazinon resistance in this species during 1993/94 followed by a steady increase in resistance between 1995 and 1997.

Table 1.1.9 Diazinon LC_{50}s for *Lucilia sericata* larvae presented by region.

Region	Number of samples	Mean LC_{50} (mg/l) (min – max.)	
2	2	0.44 (0.30 – 0.57)	
3	30	0.67 (0.28 – 1.11)	NS
4	12	0.69 (0.36 – 1.25)	NS
5	16	0.99 (0.32 – 2.06)	**
6	39	0.55 (0.18 – 1.06)	NS

Table 1.1.10 Diazinon LC_{50}s for *Lucilia sericata* larvae presented by year.

Year	Number of samples	Mean LC_{50} (mg/l) (min – max.)	
1991	7	0.51 (0.32 – 0.63)	
1992	9	0.84 (0.47 – 1.29)	*
1993	3	0.44 (0.28 – 0.69)	NS
1994	17	0.45 (0.30 – 0.83)	NS
1995	11	0.64 (0.33 – 1.11)	NS
1996	36	0.75 (0.18 – 2.06)	**
1997	16	0.77 (0.30 – 1.31)	*

Frequency distributions Frequency distribution histograms show that response to diazinon (LC_{50}s) was broad but normally distributed for both *L. sericata* and *L. cuprina* larvae (see Appendix 1.1.13 for frequency distribution histograms). Note that the OP-susceptible strain was well separated from the field distribution range in both species.

(ii) Ldp-lines and slope

The slopes of diazinon ldp-lines for *L. sericata* larvae were predicted by the LC_{50} of diazinon ($p = 0.001$) but were not influenced by season or region. Slopes of deltamethrin ldp-lines were similarly predicted by deltamethrin LC_{50}s for *L. cuprina* larvae. In contrast, LC_{50}s of all OPs measured for *L. cuprina* larvae failed to predict the slope of their ldp-lines. Season however, had a significant influence on slope with respect to diazinon ($p<0.0001$). (See Appendix 1.1.14 for ANOVA tables). Examples of regression diagnostics are available in Appendix 1.1.15.

Figures 1.1.1 – 1.1.4 are examples of plots of the response of susceptible and field strains of *L. cuprina* larvae to five OPs. Ldp-lines for the OP susceptible strains (Figure 1.1.1) are parallel but have differing horizontal intercepts compared with field strains. The differences in these lines are due to the variable insecticide

concentrations to which larvae respond. Diazinon, chlorpyrifos and dichlofenthion ldp-lines of field strains are near parallel but chlorfenvinphos and propetamphos lines are far from parallel (Figures 1.1.2 - 1.1.4).

Figure 1.1.5 show an example plot of the response of susceptible and field strains of *L. cuprina* for diazinon. The plots for susceptible strains lie some distance to the left of field strains suggesting that there might be quantitative differences in detoxification enzymes while those for field strains are neither parallel nor equal (within the same concentration range) suggesting additional qualitative differences in enzymes.

(iii) Side- and cross-resistance between insecticides

LC_{50}s of chlorpyrifos, dichlofenthion and propetamphos significantly predicted diazinon LC_{50}s (Figures 1.1.6 – 8, Appendix 1.1.16). The relationship was weakest for propetamphos ($p = 0.0001$) (Figure 1.1.9, Appendix 1.1.16). Region was also a significantly contributing factor of side-resistance with these diazinon/OP combinations. All combinations of pairs of the above OPs, with the exception of diazinon generated significant correlations but resistance was not generally influenced by region. Resistance to chlorfenvinphos was significantly related to propetamphos resistance ($p = 0.002$) but region did not influence this relationship (Appendix 1.1.16). As with chlorfenvinphos, deltamethrin LC_{50}s showed no relationship with those for diazinon. (Appendix 1.1.16). Note that p-values in figures (1.1.6 – 1.1.9) differ from those in ANOVA tables as the graphs are unable to express the influence of region.

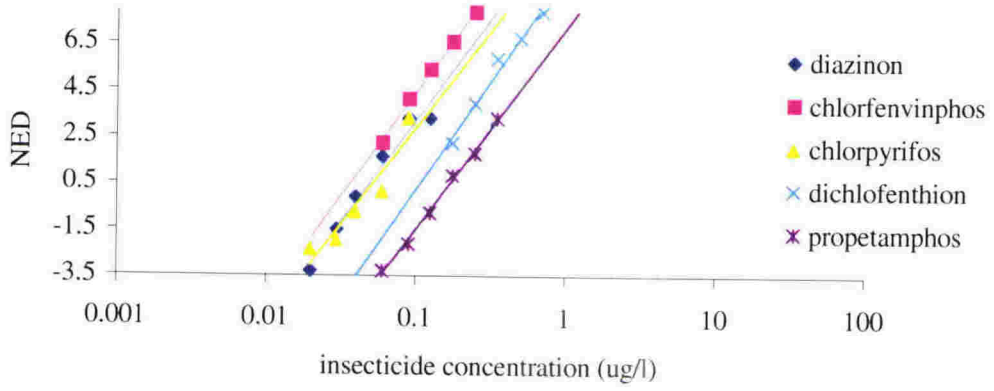


Figure 1.1.1. Relative toxicity of five organophosphates to an insecticide susceptible strain of *Lucilia cuprina* larvae. (NED Normal equivalent deviation *see Glossary*)

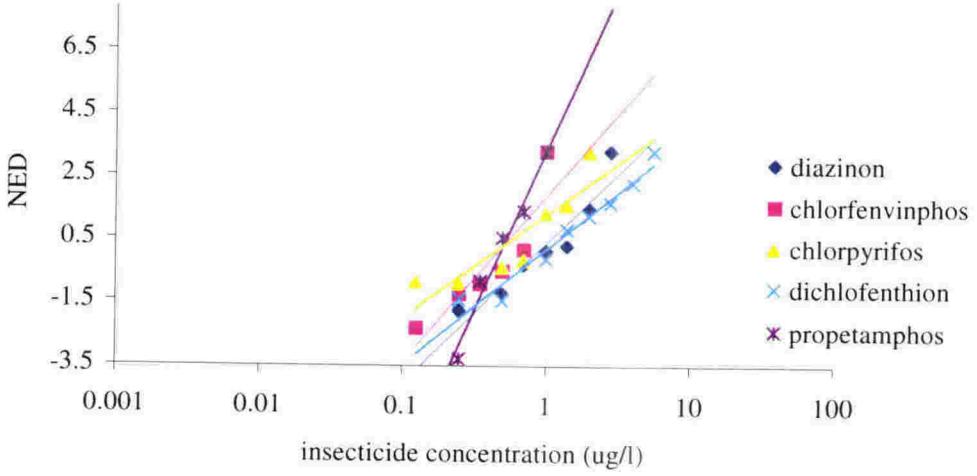


Figure 1.1.2 Relative toxicity of five organophosphates to a field strain of *Lucilia cuprina* larvae from Kaiwaka (Farm 036, 14 March 1994). (NED Normal equivalent deviation *see Glossary*)

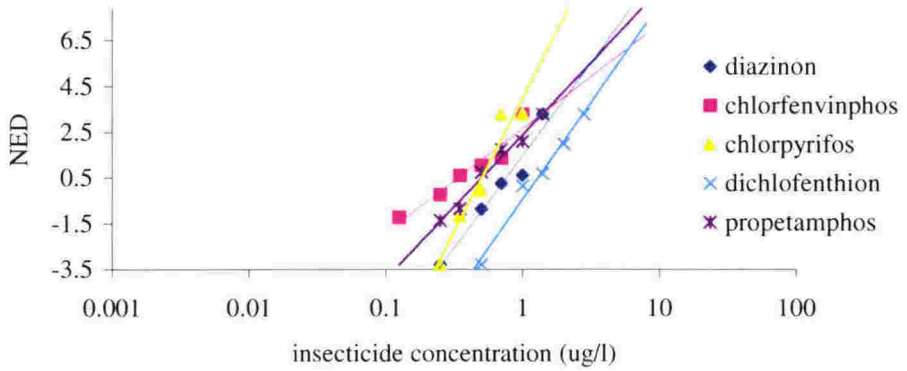


Figure 1.1.3 Relative toxicity of five organophosphates to a field strain of *Lucilia cuprina* larvae from Levin (Farm 035, 11 April 1994). (NED Normal equivalent deviation *see Glossary*).

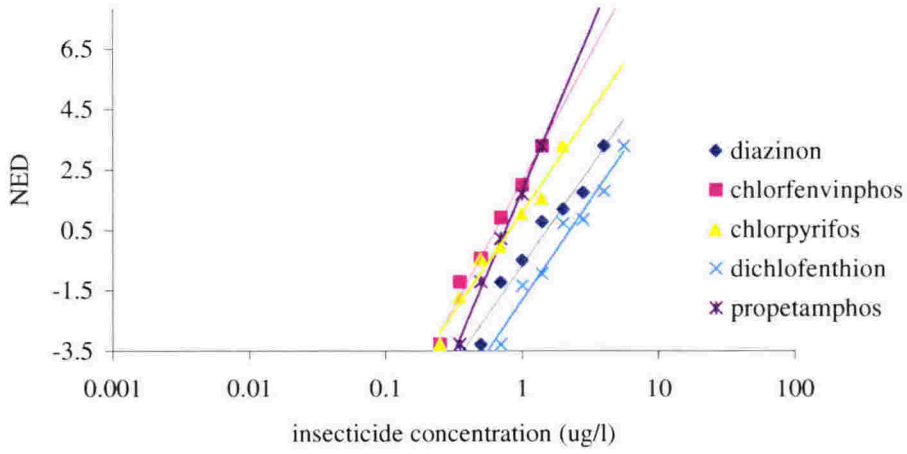


Figure 1.1.4 Relative toxicity of five organophosphates to a field strain of *Lucilia cuprina* larvae from Hastings (Farm 012, 8 April 1994). (NED Normal equivalent deviation *see Glossary*).

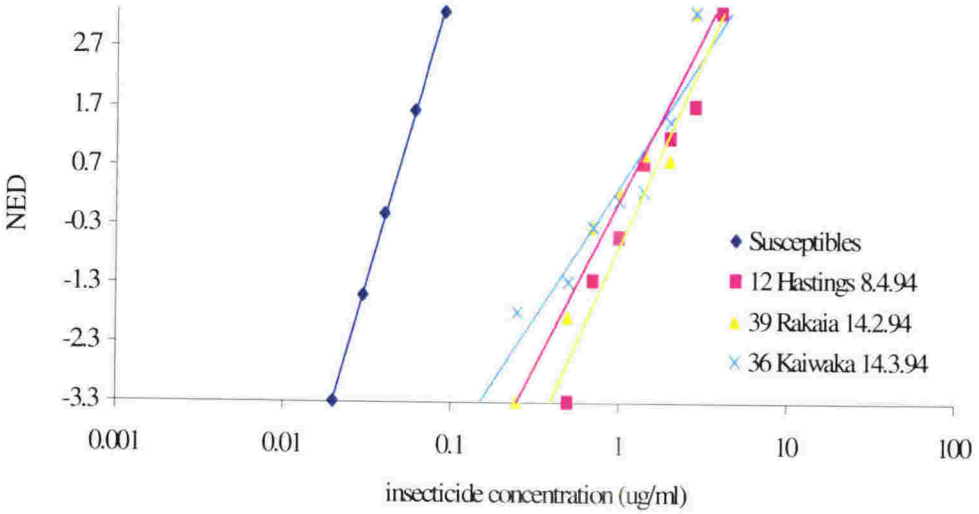


Figure 1.1.5. Dosage mortality lines for four strains of *Lucilia cuprina* larvae with diazinon. (NED Normal equivalent deviation *see Glossary*).

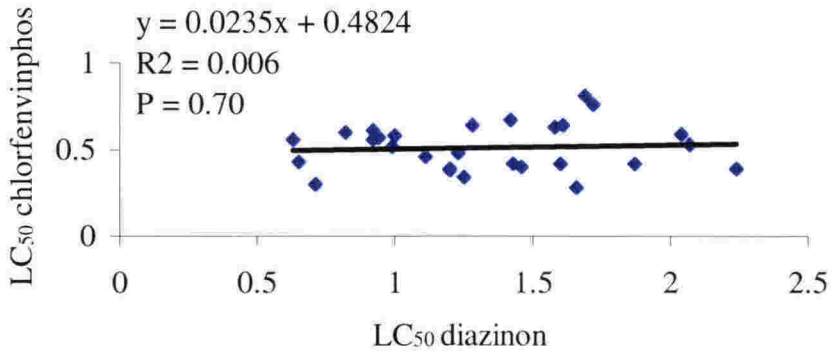


Figure 1.1.6 Regression plot of the relationship between diazinon and chlorfenvinphos in the absence of regional influence.

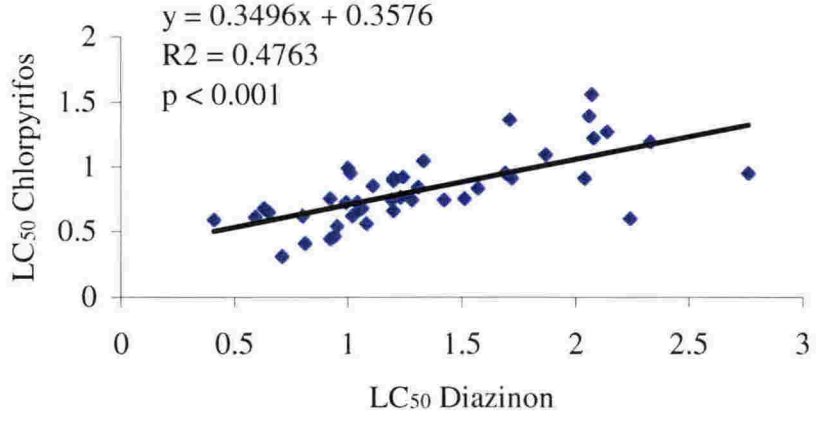


Figure 1.1.7 Regression plot of the relationship between diazinon and chlorpyrifos in the absence of regional influence.

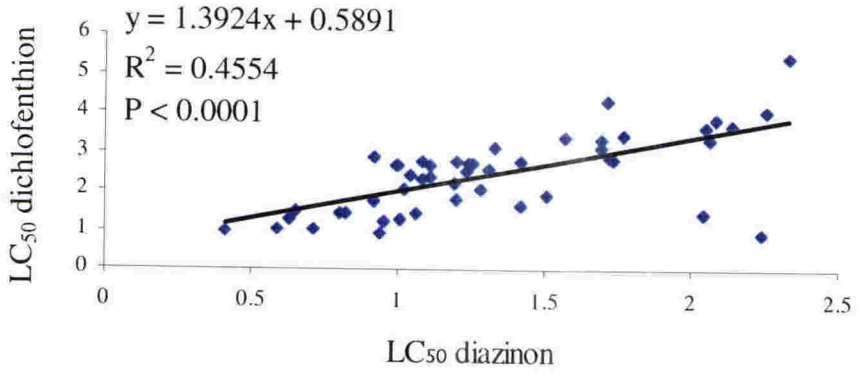


Figure 1.1.8 Regression plot of the relationship between diazinon and dichlofenthion in the absence of regional influence.

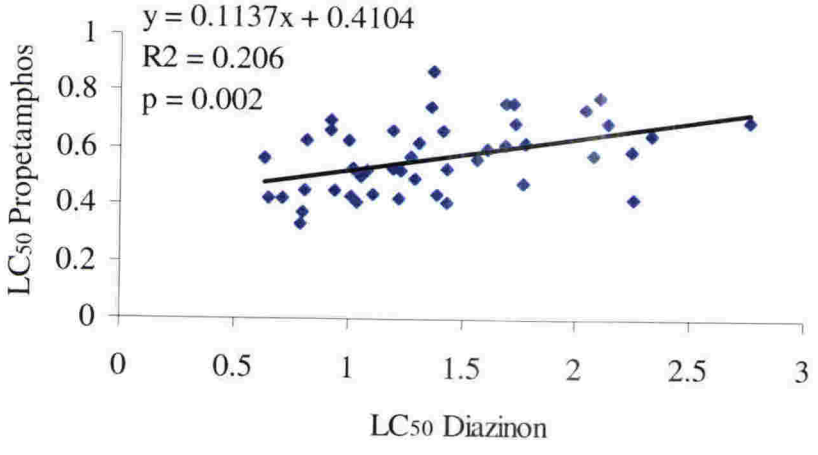


Figure 1.1.9 Regression plot of the relationship between diazinon and propetamphos in the absence of regional influence.

(1) Adult topical application bioassays

Table 1.1.11 shows the response of female *L. cuprina* adults to a discriminating dose of diazinon (0.5 µl of 0.02%). The laboratory OP-susceptible strain showed high mortality (99.3%) compared with field strains. Mortality within field strains ranged from 0 – 37.1%. Analysis of variance (ANOVA) showed a strong negative relationship ($p < 0.005$) between mean fly weight and % mortality within field strains. It was not surprising and this suggested that heavier flies were more able to survive the discriminating dose than lighter ones.

Table 1.1.11 Response of female *Lucilia cuprina* adults to topical application of 0.5 µl of 0.02% diazinon in relation to mean body weight.

Farm	Area	Sample date	N	% Mortality	Mean Weight (mg)
000	Laboratory	00.00.00	140	99.3	11.00
004	Bulls	25.03.91	100	0	33.83
119	Levin	27.03.91	56	0	43.93
016	Kaikohe	01.04.91	240	1.7	38.05
006	Wairoa	03.04.91	120	0	37.45
111	Masterton	07.04.91	220	21.8	17.42
067	Manutuke	08.04.91	140	0	40.02
004	Bulls	15.04.91	200	23.0	15.29
118	Hastings	15.04.91	180	0.6	37.36
050	Levin	16.04.91	120	5.0	30.99
131	Takapau	21.04.91	140	37.1	14.40
043	Waingaro	21.04.91	200	7.0	37.37
018	Waipawa	21.04.91	140	0.7	37.60
004	Bulls	24.04.91	100	3.0	38.68
004	Bulls	29.04.91	100	6.0	36.14
118	Hastings	19.07.91	40	31.2	31.20
023	Masterton	30.07.91	120	0	40.50

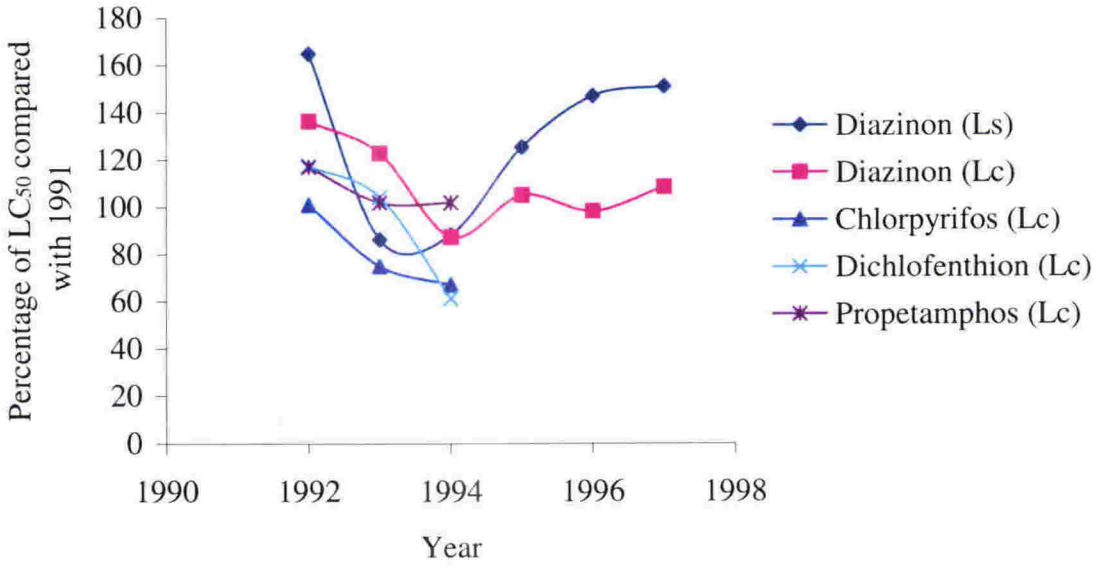


Figure 1.1.10 Yearly trend in response of *Lucilia cuprina* (Lc) and *Lucilia sericata* (Ls) larvae to organophosphorus compounds expressed as percentage of mean LC_{50}s compared with those recorded in 1991.

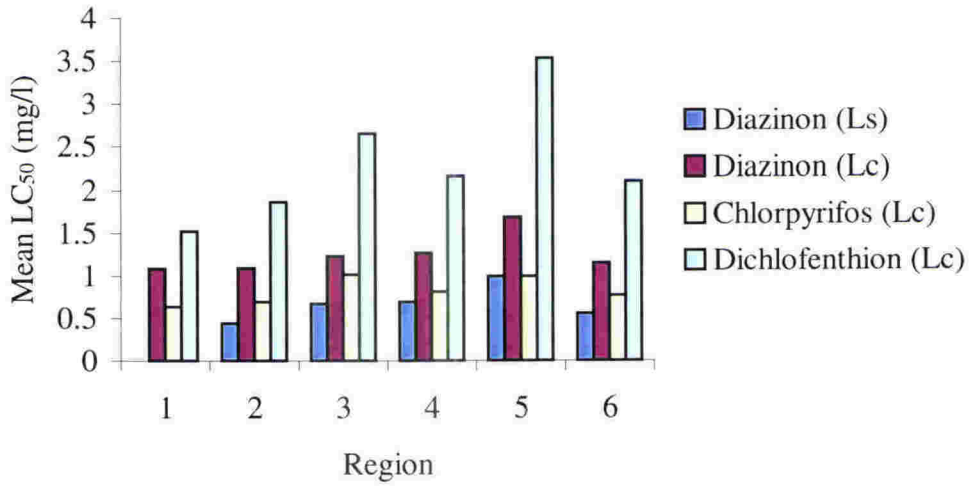


Figure 1.1.11 Regional trend in response of *Lucilia cuprina* (Lc) and *Lucilia sericata* (Ls) larvae to organophosphorus compounds.

Discussion

Circumstances conducive to the evolution of resistance to organophosphate insecticides in blowflies have been present in New Zealand and Australia for over 30 years. Diazinon was first introduced in the early 1960s and was followed by a range of now veteran OPs. More recently, propetamphos became available: in 1984 in New Zealand and in 1986 in Australia. A history of insecticide use has therefore laid the foundations of OP-resistance in *L. sericata* in New Zealand. The high level of resistance that is observed in *L. cuprina* towards OPs is thought to stem from the species' origin of colonisation, since this fly has only been established in New Zealand since the late 1970s yet exhibits higher OP resistance than the indigenous *L. sericata*. There is strong evidence to suggest that *L. cuprina* was introduced from Australia (Gleeson 1995) where OP resistance was detected in a relatively short time after diazinon was first registered for fly control (Shanahan 1966). If, as evidence suggests, *L. cuprina* was introduced from Australia, it would be safe to assume the alleles conferring OP resistance would have been present in the founding population at a high frequency.

Two genetically distinct forms of OP-resistance have been identified in Australian strains of *L. cuprina*. A single locus on chromosome 4, designated R_{OP}-1, is known to confer a non-specific OP-resistance to several OP insecticides including diazinon and chlorfenvinphos (Hughes 1981). Encoded by R_{OP}-1 is a mutant form of esterase that is able to degrade OPs more effectively than the original form, by an enhanced hydrolysis of the phosphoester linkage. This mutation has been attributed to a single amino acid substitution in the active site (Campbell *et al.* 1997, 1998). Hughes (1981) reported that >98% of field populations of *L. cuprina* carried alleles for

resistance at R_{OP}-1 in Australia. Levot (personal communication) first confirmed the presence of R_{OP}-1 in a New Zealand population of *L. cuprina* and measured its frequency at 100%. Observations in this thesis show the frequency to be approximately 97% of the field population tested (Chapter 2.2). The second form of resistance is similarly located on chromosome 4 and is specific to malathion and phenthoate (R_{mal}) and is closely linked to R_{OP}-1 (Raftos & Hughes 1986, Smyth *et al.* 1994, Campbell *et al.* 1997).

(1) OP-resistance in larvae

The results in this thesis show that resistance to OPs is widespread in field populations of *L. cuprina* and *L. sericata* larvae in New Zealand. The highest and broadest range of resistance factors was observed with response to diazinon for both species (*L. cuprina* RFs 7.8 – 55.2, *L. sericata* RFs 3.0 – 34.3). It is not surprising that high level resistance was observed towards diazinon, as this was the first OP used to combat flystrike in New Zealand as well as Australia. It has, therefore, probably been the main selecting agent for OP-resistance for both species in this study and certainly for *L. cuprina* in Australia (Hughes 1981). In marked contrast is the low and narrow range of resistance factors towards propetamphos observed for *L. cuprina* (RFs 2.2 – 5.9). Propetamphos is the most recently registered OP for fly control in both Australia and New Zealand. The level and range of resistance does not however, directly relate to the date of registration but is more likely to reflect factors such as the structure of the OPs and their route of metabolism in the insect. These differences are discussed in more detail when referring to side- and cross-resistance and in a later chapter (Chapter 4.1).

Hughes (1981) considered that variation in resistance factors in field samples was indicative of the genetic variability in populations, the high end of this variation exemplifying the potential for selection for increased resistance in the presence of insecticide pressure (Hughes 1981). The current study has considered variation in resistance in terms of genetic variability (see *population homogeneity* later in this discussion) and has also determined that environmental factors such as season, region, sheep density and yearly trends are linked to the phenotypic expression of resistance. An attempt is made below to explain the influence of environmental factors on resistance and the subsequent selection pressures involved.

Yearly, seasonal and regional trends in resistance

Information on the regional, seasonal and yearly purchase of OPs in New Zealand is available in FMI Farmers' Panels, Ovine Ectoparasiticides, Year to end January 1999 (AC Nielsen). These figures are a result of a random survey of 1200 farmers in New Zealand. Unfortunately regional and seasonal designations differ slightly from that used in the analysis of this study. Assuming however, that these statistics relate in large part to actual insecticide usage (and therefore insecticide pressure) some interesting trends can be identified. Table 1.1.12 is a summary of yearly sheep insecticide purchases (in $000s) extracted from Neilsen's market research survey. It shows that an increase in IGR and 'other' purchases over successive years has paralleled a rise in the number of IGR products registered and a consequential decrease in OP purchases (see Appendix 1.1.17 for a summary of current product trade names and active ingredients).

Table 1.1.12 also shows that, with the exception of 1998, total spending on dips has increased very little over the last seven years and any increase may be more likely an indicator of inflation than increased use of insecticides. Total insecticide pressure could be assumed to have remained fairly similar over time given unchanged expenditure but changed from predominantly OP to IGR induced selection pressure from 1994 onwards (Table 1.1.12). This may be significant when considering that chlorpyrifos and dichlofenthion resistance in *L. cuprina* and diazinon resistance in *L. sericata* were significantly different between years in this study. Peak resistance to each of these insecticides, expressed as high LC_{50}s (mg/l), occurred in 1992. Further, 1992 was also the year of highest OP insecticide purchases suggesting it could be a year of higher than normal insecticide selection pressure (Figure 1.1.10). Resistance towards chlorpyrifos and dichlofenthion decreased after 1992 in *L. cuprina,* as did the purchase of OP insecticides. Diazinon resistance was more variable with respect to *L. sericata* however, suggesting a differential fitness in resistance both between species and with insecticide that might be dependent on variable OP insecticide pressure.

Region was also shown to influence resistance to diazinon (Table 1.1.1), chlorpyrifos (Table 1.1.3) and dichlofenthion (Table 1.1.6) in *L. cuprina* and to diazinon (Table 1.1.9) in *L. sericata.* That is, resistance was generally higher in region 5 (which incorporated Nelson, Marlborough and Kaikoura) for all the above OPs (Figure 1.1.11). Lack of concordance of regional designations with these in the FMI survey, especially with respect to regions 5 and 6 in this study, make it difficult to draw comparisons and comment on resistance levels.

One significant feature of the FMI survey (summarised in Table 1.1.13) was to show that more OPs were purchased in the South Island than in the North Island. Complementary to this was that far fewer IGRs were purchased in the South compared with the North Island despite the greater density of sheep being in the South. Flystrike is however, more common in the North Island than the South where lice pose the largest problem for farmers (A. Heath personal communication). In the higher flystrike regions of the North Island, farmers favour the longer protection periods that the IGRs can provide.

It is considerably more difficult to draw a seasonal comparison between the FMI survey and this study in respect of seasonal differences in insecticide use. This is because the FMI survey is categorised bimonthly rather than by season (Table 1.1.14). The present study showed that chlorpyrifos resistance was highest in spring (4 = September, October, November) (Table 1.1.4) whereas that for dichlofenthion was highest in summer (1 = December, January, February) (Table 1.1.7). It is logical to assume that insecticide pressure would be greatest during summer months when blowflies are most active and dipping most frequent. The differing results between the above two insecticides may be an artefact of small sample size, which may have detected random events rather than a consistent trend.

Table 1.1.12 Yearly sheep insecticide purchases in $000s based on a survey of 1200 farmers (extracted from FMI Farmers' Panels, Ovine Ectoparasiticides, Year to end January 1999, produced by AC Nielsen)

Ingredient	**1991**	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**
OP	40	43	38	32	30	28	26	28
IGR	18	16	18	27	31	35	40	46
Other	2	4	4	14	15	18	14	12
Total	**61**	**62**	**62**	**63**	**63**	**65**	**68**	**77**

Table 1.1.13 Regional sheep insecticide purchases in $000s based on a survey of 1200 farmers (extracted from FMI Farmers' Panels, Ovine Ectoparasiticides, Year to end January 1999, produced by AC Nielsen)

Ingredient	Northland Auckland Waikato Bay of Plenty	Gisborne Hawkes Bay Wairarapa	Manawatu Taranki Wanganui Wellington	Nelson, Marlborough Canterbury	Otago Southland
OP	27	22	21	29	52
IGR	32	34	34	14	4
Other	2	-	1	1	2
Total	**61**	**56**	**56**	**44**	**58**

Table 1.1.14 Bimonthly sheep insecticide purchases in $000s based on a survey of 1200 farmers (extracted from FMI Farmers' Panels, Ovine Ectoparasiticides, Year to end January 1999, produced by AC Nielsen)

Ingredient	Dec/Jan 97/98	Feb/Mar 1998	Apr/May 1998	Jun/Jul 1998	Aug/Sep 1998	Oct/Nov 1998	Dec/Jan 98/99
OP	36	39	26	9	29	19	30
IGR	23	16	-	8	1	3	12
Other	3	2	-	-	-	-	1
Total	62	57	26	17	30	22	43

Population homogeneity

ANOVA results indicate that it is likely the slope of the ldp-line is an indicator of large genetic variation with respect to deltamethrin resistance in field strains of *L. cuprina* and diazinon resistance in *L. sericata* (Appendix 1.1.14) (see *Statistical analysis* in methods for interpretation of slope). Slope of the ldp-line was either not an indicator of genetic variation or strains were homogeneous in their expression of resistance with respect to OP and benzoyl urea resistance in field strains of *L. cuprina* (Appendix 1.1.14). Variation in slope appeared instead to be dependent on environmental factors such as season. Seasonal trends may be a collective result of both activity of flies (and oviposition) and insecticide pressure. Fly activity (and flystrike) increases with increasing mean temperature (i.e. in spring and summer) and as a consequence insecticide pressure increases as farmers attempt to prevent or control flystrike. It is difficult to predict the effect that increased insecticide pressure

may have on the slope of the ldp-line from the current data set. It is possible however that this would increase the slope, as increased pressure would select for more highly resistant individuals. Testing this hypothesis may require a controlled laboratory environment or an intensive longitudinal study on one or more properties to determine the variability of insecticide pressure, mobility of fly populations and competitive relationships between fly species. (Genetic variation of this species is examined in more detail in Section 3 by a RAPD PCR technique).

An explanation for the lack of relationship between slope and LC_{50} for OPs and *L. cuprina* compared with the significant relationship observed with deltamethrin might be that genetic variation for OP resistance has reached a state of equilibrium or homogeneity in this species. Normal distribution histograms (Appendix 1.1.13) might also indicate this although a fall off in resistance factor after 1992 (Figure 1.1.10) argues against a state of homogeneity. It is therefore more likely that slope of the lpd-line is not an indicator of genetic variation with respect to OP resistance in *L. cuprina.*

(2) Side- and cross resistance

Diazinon, chlorpyrifos, dichlofenthion and propetamphos were all shown to be strongly side-resistant in the present study. It would appear that some degree of non-specific OP resistance is conferred by R_{OP}-1 to each of these insecticides. All share structural similarities that decide their metabolic fate. That is, they all contain a phosphorothionate residue, which is desulfurated to a phosphate by the action of the mixed function oxidases (Eto 1974), before they can exert their toxicity. Phosphate linkages may then be hydrolysed by phosphatase activity. The relationship between

propetamphos and diazinon was slightly weaker than with the other OPs, which may be a reflection of the additional potential routes of detoxification via the amido bond and the carboxyl ester linkages, that propetamphos contains. The carboxyl ester links allow propetamphos to be either degraded by carboxylesterase, phosphatase or amidase activity following desulfuration (Eto 1974).

Smyth *et al.* (1998) found, however, that propetamphos resistance did not appear to be conferred by R_{OP}-1 or R_{mal} alleles in *L. cuprina*. Instead they suggested that the minor diazinon resistance locus R_{OP}-2 (Arnold & Whitten 1976) might encode a mixed function oxidase that confers this resistance. Campbell *et al.* (1998) support this suggesting that the active oxon form of propetamphos can either inhibit or is unreactive with the mutant aliesterase encoded by R_{OP}-1.

R_{OP}-1 has been shown to confer resistance to chlorfenvinphos (Hughes 1981) but structural differences may account for the lack of side-resistance with other OPs in this study. Chlorfenvinphos differs from these other OPs by already being in the active oxon form. It therefore does not need to undergo desulfuration by mixed function oxidases. It is also relatively stable to hydrolysis compared with the other OPs (Eto 1974). Figures 1.1.2-1.1.4 provide some support for the suspected differences in metabolic fate of propetamphos and chlorfenvinphos compared with diazinon. Diazinon, chlorpyrifos and dichlofenthion ldp-lines were often near parallel compared with those of chlorfenvinphos and propetamphos suggesting the first three might share a common metabolic fate other than that for the later two. Differing ldp-lines for propetamphos and chlorfenvinphos might therefore suggest

these OPs have divergent fates although this can only be confirmed by specific metabolic studies.

There is a suggestion that cross-resistance exists between diazinon and diflubenzuron in *L. cuprina.* It has been reported that such a cross-resistance relationship exists in Australian strains of *L. cuprina* (Levot & Shipp 1983, 1984, Hughes & Levot 1987). The involvement of both esterases and mixed function oxidases has been implicated in diflubenzuron resistance (Kotze & Sales 1994) although the differing chemical structures of the two insecticide groups provide no reason to expect cross-resistance. The small sample size in this study (n = 16) did not allow for an accurate analysis of cross-resistance with diazinon.

No cross-resistance was detected between diazinon and deltamethrin, which was consistent with Australian findings (Sales *et al.* 1989). Other pest species have shown a cross-resistance between SP and OP groups of insecticides. The diamondback moth (*Plutella xylostella*) showed significant cross-resistance between diazinon and four SPs, permethrin, deltamethrin, cypermethrin and fenvalerate (Liu *et al.* 1981). Strains of the brown plant hopper (*Nilaparvata lugens*) also showed OP/SP cross-resistance between diazinon and SPs, deltamethrin, cypermethrin and fenvalerate (Park & Choi 1991). The lack of detectable cross-resistance in *L. cuprina* may be a function of low SP-insecticide pressure in the field compared with OP pressure particularly as the highest SP pressure is in areas where *L. cuprina* has only just arrived. Deltamethrin is currently not registered for fly control although cypermethrin is, as a combination with chlorpyrifos (Flypel®) or by itself as Cypafly®.

Synthetic pyrethroid residues are however regularly available in the fleece as they are the predominant group of insecticides used for louse control. This would suggest that they provide conditions that are conducive to contact by blowflies and offer a form of selection pressure. Larval toxicology results show a range of responses but without a known insecticide-susceptible, SP-naive strain reliable inferences cannot be drawn. It is likely that if the variation in response observed can be described as resistance and no relationship exists with diazinon resistance, then perhaps larvae employ a different detoxification system than that for OP detoxification. Evidence of mechanisms of deltamethrin resistance in other studies suggests that this is species-specific. The housefly, *Musca domestica*, exhibits mechanisms involving site-insensitivity (Osborne *et al.* 1995) while other species such as *P. xylostella* have esterases that play an important role (Kim *et al.* 1996). The mixed function oxidases also play a role in deltamethrin resistance in some species of mosquitoes, i.e. *Culex quinquefasciatus*, *Aedes aegypti*, *Anopheles stephensi*, and also with the codling moth, *Cydia pomonella.*

(2) OP-resistance in blowfly adults

It was evident from the results obtained through topical application of a discriminating dose of insecticide that adult *L. cuprina* field strains were resistant to diazinon compared with the OP-susceptible laboratory strain. There was however a significant relationship observed between adult body weight and response to insecticide, that is, lighter insects were more susceptible to the dose than heavier ones. The topical application method used in this study needed either to standardise the weight of insects tested by increasing consistency in larval diet and rearing

conditions or by compensating for weight differences by altering the dose of the insecticide applied to each insect. Reducing this type of weight ratio error is essential if adult and larval resistance factors are to be compared. The method employed in this study followed that of Arnold & Whitten (1975) who failed to find a correlation between resistance factors to diazinon in larvae and adults of *L. cuprina* using a range of dose concentrations. This lack of correlation may have been due to low sample size (n = 6 strains) or to lack of recognition that insect body weight was a significant variable as they do not report standardising insect weight in any way. Shanahan (1966) made reference to size variation but did not give specific size ranges to be used within bioassays, instead variation was accommodated by using a discriminating dose of 0.02 % diazinon rather than 0.014 % as used by G. Levot (NSW Department of Agriculture, Sydney; personal communication). Insect weight is frequently a consideration in insecticide resistance bioassays (Gast 1959, Robertson & Preisler 1992) although it also appears to be species variable (Robertson & Preisler 1992) with the response by some species being unaffected by weight variation (Bliss 1936, Way 1954).

However, a more detailed study of mode of insecticide application by Busvine & Iwuala (1975) reinforced Arnold & Whitten's (1975) findings of a lack of an adult/larval resistance relationship, although Busvine & Iwuala (1975) ignored insect weight. They showed that the mode of insecticide administration affected the relative potency of an insecticide in three Diptera, *L. sericata* and *Chyrosomya putoria* (which are phylogenetically related to *L. cuprina* (Erzincliogl u 1984)), and *Musca domestica.* Contact treatments provided the highest LC_{50}s followed by oral treatments and then by injection of insecticide. Busvine & Iwuala (1975) also found

a strong correlation between mode of application (oral versus contact) within the same stage ($p<0.05$; larvae $r = 0.941$, adult $r = 0.837$) but none between different stages (oral larval/adult $r = -0.222$ and contact larval/adult $r = -0.174$). This was consistent with Arnold & Whitten's (1975) findings. In contrast, a more comprehensive test of larval/ adult resistance, based on both toxicological response and *in vitro* metabolism of DDT in the mosquito *A. aegypti* showed significant correlation ($p<0.05$; toxicological $r = 0.62$, *in vitro* metabolism $r = 0.82$)(Rathor & Wood 1981). This may only be indicative of a species-specific response although Hughes & Levot (1985) also note that wide larval variation in response to diazinon compared with adult *L. cuprina*, may be a reflection of test method.

Because of the effects of weight on response, and inadequate methods for compensating for this a decision was made not to compare the relative adult and larval resistance factors in this study. The lack of relationship observed in toxicological tests by Arnold & Whitten (1975) was deemed to have some validity. Arnold & Whitten (1975) pointed out however, that single comparison of resistance factors based on toxicological assays alone is not as meaningful as when accompanied by comparisons of the genetic and biochemical mechanisms of resistance. In particular, they referred to the relative production of detoxifying enzymes and the interaction between resistance alleles. Following this reasoning this thesis focuses more on observed relationships between the relative expression of various larval and adult detoxifying enzymes, the findings being presented in the following chapters. Other potential influences such as isoenzymes and sex-linked resistance traits (e.g. Rathor & Wood 1981) have not been considered.

Summary

The key points arising from the toxicological monitoring of insecticide resistance in *L. cuprina* and *L. sericata* were:

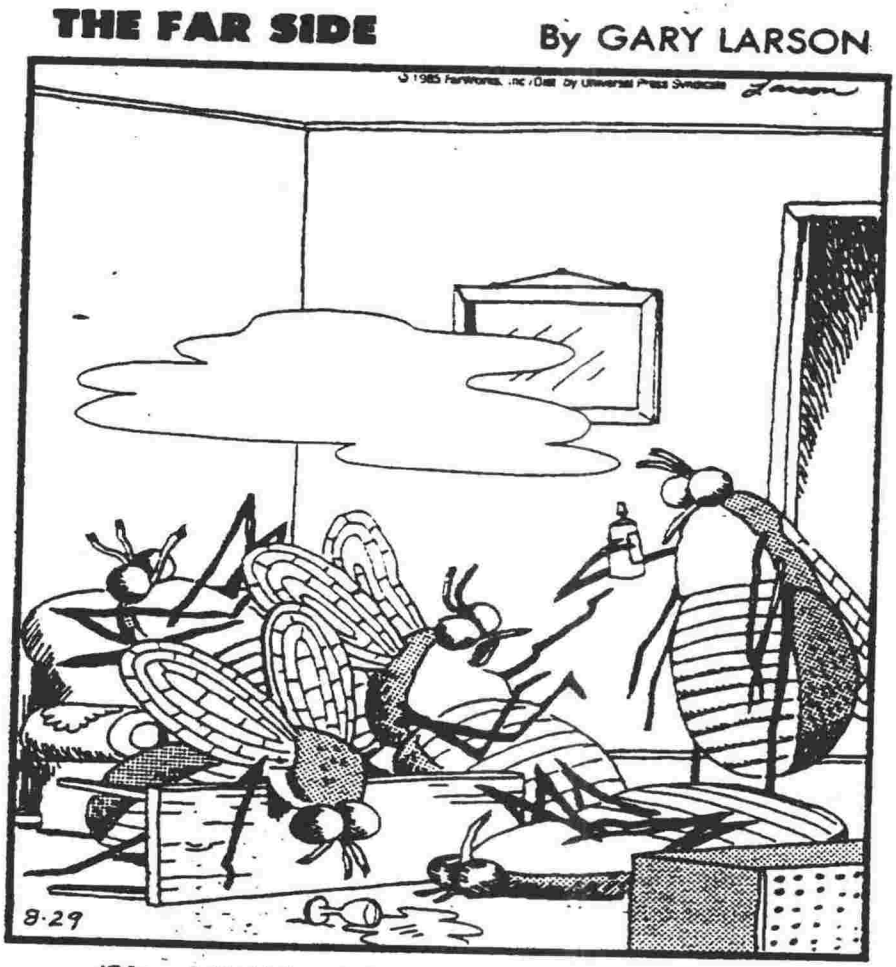
(1) Organophosphate resistance is widespread in both species of blowfly. Phenotypic expression of resistance appeared to be influenced by environmental factors such as season, region, sheep density and year, and these may influence insecticide selection pressure.

(2) The slope of the ldp-line was suggested to be an indicator of genetic heterogeneity with respect to deltamethrin resistance in *L. cuprina* and diazinon resistance in *L. sericata.* This was not the case with OP resistance in *L. cuprina* suggesting that OP resistance had reached equilibrium or homogeneity within the species.

(3) Side-resistance exists between diazinon, chlorpyrifos, dichlofenthion and propetamphos in *L. cuprina* suggesting that the same or similar mechanisms of resistance might operate on structurally similar insecticides. No cross-resistance could be demonstrated with diazinon and deltamethrin, or between diazinon and diflubenzuron.

(4) No inferences regarding relationship can be drawn between adult and larval resistances due to differences in the test methods.

The question now remains of why two such closely related species differ so markedly in their expression of resistance. Section 2 attempts to elucidate these differences by assessing the contribution of various detoxification enzymes in resistance in the two species. These as well as the ecological habits of the two species are considered in following chapters.



"You idiot! I said get the room freshener! That's the insecticide!"

CHAPTER 1.2

Relative efficacy of organophosphorus insecticides against susceptible and resistant strains of *Lucilia cuprina*

Introduction

The blowflies involved in flystrike of sheep in New Zealand are more heterogeneous than that in Australia, despite the presence of numerous Calliphoridae in both countries. In addition, mono-specific strikes are less common in New Zealand. For example, during September-April 1991-92 *L. cuprina* was found singly in 21.8% of strike samples submitted to the Wallaceville Animal Research Centre, *Calliphora stygia* in 14.9%, *L. sericata* in 7.9%, *Chrysomya rufifacies* and 2.6% of samples comprised unidentified and non-primary strike species. The remaining strikes were of mixed species, comprising 51.3% of samples during 1991-92 (A. Heath & D. Bishop, unpublished data). The high participation of *L. cuprina* in field strikes in New Zealand, together with its known resistance to organophosphates (OPs), determined in the previous chapter (1.1), stimulated this investigation into its response to currently available sheep dips. Resistance factors alone do not provide an indication of how resistance affects the protective value of dips used on the farm. In order to make comparative evaluations artificial flystrike was established by implanting laboratory bred larvae onto treated sheep. The larval implant method (McLeod 1937, Fiedler & du Toit 1951, Harrison & Johnson 1961) is an established bioassay for assessing the efficacy of insecticides for protecting sheep against flystrike. In New Zealand, various studies have employed the technique using larvae from field strains of *Lucilia sericata* maintained in culture for many generations (Greenwood 1964, Heath *et al.* 1986). In contrast, efficacy tests in Australia use *L. cuprina* (Hughes &

Shanahan 1978) but latterly this species has also been used in New Zealand (Wilson *et al.* 1996).

In order to gain some insight into the relative protective nature of OPs and the extent to which the OP resistance status of *L. cuprina* has affected this, a susceptible strain of *L. cuprina*, and a strain known to exhibit side-resistance to at least four OPs, were used in a series of *in vivo* bioassays. It was intended that the results would provide a basis for establishing the relative efficacies of currently available flystrike preventatives.

Methods

Insecticides

Commercial formulations of chlorfenvinphos, dichlofenthion, diazinon and propetamphos were applied by plunge dipping at concentration in accord with the manufacturers' recommendations (Table 1.2.1).

Insects

First instar larvae (< 4 hours old) of two strains of *L. cuprina* were used in the bioassay. These strains are described below.

OP susceptible strain

See chapter 1.1 for a description of the *L. cuprina* susceptible strain.

Table 1.2.1 Insecticides applied to sheep.

Active Ingredient	Dilution	Application Method	Trade Name	Manufacturer
Chlorfenvinphos (1000 g/l)	1:2000	Plunge dip	Supreme DFF®	Coopers Animal Health NZ Ltd
Diazinon (400 g/l)	1:2000	Plunge dip	Diazinon 40®	Young's Animal Health NZ Ltd
Dichlofenthion (100 g/l)	1:2000	Plunge dip	Trigon DFF®	Coopers Animal Health NZ Ltd
Propetamphos (160 g/l)	1:500	Plunge dip	Seraphos 500®	Young's Animal Health NZ Ltd

Combined field strain

A combined field strain (CFS) of *L. cuprina* was used which had been derived from instances of flystrike occurring in New Zealand and maintained in culture for no more than five generations. Resistance factors for the strain were determined by a method described in chapter 1.1 and ranged between 21.8 - 24.0 x resistance to diazinon (compared with the OP susceptible strain) for each generation used in the implant procedure.

Sheep

Four groups each of five Romney lambs (10 months old) born in 1991 were treated in a plunge dip in May 1992. A fifth group acted as a dry, insecticide-free control. All sheep were snow-combed (i.e. shorn but leaving about 1 cm of wool) 4 weeks prior to treatment to provide the recommended wool length for insecticide uptake and retention. Each treatment group was grazed separately.

Larval implants

The bioassay employed the implant procedure as described by Hughes and Shanahan (1978). Briefly, the skin surface was scraped to provide tissue exudates. About 200 first instar larvae of each of the CFS strain and the insecticide susceptible strain were deposited respectively onto separate areas on the skin (at least 20 cm apart). The same CFS strain was used throughout the course of the trial. Each implant was covered by a cotton dental plug moistened with water, and 1.5 ml of sheep serum was pipetted on to and around the plug. The surrounding fleece was drawn up over the cotton plug and held in place by a rubber ring ('Elastrator') normally used for docking. The implanted areas were examined 24 hours later for live larvae.

Surviving larvae were killed with 100% ethanol. Positions of the implant sites were changed weekly with the assumption that the deposition and decay rates would be the same at each site along the backline. Each sheep was implanted with *L. cuprina* larvae 2 weeks after dipping and then weekly until such time as strikes remained alive on four of five sheep in the group. Implants were considered positive if at least one larva was alive at the 24 hour inspection period. The minimum protection period was defined as weeks post-treatment when at least one larva remained alive on one animal and maximum protection period as weeks post-treatment when at least one larva remained alive on four animals.

Results

Both susceptible and resistant strains of *L. cuprina* larvae established active strikes in control animals each week of the trial. All four insecticides remained toxic to susceptible larvae for between 19 and 21 weeks. Results with the resistant strain were more variable, ranging from a minimum of 9 weeks with diazinon to 17 weeks with chlorfenvinphos (Table 1.2.2).

Table 1.2.2 Periods of protection against larval implantation provided by four registered insecticides.

Formulation	Period of Protection[a] (weeks)	
	CFS[b]	susceptible [c]
Chlorfenvinphos (1000g/l)	16 - 17	21
Diazinon (400g/l)	9 - 13	19
Dichlofenthion (100g/l)	10 – 13	20
Propetamphos (160 g/l)	15 – 16	19
Control	0	0

[a] as defined in text
[b] Combined resistant field strain
[c] OP-susceptible *L. cuprina*

Discussion

Insecticide resistance in *L. cuprina* has, on anecdotal evidence, resulted in OP insecticides providing a reduced period of protection against flystrike. In Australia, diazinon resistance was detected in 1965 in *L. cuprina* (Shanahan 1966). It is likely that *L. cuprina* entered New Zealand from Australia, so it is not surprising that New Zealand strains are also resistant (Wilson & Heath 1994). No New Zealand field populations of *L. cuprina* are as susceptible to OPs as the strain imported from Australia for this trial.

Although *Lucilia sericata* has been used in New Zealand larval implant studies previously (Greenwood 1964, Heath *et al.* 1986) the now widespread occurrence of *L. cuprina* makes it the logical choice for efficacy trials. Furthermore, *L. cuprina* has exhibited the highest resistance to the flystrike insecticides. Most field strains of the other main strike species, *C. stygia* and *C. rufifacies* have been found to have a comparable susceptibility to diazinon as the susceptible strains of *L. cuprina*. The resistance factors of tested field strains have not exceeded three times that of the susceptible *L. cuprina* larvae used in this trial (N. Haack & J. Wilson, unpublished data). Field strains of *L. sericata* have been found to exhibit moderate resistance to diazinon (mean RF=11.2; n = 99)(N. Haack & J. Wilson, unpublished data) compared with field strains of *L. cuprina* (mean RF = 25.7; n = 359) (Chapter 1.1).

To provide an accurate representation of efficacy against a flystrike fauna, mixed species bioassays, comprising either separate species at each site or a composite of species at one site, should be used and in fact have been tested successfully (C. MacKay, N. Haack & J. Wilson, unpublished data). The main disadvantage of

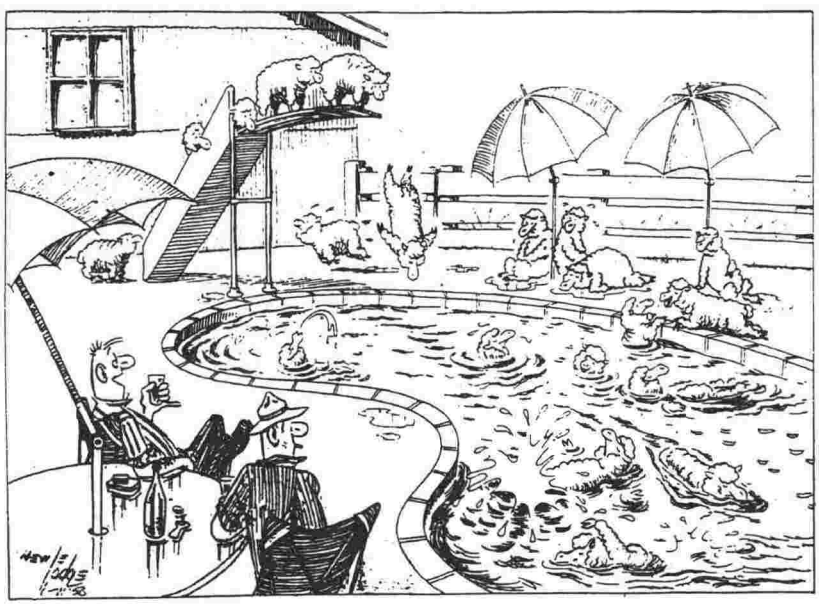
separate species' sites is that the number of sites per sheep is increased providing an animal welfare problem. Handling time for each sheep is also increased. Furthermore, known resistant and susceptible strains of some species are not available, which limits information on the range of protection conferred against each species.

The larval implant technique is considered a suitable and convenient method for initiating experimental fly strikes on sheep to assess the efficacy of flystrike preventatives. Although it is not compulsory for agrochemical companies to use the method for establishing insecticide label claims (Animal Compounds Unit 1994) it is still widely used to provide data to support product registration. It has the advantage of standardising conditions under which an insecticide is challenged - something that is more difficult to achieve in the field. Variations in seasonal and local activity of flies and the influence of predisposing factors such as fleece rot on the susceptibility of sheep mean that flystrike can be unpredictable in the field (Dallwitz *et al.*1984), Merritt 1978).

An explanation for the differences in the relative efficacy of the insecticides tested here cannot be readily determined. It is likely however, to be based on a variety of factors, including deposition and decay rates (Rammell & Bentley 1989), chemical reactivity (Eto 1974) and the relative ability of the *L. cuprina* resistant strain to detoxify each insecticide (Wilson & Heath 1994).

Toxicities for susceptible larvae were similar for all four insecticides with protection periods within a narrow range of 19-21 weeks. The combined field resistant strain

demonstrated variation in susceptibility with each OP formulation. The relative toxicity of each insecticide as determined in this study are unlikely to be similarly reflected in practical usage on sheep. Variations in wool length, dipping apparatus and individual farmer competency and knowledge would largely account for an insecticide's performance in the field. In general, field protection periods against flystrike are invariably shorter than those obtained with carefully controlled bioassays (Heath *et al.* 1991). These results highlight the value of using both susceptible and resistant strains in comparative tests to improve the confidence with which practical advice on product choice is given to farmers.



"So I decided, as we had to have the dip done up only the best was good enough for 'em."
(Cartoon by Neville Lodge)

SECTION 2

Mechanisms of insecticide resistance in adult and larval *Lucilia cuprina* and *Lucilia sericata.*

Introduction

Resistance to insecticides has been shown to result from four possible mechanisms, one or more of which can develop against a particular insecticide. These mechanisms include (1) behavioural changes; (2) reduced uptake and penetration; (3) enhanced detoxification due to metabolism; and (4) altered target site.

Behavioural Resistance

Behavioural resistance is often subtle and difficult to measure. It normally manifests itself as an enhanced avoidance behaviour that reduces an insect's exposure to a toxin (Sparks *et al.* 1989). It was first recognised as a resistance mechanism towards DDT in mosquitoes in Africa (Muirhead - Thompson 1960). Early malaria eradication programs in Africa involved spraying interior walls of huts with a DDT-solution that acted as a long-term residual treatment. At first this treatment worked well but it was later noticed that adult mosquitoes began avoiding walls and sought alternative untreated areas, such as ceilings or exterior walls, on which to rest (Muirhead - Thompson 1960).

Numerous examples of behavioural resistance have arisen since this initial observation, and include mosquitoes in different geographic areas. For instance, changes in the behaviour of the mosquito, *Anopheles farauti*, has been implicated in

resistance after DDT house-spraying in the Solomon Islands whereby mosquitoes showed a preference for untreated surfaces (Taylor 1994). Behavioural resistance did not appear to develop however, in *Anopheles darlingi* towards DDT-wettable powder in huts in Suriname, South America (Rozendaal *et al.* 1989). This was confirmed using a choice-box experiment in which mosquitoes showed no preference for either sprayed or unsprayed resting surfaces (Rozendaal *et al.* 1989).

Other examples of behavioural resistance involving avoidance include; the red flour beetle, *Tribolium castaneum*, with malathion and fenitrothion (Pinniger 1975), *Drosophilia melanogaster*, with malathion (Pluthero & Threlkeld 1981); the horn fly, *Haemotobia irritans*, with fenvalerate and permethrin (Lockwood *et al.* 1985, Byford *et al.* 1987); houseflies, *Musca domestica*, with methomyl and asamethiphos (Learmount *et al.* 1996) and the German cockroach, *Blattella germanica*, with cypermethrin (Hostetler & Brenner 1994).

A key point arising from these studies is that behavioural and physiological mechanisms of resistance may be genetically independent (Pluthero & Threlkeld 1981) but may often coexist (Lockwood *et al.* 1984). For instance, in some species, behavioural resistance may be physiologically potentiated or stimulus-dependent (where the insecticide is the stimulus) (Lockwood *et al.* 1980) while in others, such as horn flies, behavioural resistance has become stimulus-independent after years of intensive pyrethroid selection (Byford *et al.* 1987). Behavioural resistance can also be insecticide-specific and sex-specific (Hostetler & Brenner 1994).

Behavioural resistance is not tested empirically in this thesis. It is, however, considered in the final discussion, with respect to differences in resistance between the two *Lucilia* species under investigation.

Reduced Uptake and Penetration

The cuticle is the main physical defence an insect has against a contact poison. Decreased or delayed penetration of an insecticide is seldom the principal mechanism of resistance employed by an insect, but can allow detoxification mechanisms to operate more effectively.

Enhanced Detoxification Due to Metabolism

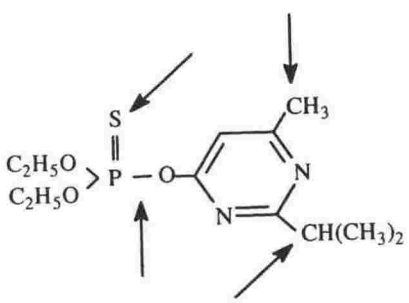
Once an insecticide has penetrated the cuticle and is inside the insect's body it is subject to metabolic processes which transform it to more water-soluble, more polar and therefore less toxic products, facilitating its removal from the organism. Metabolic detoxification systems are diverse, are often integrated and can occur either as a result of modifying existing enzymes or the increased production of an enzyme already available at lower levels (Oppenoorth 1976). The following section describes and discusses the known detoxification systems.

(1) Oxidation

Insect microsomal monooxygenases (MOs) function as non-specific metabolising systems. They have a characteristically wide range of substrates, which include many of the available classes of flystrike preventatives such as organophosphates and synthetic pyrethroids. They are important mechanisms in resistance of insects to insecticides and are also involved in insect growth and development.

MOs are localised mainly in the endoplasmic reticulum in insect cells and consist of two components, cytochrome P-450 and NAPDH-cytochrome P-450 reductase. Cytochrome P-450 is a b-cytochrome-type haemoprotein and is named from its characteristic wavelength (450 nm) of absorption of the CO derivative of the reduced form (Ray 1967). Cytochrome P-450 has been shown to occur in the greatest concentration in the midgut of insects. It also occurs in the fat body and the malpighian tubules (Lee & Scott 1992). NADPH-cytochrome P-450 reductase is a flavoprotein enzyme that acts by transferring a reducing equivalent from NADPH to cytochrome P-450.

Most organic insecticides are subject to the action of microsomal monooxygenases and many possess multiple sites at which oxidations can occur. Diazinon, for example, can be attacked at 4 different sites (Figure 2.0.1).



Diazinon

Figure 2.0.1 Possible sites by which oxidation can occur in the OP, diazinon. (from Nakatsugawa & Morelli 1976)

As a consequence of multiple oxidation sites a combination of many biotransformations can occur with any one compound. Many of these reactions are involved in detoxification while others are involved in activation where a product is

more toxic than the parent compound (see desulfuration). Specific examples of oxidative reactions of significance to insecticide action and degradation are described below.

Desulfuration

Many OPs and specifically phosphorothioates and phosphorodithioates, are not potent toxins unless they are first oxidatively desulfurated. Desulfuration increases the ability of the insecticide to inhibit the target enzyme, namely cholinesterase. Desulfuration resulting in increased toxicity in the oxygen-analogue has been demonstrated in a number of insect species including cockroaches (Nakatsugawa & Dahm 1965) and houseflies (El-Bashir & Oppenoorth 1969) with the conversion of parathion to paraoxon in the presence of NADPH and molecular oxygen (Figure 2.0.2). Nakatsugawa & Dahm (1967) showed that desulfuration of parathion lead to the covalent binding of the sulphur atom to microsomes *in vitro*. The detached sulphur was excreted as inorganic sulphate *in vivo* (Nakatsugawa & Dahm 1967). Oxons are normally more toxic than their stable parent -thions and break down more readily. This is not always the case however and Nomeir *et al.* (1980) demonstrated a contrary effect resulting from oxidative desulfuration of EPN (*O*-ethyl-*O*-*p*-nitrophenyl phenylphosphonothioate) to its oxygen analogue EPNO in houseflies. In this example EPNO was found to be less toxic to resistant houseflies (to a lesser extent in susceptible strains) compared to EPN (Nomeir *et al.* 1980). It would appear that desulfuration contributes in part, to resistance in these strains of houseflies.

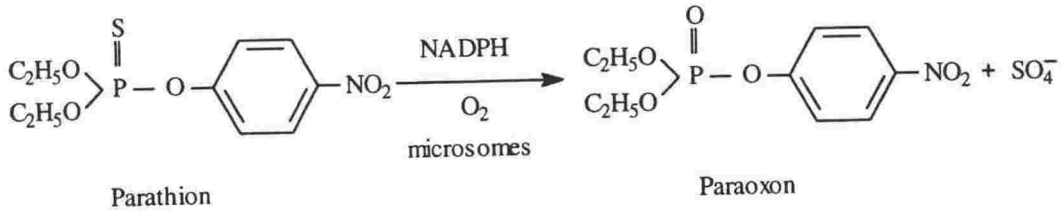


Figure 2.0.2 Desulfuration of parathion (from Dahm 1970)

Epoxidation

Epoxidation of the unchlorinated double bond is an important oxidative reaction in cyclodiene insecticides such as aldrin. Epoxidation results from the conversion of alkenes to form stable epoxides, as well as arene oxides (aromatic ring epoxides) which act as intermediates in aromatic hydroxylations. The conversion of aldrin to the stable epoxide, dieldrin, is the best known example of epoxidation (Figure 2.0.3). Aldrin shows the same toxic effects as dieldrin but dieldrin is far more persistent (Matsumura 1975).

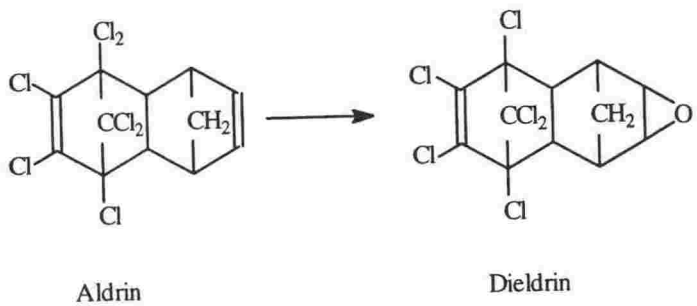


Figure 2.0.3 Conversion of aldrin to dieldrin by epoxidation.

(from Hodgson & Guthrie 1980)

Kotze *et al.* (1997) measured the rate of aldrin epoxidation to show a significant relationship between MO activity and diflubenzuron and diazinon resistance in *L. cuprina* larvae. A similar trend was seen with fenvalerate-resistant diamondback

moth larvae where resistant strains showed higher rates of aldrin epoxidation than susceptibles (Yao *et al.* 1988).

O-, N-, S-dealkylation

O-dealkylation occurs in a variety of vinyl, phenyl, phenylvinyl, naphthyl phosphates and thionophosphate triesters. During *O*-dealkylation an unstable α-hydroxy intermediate is produced. The α-hydroxy then spontaneously releases an aldehyde, if from a primary alkyl group, or a ketone, if from a secondary alkyl group (Figure 2.0.4). *O*-dealkylation has been shown to occur with chlorpyrifosmethyl in the tobacco budworm (*Heliothos virescens*) (Whitten & Bull 1974). Although the reaction of chlorpyrifosmethyl was not specifically linked to resistance, other studies of MO activity in this species link enhanced *O*-dealkylation activity towards dimethyl *p*-(methylsulphonyl) phenyl phosphate and aldrin with resistance (Bull & Whitten 1972).

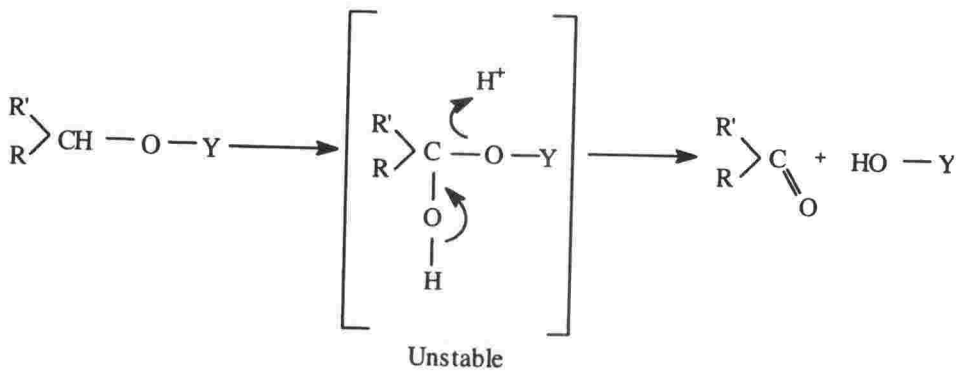


Figure 2.0.4 Dealkylation of *O*-alkyl groups of ester or ether structures of insecticides (from Nakatsugawa & Morelli 1976).

N-dealkylation commonly occurs with *N*-dialkyl carbamate insecticides and other *N*-containing substituents. *N*-dealkylation differs from *O*-dealkylation in that it often

produces a stable *N*-α-hydroxyalkyl derivative. This may be because nitrogen is less electronegative than oxygen (Nakatsugawa & Morelli 1976). *N*-dealkylation has been shown to occur with the 2-step bioactivation of isofenphos to *N*-desisopropylisofenphos oxon in *M. domestica* (Gorder *et al.* 1986). It is thought that this reaction occurred via an α-carbon oxidation without the formation of cholinesterase inhibiting intermediates (Gorder *et al.* 1986). As with desulfuration reactions, removal or modification of *N*-substituents may have a range of effects on insecticide toxicity.

Di- and tri-alkyl thiophosphates and thiophosphoramidate esters are subject to *S*-dealkylation (Coats 1991).

Sulphoxidation

Sulphoxidation (Figure 2.0.5) is a major metabolic route of carbamates and aromatic alkyl thioethers such as measurol and methiochlor (Kapoor *et al.* 1972). Microsomal sulphoxidation has been implicated in resistance to phorate in the fall armyworm, *Spodoptera frugiperda* (Yu 1985) (Figure 2.0.6). Oral toxicity of phorate to larvae decreased with an increase of phorate sulphoxidation, which was induced by allelochemicals and host plants (Yu 1985). In particular, the food plants cotton and parsley were shown to be the best inducers of organophosphate sulphoxidation (Yu 1986). With respect to cotton, induction was associated with decreases in the toxicity of diazinon and azinphosmethyl to fall armyworm larvae (Yu 1986).

$$R\text{-}S\text{-}R' \rightarrow R\text{-}SO\text{-}R' \rightarrow R\text{-}SO_2\text{-}R'$$

Figure 2.0.5 Sulphoxidation of xenobiotics catalysed by monooxygenases (from Eto 1974)

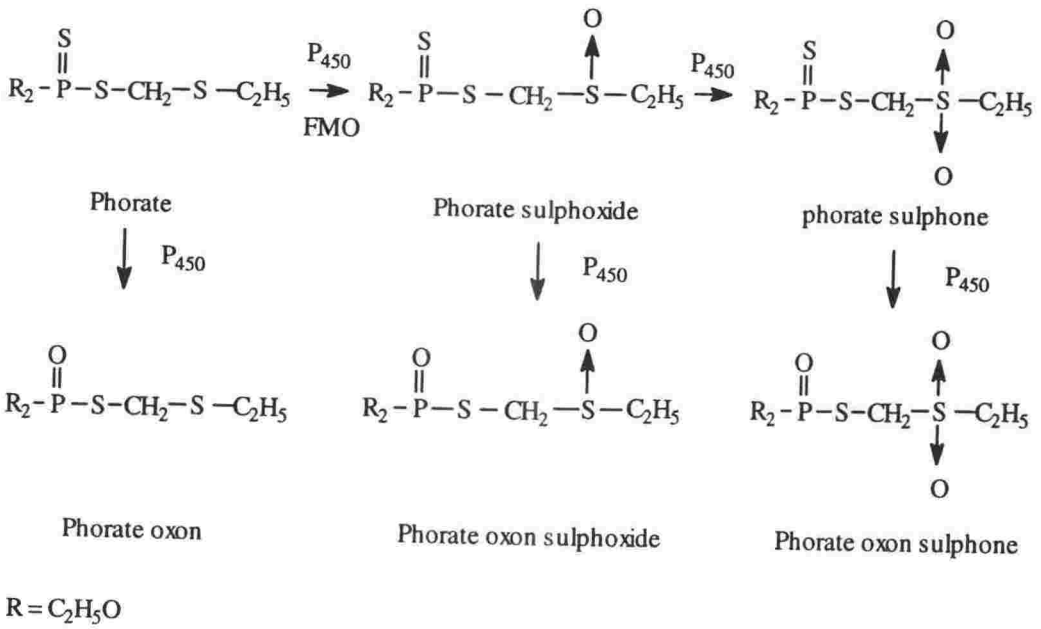
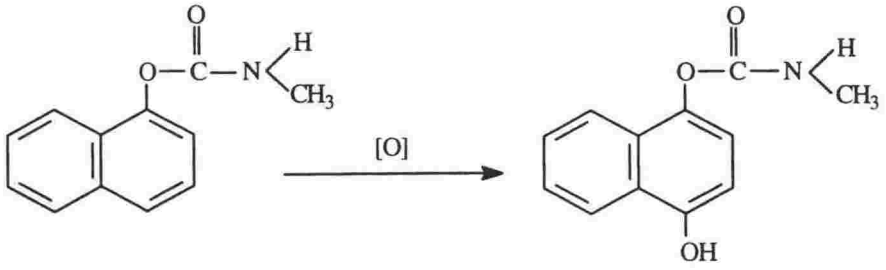


Figure 2.0.6 Oxidative metabolism of phorate. Sulphoxidation to phorate sulphoxide is catalyzed by both P_{450} and flavin-containing monooxygenases (FMO). Other oxidations are catalysed by P_{450} only (from Levi & Hodgson 1992).

Ring hydroxylation

Ring hydroxylation occurs on aromatic rings in pesticides of several types including polycyclic aromatic hydrocarbons (Coats 1991). The ring hydroxylation of carbaryl is shown below (Figure 2.0.7).

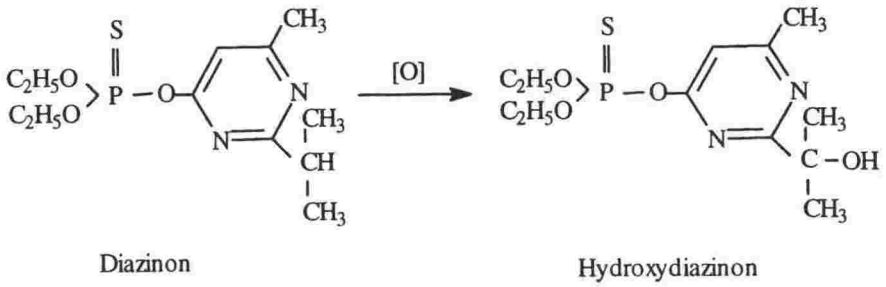


Carbaryl 4-hydroxy carbaryl

Figure 2.0.7 Ring hydroxylation of carbaryl (from Coats 1991)

Side-chain oxidation

Methylene groups in aliphatic side chains are susceptible to oxidation. Diazinon for example is converted to hydroxydiazinon via this type of oxidation (Figure 2.0.8). Terminal methyl groups may also undergo side-chain oxidation.



Diazinon Hydroxydiazinon

Figure 2.0.8 Side-chain oxidation of diazinon (from Coats 1991).

Almost all these reactions increase the water solubility of the original xenobiotic and either convert it to a directly excretable form or to one that can be further conjugated before excretion.

In this study the importance of MO activity in resistance to OP pesticides has been investigated by the use of a synergist. Synergists act by inhibiting metabolic

pathways that may have been altered or amplified in resistant species. The result is a more-than-additive increase in the toxicity of an insecticide when used in combination with the synergist (Price 1991). Certain synergists are known to act on specific metabolic pathways involved in detoxification. Piperonyl butoxide is a methylene dioxyphenyl compound that is widely used as an inhibitor of the monooxygenases. The use of this synergist provides an initial clue to whether MOs are involved in resistance in *L. cuprina*.

(2) Hydrolysis

Hydrolysis is the addition of water in order to split a bond. Esterases are types of hydrolases which catalyse cleavage of ester bonds by addition of water to give an acid and an alcohol (Healy *et al.* 1991). Organophosphate, carbamate and pyrethroid insecticides may all contain carboxylic ester bonds susceptible to attack by hydrolytic enzymes or hydrolases (Hutson & Roberts 1985).

Another type of hydrolysis can occur as the result of epoxide hydrolyases. Epoxide rings of certain insecticides such as the cyclodienes (e.g. dieldrin) can be cleaved and hydrated by epoxide hydrolases to form *trans*-dihydrodiols resulting in a reduced insecticidal activity (Brooks 1973, Slade *et al.* 1975).

One well-known example of hydrolysis is the carboxyesteratic cleavage of malathion or malaoxon to the corresponding alpha or beta carboxylic acid (Figure 2.0.9). Note that malathion is a unique OP possessing two carboxylester residues. The presence of an ester linkage in these insecticides therefore allows esterases to play an important

role in the development of resistance to these pesticides (Oppenoorth 1985, Hughes & Devonshire 1982, Hughes & Raftos 1985).

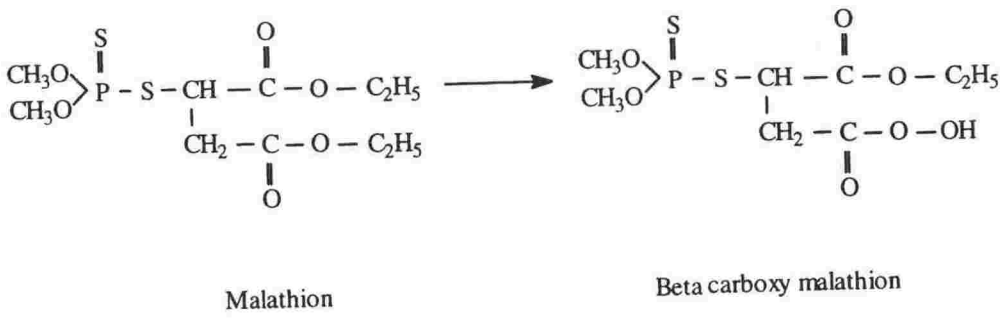


Figure 2.0.9 Carboxyesteratic cleavage of malathion (from Coats 1991)

Esterase-mediated OP resistance by mutation, gene amplification or increased esterase activity is discussed in Chapter 2.2.

(3) Glutathione conjugation

The glutathione S-transferases (GSTs) are a multifunctional group of proteins that have been implicated in the detoxification and biotransformation of many xenobiotics. They are of particular importance in the detoxification of phosphorothionate and phosphate insecticides in which either the alkyl or aryl components can be removed as glutathione adducts (Dauterman 1985, Wilkinson 1985) (Chapter 2.3, Figure 2.3.2). Glutathione S-transferases catalyse the conjugation of these groups to the sulphur atoms of the tripeptide glutathione (GSH) (γ-L-glutamyl-L-cysteinyl-glycine). The conjugate can either be excreted directly or may be modified further before excretion (Pickett & Lu 1989). Resistance has been associated with increased levels of GST activity toward particular insecticides

(Oppenoorth & Welling 1976). Detail and examples of this mechanism's contribution to resistance in insect species is presented in chapter 2.3.

DDT-dehydrochlorinase

DDT-dehydrochlorinase is a specialised form of GST (Clark & Shamaan 1984, Wilkinson 1985). DDT-dehydrochlorinase converts DDT (2,2-(*p*-chlorophenyl)-1,1,1-trichloroethane) to the less toxic DDE (2,2-(*p*-chlorophenyl)-1,1-dichloroethylene) and is a principal mechanism of resistance to DDT in houseflies (Yang 1976, Mahmood & Motoyama 1994). The dehydrochlorination of DDT to DDE occurs only in the presence of GSH (Valdes 1971).

Altered Target Site

The best known examples of altered target site associated with insecticide resistance are altered acetylcholinesterase and 'knockdown' resistance. The first example affects OPs and carbamates and is investigated in this study. The role of acetylcholinesterase (AChE) is briefly described below. Specific examples in insects of reduced sensitivity of AChE to OPs are dealt with in more detail in chapter 2.4. The second example of altered target site (*Kdr*-resistance) affects DDT and pyrethroids. This, and a third example involving the site of action of the cyclodienes are described below.

Altered Acetylcholinesterase

Acetylcholinesterase (AChE) acts by catalyzing the removal of acetylcholine from nerve synapses after nerve impulse transmission in order to prepare the synapse for another transmission. Organophosphate and carbamate insecticides are neurotoxins

that act by inhibiting the action of acetylcholinesterase. Acetylcholinesterases (AChE) consist of active esteratic and anionic subsites. They also contain a hydrophobic site, an indophenyl site, charge transfer sites and peripheral allosteric anionic sites (Eldefrawi 1985). Inhibition of AChE can occur by binding of xenobiotics at any of these sites either reversibly or irreversibly. Most OPs bind to the esteratic subsite and are effectively irreversible inhibitors. Certain carbamates also bind to the esteratic subsites but reactions are often reversible. The result of this inhibition is an increase in time that the transmitter, acetylcholine (ACh) is at the cholinergic synapse resulting in a repetitive nerve firing and eventually death (Soderlund & Bloomquist 1990, Scott 1991). Several resistant strains of insects have an altered form or forms (or isozyme) of this AChE which is less sensitive to inhibition by certain structurally similar OPs and carbamates (Scott 1991).

Neuronal insensitivity - DDT and Pyrethroids

DDT and pyrethroids predominantly act upon sodium channels of the insect nerve membranes resulting in a rapid paralytic effect (Soderlund & Bloomquist 1990). A resistance to early knockdown by DDT was first identified in houseflies (Busvine 1951). The resistance, which did not occur by dehydrochlorination, was found to be the result of a decrease in nerve sensitivity to the insecticide and to be associated with a recessive gene named *kdr* from knock-down resistance. The gene is located on the third chromosome of the housefly (Farnham 1977) and several alleles have since been mapped (Farnham *et al.* 1987). A similar *kdr*-type resistance to DDT and pyrethroids has been shown to be a major mechanism of resistance exemplified by reduced sensitivity in the German cockroach (*B. germanica*) (Scott & Matsumura 1983, Hemingway *et al.* 1993) as well as mosquitoes (*Culex quinquefasciatus, Aedes*

aegypti) (Priester & Georghiou 1980, Brealey *et al.* 1984), soybean looper (*Pseudoplusia includens*) (Rose *et al.* 1990), horn fly larvae (*H. irritans*) (Crosby *et al.* 1991), citrus thrips (*Scirtothrips citri*) (Immaraju 1990) and Eygptian cotton leafworm (*Spodoptera littoralis*) (Gammon 1980).

Explanations for this reduced sensitivity include modifications of the sodium channel (Salgado *et al.* 1983, Schuler *et al.* 1998, Lee *et al.* 1999), changes in nerve membrane lipids and proteins (Chialiang & Devonshire 1982) and reduction in the number of receptor sites or sodium channels in resistant strains compared with susceptibles (Chang & Plapp 1983, Rossignol 1988).

Neuronal insensitivity - Cyclodienes

GABA (4-aminobutyric acid) is an inhibitory neurotransmitter present in post-synaptic membranes and associated with chloride channels. Activating the GABA receptor-channel increases membrane permeability to chloride ions. GABA receptors appear to be the target of lindane, cyclodienes, and cyclodiene-type insecticidal compounds (Soderlund & Bloomquist 1990). These compounds act by inhibiting GABA-regulated chloride ion channels in the insect nervous system. Modification of the GABA receptor-chloride ionophore complex at the synapses of the CNS results in a reduced affinity of an insecticide for a receptor. This type of modification has been suggested as the cause of resistance to the above compounds in several insect species (Soderlund & Bloomquist 1990). Resistance to picrotoxinin (a cyclodiene-type compound) in the German cockroach (*B. germanica*) and housefly (*M. domestica*) is due to alterations of the nerve receptor (Kadous *et al.* 1983, Anthony *et al.* 1991). This type of alteration has also been demonstrated in *D. melanogaster* and is

conferred by a single gene that has a single amino acid substitution (ffrench-Constant *et al.* 1993). This monogenic phenomenon is the basis of lindane resistance in the red flour beetle (*T. castaneum*) (Beeman & Stuart 1990).

This section consists of six chapters, five of which investigate specific biochemical mechanisms of resistance. These include penetration of insecticide, various metabolic detoxification systems (MOs, esterases and GSTs) and lastly acetylcholinesterases as having either an altered sensitivity to OPs or an increase in activity. The GSTs are identified as playing a role in *L. cuprina* OP-resistance and are partially purified to determine charactersistics of specific isozymes involved (Chapter 2.3). This section concludes (Chapter 2.6) with a discussion of the relative contribution of each mechanism to resistance and a comparison between species.

CHAPTER 2.1

Penetration of [^{14}C] methyl parathion in organophosphate resistant and susceptible larvae of *Lucilia cuprina* and *L. sericata*

Introduction

The first defence an insect has against a contact insecticide is the physical barrier of its cuticle. The cuticle is however, a complex organ and it is unresolved how insecticides and other substances penetrate through it to the haemolymph of the insect. Organophosphorus compounds (OPs) primarily act as contact poisons and to a lesser but still significant extent as stomach poisons (Thompson 1992, Meadows 1993).

Gerolt (1969) suggested that insecticides diffuse laterally in the integument to the tracheae to reach the target site, namely the central nervous system (CNS) with respect to OPs. Welling & Paterson (1984) and Benezet & Forgash (1972a) rejected this theory primarily because autoradiographic experiments of the housefly were unable to demonstrate radioactivity in tracheae (Benezet & Forgash 1972a). In a review, Noble-Nesbitt (1970) suggested other alternatives including penetration firstly through wax canals then through pore canals to the interior. Thin non-sclerotised regions including intersegmental membranes and permeable areas on sensillae and around setae of some insects may also be possible areas for penetration (Noble-Nesbitt 1970).

Decreased or delayed penetration has been implicated as an important selection mechanism for resistance in some arthropod species although it is not normally a sole

factor. Reduced rates of penetration enables an insect's other defence mechanisms to detoxify incoming insecticide more easily (Price 1991). Examples can be found in numerous species and include reduced penetration to DDT in the spotted root maggot, *Euxesta notata* (Hooper 1965), the tobacco budworm, *Heliothis virescens* (Vinson & Brazzel 1966, Vinson & Law 1971), and the bollworm *H. zea* (Pate & Vinson 1968). A strain of the lepidopteran, *Spodoptera exigua,* with a resistance factor of over 100 times to the synthetic pyrethroid, deltamethrin, was shown to contain less than half the amount of [^{14}C] deltamethrin compared with a susceptible strain after one hour, indicating delayed penetration of [^{14}C] deltamethrin (Delorme *et al.* 1988). Associated with this was its ability to degrade deltamethrin 17 times faster than a susceptible strain, which was also correlated with an increase in esterase activity explaining a large proportion of the resistance (Delorme *et al.* 1988). Reduced cuticular penetration is an important mechanism of fenvalerate resistance in diamondback moth (*Plutella xylostella*) larvae, resulting in lower internal accumulation of *S*-[^{14}C] fenvalerate than in a susceptible strain (Noppun *et al.* 1988). Decreased rate of penetration has been found to be a minor factor of resistance in the tobacco budworm (Konno *et al.* 1988), with a lower rate of [U-^{14}C]-methyl parathion penetration in a resistant strain than a susceptible strain after 12 hours, but not after 24 hours. A slower rate of penetration was found to be a major factor in the resistance of *Heliothis virescens* larvae to an amidinohydrazone (AC 217,300; AMDRO). Resistant western corn rootworms, *Diabrotica virgifera virgifera*, also showed a 40% slower penetration of aldrin compared with susceptible strains (Hollinghaus & Little 1984), as well as an increase in excretion of the insecticide (Siegfried & Mullin 1990).

There are numerous examples of the housefly, *Musca domestica,* exhibiting reduced insecticide penetration in resistant strains. Forgash *et al.* (1962) demonstrated that absorption of diazinon in the Rutger's OP resistant strain took twice as long as in a susceptible strain, indicating that slower penetration contributed to resistance. Scott & Georghiou (1986) showed that decreased penetration was also a minor mechanism of resistance in the Learn-PyR strain, the mixed function oxidase (MFO) system being the major contributor. A more striking decrease in penetration was found in the 147-R strain which was shown to be 67-fold less sensitive to (1R,S)-trans[14C] permethrin than a susceptible strain. The former strain was also 2.6 - 43 fold less sensitive to other pyrethroids (De Vries & Georghiou 1981). Decreased penetration of [^{14}C]-malathion in a resistant compared with a susceptible strain of housefly was shown by Benezet & Forgash (1972a). They also compared epicuticular waxes between these strains and found no quantitative differences but suggested that differences in sterol, ester, and fatty acid composition might be responsible (Benezet & Forgash 1972b).

The penetration-delaying factor has been genetically isolated from various strains of housefly (Plapp & Hoyer 1968, Hoyer & Plapp 1968, Sawicki & Lord 1970). The *pen*-gene (also known as organotin-R in the above references) is located on chromosome III. Organophosphate resistance in certain housefly strains (e.g. SKA) is caused by the interaction of this minor mechanism with other major resistance genes on chromosome II (Plapp & Hoyer 1968, Sawicki & Farnham 1968, Sawicki & Lord 1970).

Penetration of [^{14}C] malathion, [^{14}C] parathion and [^{14}C] paraoxon has been measured in two strains of adult Australian sheep blowfly *L. cuprina* in Australia (Hughes & Devonshire 1982, Raftos 1986). Hughes & Devonshire (1982) found no significant differences between penetration of [^{14}C] parathion in a resistant (Q, OP resistant strain derived from the field) and a susceptible strain (LS, laboratory susceptible strain) but found that resistant flies excreted more [^{14}C] than susceptibles. Raftos (1986) similarly found no differences in rate of penetration of [^{14}C] malathion in two strains of *L. cuprina* adults (RM - malathion resistant, LS as above).

The current study aimed to investigate penetration of [^{14}C] methyl parathion into wandering third instar larvae of *L. cuprina* and *L. sericata* rather than adults as in previous studies (Hughes & Devonshire 1982, Raftos 1986). Larvae cause the mechanical damage of strike and are in contact with insecticide for a greater period of time than adults and are therefore a more appropriate choice of experimental subject. Field strains of larvae derived from instances of flystrike and established in culture have been compared with laboratory-maintained susceptible strains of both species, as described below.

Materials and methods

Chemicals

[^{14}C] Methyl parathion was obtained from Amersham (Braunschweig, Germany), OptiScint 'Hi Safe' scintillation fluid was from Pharmacia-Wallac (UK) Ltd (Milton Keynes, United Kingdom).and Hyamine 10-X hydroxide solution (scintillation grade) was from BDH Chemicals Ltd (Palmerston North, New Zealand). Details of other chemicals can be found in Appendix A.

Penetration bioassay

Method 1

Third instar larvae of 13 field strains of *L. cuprina* and *L. sericata* were treated topically in batches of 20 with 0.5 µl of an acetonic solution of [^{14}C] methyl parathion (2.4 mM, 10µCi/ml) which had been previously determined to be sublethal. Treated larvae were kept at room temperature (20-25°C) in screw-top 20 ml Beckman Poly Q™ vials for two hours. An optimal two-hour insecticide exposure time was determined by a preliminary investigation of four strains measuring penetration at 0, 1, 2, 4 hours and two strains with an additional measurement at 7 hours following the same procedure. After two hours larvae were rinsed in 1 ml of AnalaR acetone and removed to another 20 ml scintillation tube. One ml of hyamine 10-X hydroxide solution was added to the acetone rinsings. Larvae were macerated in 1 ml 30 % w/v AnalaR H_2O_2 (BDH Chemicals Ltd.) and incubated for 30 minutes at 45°C to allow for bleaching. After 30 minutes 1 ml of hyamine 10-X hydroxide solution was added to each vial and they were further incubated for 7 days. Ten ml of OptiScint 'Hi Safe' scintillation fluid was added to all vials. Disintegrations per minute (DPM) were determined for absorbed (larvae) and unabsorbed (rinsings) samples on a Wallac 1409 liquid scintillation counter.

Method 2

An alternative to the above method was performed at a later date using the 000 susceptible strain and a highly resistant field strain (058, Takapau). This method replaced the acetone wash of the above method with a dH_2O followed by a hexane wash in an attempt to account for excretion of organic metabolites, which were not explicitly determined in *Method 1*.

Statistical Analysis

Results were analysed using paired t-tests to determine differences between penetration means of field and susceptible strains and by ANOVA to determine the relationship between resistance and penetration of [^{14}C] methyl parathion.

Results

Method 1

The apparent rate of penetration of [^{14}C] methyl parathion was greatest in the insecticide-susceptible strain of *L. cuprina* at all time periods tested (0 – 4 hours) (Figure 2.1.1). Penetration of [^{14}C] methyl parathion in this strain showed a linear increase during the 0 – 4 hour time period ($y = 5.798x - 0.305$; $r^2 = 0.96$) suggesting that penetration was of first order. This differed from field strains that exhibited fluctuating penetration rates over time. Differences between susceptibles and field strains were greatest at longer exposure periods. For example, at 4 hours internal radioactivity was as much as 4.3 times greater in the susceptibles compared to the Levin strain (Figure 2.1.1). The variability in apparent penetration rates in field strains compared with susceptibles was reflected by considerable variation in individual responses in the different strains. This was apparent from the large standard errors observed (Table 2.1.1).

Table 2.1.1. Mean percentage internal radioactivity of [^{14}C] methyl parathion through *Lucilia cuprina* and *Lucilia sericata* cuticle two hours after application using *method 1*.

Farm	Area	Date	Mean % Penetration	N	SE	RF
L. cuprina						
000	Lab	00.00.00	17.9	5	2.7	1.0
046	Lab	00.00.00	18.6	20	2.7	1.0
039	Rakaia	14.02.94	21.0	20	2.8	18.4
024	Masterton	31.02.94	6.7*	20	0.8	15.2
008	Kihikihi	07.03.94	3.8*	20	0.7	11.8
036	Kaiwaka	14.03.94	10.2*	20	1.8	18.8
037	Levin	15.03.94	4.4*	20	0.6	16.0
003	Turakina	16.05.94	10.3*	20	1.5	-
035	Levin	11.04.94	6.6*	20	0.9	14.2
034	Leeston	16.04.94	1.3*	20	0.1	8.2
004	Bulls	05.04.94	14.6*	5	2.1	11.6
L.sericata						
001	Lab	00.00.00	11.0	14	1.4	1.0
034	Leeston	18.04.94	1.5*	20	0.1	6.2
009	Blenheim	25.04.94	2.0*	20	0.2	12.5

SE Standard Error

RF Resistance factor (LC_{50} field strain/ LC_{50} susceptible strain)

* Significantly different from Lab susceptible strains ($p < 0.01$). Note that t-tests were performed using the 046 Lab strain for *L.cuprina* as N matched that of the field strains

The standard two-hour exposure adopted for determinations of penetration versus resistance in this study was based on the greatest between-strain differences with the lowest within-strain standard error from the time-optimisation bioassay. That is, with the exception of the 004, Bulls strain ($p = 0.4$) the mean apparent penetration of [^{14}C] methyl parathion was significantly lower $p<0.01$ than that of the 000 (NSW) susceptible strain. In general, rate of [^{14}C] methyl parathion penetration in field strains was found to be significantly lower in most field strains than in lab susceptible strains (Table 2.1.1). Differences in penetration were not however, related to resistance factors in field strains of *L. cuprina* ($p = 0.40$, with susceptibles, $p = 0.20$ without susceptibles) (Tables 2.1.2 & 2.1.3) (Figure 2.1.2). There were not enough data points to determine a relationship between resistance and rate of

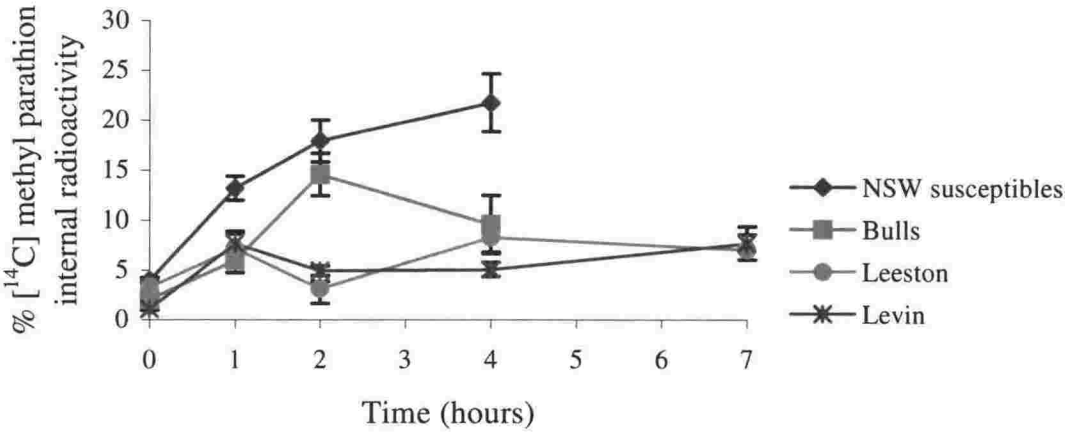


Figure 2.1.1 Comparative internal radioactivity of [^{14}C] methyl parathion in larvae of three resistant field strains and one organophosphate susceptible strain of *Lucilia cuprina* using *method 1.*

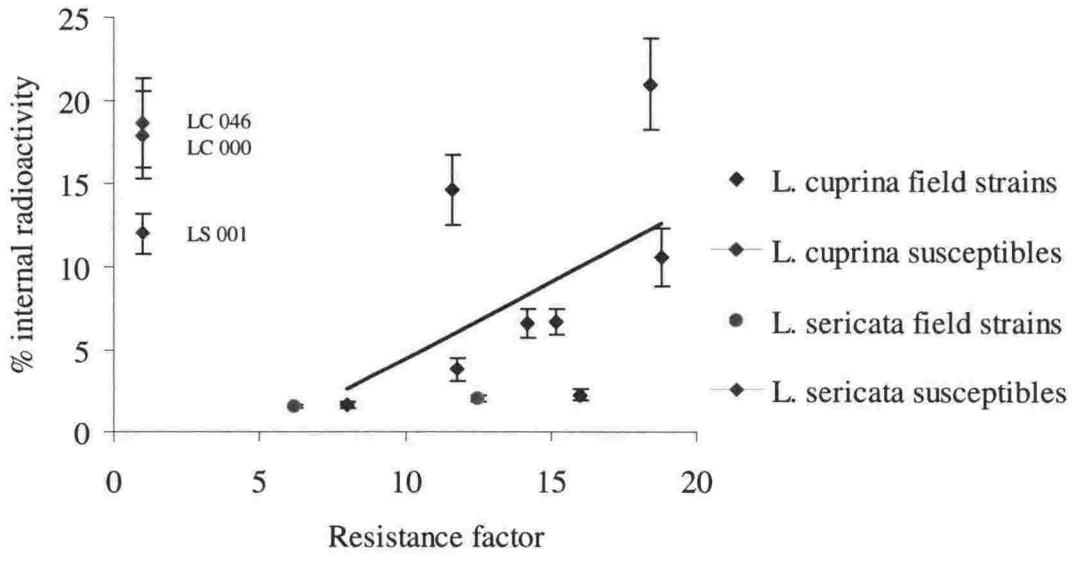


Figure 2.1.2 Relationship between percentage internal radioactivity of [^{14}C] methyl parathion and resistance to diazinon in field resistant and laboratory susceptible strains of *Lucilia cuprina* and *Lucilia sericata* using *method 1.* A regression line has been fitted to the field strains of *L. cuprina.*

penetration of [^{14}C] methyl parathion by ANOVA in field strains of *L. sericata.* These points, however, have been included in figure 2.1.2 as a comparison with strains of *L. cuprina*. The initial apparent rate of uptake by *L. sericata* susceptibles (11.9 % at 1 hour) differed from that observed in susceptible strains of *L. cuprina* (17.9 % in the NSW strain and 18.6 % in the CSIRO at 1 hour).

Table 2.1.2 Analysis of variance table showing the relationship between % [^{14}C] methyl parathion penetrated through the cuticle of *Lucilia cuprina* larvae and resistance factor using *method 1*. Note that susceptible *Lucilia cuprina* strains are included in this analysis.

	DF	Sum of Squares	Mean Sq.	F value	Pr (F)
Regression	1	40.140	40.14	0.75	0.412
Residual Error	8	428.60	53.58		
Total	9	468.74			

Table 2.1.3 Analysis of variance table showing the relationship between % [^{14}C] methyl parathion penetrated through the cuticle of *Lucilia cuprina* larvae and resistance factor using *method 1*. Note that susceptible *Lucilia cuprina* strains are not included in this analysis.

	DF	Sum of Squares	Mean Sq.	F value	Pr (F)
Regression	1	79.910	79.91	2.06	0.201
Residual Error	6	232.58	38.76		
Total	7	312.49			

Method 2

The rate of penetration of [^{14}C] methyl parathion into individuals of the 000, susceptible strain with this method did not significantly differ from that observed with *method 1* (Appendix 2.2.1, mean = 19.27 compared with mean =17.91 in *method 1*). What was not detected in *method 1* however was a 24.57 % conversion of penetrated insecticide into excreted organic metabolites in susceptible larvae and 20.45 % in the resistant strain. Penetration of [^{14}C] methyl parathion was not significantly lower in the highly resistant 058, Takapau strain (16.71) compared with the susceptible strain. There was also no significant difference in percentage of

penetrated insecticide converted into excreted organic metabolites between the two strains (Appendix 2.1.1). Note that standard errors were higher with *method 2* than with *method 1* (Appendix 2.1.1).

Discussion

The contribution of reduced penetration, as a mechanism of OP insecticide resistance is unclear from this study. Preliminary experiments monitoring penetration over time suggested significant differences in penetration between susceptible and resistant strains of *L. cuprina* larvae (Figure 2.1.1). Analysis of variance (ANOVA) of mean percentage penetration and resistance factors of field strains of *L. cuprina* larvae at 2 hours (using *method 1*) suggested that although rate of penetration differed between strains it was not related to level of resistance (($p = 0.41$, with susceptibles; $p = 0.20$ without susceptibles) (Figure 2.1.2). It is unlikely that testing a greater number of strains would clarify the significance of penetration rate as a mechanism of resistance in both *L. sericata* and *L. cuprina* populations however, as this method (*1*) failed to adequately account for external water-soluble metabolites of penetrated insecticide. In contrast, to the results of *method 1* was the lack of a significant difference shown in percentage insecticide penetrated (internal radioactivity) between the susceptible and resistant strains observed with *method 2*. Parallel to this was a lack of a significant difference in the percentage of water-soluble metabolites produced by either strain (Appendix 2.1.1). Time did not permit comparison of a range of resistant field strains, which may have clarified these results. Of concern with both methods was the source of variation within strains, highlighted by high standard errors (Table 2.1.1, Appendix 2.1.1).

The most important determinant of within-strain variation maybe the resistance heterogenity of field populations. Previous toxicological experiments (Chapter 1.1) measured the mean resistance response of a population. This study on the other hand, has measured individual response to determine a population mean. The variation is a reflection of the range of individual resistance responses within each strain. To overcome within-population variation, resistant field strains need to be back-crossed to an appropriate balancer stock (Parker, PhD thesis 1993). This may clarify the possibility of the contribution of reduced penetration as a mechanism of resistance although the value of a populational response may be lost. If a populational response is to be retained however, avenues other than back-crossing, for reducing standard error need to be explored. Standardising factors that affect toxicity of insecticide to individuals may be a possible alternative to this. For example, insecticide toxicity studies show that variation may be due to individual weight variations of third instar larvae within strains, or differences between female and male response among other factors (Robertson & Preisler 1992). Variations in toxicity were seen to occur with weight of insect in topical application experiments where a discriminating dose of insecticide was applied to adult *L. cuprina* (Chapter 1.1). These factors have similarly been documented in other studies of adult insects (Potter & Way 1958, Busvine 1957). The sex of blowflies cannot be determined from larval anatomy so was discounted as a factor in this experiment. One can speculate however, that the above considerations may have little difference in reducing error within a strain. Differences could be due to individual differences in physical attributes such as cuticular structure or the degree of sclerotization of the cuticle (Vinson & Law 1971).

Olson (1970) described the main barrier of acetonic solutions into the lipoidal epicuticle layer of cockroach integument to be the proteinaceous component of the cuticle. Polar rather than apolar compounds were therefore expected to pass through cockroach cuticle more easily (Olson 1970). Supporting this was the observation of higher cuticular protein content in the cuticle of chlorinated hydrocarbon-resistant tobacco budworm larvae compared with susceptible larvae (Vinson & Law 1971). In addition, more of this protein in resistant forms was shown to be sclerotized, rendering it more apolar. This lead to the assumption that apolar cuticle acted as a 'sponge' for apolar substrates that reduced penetration of hydrocarbons into these insects (Vinson & Law 1971). This assumption supports early work by Treherne (1957) and Olson & O'Brien (1963) who determined that penetration rate was proportional to the polarity of an applied compound.

With this in mind it would be interesting to explore differences in penetration rate of the OPs tested in the *in vitro* toxicology bioassay (Chapter 1.1) to determine the correlation between polarity and relative resistance. The availability of the appropriate radio labelled insecticides did not permit this at the time of the initial experiment. It is expected that the order of highest to lowest penetration rate for these OPs would range from chlorfenvinphos > propetamphos > methyl parathion > diazinon > dichlofenthion > chlorpyrifos. This order is based on polarity determined by thin layer chromotography (M. Clear, AgriQuality, Upper Hutt, unpublished data) (also refer to solubility for each insecticide in the Merck Index, Budavari *et al.* 1996).

Differences in the developmental stages examined make comparisons with Australian studies of insecticide penetration in *L. cuprina* more difficult as metamorphosis leads

to marked changes in the physical properties of the cuticle. Most notably, sclerotization increases during the pupal stage and continues through to adults, affecting the polarity of the cuticle (Silvert 1985). Adult cuticle is therefore more apolar than larval cuticle. The likely effect of this is that the rate of penetration of insecticide into adults would be slower than in larvae. Variations in the amount of sclerotization in larvae may result in a more pronounced effect on insecticide penetration than in adults that have shown no significant differences in penetration of [^{14}C] malathion, [^{14}C] parathion and [^{14}C] paraoxon between susceptible and resistant strains (Hughes & Devonshire 1982, Raftos 1986). Examination of the properties of larval and adult cuticular sclerotization and insecticide-induced changes in sclerotization may be appropriate to determine such a relationship. There are studies of cuticular proteins in developmental stages of *L. cuprina* (Retnakaran & Hackman 1985, Binnington 1985, Barratt 1987 Skelly & Howells 1987, Skelly & Howells 1988) but none that specifically investigate tanning mechanisms in relation to sclerotization.

The method of insecticide administration may also be an important consideration in addition to cuticle structure. There is evidence to suggest that acetone may not be an appropriate application solvent and that choice of solvent may show strain-specific differences. For example, Sawicki & Lord (1970) showed that application of an OP with the hydrocarbon *n*-dodecane had a higher initial rate of penetration than when using acetone as the solvent. In the same study *n*-dodecane increased the initial penetration rate by 5-fold in a resistant housefly strain containing the *pen*-factor but only by two-fold in a strain without this factor when compared with acetone. It is thought that the high volatility of acetone ensures that it does not spread far from the

origin of application. This is a disadvantage in a bioassay where an increase in surface-area of application increases the likelihood of a higher initial penetration rate if acetone is used as the solvent. Selection of the two-hour exposure time in this study may have overcome some of these initial differences experienced by other workers.

The lack of optimisation of dose rate may also have a bearing on the overall efficiency of the assay. In houseflies, Sawicki & Lord (1970) were able to show more pronounced differences between susceptible and resistant strains using lower doses per individual (0.2μg / individual) compared to higher doses (20 μg / individual).

Although this has not been studied in the present work, it is worth mentioning microorganisms that are able to metabolise insecticides. Singh (1981) identified several species of bacteria and yeast inhabiting the surface of the blowfly *Calliphora erythrocephala*. These organisms were shown to metabolise between 0.8% (*Streptococcus* spp.) and 29% (*Micrococcus* spp.)[^{14}C] dieldrin in their culture media. Singh (1981) also detected a dieldrin metabolite in the epicuticular layer of the blowfly that was the result of bacterial activity. It is therefore possible that such microorganisms may mask the effect of penetration through insect cuticle by their metabolism of insecticide. The rate of microorganism activation and the effect of the application solvent alone on microorganism metabolism are unclear however.

Microorganism activity might well have relevance to the metabolism of insecticides in the present study. An autoradiograph of a hexane wash of unabsorbed diazinon

from the surface of *L. cuprina* flies revealed several metabolites, including diazoxon and hydroxy-diazinon (Duncan 1999). These metabolites were assumed to have leeched from the cuticle (Duncan 1999) but might also be due to microbial metabolism.

The fluctuating non-linear form of the kinetics of penetration over time in the resistant strains compared with that in the susceptible in this study (Figure 2.1.1) was an interesting result that also highlighted shortcomings in experimental design of *method 1*. An early study by Uchida *et al.* (1965) showed a smooth, rather than a fluctuating, non-linear response for the penetration of OPs into various insect cuticles. Uchida *et al.* (1965) described this non-linear penetration as phasic beginning with an initial monophasic fast penetration followed later by a slow phase. All strains in the preliminary time-course study (Figure 2.1.1) showed an initial increase in penetration from 0 - 1 hour, beyond this two field strains (Levin and Leeston) declined in penetration rate while the 000, susceptible and 004, Bull's strains continued to increase. Uchida *et al.* (1965) suggested the initial increase is a consequence of rapid saturation into a cuticular zone due to the acetonic nature of the compound, which is then followed by slower penetration into other areas. Fluctuating penetration rates in the initial experiment (*method 1*) might be due to the differential activation of various detoxification mechanisms and excretion of the toxin at differing time periods. Detoxification within larvae would indicate penetration of the insecticide whereas reductions in penetration rates may be indicative of elimination of the toxin. Excretion would increase total larval external washings and reduce the apparent percentage internal radioactivity as described earlier. *Method 1* failed to adequately account for external water-soluble metabolites

and penetration was reassessed using *method 2*. Timing of probable elimination of [^{14}C] methyl parathion metabolites is also unknown and metabolic studies of [^{14}C] diazinon as well as repetition of *method 2* with more field strains and over time is therefore necessary to determine the extent of excretion and organic metabolites.

The time-course study has indicated differences between rate of penetration of [^{14}C] methyl parathion in strains of *L. cuprina* larvae despite the apparent lack of relationship between penetration rate and resistance in field strains of *L. cuprina* at two hours. To clarify the contribution of the penetration mechanism a number of experimental issues need to be addressed. These include standardisation of larval weight, optimisation of dose per individual, determination of an appropriate solvent vehicle, an increase in number of strains using *method 2*, and appropriate supportive metabolic studies. Other factors for consideration include toxicological issues such as determination of resistance to methyl parathion as the appropriate dependent variable or alternatively determination of the side-resistance relationship between diazinon and methyl parathion, or repetition of this experiment with [^{14}C] diazinon.

CHAPTER 2.2

The role of non-oxidative metabolism in organophosphate resistance in strains of *Lucilia cuprina* and *Lucilia sericata*

I. Esterases

Introduction

Esterases in insects are a large, ubiquitous group of enzymes that are able to metabolize a wide variety of substrates. Among them are esterases able to catalyze the cleavage of the different types of ester bonds (Healy *et al.* 1991) found in OP and carbamate insecticides (Hutson & Roberts 1985). It is therefore not surprising that elevated levels and mutated forms of esterases feature in mechanisms of resistance to these insecticidal groups.

Increased levels of esterases have been implicated in resistance to organophosphorus insecticides in sweet potato whitefly, *Bemisia tabaci* (Dittrich *et al.* 1990), light brown apple moth, *Epiphyas postvittana* (Armstrong & Suckling1990), the mosquitoes, *Culex pipiens* and *C. quinquefasciatus* (Qiao *et al.* 1996, Severini *et al.* 1994, Fournier *et al.* 1987, Mouches *et al.* 1986) and the peach potato aphid, *Myzus persicae* (Sudderuddin 1973, Needham & Sawicki 1971).

Increased levels of esterases can in some instances, be attributed to gene amplification. Gene amplification occurs when alterations of gene linkage result in multiple copies of structural genes. In this context, increased copies of the genes produce an increase in the enzyme able to metabolize the insecticide (Terriere 1983).

Amplification of one esterase (E_4) is responsible for resistance in *M. persicae* (Needham & Sawicki 1971). Concentrations of E_4 were shown approximately to double between seven strains of resistant aphids, based on measurements of catalytic centre activity (Devonshire 1977) and polyacrylamide gel electrophoresis (Devonshire & Moores 1982). A highly resistant strain was thought to have 64 times more copies of the gene than a susceptible strain (Field 1989, Devonshire & Sawicki 1979). Devonshire & Sawicki (1979) concluded that this was a result of a series of duplications of the structural gene for the enzyme. More recent work suggested a more complex mechanism in operation in which expression of amplified esterase genes is modulated by DNA methylation (Pickett & Devonshire 1998). As a result of the multiple gene copies the E_4 esterase constitutes a significant proportion of total protein and in the most resistant strains may account for as much as 3% of total protein (Devonshire & Moores 1982).

The E_4 esterase has high hydrolytic activity towards α- and β-naphthyl acetate but hydrolyses OP insecticides more slowly. The esterase confers resistance to OPs in the first instance by sequestration of the toxicant. Sequestration is the binding prior to the slow hydrolysis of the insecticide. The synthesis of larger amounts of the protein enhances the sequestering capacity in addition to its phosphatase properties (Devonshire & Moores 1982, Devonshire 1977).

There is evidence to suggest that, in the absence of insecticide pressure, there is a loss of resistance (Devonshire & Moores 1982, Devonshire & Sawicki 1979, Sudderuddin 1973). This was first thought to be due to a loss of gene copies

(Devonshire & Moores 1982). Instead it now appears that DNA methylation of the amplified esterase genes leads to reduced transcription of these genes, which results in loss of resistance (Field *et al.* 1989).

Qualitative differences in overproduced forms of esterase have also been detected in *M. persicae*. Two related forms designated E_4 and FE_4 with molecular weights of 65 and 66 Kda respectively, show catalytic activity against different substrates (Devonshire *et al.* 1983). Overproduction of FE_4 occurs in resistant wild-type karyotypes, and E_4 in heterozygotes, with a translocation between the first and third autosomes (Blackman *et al.*1978).

Overproduction of a number of detoxifying esterase isoenzymes also results in OP resistance in culicid mosquito species. Esterase genes tend to occur on closely linked loci or clusters and are classified as 'A' or 'B' based on their substrate preferences for either α- or β-naphthyl acetate in several insect species (Raymond *et al.*1987, Newcomb *et al.* 1996). Isoenzymes in these classes are identified by numerical subscripts according to their electrophoretic mobility. A common resistance phenotype in Culicidae involves the elevation of both A_2 and B_2 esterases in the same individual. Genes coding for these enzymes show almost complete linkage disequilibrium. This type of resistance has been found in mosquitoes in over 30 countries in three continents (Raymond *et al.* 1991). Ketterman *et al.* (1993) also demonstrated significant qualitative differences in the kinetic characteristics of these esterases in resistant strains of *C. quinquefasciatus* indicating polymorphisms in these enzymes.

Another phenotype observed in resistant culicids is elevated B_1 activity due to gene amplification (Mouches *et al.* 1986). A comparison of isoenzymes in strains of *C. pipiens* showed that 99% of esterase activity in a susceptible strain was due to the A_1 esterase in contrast to only 5% in a resistant strain (MSE strain) which showed elevated levels of B_1 esterase (Fournier *et al.* 1987). This was also consistent with findings in the closely related species *C. quinquefasciatus*. In this latter species the B_1 esterase was estimated to be 500-fold more abundant in a resistant strain (Tem-R) than in a laboratory susceptible strain. The gene coding for this enzyme was thought to be amplified at least 250-fold (Mouches *et al.* 1986). In such strains, the B_1 esterase is estimated to account for an extraordinary large amount (6-12%) of the total soluble protein (Fournier *et al.* 1987). This B_1-type esterase resistance occurs in field populations of mosquitoes throughout the USA, Central America, Asia and the Caribbean (Vaughan *et al.* 1995).

These overproduced esterases allow sequestration of the OP insecticides followed by hydrolysis in much the same way as occurs in *M. persicae*. One major difference between the esterases in these species is however, that E_4 in *M. persicae* is membrane bound whereas the culicid esterases studied were water-soluble. The significantly higher proportion of protein composed of esterases seen in culicid species (6-12%) compared with *M. persicae* (1-3%) relate to this difference (Fournier *et al.* 1987).

Resistance to malathion in an Australian strain of the blowfly *Lucilia cuprina* (Calliphoridae) has also been shown to be due to a quantitative increase in malathion carboxylesterase (MCE) activity. Resistant strains were shown to have 9 times more MCE activity than a susceptible strain (Whyard *et al.* 1994). Smyth *et al.* (1996)

however, identifed three MCE phenotypes in *L. cuprina* of low, intermediate and high resistance. The intermediate phenotype had a 21-fold higher MCE activity and the high phenotype 33-fold. The high phenotype was 1000-fold more resistant to malathion compared with the other phenotypes. The enzyme produced in this high resistance phenotype was thought to be structurally different from that in other phenotypes (Smyth *et al.* 1996). Malathion carboxylesterase (MCE) acts in a somewhat different way to that observed in *M. persicae* and the culicids. Rather than sequestering the insecticide, MCE is able specifically to hydrolyze the activated form of malathion (malaoxon; produced by MFO action) into less toxic, more easily excreted monoacids and diacids (Whyard *et al.* 1994, Eto 1974).

There are other instances of mutant forms of esterases playing a role in resistance by metabolizing insecticide more effectively. Oppenoorth & van Asperen (1959) were first to demonstrate a correlation between resistance and a *decrease* in ali-esterase activity, measured using the aliphatic ester substrate, methyl butyrate. From this observation emerged the 'mutant ali-esterase hypothesis' in which it was proposed that the susceptible form of ali-esterase had mutated in resistant strains to a phosphatase which was able to confer resistance. The aliesterase had lost or had reduced ability to hydrolyze carboxylesters, such as the methyl butyrate substrate, but was able to hydrolyze the OP oxon insecticides more efficiently.

The key mechanism of resistance to OPs (with the exception of malathion) in Australian strains of *L. cuprina* appears to involve a specific carboxylesterase (E_3) which is located on the $R_{OP\text{-}1}$ α-esterase gene cluster on chromosome four (Hughes & Raftos 1985, Hughes & Raftos 1983, Parker *et al.* 1996, Spackman *et al.* 1994). The

mutated E_3 is thought to be responsible for much of the hydrolysis of OP insecticides in *L. cuprina.* Hughes & Raftos (1985) consistently found E_3 in susceptible and heterozygous resistant flies but the mutant form of E_3, found in homozygous resistant forms of *L. cuprina*, could not be visualized on a native polyacrylamide gel. The mutant form of the E_3 had lost the ability to hydrolyze α- and β-naphthyl acetate but could detoxify OPs more efficiently. Newcomb (1996, PhD thesis) showed that there is no evidence of gene amplification or any increase in levels of transcription resulting in the overproduction of the mutant E_3 form in resistant strains. Rather than the esterase accounting for a significant proportion of total soluble protein as in the culicids (6-12%) or E_4 in *M. persicae* (up to 1%), the mutant E_3' represents 10-fold less of the normal level of E_3 protein but has a greater OP hydrolytic ability (Parker *et al.* 1996).

The OP-resistant alleles of the gene that encodes E_3 has been shown to differ by five amino acids compared with the OP-susceptible allele (Newcomb *et al.*1997). Only one of these substitutions however, is responsible for resistance. The $Gly^{137} \rightarrow$ Asp substitution was shown to be responsible for loss of E_3 carboxylesterase activity and gain of OP-hydrolase activity (Newcomb *et al.* 1997).

This study assesses possible increases in non-specific esterase activity in susceptible and resistant strains of *L. cuprina* and *L. sericata.* Electrophoretic analyses of esterases were also undertaken to determine the presence of the mutant E_3 esterase in New Zealand populations of *L. cuprina.* An electrophoretic profile of esterases from *L. sericata* field and lab strains is presented and compared with *L. cuprina.* Ali-

esterase activity in strains of *L. sericata* was also assessed in an assay using methylthiobutyrate (MtB) as substrate.

Materials and Methods

Chemicals

Methylthiobutyrate was obtained from Aldrich Chemical Co. (Milwaukee, USA), α-naphthyl acetate was from Ajax Chemicals Ltd (Sydney, Australia) and β-naphthyl acetate from Sigma Chemical Co. (St Louis, USA). Details of other chemicals can be found in Appendix A.

Non-specific esterase activity

Non-specific esterase activity from whole body homogenates was determined using α- and β-naphthyl acetate as substrates, using an adaptation of the method of Motoyama *et al.* (1980). Flies were homogenized in batches of three in 3 ml of 0.1 *M* Tris-HCl buffer, pH 9.0, using a Polytron Kinematica® motorized homogenizer. Larvae were homogenized in the same buffer containing 10% v/v glycerol, 1m*M* phenyl thiourea, 5 m*M* EDTA, and 5% (w/w of larvae) polyvinylpolypyrrolidone. The homogenates were centrifuged at 10,000 g for 10 minutes at 4°C. The supernatant was used as the enzyme solution. The reaction mixture consisted of the above supernatant (100 µl for α-naphthyl acetate and 250 µl for β-naphthyl acetate), 1.0 ml of 6 x 10^{-4} *M* aqueous solution of the ester (added from a stock solution of 0.15 *M* in ethanol), and 2.0 ml of 1.0 *M* sodium phosphate buffer, pH 7.0. The reaction mixtures were incubated at 27°C in 40 ml stoppered glass tubes for 5 minutes. The reactions were stopped and the colour was developed with Fast Blue B

salt as described by Motoyama *et al.* (1980). Absorbance was measured at 410 nm where α-naphthyl acetate was used as substrate and 510 nm for β-naphthyl acetate. Non-specific esterase activities were expressed as μmol/min/mg protein with protein concentrations determined according to the method of Bradford (1976) using bovine serum albumin as standard (described in Appendix C).

Specific esterases

The discontinuous native polyacrylamide gel electrophoresis (PAGE) system employed by Hughes & Raftos (1985) was used to separate specific esterases. The protocol was as follows (D. Gleeson, personal communication). The stacking gel was 3% acrylamide in 0.014 *M* Tris-HCl buffer, pH 7.12, containing 0.17% v/v Triton X-100. The separating gel was 7.9% acrylamide in 0.12 *M* Tris-HCl buffer pH 7.3, containing 0.34% v/v Triton X-100. Tris barbitone buffer (0.03 *M* in barbitone) pH 8.6, was used as electrode buffer. Adult flies (4-6 days old) or clear-gut third instar larvae were homogenised individually in 100 μl of ice-cold distilled H_2O containing 0.15% w/v cysteine. The insects were generally processed in batches of 30, 28 individuals from the resistant strain under scrutiny and 2 from the insecticide susceptible strain. The homogenates were centrifuged at 13,000 rpm for 30 seconds. A 10 μl aliquot from each homogenate was added to 10 μl of 0.01 *M* phosphate buffer containing 1 m*M* EDTA, 0.5% w/v Triton X-100, and 20% w/v sucrose. Bromophenol blue was added to each sample as a tracking dye and the mixtures were mixed by brief vortexing. Aliquots of 10 μl were loaded into the sample wells.

After development, gels were incubated at 25°C for 20 minutes with 200 ml of 0.1 *M* phosphate buffer, pH 6.8, containing a mixture of 50 mg α-naphthyl acetate and 50 mg of β-naphthyl acetate (added in 1ml acetone) and 100 mg of Brentamine Fast Blue BB salt. The presence of the numerous esterase bands was recorded for each sample and, in particular, the presence or absence of the E_3 esterase (Hughes & Raftos 1985, Parker *et al.* 1991) was noted. No attempt was made to discriminate between homozygous E_3 positive individuals and heterozygotes in field samples. Some examples of gels were photographed and gels were then discarded.

Aliesterase activity (Methylthiobutyrate Assay)

Enzyme preparation

Individual flies or larvae were homogenised in 250 µl 0.1 *M* sodium phosphate buffer pH 7.0 containing 0.5 % v/v triton X-100 and a few specks of PTU. The homogenate was centrifuged at 14,000 g for 10 mintues at 4°C. The supernatant was used as the source of enzyme.

MtB Assay

Reactions with methylthiobutyrate (MtB) as substrate were used to determine aliesterase activity in strains of *L. sericata* using an adaptation of a method of Kao *et al.* (1985). Briefly, 60 µl of 0.01 *M* DTNB (5,5'-dithiobis(2-nitrobenzoic acid)) (in 0.1 *M* sodium phosphate buffer pH 7.0) was added to 2.92 ml of 0.3 mM methylthiobutyrate (in 0.1 *M* sodium phosphate buffer pH 8.0). The enzymatic reaction at 25°C was started with the addition of 20 µl of enzyme. Absorbance measurements were taken at 30 second intervals over 5 minutes at 412 nm.

Statistical Analysis

Non-specific esterase activity

Non-specific esterase activities, using α- and β-naphthyl acetate as substrates, were analyzed by analysis of variance (ANOVA) methods in S-Plus (Everitt 1994). Non-specific esterase activity was used as the predictor of diazinon resistance for each strain. The influence of region and season was also considered in the model.

The relationship between larval and adult non-specific esterase activities was determined using regression analysis in Microsoft Excel®.

Aliesterase activity

Two sample t-tests were performed to determine differences between insecticide susceptible and resistant strains of both species, using Minitab® analysis tools. Regression analysis was also used to determine whether there was a relationship between MtB activity and level of diazinon resistance.

Results

Lucilia cuprina

Non-specific esterase activity

Level of resistance to diazinon was not predicted by non-specific esterase activities using α- and β-naphthyl acetate as substrates in adult or larval strains of *L. cuprina*. Region and season also had no influence on activities. Tables of mean esterase activities for strains of each species and life stage are presented in Appendix 2.2.1 and ANOVA tables are shown in Appendix 2.2.2.

Specific esterases

Presence or absence of E_3 was scored from native polyacrylamide gels for strains of *L. cuprina* and percentage occurrence is shown in Appendix 2.2.3. Incidence of the E_3 band in resistant strains was low in comparison with that in the OP-susceptible laboratory strain that showed a 100% occurrence of the band. In most field strains the E_3 band failed to stain. Strains from 009 Blenheim and 022 Te Kuiti however showed a 17.9% occurrence of the E_3 staining form. Visualisation of the E_3 band was more apparent in samples from 1992 than samples from 1994 (Appendix 2.2.3). Examples of native PAGE for the OP-susceptible and field strains are presented in Appendix 2.2.4. A diagrammatic representation of esterase bands from 20 strains of *L. cuprina* is shown in figure 2.2.1. Numbers were assigned to esterases (1 – 16) based on Hughes & Raftos (1985). Ten esterase bands were visualised in the OP-susceptible strain. Number of bands visualised from field strains was variable and ranged from 7 – 11 (Figure 2.2.1).

Aliesterase activity (MtB assay)

There were too few data points to make a reliable observation of the relationship between level of resistance to diazinon and activity towards the substrate MtB in larval and adults strains of *L. cuprina* (Tables 2.2.1 & 2.2.2). Significant differences were observed when comparing susceptible and resistant strains of *L. cuprina* using a two-sample t-test (Tables 2.2.1 & 2.2.2). The absence of the E_3 band from field strains in the native PAGE confirmed the significant differences shown between resistant and susceptible strains in the MtB activity. *Lucilia cuprina* MtB activities provided a comparison with those of *L. sericata* and showed that the response of OP-susceptible laboratory strains was similar in both species (Tables 2.2.1 – 2.2.4).

Table 2.2.1 Aliesterase activity for *Lucilia cuprina* adults using methylthiobutyrate as substrate

Farm	Area	Date	Activity (min-[1])	StDev	RF	
046	Laboratory	00.00.00	0.02367	0.00058	1.0	
000	Laboratory	00.00.00	0.03050	0.00920	1.0	NS
038	Rangiora	01.04.93	0.00600	0.00100	22.2	**
035	Levin	11.04.94	0.00633	0.00058	14.2	**
034	Leeston	18.04.94	0.00733	0.00058	8.1	**
038	Marton	09.05.94	0.00733	0.00058	11.8	**
031	Amberley	15.02.96	0.00833	0.00058	25.8	**

Table 2.2.2 Aliesterase activity for *Lucilia cuprina* larvae using methylthiobutyrate as substrate

Farm	Area	Date	Activity (min-[1])	StDev	RF	
046	Laboratory	00.00.00	0.09750	0.00378	1.0	
000	Laboratory	00.00.00	0.05600	0.00713	1.0	NS
038	Marton	09.05.94	0.00567	0.00058	11.8	**
058	Hawkes Bay	03.04.95	0.01767	0.00208	42.6	**

NS not significant at the 95% confidence level
* significant at the 95% confidence level
** significant at the 99% confidence level

Lucilia sericata

Non-specific esterase activity

As with *L. cuprina*, level of diazinon resistance was not predicted by non-specific esterase activities using α- and β-naphthyl acetate as substrates in adult or larval strains of *L. sericata*. Region and season also failed to influence non-specific esterase activities. Tables of mean esterase activities for strains of each species and life stage are presented in Appendix 2.2.1 and ANOVA tables are shown in Appendix 2.2.2.

Specific esterases

A direct comparison of esterase patterns between *L. cuprina* and *L. sericata* was difficult to make despite running samples of both species on the same gel (Appendix

2.2.5, Figures 1 & 4). There were obvious differences in the mobility of some bands between species but without additional biochemical, physiological and molecular information a direct comparison could not be made. Appendix 2.2.5 also shows a comparison of two OP-susceptible strains of *L. sericata* (Appendix 2.2.5, Figure 2.).

There was no clear indication from the native PAGE of a mutated esterase (as occurs in *L. cuprina*) or an over-expressed esterase as the cause of organophosphate resistance in field strains of *L. sericata* (Appendix 2.2.5, Figure 3 & 4). There was however, clear evidence of an over-expressed esterase with no effect on resistance in the OP-susceptible 152 WE strain (Appendix 2.2.5, Figure 2). This strain is a white-eyed mutation of the OP-susceptible 001 ICI strain (Appendix 2.2.5, Figure 2). A schematic diagram of the type of combinations of esterases found in *L. sericata* is presented in Figure 2.2.2 (Note that this is only a subset of strains tested in this thesis but is representative of the types of combinations visualised). Information for this diagram was drawn from 20 strains and esterases matched by eye based on the substrate preferences and distance from origin of application. It is noteworthy that the LsE8 was absent from the OP-susceptible strains as well as lanes 12 and 13 which showed low resistance to diazinon (i.e. RF 5 and 6.2 respectively) compared with other field strains. The significance of this observation is uncertain however, and requires further investigation. Distances between bands and width of bands are only generalisations of those visualised on gels.

A greater number of bands were visualised in *L. sericata* field strains compared with the OP-susceptible strain. A total of 16 bands were visualised from *L. sericata* (Figure 2.2.2) which is the same as that observed by Hughes & Raftos (1985) for *L.*

cuprina. Similar numbers of bands were expected considering the taxonomic homology of the two species (Stevens & Wall 1996).

Aliesterase activity (MtB assay)

Resistance to diazinon was not significantly predicted by aliesterase activities, using MtB as substrate, in larval strains or adult strains of *L. sericata* when susceptible larvae were excluded from analysis (larvae $p = 0.29$, adults $p = 0.47$) (Appendix 2.2.6) but was significant for larvae with their inclusion ($p = 0.019$). Susceptible larvae were clearly outliers from the normal population when standardised residuals of the regression were examined (Appendix 2.2.8). This indicated that there was not a gradation of MtB activity associated with level of diazinon resistance. T-tests showed that in general resistant field strains of *L. sericata* showed significantly lower levels of measurable MtB activity than the laboratory susceptible (Tables 2.2.3 & 2.2.4). Tables of mean aliesterase activities are shown in tables 2.2.3 and 2.2.4. It is likely that aliesterase activity is either present or absent, rather than at a distinguishable level based on resistance or susceptibility. ANOVA tables showing the inclusion of the OP-susceptible strains are also shown in Appendix 2.2.6.

Table 2.2.3 Aliesterase activity for *Lucilia sericata* adults using methylthiobutyrate as substrate.

Farm	Area	Date	Activity (min-^{1})	StDev	RF	
001	Laboratory	00.00.00	0.02167	0.00413	1.0	
066	Laboratory	00.00.00	0.02733	0.00289	1.0	NS
009	Blenheim	10.12.96	0.00730	0.00250	15.8	**
060	Wairoa	14.12.96	0.00700	9.5E-11	14.5	**
021	Amberley	14.12.96	0.01067	0.00208	13.8	**
059	Wairoa	08.01.97	0.01233	0.00115	12.2	*
014	Blenheim	12.01.97	0.00833	0.00153	21.8	**
057	Cheviot	13.01.97	0.01200	0.00625	13.3	**
056	Carterton	15.01.97	0.02467	0.00306	11.3	NS
061	Masterton	15.01.97	0.01500	0.00346	11.8	*
032	Blenheim	20.01.97	0.00667	0.00058	14.2	**
064	Ashburton	16.02.97	0.00900	1.3E-10	9.5	**
062	Timaru	27.02.97	0.00800	0.00100	10.8	**
065	Ashburton	14.04.97	0.01667	0.00306	9.3	*
012	Hastings	18.04.97	0.03667	0.01069	17.7	NS
055	Ashburton	29.04.97	0.00433	0.00058	5.0	**

Table 2.2.4 Aliesterase activity for *Lucilia sericata* larvae using methylthiobutyrate as substrate.

Farm	Area	Date	Activity (min-^{1})	StDev	RF	
001	Laboratory	00.00.00	0.14750	0.01046	1.0	
009	Blenheim	10.12.96	0.02167	0.00058	15.8	**
060	Wairoa	14.12.96	0.00833	0.00058	14.5	**
014	Blenheim	12.01.97	0.03233	0.01626	21.8	**
057	Cheviot	13.01.97	0.05900	0.02524	13.3	**
056	Carterton	15.01.97	0.04633	0.01955	11.3	**
061	Masterton	15.01.97	0.08300	0.00300	11.8	**
064	Ashburton	16.02.97	0.01233	0.00216	9.5	**
063	Wakefield	26.02.97	0.01167	0.00351	12.7	**
062	Timaru	27.02.97	0.01100	0.00346	10.8	**
009	Blenheim	14.04.97	0.03267	0.00971	19.2	**
065	Ashburton	14.04.97	0.03833	0.00513	9.3	**
012	Hastings	18.04.97	0.06533	0.00513	17.7	**
055	Ashburton	29.04.97	0.10467	0.01266	5.0	*
021	Amberley	14.12.97	0.03133	0.00551	13.8	**

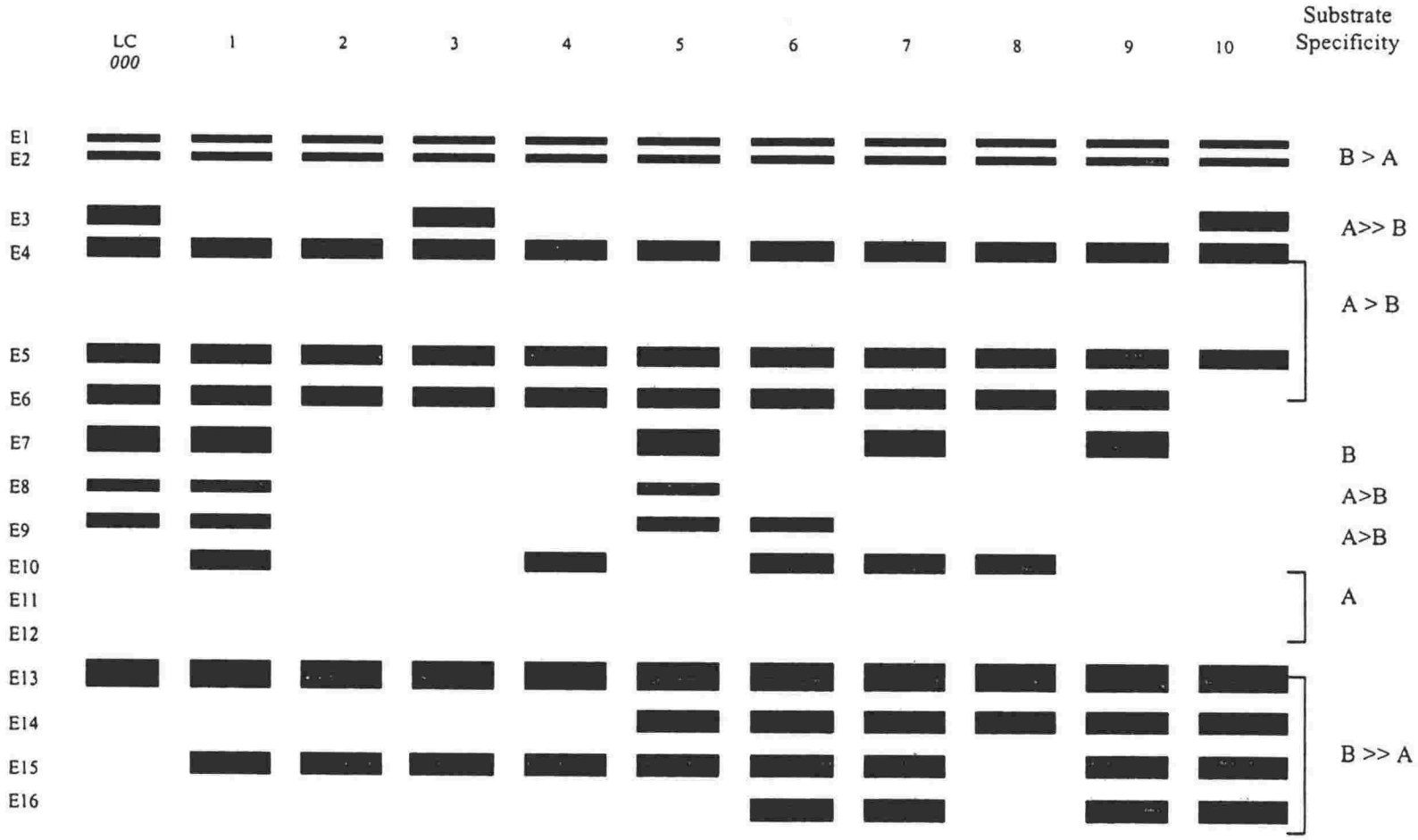


Figure 2.2.1 Electrophoretic esterase patterns of laboratory OP-susceptible and field resistant strains of *Lucilia cuprina*. Numbers have been assigned to esterases (1-16) based on Hughes & Raftos (1985). The predominant substrate specificity, either α- or β-naphthyl acetate (A or B respectively), is noted at the right of the diagram. Note that distance and width of band are not necessarily an accurate representation of that occurring on native PAGE.

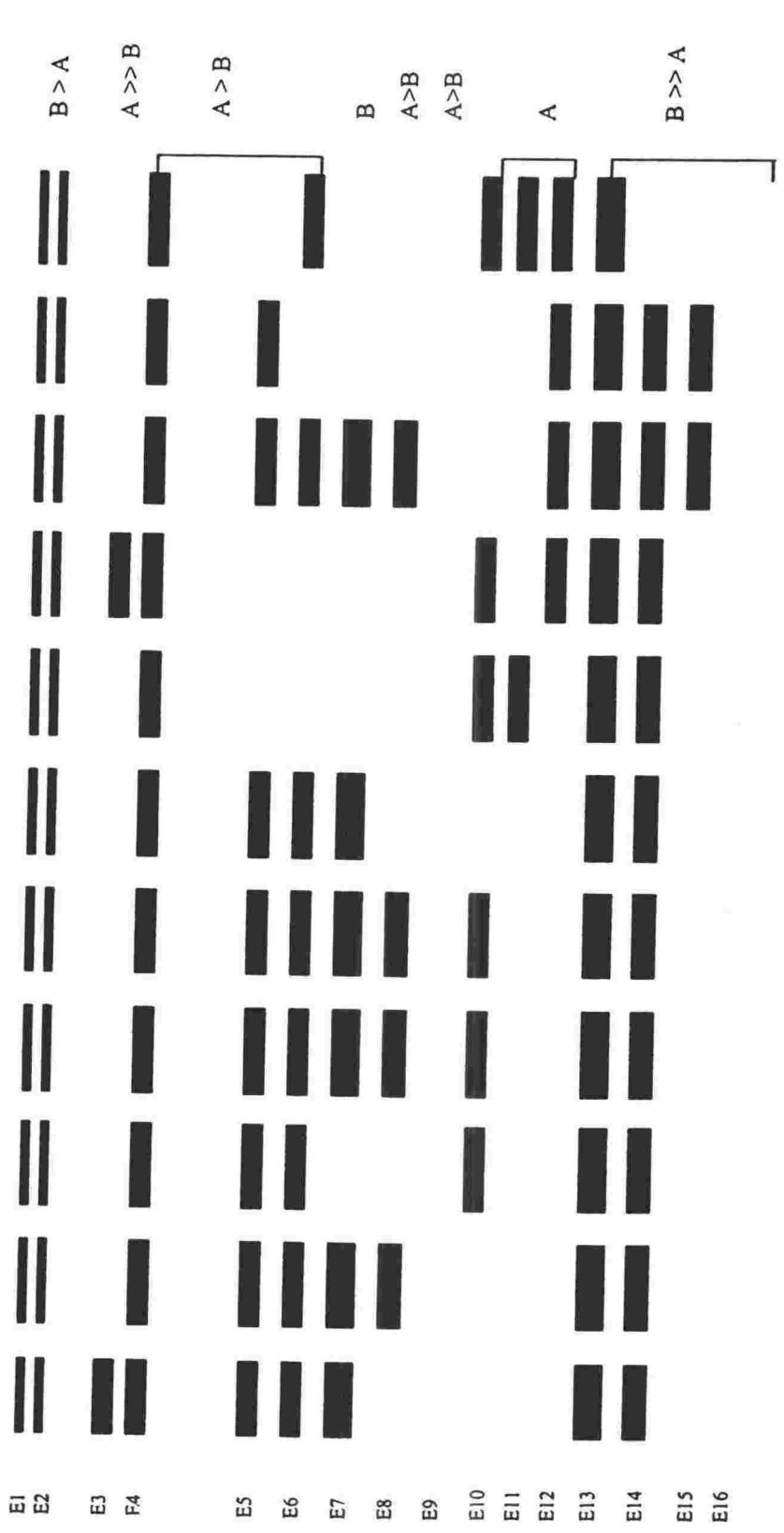


Figure 2.2.1 (continued)

Key to *L. cuprina* strains used for representation of electrophoretic banding patterns in **Figure 2.2.1**.

#	Farm	Area	Sample Date	RF
	000	Laboratory	00.00.00	1.0
1	020	Hastings	21.01.92	28.6
2	038	Gisborne	19.03.92	34.0
3	010	Te Kuiti	16.02.92	30.0
4	007	Silverstream	23.04.92	27.8
5	012	Hastings	14.01.92	35.4
6	024	Masterton	29.12.91	27.6
7	008	Kihikihi	07.03.94	11.8
8	035	Levin	11.04.94	14.2
9	023	Masterton	28.02.94	-
10	026	Foxton	27.01.92	34.6
11	003	Wanganui	16.05.94	-
12	038	Marton	09.05.94	11.8
13	036	Kaiwaka	14.03.94	-
14	012	Hastings	08.04.94	-
15	043	Waingaro	31.01.94	24.0
16	014	Blenheim	15.12.91	27.6
17	004	Bulls	13.01.92	42.8
18	045	Feilding	13.04.94	-
19	005	Waiau	18.01.92	20.8
20	004	Bulls	14.02.92	33.4

Key to *L. sericata* strains used for representation of electrophoretic banding patterns in **Figure 2.2.2**.

#	Farm	Area	Sample Date	RF
	001	Laboratory	00.00.00	1.0
1	055	Ashburton	16.01.95	8.5
2	052	Hakataramea	09.01.96	-
3	049	Wairoa	10.01.96	12.7
4	054	Alexandra	17.01.96	-
5	003	Turakina	22.01.96	20.8
6	040	Wanganui	25.03.96	11.2
7	018	Waipawa	28.12.95	9.8
8	048	Kurow	10.01.96	-
9	056	Carterton	19.11.95	14.0
10	012	Hastings	04.11.95	10.7
11	009	Blenheim	25.04.94	12.5
12	043	Waingaro	31.01.94	5.0
13	066	Dorie	20.04.94	6.2
14	060	Wairoa	14.12.96	-
15	125	Timaru	23.11.96	-
16	061	Masterton	15.01.97	9.3
17	057	Cheviot	13.01.97	13.3
18	014	Blenheim	12.01.96	21.7
19	059	Wairoa	08.01.97	12.2
20	009	Blenheim	10.12.96	15.8

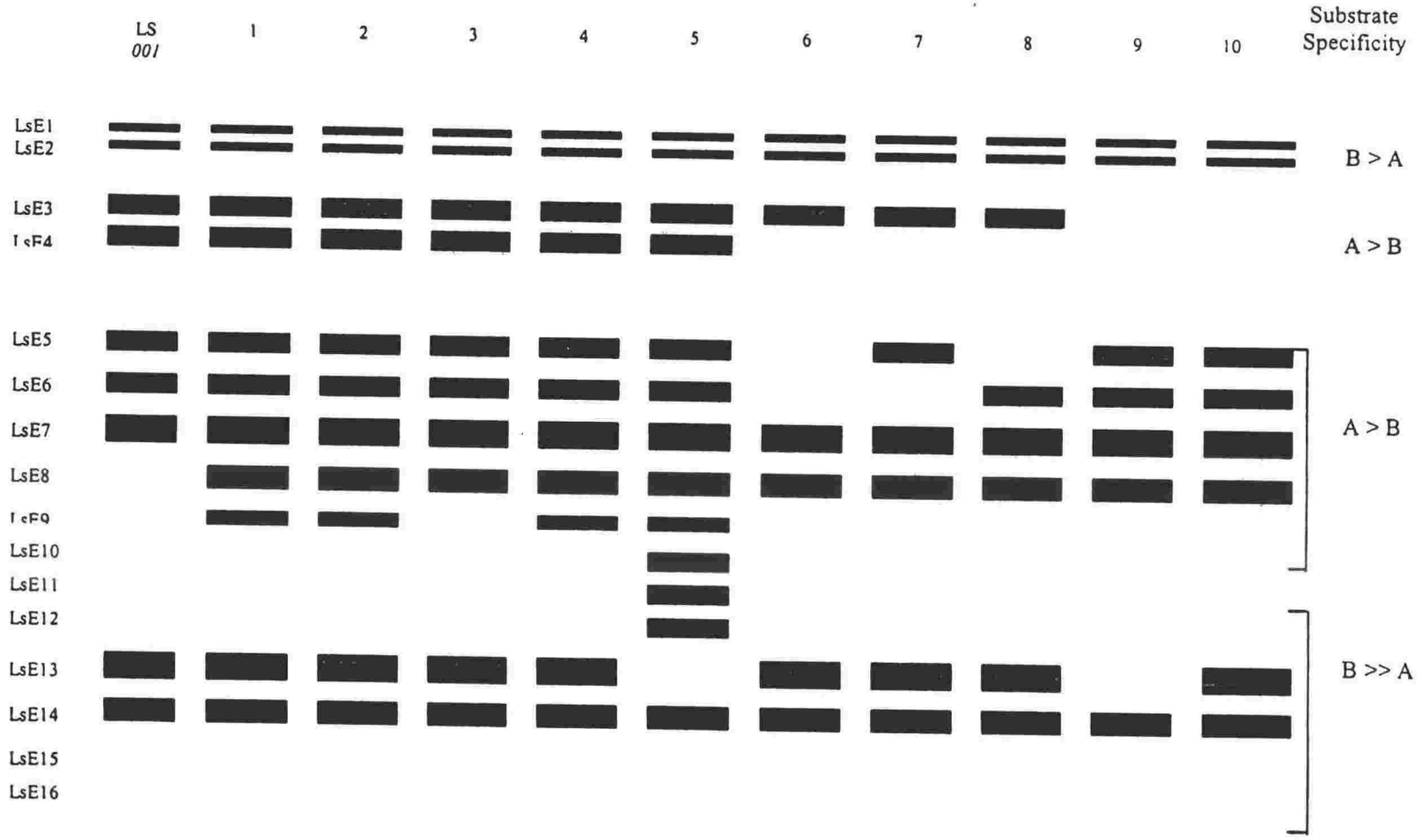
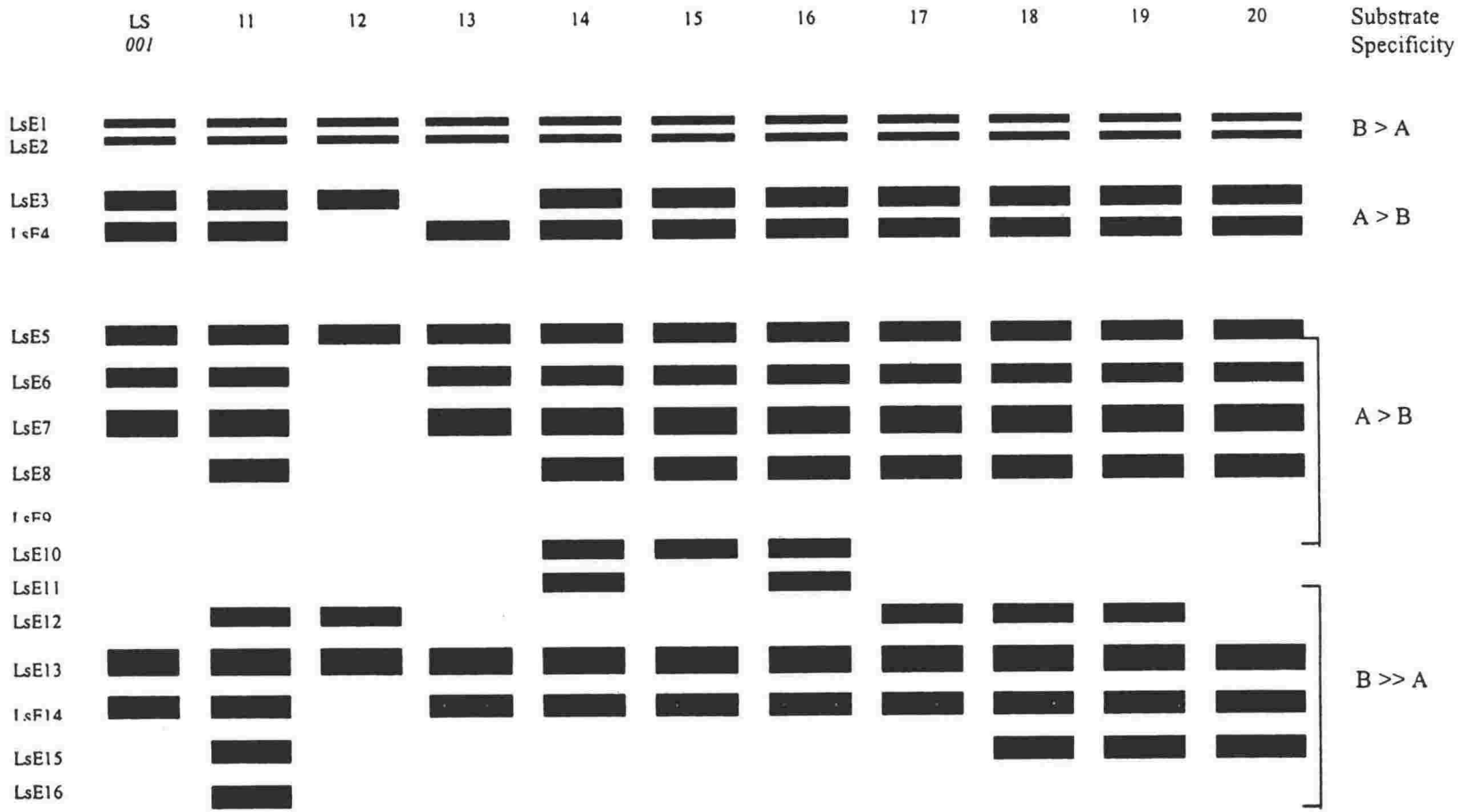


Figure 2.2.2. Electrophoretic esterase patterns of laboratory OP-susceptible and field resistant strains of *Lucilia sericata*. Numbers have been arbitrarily assigned to esterases (1-16) and the predominant substrate specificity, either α- or β-naphthyl acetate (A or B respectively), is noted at the right of the diagram. Note that distance and width of band are not necessarily an accurate representation of that occurring on native PAGE.

Figure 2.2.2. (continued)



Larval vs adult non-specific esterase activities

There was no relationship observed between adult and larval non-specific esterase activities towards either α- or β-naphthyl acetate. Analysis of variance tables of the regression are shown in Appendix 2.2.7.

Discussion

Native PAGE results clearly confirm the importance of the mutant E_3 esterase as a mechanism (or associated with a mechanism) conferring resistance in New Zealand strains of *Lucilia cuprina*. Observations of the wild type (or staining) form of E_3 were limited to approximately 3% of the field population tested (Figure 2.2.1, Appendix 2.2.3). It is likely that the average may be lower than 1% however, since there was no attempt made to discriminate between homozygous resistant and heterozygous representation of the mutant esterase. Organophosphate insecticide use has clearly selected for this mutation and it appears that the mutant form is entrenched in field populations in New Zealand as it is in Australia (Hughes & Raftos 1985).

Although only presence or absence of the E_3 has been recorded in this study and diagrammatic representation of esterases from 20 field strains, there is a wealth of information available from esterase patterns. Such patterns may be indicative of within or between population variation. Booy & van Raamsdonk (1998) have recently investigated inter- and intra-specific variation in bulb scale esterases of *Tulipa* species to determine genetic relationships at different taxonomic levels. They found that at a populational level, esterases, separated by native PAGE, acted as suitable markers when subjected to cluster analysis by defining closely related

species (Booy & van Raamsdonk1998). This type of analysis was not undertaken in the present study of *L. cuprina* as it only considers esterases as a contributor to OP-resistance. An attempt to define population variation is made in chapter 3 by using a RAPD PCR method. This may be a useful tool for exploring *L. sericata* population variation as this species was not subjected to RAPD PCR in this thesis.

Identification of the roles of various esterases may also be useful when comparing species and their homology. Hughes & Raftos (1985) have identified E_1 and E_2 as isozymes of acteylcholinesterase. Parker *et al.* (1991) support this finding and also suggest that E_8 may be a cholinesterase. E_1 has been shown to be the embryonic or juvenile form of AChE by a number of workers (Morton & Singh 1980, Zador & Maroy 1987) although it also occurs in adults (Parker *et al.*1991). The α-staining E_3 and E_9 are both carboxylesterases that are sensitive to inhibition by OPs. The E_3 esterase occurs throughout the life stages of the blowfly and is abundant in many tissues compared with E_9, which tends to be confined to pupae (Parker *et al.* 1991). The current study confirms that E_3 occurs in susceptible forms of both third instar larvae and 3-6 day old adult *L. cuprina.* Parker *et al.* (1991) also showed that E_9 is only detectable in OP-susceptible insects although it is thought to be unlikely to confer major gene resistance in feeding stages of *L. cuprina.* E_4 and E_{13} have also been identified as carboxylesterases but are only moderately sensitive to OP inhibition compared with E_3 and E_9 (Parker *et al.* 1991).

Information on the function of esterases in *L. sericata* could be used to make a useful comparison with *L. cuprina* and may clarify differences between the expression of resistance between the two species. This is especially important since results with

native PAGE were inconclusive in showing whether a mutant aliesterase is responsible for resistance in *L. sericata*. This may be because the method in current use failed to detect subtle differences in quantity of esterases as gel loadings were not based on standard protein determinations but rather on a 10 µl aliquot of each homogenate. This may mean that a contribution from gene dosage may be missed, although significant increases in quantities of esterases were not detected in resistant strains of either species of *Lucilia* in non-specific esterase assays. Field strains of *L. sericata* exhibiting OP-resistance may also possess a similar mutation to *L. cuprina* but in a smaller proportion of the population. The mutation may exist more readily in a heterozygous form in *L. sericata* rather than the homozygous form that typically occurs in *L. cuprina*. The homozygous form of the mutation in *L. cuprina* has lost the ability to hydrolyse naphthyl acetate substrates and therefore does not stain. The lack of an apparent gradation of MtB activity associated with level of resistance in field strains of *L. sericata* may be further complicated by pooling of individuals for native PAGE analysis. It is likely that the effect of pooling would fail to detect any non-staining individuals in the population. T-tests however, showed that in general resistant field strains of *L. sericata* showed significantly lower levels of measurable MtB activity than the laboratory susceptible strain (Tables 2.2.3 & 2.2.4). It is therefore, possible that aliesterase activity is either present or absent, rather than at a distinguishable level based on resistance or susceptibility in *L. sericata*. The likelihood of aliesterase activity occurring in *L. sericata* is reinforced by the comparable data sets for field and laboratory strains (adult and larval) for *L. cuprina*, which is known to show this activity.

Conclusions

Despite the confirmation that a mutated E_3 esterase is responsible in part for resistance of *L. cuprina* towards OP insecticides in New Zealand, there is much work to be achieved before a valid comparison can be made with its principal flystrike competitor *L. sericata.* A thorough screening of individuals from many populations needs to be undertaken to estimate the number of homozygous resistant flies that occur naturally. It is also necessary to determine a method of accurately scoring heterozygotes from native PAGE if a valid comparison is to be made with *L. cuprina.* Biochemical, physiological and molecular characteristics of *L. sericata* esterases also need to be considered to determine homology with *L. cuprina.* Campbell *et al.* (1997) have already shown that the E_3 esterase in *L. cuprina* is biochemically similar to methylbutyrate hydrolysing aliesterases found in *Drosophilia melanogaster*, *Musca domestica*, and *Chrysomya putoria.* In each instance, the esterases have similar electrophoretic mobility, inhibitor insensitivity, developmental profile and tissue distribution and they all map to a cluster of esterases on orthologous chromosome arms (Campbell *et al.* 1997). Comparisons of this type may provide a clearer picture of the contribution of esterases as the principal biochemical mechanism of resistance in the two species.

CHAPTER 2.3

The role of non-oxidative metabolism in organophosphate resistance in strains of *Lucilia cuprina* and *Lucilia sericata*

II. Glutathione *S*-transferases

Introduction

The glutathione *S*-transferases are a group of enzymes found in all mammalian tissues as well as insects, protozoa, algae, fungi and bacteria (Jakoby 1978). The glutathione *S*-transferases serve an intracellular protective function by detoxifying electrophilic compounds that can react with the nucleophilic centres of nucleic acids, proteins and cell membranes. The glutathione *S*-transferases do this by catalysing the covalent linkage of hydrophobic compounds containing electrophilic centres with the thiol group of the nucleophilic tripeptide, glutathione (L-γ-glutamyl-L-cysteinyl-glycine; GSH) (Boyland & Chasseaud 1969, Chasseaud 1979, Dauterman & Hodgson 1978, Pickett & Lu 1989, Walker *et al.*1993, Meyer *et al.*1995, Yu 1996). An electrophilic centre can often be introduced into a compound by another reaction; for example, by the action of the mixed function oxidases to form an epoxide which then reacts with glutathione (Jakoby 1978). These electrophilic compounds may be generated endogenously e.g. lipid peroxides (Litwak *et al.* 1971, Kamisaka *et al.* 1975), or may be of an exogenous xenobiotic nature (Fukami 1980, Motoyama & Dauterman 1980). Conjugation of a foreign compound with GSH may be followed by the removal of the glutamic acid and glycine to yield a cysteine conjugate and lastly by the acetylation of the cysteine amino group. The glutathione conjugate is less toxic and more water-soluble than the original hydrophobic compound. This product can either be excreted immediately or may undergo further transformation, as described, before excretion

from biological tissues (Boyland & Chasseud 1969). These transformation reactions typically involve a γ-glutamyl transferase, a dipeptidase and an acetyl-coenzyme A linked acetylase that results in the formation of mercapturic acids (Boyland 1971, Tate 1980, Devonshire & Hodgson 1978). Figure 2.3.1 outlines the formation of mercapturic acid from an alkyl halide (Boyland & Chasseaud 1969). Mercapturic acid biosynthesis has been shown to occur in insects (Gessner & Smith 1960, Cohen & Smith 1964, Dystra & Dauterman 1978, Dauterman 1983).

The GSTs are of particular interest in insect pest species, due to their ability to conjugate a wide range of insecticides. There are several avenues of detoxification with GSH with respect to organophosphorus insecticides (OPs). These have been described by Motoyama & Dauterman (1980) (Figure 2.3.2) and include alkyl, aryl and phosphonate conjugations. The resulting products are more water soluble, less toxic and easily excreted. It is the activity of these enzymes that has led to the acquisition of resistance in a number of insects (Fukami 1980, Motoyama *et al.* 1972, Clark 1989). Early work by Lewis (1969) showed evidence that GSTs played an important role in metabolism of OPs. Lewis (1969) reported that *in vitro* GSH increased reaction rates five-fold when diazinon was cleaved by extracts from houseflies, *Musca domestica*, to give diethyl phosphorothioic acid. GSH was also shown to be an important co-factor in the desethylation of diazinon to desethyldiazinon (Lewis & Sawicki 1971).

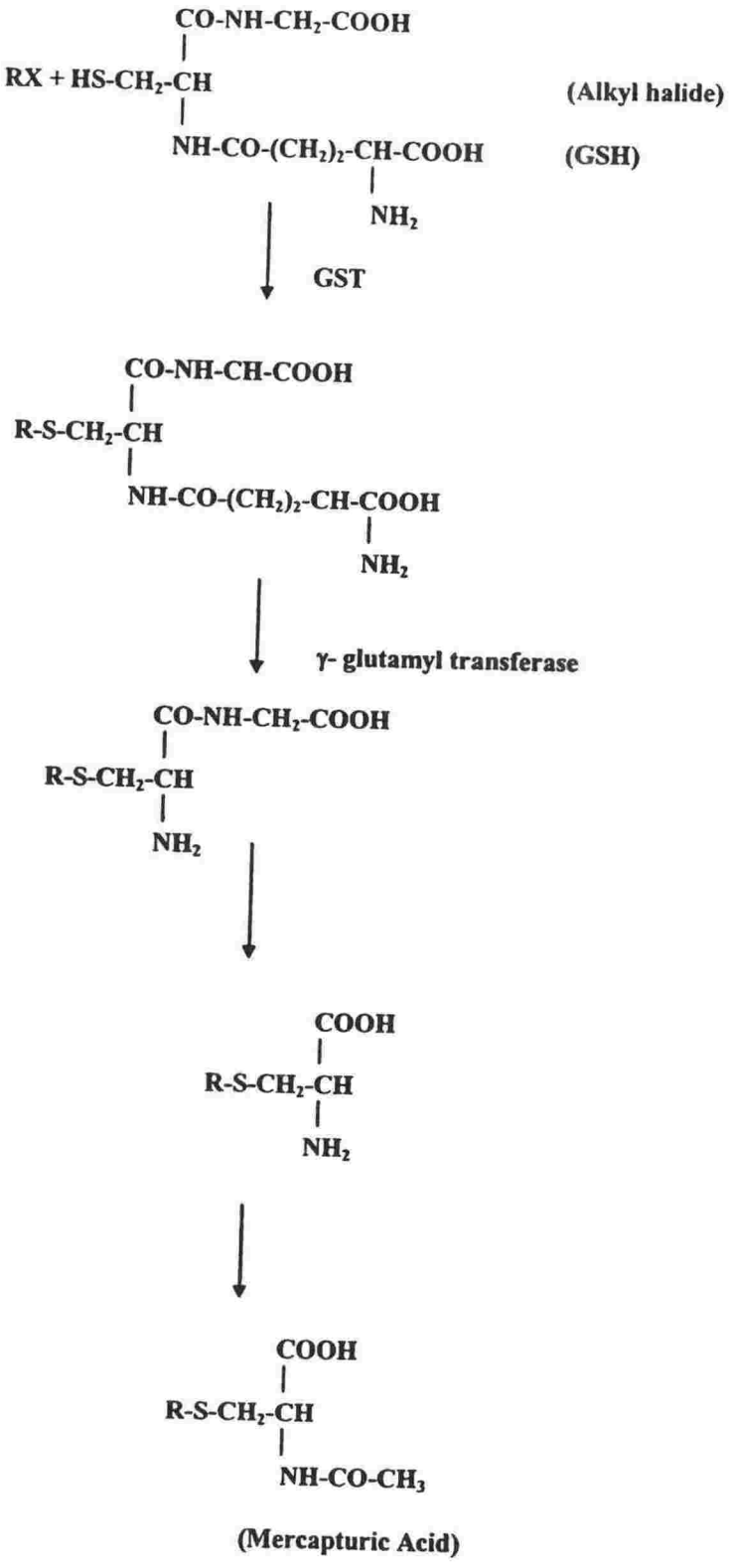
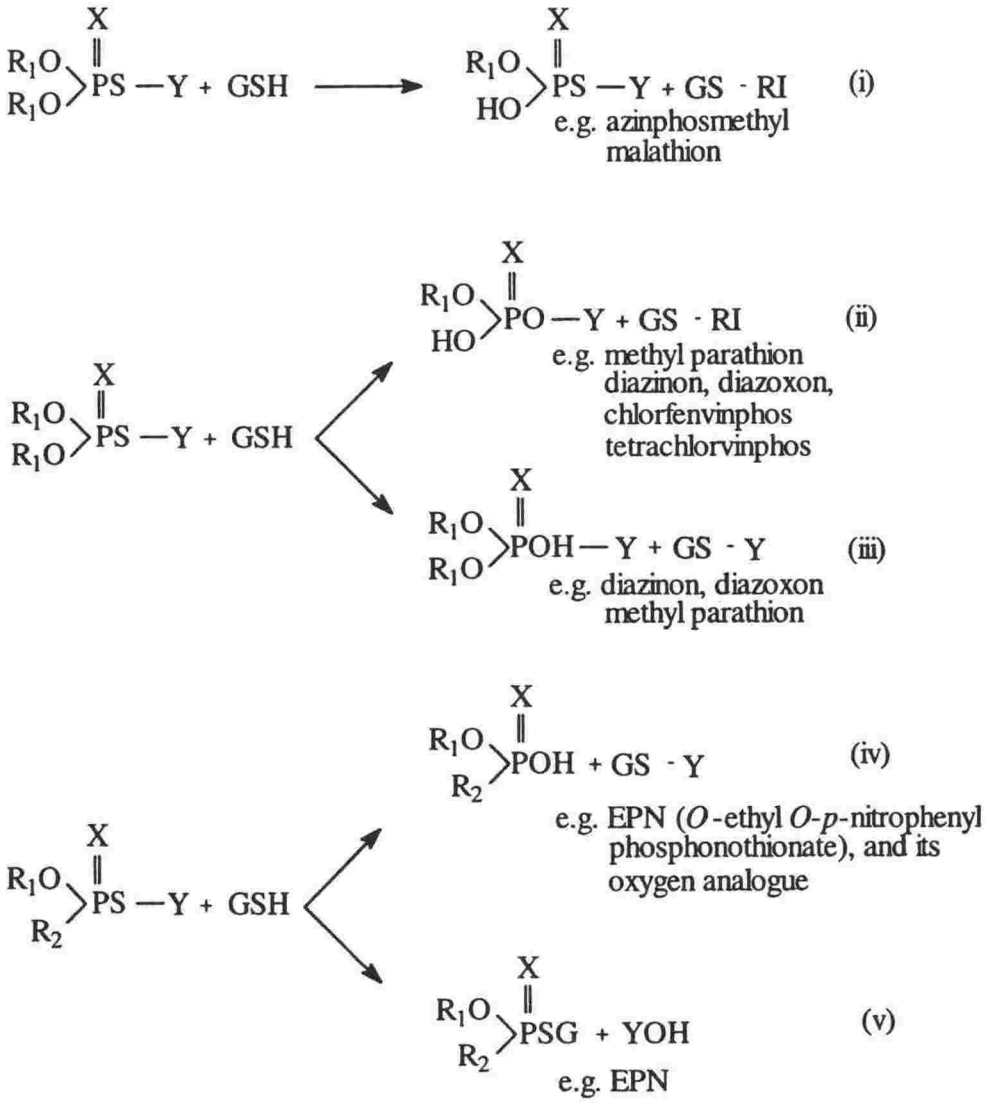


Figure 2.3.1. Mercapturic acid formation from an alkyl halide (from Boyland & Chasseaud 1969).



R_1 = alkyl
R_2 = aryl
X = S or O, Y = leaving group

Figure 2.3.2 Types of glutathione *S*-transferase reactions that occur with organophosphorus insecticides (from Motoyama & Dauterman 1980).

Elevated levels of GST activity have been associated with the development of resistance to other insecticides. Oppenoorth *et al.* (1972) found a correlation between the rate of GSH-dependent parathion detoxification and the level of malathion resistance in strains of *M. domestica*. The rate of detoxification was highest in the most highly resistant strains. The gene for glutathione-dependent degradation was located on the second chromosome which is the same chromosome that the gene for low aliesterase activity and hydrolytic detoxification of paraoxon is located (Oppenoorth 1972). Oppenoorth *et al.* (1977) later reported that a tetrachlorvinphos-resistant strain of houseflies showed GSH-dependent detoxification of certain organophosphate insecticides to be 9- to 120-fold higher than in a susceptible strain. Quantitative differences in GSTs have also been shown to occur in resistant strains of *M. domestica* from Japan (Motoyama *et al.* 1980, Ugaki *et al.* 1985). A three-fold elevation in GST activity is also shown in a malathion resistant strain of *Drosophilia* compared with a susceptible strain (Cochrane *et al.* 1992). Other examples of increased production of GSTs contributing to resistance are seen in the predacious mite, *Neoseiulus fallacis* (Motoyama *et al.* 1971), granary weevils, *Sitophilus granarius* (Starratt & Bond 1981) and in the diamondback moth, *Plutella xylostella*, (Kao & Sun 1991). This phenomenon is not restricted to insects but has also been shown in the nematode *Haemonchus contortus*. Increases of GST activity were 1.5 - 1.8 xs higher in a cambenazole resistant strain than a susceptible strain (Kawalek *et al.* 1984). Fournier *et al.* (1992) suggested that resistance in houseflies, with known elevations of GST activities, was due to an over-transcription of the GST1 gene. Syvanen *et al* (1996) showed however, that resistance in houseflies was not only due to over-transcription but also to divergence of sequence by a few nucleotides between amplified copies. Conjugation with glutathione represents an efficient metabolic

detoxification pathway for insecticides.

Further evidence for the role of glutathione *S*-transferases in insecticide resistance comes from induction studies by various workers. Glutathione *S*-transferase activity increased in strains of adult houseflies when induced by phenobarbital, chlorinated hydrocarbons and to a lesser extent with OPs (Hayaoka & Dauterman 1982). Similarly, *L. cuprina* responds to induction by phenobarbital with a 3-4 x increase in 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-dichloro-4-nitrobenzene (DCNB) activity (Kotze & Rose 1987). Caution must be exercised when interpreting these results however, since phenobarbital and various chlorinated hydrocarbons have been shown to induce mixed function oxidases as well as DDT-dehydrochlorinase activity in houseflies (Perry *et al.* 1971, Yu & Terriere 1973, Terriere *et al* 1974, Plapp & Rhee 1979, Ottea & Plapp 1981).

Naturally occurring chemicals and allelochemicals from host plants such as umbellifers and crucifers can also be potent inducers. This has been demonstrated in studies of phytophagous insects where dietary components influence GST synthesis (Lee 1991, Yu 1982, 1983, 1984). Yu (1982) reported differences in insecticide susceptibility of soybean- or mustard-fed fall armyworm larvae, *Spodoptera frugiperda*. Mustard fed larvae showed a 10-fold increase in GST activity compared with soybean fed larvae. The increase in GST activity was attributed to induction by certain mustard plant constituents. In the same study it was reported that the rate of detoxification exceeded the activation rate where phosphorothiolate organophosphates were used as inducers thus illustrating the protective function of GSTs and other mechanisms. Generalist phytophagous insects are thought to rely on

GST pathways of detoxification for metabolism of allelochemicals more than specialist feeders (Yu 1992). Induction appears to be an environmentally dependent response that underlies detoxification by GSTs and other mechanisms of various xenobiotic compounds (Nebert 1979).

The success of the GSTs as mechanisms of detoxification is attributed to the differing properties of individual GSTs, their number, and their overlapping specificities (Clark 1989). This is based on numerous studies of the characterisation and purification of the GSTs in a number of insect species. These include the greater wax moth, *Galleria mellonella* (Clark *et al.* 1977), the porina moth, *Wiseana cervinata* (Clark & Drake 1984), the diamond back moth, *Plutella xyostella* (Cheng *et al.* 1984), houseflies, *Musca domestica* (Motoyama & Dauterman 1977, Clark *et al.* 1984, Fournier *et al.* 1992, Franciosa & Berge 1995), the fall army worm, *Spodoptera frugiperda* (Kirby & Ottea 1995) and the Australian sheep blowfly, *Lucilia cuprina* (Kotze & Rose 1989).

Kirby & Ottea (1995) separated four GSTs from fat body homogenates of *Spodoptera frugiperda* larvae. Two were shown to have activity with DCNB and CDNB and two with only CDNB. Peaks of activity for these occurred at pH 7.8 and at pH 9.4 for DCNB active fractions and at pH 7.8 and pH 9.0 for CDNB active fractions. Kirby & Ottea (1995) also postulated that the expression of GST activities was regulated by multiple mechanisms. This was based on induction studies where injection of larvae with 8-methoxypsoralen (8-MOP) increased DCNB activity but not CDNB. Injection with pentamethylbenzene (PMB) resulted in increased CDNB activity but not DCNB. Induction by 8-MOP was shown to be inhibited by PMB in a dose-dependent fashion. In contrast, activity towards CDNB increased following PMB but subsequent 8-MOP

injection led to a reduction in one of the CDNB activities but not the other.

Kotze & Rose (1989) identified several forms of GSTs in *L.cuprina* larvae that they separated into two clearly distinguishable fractions based on their substrate specificities. Fraction II showed activity with 1-chloro-2,4-dinitrobenzene (CDNB) and had a molecular weight (MW) of 44 000. Fraction I showed activity towards CDNB, 1,2-dichloro-4-nitrobenzene (DCNB) and *p*-nitrobenzyl chloride (*p*-NBC) and had a MW of 45 000. Molecular weights of these fractions were similar to other insect GSTs. Franciosa & Berge (1995) also located two serologically distinct families of GSTs (GST 1 and GST 2) from adult houseflies (Fournier *et al.* 1992). It is thought that GST1 plays an important role in detoxification of insecticides as increases in its production parallel increases in insect resistances (Clark 1990, Fournier *et al.* 1992, Cochrane *et al.* 1992). Cochrane *et al.* (1992) also showed the involvement of GST2 in malathion resistant strains of *Drosophilia melanogaster.* Franciosa & Berge (1995) showed each family was distributed in distinctly different tissues. GST1 was found in the cytosol of haemocytes while GST2 was predominant in the cortex of the central nervous system (brain and ganglia of the abdomen and thorax) and in the indirect flight muscles. Franciosa & Berge (1995) reported no difference in GST distribution between susceptible and resistant strains.

The results from *M. domestica* differ from those observed in *L. cuprina* by Kotze and Rose (1989) who showed GST activity to be predominantly localised in the fat body and lower activities in the cuticle, gut and blood of larval *L.cuprina.* Other studies show a general distribution of GST activity in insect organs (Cohen et al. 1964, Wood *et al.* 1986). Wood *et al.* (1986) also report that the genitalia of male *Triatoma*

infestans was a rich source of GST activity compared with genitalia of females of the species. Franciosa & Berge (1995) attribute these differences to either contamination of tissues during preparation or to haemolymph in the tissues although this does not explain sex differences in *T.infestans*. Franciosa & Berge's (1995) findings of GST activity distributed in the haemolymph and insect central nervous system does however reflect the protective function of these enzymes. The insect's haemolymph is a major transport system for insecticides such as OPs that target the acetylcholinesterase of the central nervous system.

The protective function is further exemplified by the observed changes in activity associated with developmental stages. Kotze & Rose (1987) report an increase in DCNB and CDNB conjugation (per mg protein) capacity throughout the development of larval *L. cuprina,* peaking early in the pupal stage. Activity decreases throughout the pupal stage and continues to decline for 6-7 days after emergence (Kotze and Rose 1987). This is of interest because OPs used for control of *L. cuprina* on sheep primarily target larval stages as feeding on the live tissue of sheep causes the most economic losses and the larvae are restricted to the host for the duration of feeding.

Methods for purification of the GSTs have in the past included enzyme precipitation, ion exchange chromatography, gel filtration and hydroxyapatite chromatography methods. More efficient techniques using affinity chromatography have since been developed. The success of affinity chromatography lies in the reversible attachment of a target protein to a specific or substrate analogue inhibitor. Ligands suitable for GST affinity include GSH and an organic dye, sulfobromophthalein (BSP). BSP acts as an analogue of the electrophilic substrate but may also partially compete with GSH

for its binding site on insect enzyme (Clark *et al.* 1977, Clark & Wong 1978). The matrix is packed into a chromatography column and an impure sample passed through it. Desired proteins are bound to the immobilised ligand while those with no affinity are removed by washing. The bound proteins are recovered by elution with the appropriate molecule such as GSH or BSP that acts as a competitor for the ligand.

This chapter examines a possible role for the glutathione *S*-transferases in OP resistance in adult and larval field strains of *L. cuprina* and *L. sericata.* It begins with an assessment of adult and larval GST activities using the model substrates, CDNB and DCNB, and compares these with larval resistance factors. An attempt is then made to identify the OP-conjugating properties of partially purified GSTs from larvae of resistant *L. cuprina* larvae.

Materials and methods

Chemicals

The substrates 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-dichloro-4-nitrobenzene (DCNB) were obtained from BDH Laboratory Supplies, Poole, England. Glutathione and sulfobromophthalein were obtained from Sigma Chemical Co. (St Louis, USA). Sepharose 4B, DEAE-Sepharose 4B, QAE Sephadex and Sephadex G-25 were from Pharmacia Fine Chemicals. The sulphoromophthalein-glutathione conjugate immobilised on Sepharose 4B was prepared by the method described by Clark & Dauterman (1977, 1990). Ampholytes (pH 4 - 9) for isoelectric focusing were obtained from Sigma Chemical Co., St Louis, USA. [^{14}C] lindane and [^{14}C] methyl parathion were from Amersham Australia Pty, Sydney, Australia and [^{14}C] diazinon

was from International Isotopes, Munich, Germany. Sources of other chemicals used in these experiments can be found in Appendix A.

GSH S-transferase activities

Enzyme preparation

Ten adult flies (3-6 days old, sugar and water fed only) or larvae (third instar, clear-gut stage) were homogenised in 3 ml of buffer. The choice of homogenate buffer was dependent on the substrate used for determining GSH S-transferase activity. Where 1-chloro-2,4-dinitrobenzene (CDNB) was used as a substrate, 0.1 *M* sodium phosphate, pH 6.5 buffer was used. Where 3,4-dichloronitrobenzene (DCNB) was the substrate 0.1 *M* Tris HCl, pH 8.0 buffer was used. Cysteine-HCl (1 m*M*) was added to buffers for adult preparations and 5 m*M* cysteine, 1m*M* ethylenediaminetetra acetic acid (EDTA), 1 m*M* phenyl thiourea (PTU) and 1 m*M* phenylmethylsufonyl fluoride (PMSF) (dissolved in 1 ml methyl cellosolve) were added as stabilising agents in larval preparations. Each assay was replicated 3 or 5 times for each substrate and for each strain of flies.

Flies were homogenised for 30-60 seconds in a Polytron Kinematica® motorized homogeniser. The homogenate was centrifuged in a Sorvall® superspeed RC2-B centrifuge at 10,000 rpm for 10 minutes at 4°C. The supernatant was used as the enzyme solution.

GSH S-transferase assays

Glutathione *S*-transferase activities were determined spectrophotometrically at 25°C using CDNB and DCNB as substrates by the variants of the method described by

Habig *et al.* (1974). In brief, the reaction mixture for CDNB consisted of 0.1 *M* sodium phosphate buffer pH 6.5 (2.85 ml), 1 m*M* CDNB (50 µl of 60 m*M* CDNB in ethanol), 1 m*M* glutathione (GSH, 50 µl of 60 m*M*), and 200 µl of sample enzyme to give a final volume of 3.15 ml. The reaction mixture for DCNB was 0.1 *M* Tris HCl buffer pH 8.0 (2.35 ml) , 0.4 m*M* DCNB (50 µl of 24 m*M* DCNB in ethanol), 200 µl of 60 m*M* GSH (4 m*M*) and 300 µl of sample enzyme to give a final volume of 2.9 ml. The change in absorbance was measured at 340 nm using a Varian-Cary® 210 spectrophotometer.

Protein determinations

Protein concentrations were determined according to the method of Bradford (1976) using bovine serum albumin as standard. See Appendix C for a description of the method.

OP-conjugating properties of L. cuprina glutathione S-transferases

Preliminary Experiments

Preliminary experiments examining the most appropriate means of GSH-transferase purification from *L. cuprina* were undertaken. These experiments compared adult and larvae of a combined resistant field (CFS) strain. One preliminary method of partial purification is described in Appendix 2.3.4. Results for this method are not reported since the method below was more efficient with respect to quantity and quality of the enzyme and was therefore adopted for this study.

Enzyme preparation

An enzyme stock was prepared by washing 230 g late third instar (clear-gut stage) *L. cuprina* larvae in 500 ml of 0.1 *M* Tris-HCl buffer pH 8.7 containing 5 m*M* GSH. Washed maggots were homogenised in 500 ml of 0.1 M Tris-HCl buffer pH 8.7 containing 10 m*M* GSH and 1 m*M* PTU (in methyl cellosolve) using a Polytron® Kinemata homogeniser. The homogenate was filtered through cotton fibre twice, to remove insect cuticle, and solid ammonium sulphate was added slowly to a concentration of 40 % of saturation. The sample was centrifuged at 10000g x 20 minutes at 4° C. The supernatant was filtered though glass wool to remove lipids and ammonium sulphate added to a total concentration of 90% of saturation. The solution was centrifuged at 10000g x 20 minutes. The pellet was retained and re-suspended in 20 m*M* Tris-HCl pH 8.2 containing a few specks of dithiothreitol (DTT). A total final volume of 100ml of enzyme preparation was frozen at -20°C until required.

Enzyme Purification

All buffers and purification procedures were performed at 4° C to minimise loss of enzymatic activity. The GSH-Sepharose (3 µmol/ml) and BSP-GSH sepharose (2 µmol/ml) affinity columns were prepared as described by Clark *et al.* (1990).

The enzyme preparation was desalted on a Sephadex G25 column (50 x 220 mm) which had been equilibrated with 20 mM Tris-HCl pH 8.2 (flow rate 5 ml/min). Glutathione *S*-transferase activities were monitored throughout the desalting procedure. Active fractions were pooled, activity measured and the pH adjusted to 7.0 with 0.1 *M* NaH_2PO_4. The sample was then applied to a GSH-Sepharose affinity column (100 x 25 mm) followed by a BSP-GSH affinity column (50 x 25mm) (flow

rate 1.5 ml/min). The columns were washed with 20 m*M* NaH_2PO_4 pH 7.0 until no protein remained in the eluent. Affinity columns were then uncoupled for the elution procedure. The GSH-Sepharose column was eluted with 10 m*M* GSH in 0.1 *M* Tris-HCl pH 9.6 and the active fractions were pooled. Protein was monitored continuously by measuring absorbance at 280 nm using an ISCO UA-5 Absorbance/ fluorescence detector.

A small Sephadex QAE ion exchange column (20 x 15 mm) was connected after the BSP-GSH affinity column to remove dye from eluted protein. The BSP-GSH column was then eluted with 2 m*M* BSP in 20 m*M* NaH_2PO_4 pH 7.0 containing 0.2 *M* NaCl. Active fractions were pooled and proteins separated to their isoelectric points using a 250 ml LKB 8100 Ampholine electrofocusing apparatus.

Isoelectrofocusing

The LKB 8100 column was run at 4°C throughout focusing. Fifty ml of 1% H_2SO_4 in 50% glycerol was used as the anode solution in the LKB apparatus. A density gradient was then applied to the column (flow rate 3 ml/min) using a gradient mixer with 40% and 10% glycerol solutions. Each solution (80 ml) contained 0.1 m*M* DTT and 10 m*M* mannitol, 1% ampholytes (pH 4-9) (2 ml) and half the pooled enzyme (17 ml). Approximately 40 ml (enough to cover the electrode) of 2% ethanolamine was used as the cathode solution.

The column was set at 200 volts (1.2 watts) and run for up to 40 hours until the current had dropped and stabilised. The column was eluted and fractions collected at a rate of 3 ml/min. pH and CDNB and DCNB activity was measured in each fraction.

Active fractions were pooled, overall activity measured and conjugation of radioactive pesticides to glutathione was measured. [^{14}C] diazinon conjugation was considered firstly, followed by [^{14}C] lindane and [^{14}C] methyl parathion if there was sufficient active sample available. A sample volume of 100 µl of each fraction was concentrated to 50 µl using filtron 3K Nanosep™ (Pall Filtron Corporation) centrifugal concentrators. Total protein of pooled fractions was determined from the concentrated samples using the Pierce Bicinchoninic acid (BCA) Protein Assay Reagent. A small sample (1.5 - 2 ml) of active fraction was retained for electrophoretic analysis.

Radioactive pesticide conjugation by partially purified L .cuprina GSH S-transferases

Incubations were carried out in plastic stoppered tubes at 37°C for 2 hours. Total assay volume was 2 ml which consisted of 1 ml enzyme preparation and 1 ml 0.1 *M* Tris-HCl pH 9.0 containing 4 m*M* GSH and 15µl [^{14}C] Diazinon (10 m*M*, 10 µci/ml) or 7.5 µl [^{14}C] lindane (20 m*M*, 5 µci/ml) or 15µl [^{14}C] methyl parathion (10 m*M*, 10 µci/ml). Assays and controls were carried out in triplicate.

Reactions were stopped by the extraction of unreacted insecticide into 2 ml of chloroform. Tubes were vortexed for 30 seconds and briefly centrifuged. One ml of the aqueous phase was transferred to a scintillation vial containing 4 ml Optiphase® scintillation fluid. Radioactivity was determined in DPM using a Wallac 1409 scintillation counter.

Electrophoretic analysis

SDS PAGE was performed according to the method of Laemmli (1970) on the active fractions used in conjugation experiments. Active fractions were first concentrated using Nanosep™ microconcentrators (Pall Filtron Corporation, Northborough, Canada). Fractions were then boiled for 4 minutes in 0.1 *M* Tris-HCl pH 6.8 buffer containing 20 m*M* DTT, 2% SDS, 10% glycerol and bromophenol blue. Iodoacetamide was added to a concentration of 260 m*M* after boiling. Proteins were separated in a 12.0% acrylamide gel with a 4% acrylamide stacking gel. Proteins were detected using a silver stain method (Merril *et al.* 1984). Molecular weight standards were used to determine subunit molecular weights of the active fractions. Protein determination in partially purified enzyme preparations was by the Lowry method (Appendix C).

Statistical Analysis

Glutathione *S*-transferase activities, using CDNB and DCNB as substrates, were analyzed by analysis of variance (ANOVA) methods in S-Plus (Everitt 1994). CDNB and DCNB conjugation activities (µmol/min/mg protein) were used as predictors of diazinon resistance for each strain. The influence of region and season were also considered in the model.

The relationship between larval and adult glutathione *S*-transferase conjugation activities was determined using regression analysis and fitted line plots in Microsoft Excel®. The relationship between diazinon resistance and activity towards DCNB are also shown in a fitted line plot in the absence of seasonal and regional influences. These results have been published (Wilson & Clark 1996).

Results

Larval vs adult GST activities

Tables of mean GST activities for larval and adult strains of *L. cuprina* and *L. sericata* with respect to the model substrates DCNB and CDNB are shown in Appendix 2.3.1. A summary of Appendix 2.3.1 is presented in Table 2.3.1. This table shows that specific activities of larval GST preparations were greater than those obtained from adults using DCNB as substrate. Larvae of both species had, on average, 2.5 x more DCNB activity than adults of the same strain. These differences were less pronounced with respect to CDNB activities (Table 2.3.1).

Table 2.3.1. Summary of mean GST activities, using CDNB and DCNB as substrates, of adult and larval strains of *L. cuprina* and *L. sericata.*

Species/ Life stage	Mean GST Activity µmol/min/mg protein (Min – Max)	Standard Error	N
CDNB			
L. cuprina adults	0.076109 (0.023118 - 0.241287)	0.01088	27
L. cuprina larvae	0.070008 (0.033408 - 0.128822)	0.00469	23
L. sericata adults	0.071039 (0.007665 - 0.338187)	0.00927	37
L. sericata larvae	0.054066 (0.009204 - 0.147250)	0.00530	43
DCNB			
L. cuprina adults	0.002275 (0.001084 - 0.004658)	0.00014	36
L. cuprina larvae	0.005974 (0.001792 - 0.015623)	0.00051	35
L. sericata adults	0.003954 (0.000763 - 0.015223)	0.00048	43
L. sericata larvae	0.010605 (0.001518 - 0.040732)	0.00117	49

GST activities towards the substrate DCNB were significantly related in larvae and adults of the same strain in *L. cuprina* ($p = 0.014$) and *L. sericata* ($p < 0.001$) (Appendix 2.3.3, Figures 2.3.3 & 2.3.4). There was no relationship between larvae and adult GST activities with respect to CDNB in either species.

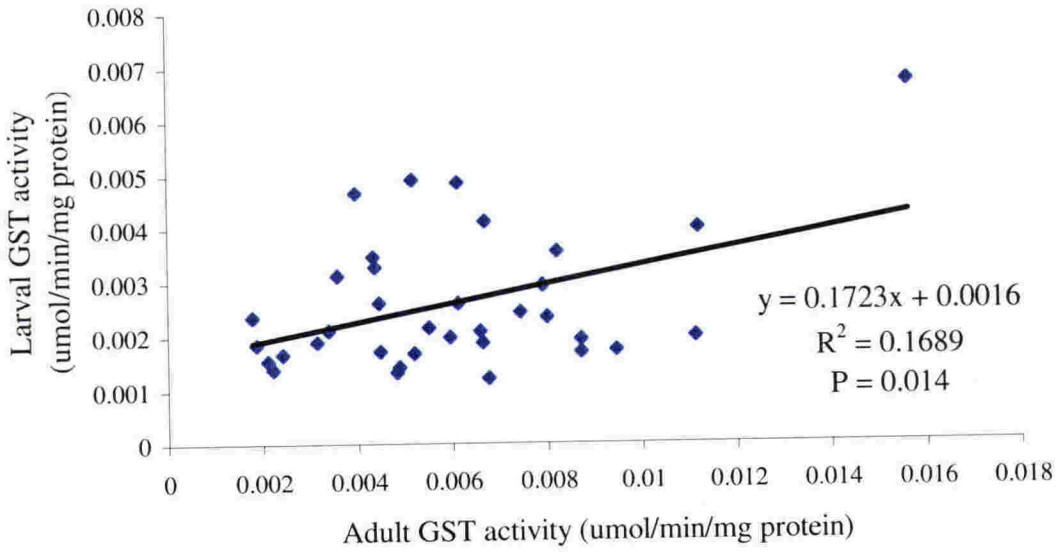


Figure 2.3.3 Relationship between glutathione *S*-transferase activities using DCNB as substrate, in adult and larval strains of *Lucilia cuprina*.

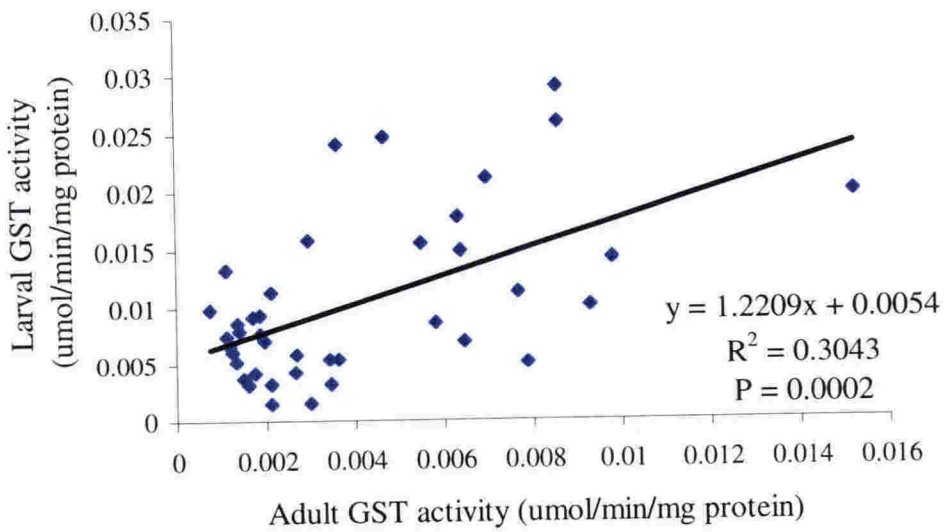


Figure 2.3.4 Relationship between glutathione *S*-transferase activities using DCNB as substrate, in adult and larval strains of *Lucilia sericata*.

GSH S-transferase activities - Lucilia cuprina

The level of resistance to diazinon was significantly predicted by glutathione *S*-tranferase activity using DCNB as substrate for both adult ($p = 0.008$) and larval ($p = 0.005$) (Appendix 2.3.2) *L. cuprina* with the inclusion of environmental factors in the ANOVA model. Season also had a significant influence in the DCNB activity model for both life stages and the adult model was additionally influenced by region. Figures 2.3.5 & 6 show the relationship between diazinon LC_{50} and glutathione *S*-transferase activity using DCNB as substrate, in larval strains of *L. cuprina* without correction for seasonal and regional influences. The relationship between adult GST activity and diazinon LC_{50} was not significant when season and region were removed from analysis showing the strong influence of these factors in the ANOVA model (Figure 2.3.6). The relationship between larval GST activity and diazinon LC_{50} was significant ($p = 0.001$) however, even when season and region were removed (Figure 2.3.5).

Activity towards CDNB did not significantly predict level of resistance although there was a suggestion that season influenced diazinon resistance in *L. cuprina* larvae ($p = 0.04$) (Appendix 2.3.2).

GSH S-transferase activities - Lucilia sericata

Glutathione *S*-transferase activity towards either DCNB or CDNB showed no significant association with diazinon LC_{50}s in both adult and larval strains of *L. sericata* either by linear regression or by ANOVA. Season however, had a significant influence on activity towards both substrates and both life stages ($p < 0.01$) (Appendix 2.3.2) using ANOVA.

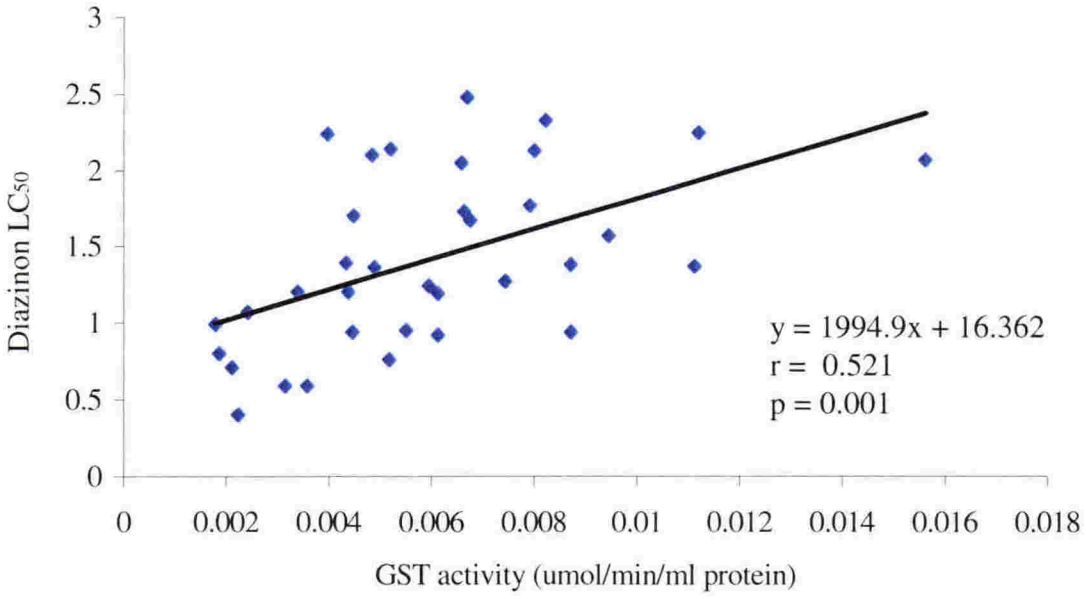


Figure 2.3.5 Relationship between diazinon LC_{50} and glutathione *S*-transferase activity using DCNB as substrate, in larvae of strains of *Lucilia cuprina* in the absence of seasonal and regional influences.

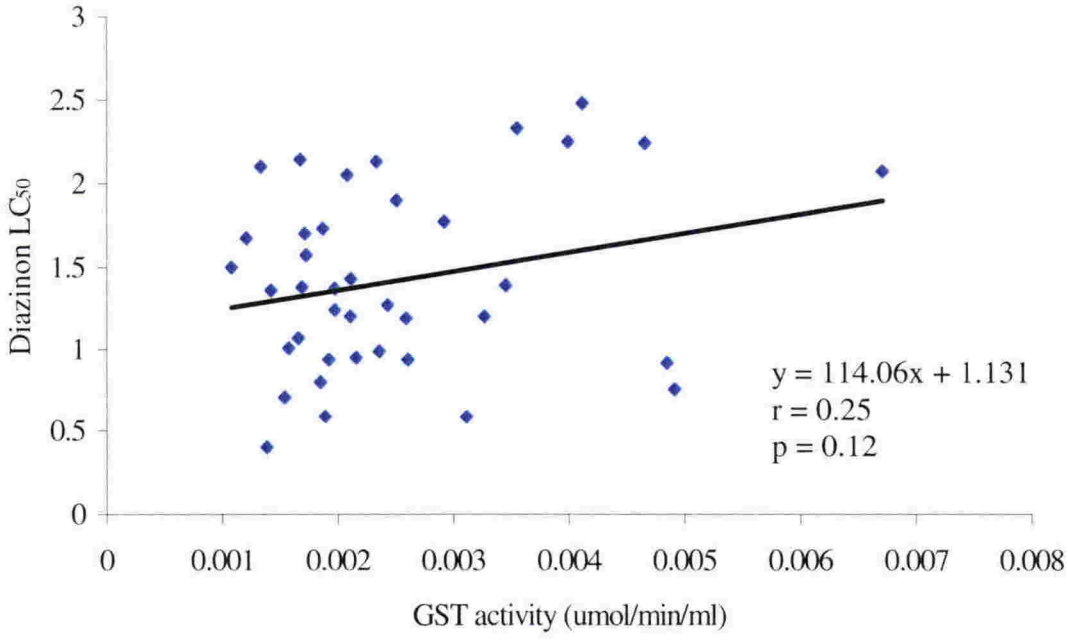


Figure 2.3.6 Relationship between diazinon LC_{50} and glutathione *S*-transferase activity using DCNB as substrate, in adults of strains of *Lucilia cuprina* in the absence of seasonal and regional influences.

OP-conjugating properties of L. cuprina glutathione S-transferases

Figure 2.3.7 shows GST enzyme activity in fractions eluted from the GSH-Sepharose column with 10 m*M* GSH in 0.1 *M* Tris-HCl, pH 9.6. The results are typical of this type of experiment. Both CDNB and DCNB activities from this column were low in comparison to active fractions eluted from the BSP-GSH Sepharose column with 2 m*M* BSP in 20 m*M* NaH_2PO_4, pH 7.0 (Figure 2.3.8). Active fractions from the BSP-GSH Sepharose column (9 – 14) were pooled and subjected to isoelectrofocusing. Isoelectrofocusing of active fractions from the elution of the GSH-Sepharose column was attempted but activities were too low to produce a meaningful result. Two GST activity peaks were observed however, from the BSP elution of the BSP-GSH-Sepharose column (Figure 2.3.9). The contents of tubes 38 – 41 (pH 6.73 – 7.47) were pooled (Fraction I) and had a pH of 7.03. Fraction I had pooled GST activities of 0.0288 µmol CDNB and 0.0069 µmol DCNB conjugated/min/ml of sample. Fraction II consisted of tubes 28 – 31 pooled (pH 4.21 – 4.91) and had a pH of 4.34. Fraction II had pooled GST activities of 0.0040 µmol CDNB and 0.0105 µmol DCNB conjugated/min/ml of sample. Analysis of these fractions using SDS-PAGE suggested that the purification was only partial. Silver staining revealed a number of proteins (Figure 2.3.10) of unexpected high molecular weight. It is expected that the proteins of interest have subunit molecular weights of 24 600 in Fraction I and 25 700 in Fraction II (Figure 2.3.10). Conjugation of radioactive OP-insecticides ([^{14}C] diazinon, [^{14}C] lindane and [^{14}C] methyl parathion) with each fraction shows that Fraction I (pH 7.03) had greater OP-insecticide conjugating capabilities than Fraction II (pH 4.34) (Table 2.3.2). The conjugation of [^{14}C] diazinon with Fraction I was less than with either lindane or methyl parathion (Table 2.3.2).

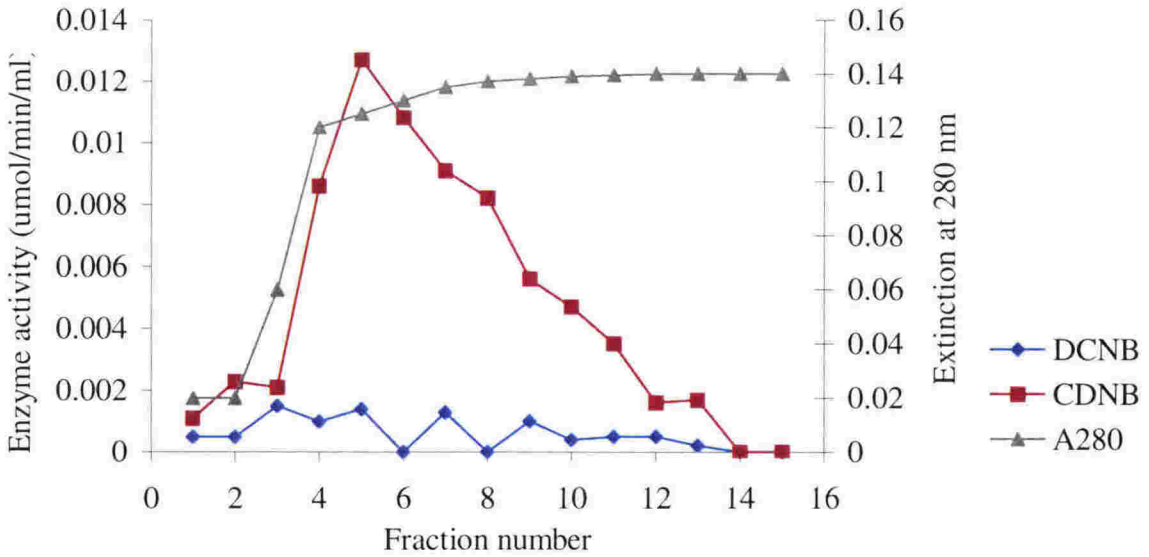


Figure 2.3.7 GSH-Sepharose chromatography of glutathione *S*-transferases from *Lucilia cuprina* larvae. Activity peaks only are presented following elution of the column with 10 m*M* GSH in 0.1 *M* Tris-HCl, pH 9.6. Enzyme activities were measured using DCNB and CDNB as substrates.

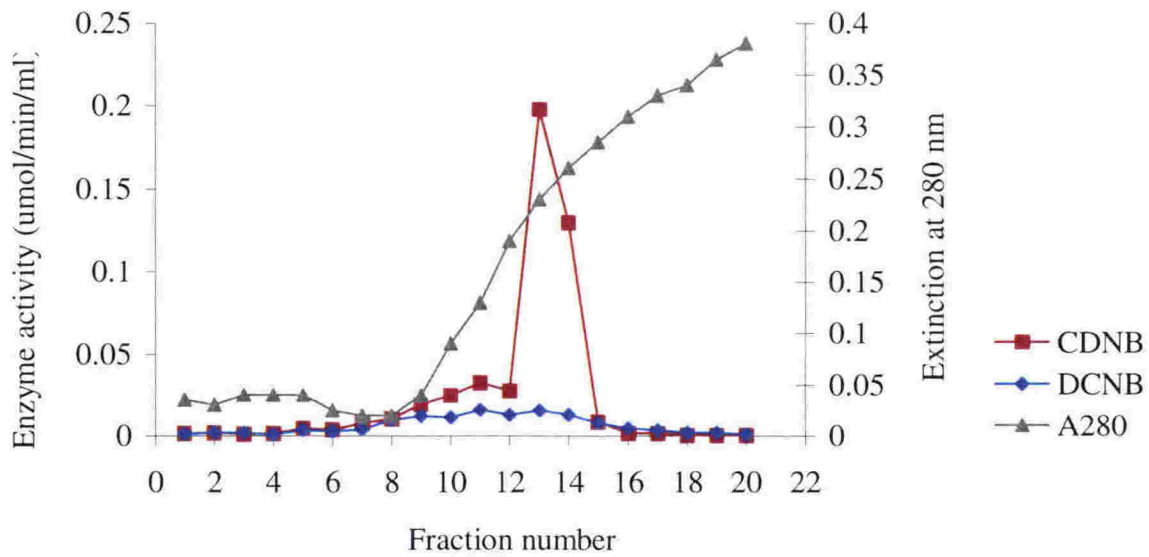


Figure 2.3.8 BSP-GSH-Sepharose affinity chromatography of glutathione *S*-transferases from *Lucilia cuprina* larvae. Activity peaks only are presented following elution of the column with 2 m*M* BSP in 20 m*M* NaH_2PO_4, pH 7.0. Enzyme activities were measured using DCNB and CDNB as substrates. Active fractions were pooled and proteins separated to their isoelectric points, results of which are shown below.

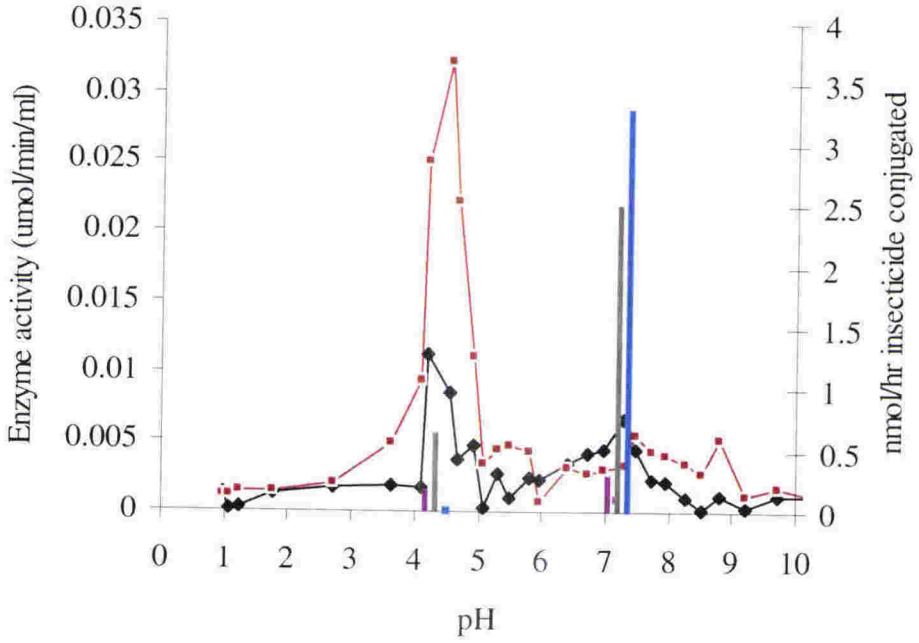


Figure 2.3.9 Isoelectric focussing of glutathione *S*-transferases partly purified by BSP-GSH affinity chromotography. Enzyme activity was measured using —◆— DCNB and —■— CDNB as substrates. Active fractions were pooled; fraction 1 pooled fractions 28-31 (pH 6.73 – 7.47) and fraction 2 pooled fractions 38 – 41 (pH 4.21 – 4.91). Radioactive insecticides conjugated by *Lucilia cuprina* glutathione *S*-transferase are shown as histograms (▪ [^{14}C] methyl parathion, ▪ [^{14}C] diazinon and ▪ lindane). Histograms are displaced for comparative purposes but represent fraction I pH 4.34 and fraction II pH 7.03.

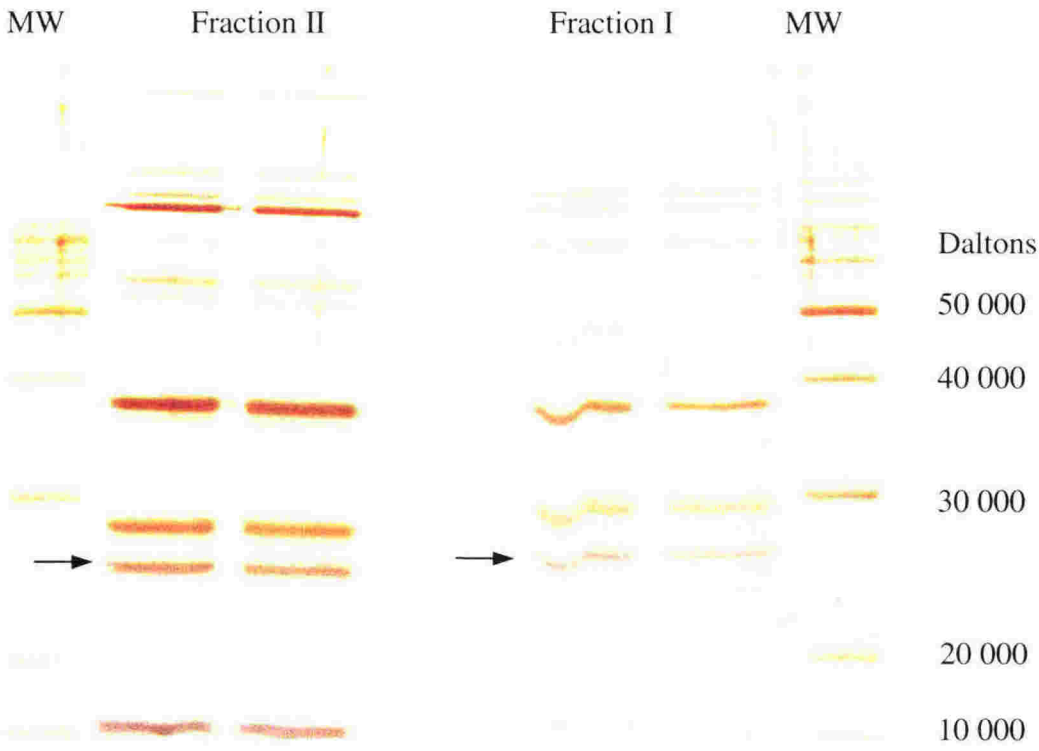


Figure 2.3.10 SDS PAGE of partially purified glutathione *S*-transferases from OP-resistant *L. cuprina* larvae prepared by affinity chromatography and isoelectric focussing. ➞Indicates band of interest. Fraction 2 has a subunit molecular weight of 24 591 and fraction 1 of 25 667.

Table 2.3.2 Radioactive pesticide conjugated by partially purified *L. cuprina* GSH *S*-transferases.

Insecticide/ Fraction	nmol/hr insecticide conjugated by GST
[^{14}C] Diazinon	
Fraction I	1.5
Fraction II	0.1
[^{14}C] Lindane	
Fraction I	15.3
Fraction II	3.8
[^{14}C] Methyl Parathion	
Fraction I	20.2
Fraction II	0

Discussion

GSH S-transferase activities

Clear differences between species and life stages have emerged from this study of GST activity and diazinon resistance in *L. cuprina* and *L. sericata*. Adult and larval strains of *L. sericata* showed no relationship between level of GST conjugating activity and resistance to diazinon, suggesting that these enzymes do not constitute a mechanism of OP-resistance in this species. Contrasting this was the observation of a strong relationship between the level of GST conjugating activity with respect to model substrate DCNB, and resistance to diazinon in both adult and larval strains of *L. cuprina* particularly when environmental factors were taken into account.

Many studies of resistant insects have shown increases in GST activities towards both CDNB and DCNB compared with susceptible strains (Reidy *et al.* 1990, Yu 1992, Yu & Nguyen 1992, Laecke 1995, Spencer *et al.* 1998) while others show increases in activity towards DCNB only (Prapanthedara *et al.* 1995, Picollo de Villar *et al.* 1990).

The relationship between GST activity and DDT resistance in the mosquito, *Anopheles gambiae* (Prapanthedara *et al.* 1995) and malathion resistance in the reduviid bugs, *Rhodnius prolixus* and *Triatoma infestans* (Picollo de Villar *et al.* 1990) for instance, show a specific relationship with activity towards DCNB, as in this study. Results of this study are consistent with these later examples and with those seen in other dipteran species (Dauterman & Hodgson 1978).

One possible mechanism that might bring about this relationship is induction. Increased insecticide pressure might increase GST activity in resistant strains of blowflies. Clear differences in inducibility of GST activity between susceptible and resistant strains of housefly have been shown with phenobarbital and various pesticides (Hayaoka & Dauterman 1982). Induction of GST activity is shown to be associated with the production of different forms of GST in resistant strains rather than increases in enzyme present in susceptible strains of housefly (Ottea & Plapp 1984, Hayaoka & Dauterman 1983). In contrast, induction of GSTs by phenobarbital in *L. cuprina* increased production of enzyme forms already present rather than inducing new or altered forms (Kotze & Rose 1987). Induction is normally a short-term event. For example, Hayaoka & Dauterman (1982) showed that the maximum amount of GST activity occurred at 96 hours after phenobarbital treatment in strains of housefly after which it decreased. With this in mind it is unlikely that increases in GSTs due to induction during periods of high insecticide pressure might continue to be high through to the F_1 laboratory generation used in this study. It seems more plausible that in populations where GSTs confer protection against OP-insecticides, individuals producing larger quantities of these enzymes have been selected for.

The contribution of season as a factor influencing resistance is a new observation for *L. cuprina*. The significant relationship between DCNB conjugating activity and resistance to diazinon in *L. cuprina* suggests that season affects GST activities with respect to DCNB. The extent of the interaction between GSTs and seasonal factors cannot be predicted from this model however, due to the limited sample size ($n > 35$) (B. Dawkins personal communication). It would appear from a comparison of linear regression and ANOVA, that seasonal effects might obscure the relationship of GST activity with resistance in *L. cuprina* adults compared with larvae. This is evidenced by the change in level of significance when seasonal influences are not considered in analysis (linear regression) (Figure 2.3.5, larvae $p = 0.001$, Figure 2.3.6, adults $p = 0.12$) compared with the ANOVA model presented in Appendix 2.3.2 (larvae $p = 0.001$, adults $p = 0.008$). It can be speculated that seasonal factors may exert more influence over adults than larvae as they experience a broader range of climatic conditions compared with larval instars that live entirely within the microclimate of the sheep fleece until late in the third instar. The consistent nature of regional influence with respect to *L. sericata* is also an interesting observation but interactive effects with GST activities are questionable. Regional influences act independently of level of GST activity as they were shown to influence the level of diazinon resistance in toxicological results (Table 1.1.10). Region may also act independently of GST activity towards DCNB with *L. cuprina* adults. The statistical interaction of these factors with enzyme activity may result from an increase in insecticide pressure within a region or during seasons due to changes in blowfly activity (see Chapter 1.1 discussion).

The other observation worthy of note in this study has been the distinct differences

between larval and adult DCNB activities. Larvae of both species showed on average 2.5 times more DCNB activity per mg protein, than adults. Differences between larval and adult CDNB activities were less pronounced. This is consistent with the results of Kotze & Rose (1987) who reported an increase in glutathione conjugating activity (conjugate/min/individual), with both CDNB and DCNB, throughout the pupal stage of *L.cuprina.* This then declined after emergence. Activities stabilized at a constant low level from 6 – 7 days after emergence (Kotze & Rose 1987). *Lucilia* adults used in the present study were 3 – 6 days old. The age of individuals used in this study may account for some unexplained error (Appendix 2.3.1).

This study also highlights striking differences in GST activities between species. *Lucilia sericata* showed twice the GST activity towards DCNB of *L. cuprina* (Table 2.3.1). This may relate to differences in ecological niche of the two species. *Lucilia cuprina* targets sheep as its primary host, whereas *L. sericata* is less specific and is frequently reported to occur on carcasses of numerous species (Dymock & Forgie 1993, Bishop *et al.* 1996). *Lucilia sericata* is therefore likely to be bombarded with an array of low molecular weight decomposition products, which the GSTs will provide protection against. Higher levels of GSTs may therefore serve a generalized protective function as an adaptation for lifestyle in *L. sericata.*

A significant relationship between adult and larval DCNB activities in both species was observed despite the lower activity levels occurring in adults. This relationship was not found with activity towards CDNB in larvae and adults. This is consistent with the presence of multiple GSTs with overlapping cross-specificities in both species. Toung *et al.* (1993) for example, have shown that in *D. melanogaster*,

members of a single gene family of GSTs may be expressed differentially during different developmental stages and may respond differently to inducing agents. Glutathione *S*-transferase activity towards CDNB may be unrelated in adults and larvae due to such differential expression of isoenzymes during life stages. The relationship of CDNB activity to insecticide resistance has been deemed to be less important than that of DCNB in this experiment and in other dipterans (Dauterman & Hodgson 1978). This suggests that isoenzymes with activity only towards CDNB serve an alternate function. This function may involve detoxification of various endogenous compounds rather than exogenous xenobiotics. The importance of endogenous toxins may change during life stages due to certain biosynthetic activities involved in metamorphosis (Mills & Lang 1976, Hazelton & Lang 1983).

OP-conjugating properties of L. cuprina glutathione S-transferases

Studies of purified housefly GSTs, show that there are at least two classes of isoenzymes, those with activity towards CDNB but not DCNB, and those with activity towards both CDNB and DCNB. In general, it is the latter of the two classes that have shown insecticide-conjugating properties (Clark *et al.* 1986). Partial purification of glutathione *S*-transferase enzymes from *L. cuprina* larvae in this study, using affinity chromatography and isoelectric focussing techniques has also revealed two distinct fractions. This supported a similar study by Kotze & Rose (1989). Kotze & Rose (1987) identified two forms of GST in *L. cuprina* larvae based on substrate specificities. Purification of fractions in this study however, revealed a number of contaminating proteins after silver staining of SDS PAGE (Figure 2.3.10). Kotze & Rose (1989) determined these GSTs to have sub-unit molecular weights of 25 500 (fraction 1) and 24 500 (fraction 2) when subjected to SDS-PAGE and 45 000 (FI)

and 44 000 (FI) when molecular weights of these enzymes were measured using gel filtration. Two bands visualised on SDS-PAGE in this study showed similar sub-unit molecular weights as those purified by Kotze & Rose (1989). These bands were therefore assumed to be the proteins of interest having molecular weights of 24 600 (Fraction I) and 25 700 (Fraction II). Fraction I from this study also showed a pH between 6.73 and 7.47 (pooled = 7.03) which was close to that determined by Kotze & Rose (1989). Fraction II was similarly comparable with that described by Kotze & Rose (1989) who showed peaks between pH 4.6 and 5.2, where this study showed a range of 4.21 – 4.91 (pooled = 4.34) after isoelectric focussing.

Fraction I in this study showed activity towards DCNB (0.105 µmol substrate conjugated/min/ml of sample) and low activity towards CDNB (0.040 µmol substrate conjugated/min/ml of sample). This result was qualitatively similar to that of Kotze & Rose (1989) who showed that, as with houseflies, this form had substantial activity towards CDNB (7.28 µmol substrate conjugated/min total) and DCNB (19.1µmol substrate conjugated/min). Higher activity of fraction II towards CDNB compared with fraction I shown in this study was consistent with Kotze & Rose (1989).

This study shows direct evidence that Fraction I, which showed high activity towards DCNB and lesser activity towards CDNB, is involved in the conjugation of OP-insecticides by *L. cuprina* GSTs. The higher quantity (nmol/min/ml) of [^{14}C] methyl parathion conjugated compared with either [^{14}C] lindane or [^{14}C] diazinon is likely to be consistent with comparable studies on GSTs from *M. domestica* (Clark *et al.* 1984).

Conclusions

That glutathione *S*-transferases appear to contribute to organophosphate resistance in field strains of *L. cuprina* has been a new observation. A contribution of GSTs to OP- resistance in *L. cuprina* was however, thought to be likely by Kotze & Rose (1987). The observed relationship between DCNB activity and expression of resistance and the apparent lack of relationship between CDNB activity and resistance in *L. cuprina* has also suggested that this substrate specificity/ insecticide conjugating relationship is the same as that occurring in houseflies. DCNB is therefore a more appropriate model substrate than CDNB for determining the insecticide detoxification capabilities of insect GSTs in this study. This observation was also extended to verify that specific isoenzyme(s) are involved in the conjugation of OP insecticides.

In the absence of a molecular investigation of these specific isoenzymes, ongoing occasional monitoring of GST activities may be of value to assess the stability of this mechanism in resistant *L. cuprina* populations. Similarly, continued monitoring would be useful to determine the possible development of this mechanism in strains of *L. sericata* with continued insecticidal pressure and inter-species competition.

CHAPTER 2.4

The role of non-oxidative metabolism in organophosphate resistance in strains of *Lucilia cuprina* and *Lucilia sericata*

III. Acetylcholinesterases

Introduction

Cholinesterase is an enzyme critical to the proper functioning of the central nervous system (CNS) of both vertebrates and invertebrates. Certain insecticides such as the organophosphates (OPs) and carbamates act by inhibiting cholinesterase activity.

In cholinergic neurons, the nerve fibres are stimulated by firing of signals across synapses which are transmitted by acetylcholine (ACh). The signals are terminated by enzymatic hydrolysis by acetylcholinesterase (AChE) (Figure 2.4.1). *In vitro*, AChE may also be hydrolysed by butyrylcholinesterase, which prefers other types of esters such as butyrylcholine and is generally thought to be a non-specific or pseudo-cholinesterase. BChE is also considered to be an 'aliesterase' as its butryl group is more aliphatic than the acetyl group of acetylcholine. The function and properties of the AChE are thought to be similar in insects and mammals. BChE is not thought to exist in insects however (O'Brien 1976), although a BChE-like enzyme has been found in the green leafhopper, *Nephotettix cincticeps* (Hama 1978). Due to the aliesterase nature of BChE and lack of evidence for its existence in insects only the function of AChE will be discussed. AChE therefore functions to break down acetylcholine hydrolytically resulting in its removal from the nerve junctions.

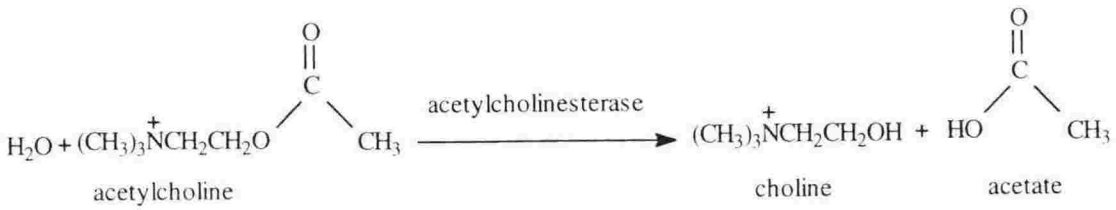


Figure 2.4.1 Hydrolysis of acetylcholine by acetylcholinesterase

The surface of the acetylcholinesterase molecule or active site contains four domains; an esteratic- and an anionic subsite (Nachmansohn & Wilson 1951), an additional peripheral anionic subsite lining the active site gorge entrance and an acyl pocket that binds the acetyl group of the substrate. The negative charge of the anionic site is able to attract the quaternary nitrogen of acetylcholine. The peripheral anionic subsite aids the transport of the ACh down to the catalytic site. The esteratic site comprises a histidine residue, a serine and glutamic acid in a catalytic triad. The key role of the serine hydroxyl is thought to be the formation of a covalent bond with the carbonyl carbon of the acetylcholine that is essential to the hydrolysis of AChE. Electrostatic charges guide positively charged ACh molecules down the active site gorge to be degraded at a catalytic centre (Baille & Wright 1984, Sattelle 1984). Detailed reviews of the structure and function of the AChE from the electric ray, *Torpedo californica* are described by Quinn (1987) and Sussman *et al.* (1991). There is a lack of similar information on insect AChE.

If a cholinesterase inhibiting pesticide such as an OP or a carbamate is present, acetylcholine can no longer be broken down by acetylcholinesterase. OP and carbamate pesticides react with the active site serine to form phosphoryl-acetylcholinesterase intermediates which prevent acetylcholinesterases from terminating neurotransmission at the cholinergic junctions. In effect the insecticide

has the ability to act as a competitive antagonist of AChE. Acetylcholine begins to accumulate in neuromuscular junctions, autonomic ganglia and cholinergic synapses in the CNS. Electrical impulses will continuously fire resulting in hyperexcitation of the CNS, causing twitching, convulsions, paralysis and eventually death (Figure 2.4.2) (Eto 1974, Hutson & Roberts 1985).

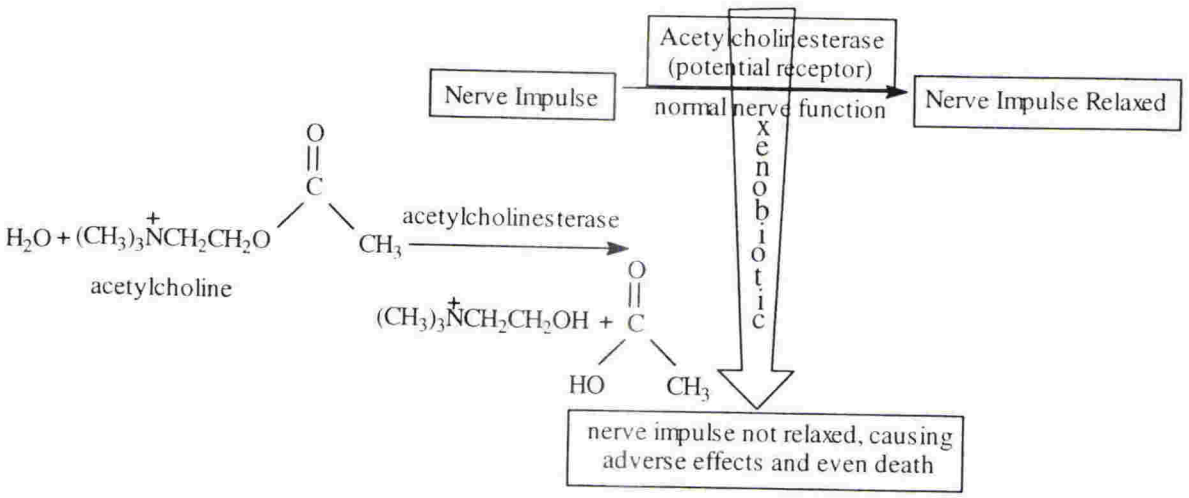


Figure 2.4.2 Inhibition of acetylcholinesterase by a xenobiotic

A reduction in the sensitivity of AChE to OPs as a mechanism of resistance was first recognised in the spider mite, *Tetranychus urticae*, by Smissaert (1964). AChE of resistant strains was less sensitive to inhibition by the OPs, paraoxon and diazoxon, than a susceptible strain. Since this first report there have been numerous reports of a reduced sensitivity of AChE as a contributor to pesticide resistance. Examples of insects possessing this resistance mechanism include strains of the house fly, *Musca domestica*, with resistance to OPs and carbamates (Tripathi 1976, Plapp & Tripathi 1978, Ugaki *et al.* 1981), the oriental housefly, *M. domestica vicina*, with resistance to paraoxon and trichlorfon (Li *et al.* 1987), the mosquitoes *Anopheles albimanus*, with resistance to paraoxon and proxur (Ayad & Georghiou 1975) and *Culex*

tritaeniorhynchus to paraoxon (Takahashi & Yasutomi 1987). Other species with a degree of AChE insensitivity to various insecticides include the rice pest, *Nephotettix cincticeps* (Hama 1980, Hama *et al.* 1980, Heong *et al.* 1982, Miyata *et al.* 1981), the cotton pest, *Spodoptera littoralis*, in Egypt (Dittrich *et al.* 1979), the brown planthopper, *Nilaparvata lugans* (Chung & Sun 1981), cotton aphids, *Aphis gossypii*, in China (Sun *et al.* 1987), strains of the sweet potato whitefly, *Bemisia tabaci* (Dittrich *et al.* 1990) and the predacious mite, *Amblyseius potentillae* (Anber & Overmeer 1988).

The reduced sensitivity of AChE is thought to be a result of a structural alteration of the active site of the enzyme. Hama *et al.* (1980) purified two types of AChE in *N. cincticeps*; a normal AChE found in the susceptible strain and an altered AChE found in a resistant strain. The altered form of AChE had a reduced affinity for the insecticide inhibitor which therefore rendered it less sensitive (Tripathi 1976, Yamamoto *et al.* 1977). Ugaki *et al.* (1981) proposed the occurrence of multiple forms of insensitive AChE in Japanese strains of *M. domestica*. This was later confirmed by Devonshire & Moores (1984), who provided evidence of at least three insensitive forms in strains of *M. domestica* from the United Kingdom.

Fournier *et al.* (1992) demonstrated qualitative differences in AChE from *Drosophilia melanogaster* strains. They found a single point mutation when comparing a wild-type resistant and a wild-type susceptible strain. The single nucleotide difference between susceptible and resistant strains resulted in the replacement of a phenylalanine by a tyrosine in the AChE protein, rendering it less sensitive to insecticide inhibition (Fournier *et al.* 1992). Fournier *et al.* (1992) also correlated

resistance in *D. melanogaster* with increases in the amount of AChE in the insect's CNS. The level of resistance conferred by this mechanism and the amount of acetylcholinesterase that was able to be tolerated before synaptic transmission was disrupted was undetermined however (Fournier *et al* 1992).

This chapter gives an account of an examination of acetylcholinesterase activity in a laboratory susceptible and field resistant strains of *L. cuprina* and *L. sericata* in relation to the substrate aceylthiocholine iodide and inhibition by the organophosphate, tetrachlorvinphos. No attempt has been made to identify differing forms of AChE.

Materials and Methods

Chemicals

Acetylthiocholine iodide and 2,2'-dithio*bis*(5-nitrobenzoic) acid were obtained from Sigma Chemical Co., St. Louis, Missouri and tetrachlorvinphos (Gardona®) was a gift from Dr A. G. Clark. Details of other chemicals can be found in Appendix A.

Acetylcholinesterase Assay

Twelve fly heads per field strain were individually sonicated in 500 µl of ice-cold 0.05 *M* phosphate buffer, pH 8.0, containing 10% v/v glycerol, 1% w/v bovine serum albumin, 1 m*M* EDTA, 5 m*M* ascorbic acid, and 5% w/w (of insects) polyvinylpolypyrrolidone in 1.5 ml plastic centrifuge tubes. In the case of larvae, the heads were homogenised in batches of five in the above buffer. Homogenates were centrifuged at 10,000 rpm for 10 minutes at 4°C. Aliquots of 200 µl were used for the determination of rates of hydrolysis of acetylthiocholine iodide in 0.1 *M* sodium

phosphate buffer, pH 8.0, at 25°C, using dithio*bis*nitrobenzoic to detect the release of product (Ellman *et al.* 1961). Rates of enzymatic release of thiocholine, in the presence and absence of tetrachlorvinphos (170 μ*M*), were measured at 1-minute intervals for a period of 10 minutes. Measurements were made with a Phillips' Pye Unicam PU 8630 kinetics' spectrophotometer. Total cholinesterase activity at time zero and an apparent first-order rate constant for the inhibition of the enzyme were calculated from a least squares regression on these data. The concentration of tetrachlorvinphos giving an appropriate rate of inhibition had been established in preliminary experiments to give greater than 90% inactivation of the enzyme preparations within a 10-minute period.

Statistical Analyses

Analysis of variance (ANOVA) methods in S-Plus (Everitt 1994) were performed using total cholinesterase activity (E_0) and pseudo-first order rate constants (Ki) as predictors of diazinon resistance for larval and adult strains of *L. cuprina* and *L. sericata.* The influence of region and season was also considered in the model.

The relationship between larval and adult total cholinesterase activities (E_0) and pseudo-first order rate constants (Ki) were determined using regression analysis in Microsoft Excel®.

Results

Total acetylcholinesterase activities and pseudo-first-order rate constants governing the inactivation of cholinesterase activity are presented in Appendix 2.4.1 (Tables 1 – 4) for adult and larval strains of *L. cuprina* and *L. sericata.*

Lucilia cuprina

The analysis of variance model showed that total cholinesterase activities (E_0) did not predict resistance to diazinon in adult and larval strains of *L. cuprina*. There was however, strong seasonal influence in the model ($p < 0.01$) (Appendix 2.4.2). Pseudo-first-order rate constants (Ki) were a significant predictor of diazinon resistance in adult strains ($p = 0.03$) although this was not the case with larvae ($p = 0.23$) in the ANOVA model (Appendix 2.4.2). Season again showed significant influence in the ANOVA model ($p < 0.01$) (Appendix 2.4.2). Figure 2.4.3 shows the relationship between diazinon LC_{50} and pseudo-first-order rate constant (Ki) in adult strains of *L. cuprina* without correction for seasonal and regional influences.

Plots of declining catalytic activity due to inactivation of AChE by tetrachlorvinphos are shown in figures 2.4.5 (*L. sericata*, adults and larvae) and 2.4.6 (*L. cuprina*, adults and larvae). The difference in scale suppresses the exponential nature of the curves in the case of the larval experiments.

Lucilia sericata

Total cholinesterase activities (E_0) and pseudo-first-order rate constants (Ki) did not predict diazinon resistance in either adult or larval strains of *L. sericata*. Region however showed significant influence in all *L. sericata* models (Appendix 2.4.2).

Adult vs larval activities

In general total cholinesterase activities and pseudo-first-order rate constants for inhibition in adult *L. cuprina* and *L. sericata* strains were greater than that obtained

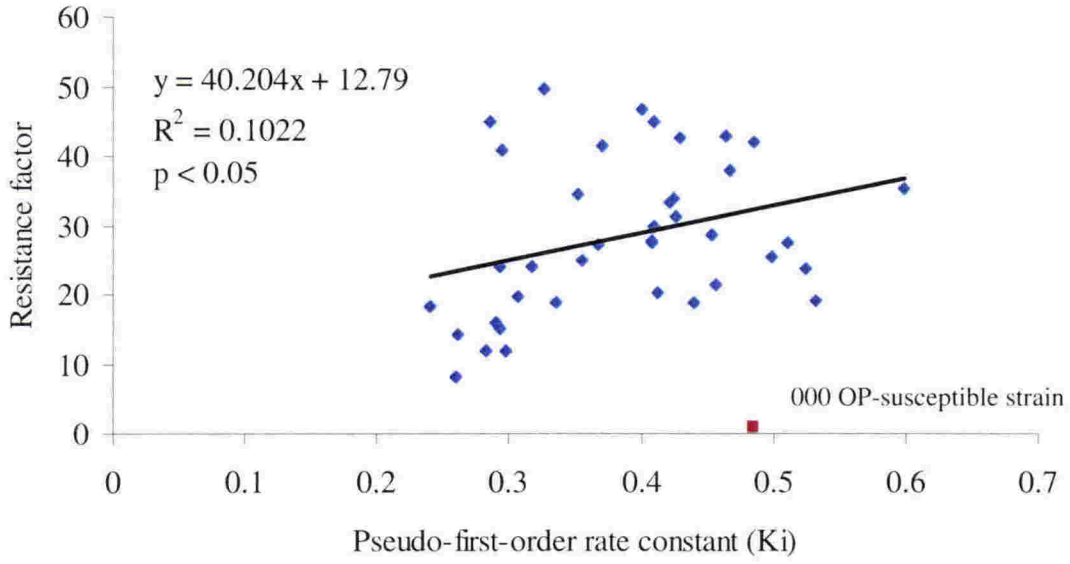


Figure 2.4.3 Relationship between pseudo-first-order rate constants and resistance to diazinon in adult strains of *Lucilia cuprina*.

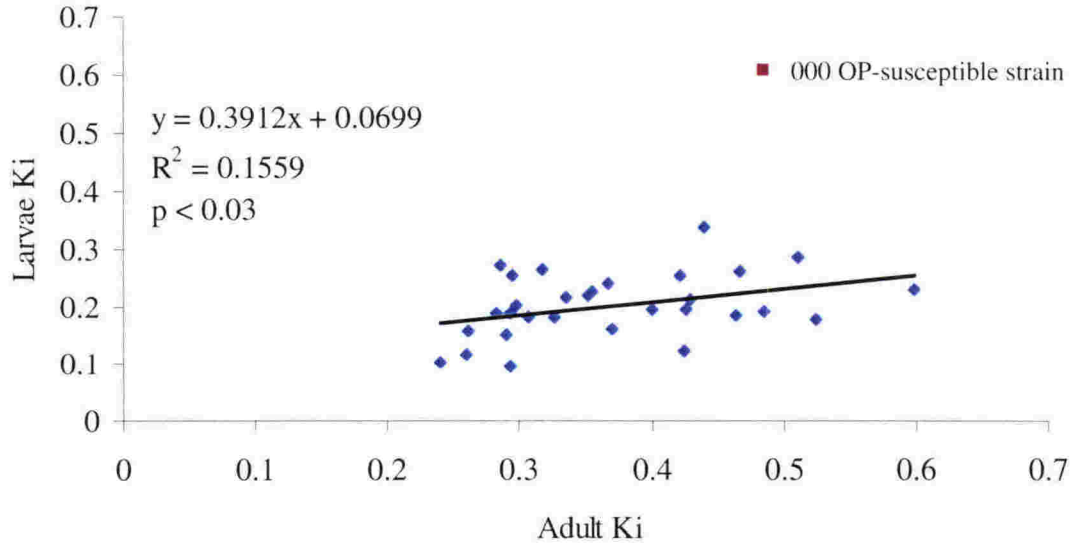


Figure 2.4.4 Relationship between pseudo-first-order rate constants in adult and larval strains of *Lucilia cuprina*.

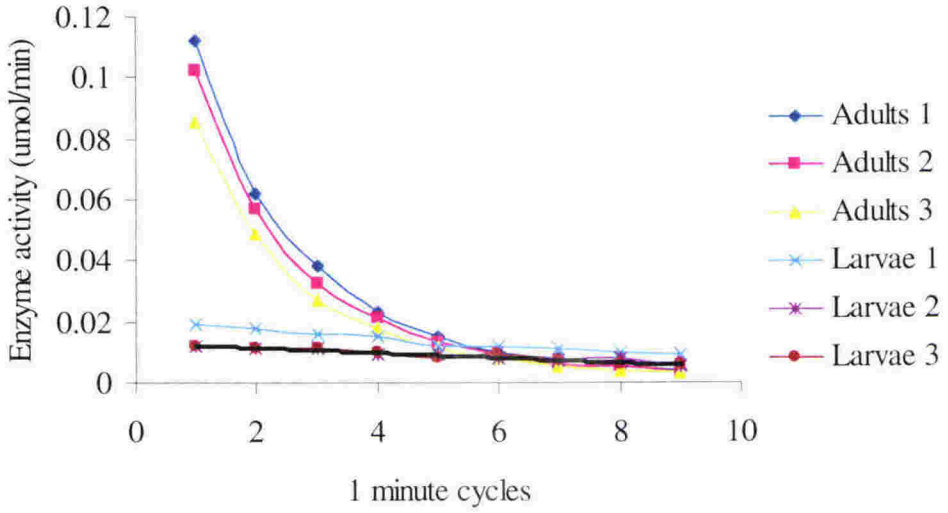


Figure 2.4.5 Plot of catalytic activity of acetylcholinesterase in larvae and adults of a strain (052, Hakataramea, 9 January 1996, RF = 7.0) of *L. sericata* over time and in the presence of tetrachlorvinphos.

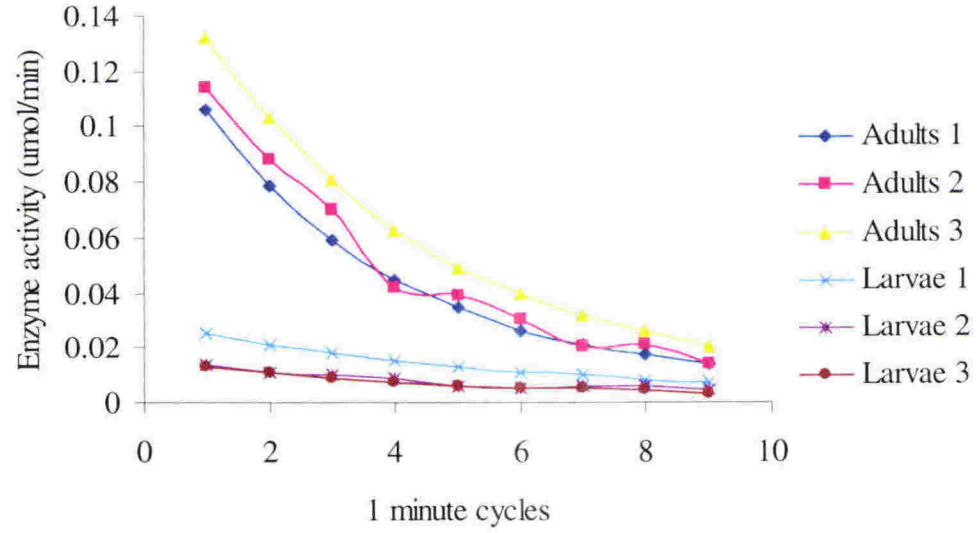


Figure 2.4.6 Plot of catalytic activity of acetylcholinesterase in larvae and adults of a strain (35, Levin, 11 April 1994, RF = 14.2) of *L. cuprina* over time and in the presence of tetrachlorvinphos.

for their corresponding larval samples (Appendix 2.4.1). Regression analysis showed that total cholinesterase activities were unrelated in adult and larval samples (Appendix 2.4.3). Adult and larval pseudo-first order rate constants were poorly related in strains of *L. sericata* ($p = 0.11$) but strongly related in strains of *L. cuprina* ($p = 0.04$) (Figure 2.4.4) (Appendix 2.4.3).

Discusssion

Neither an increase in acetylcholinesterase content nor an insensitive AChE in the central nervous system of larvae of *L. cuprina* and larvae or adult *L. sericata* appear to have been selected for in the resistant field strains examined. This result confirms that of Schuntner & Roulston (1968) who determined that AChE from *L. cuprina* fly heads showed no differences in sensitivity between susceptible and resistant strains. In contrast, however, they determined that a thoracic AChE in a resistant strain of *L. cuprina* showed a reduced sensitivity to diazoxon (Schuntner & Roulston 1966).

Thoracic AChE was not tested in this study as it was assumed the greatest concentration of all isoenzymes of AChE is located in the brain which integrates the activities of the central nervous system (Chapman 1972). Results of this study show total cholinesterase activity was high with respect to adult heads but greatly reduced in larval forms. Dissections of larvae in this study were sufficiently generous to include the head and metathorax, the latter of which is known to contain the brain in species of calliphorids (Richards & Davies 1977). Developmental increases in AChE activity from larvae through to adults have been reported in numerous insect species (Smallman & Mansingh 1969). It is therefore not surprising that such differences between life stages in these two *Lucilia* species has been shown. The weak

correlation ($p = 0.11$) between adult and larval strains of *L. sericata* is puzzling however, when compared to that found in *L. cuprina* ($p = 0.04$) (Appendix 2.4.3). This may be a function of inconsistencies in insect size due to food availability during larval rearing although there is no evidence to support this, or it may reflect differences in developmental variation in isoenzyme composition.

There was one observation worthy of note; the relationship between resistance to diazinon and susceptibility of AChE inactivation by tetrachlorvinphos in *L. cuprina* adults using the ANOVA model ($p = 0.03$ Appendix 2.4.2). Examination of this relationship by linear regression (Figure 2.4.3) shows that this relationship, in the absence of environmental factors, does not support the development of an acetylcholinesterase insensitive to inhibition by OPs. Instead it shows an enhanced rate of inactivation by OPs. This is entirely counter-intuitive and makes little mechanistic sense. It is also puzzling that this relationship occurs in adults and not in larvae as there was a just significant relationship observed between adult and larval pseudo-first-order rate constants (Figure 2.4.4). It can be speculated that developmental changes associated with metamorphosis (Richards & Davies 1977) produce different isoforms of AChE. A study of adult and stage 3 and 4 larvae of the cattle lungworm, *Dictyocaulus viviparus*, revealed that various isoforms of AChEs were only produced in later stages of the parasite (McKeand *et al.* 1994). Unfortunately no examples from insects could be found as most emphasise only adult AChE.

It would also be useful to test more than one oxon in this assay. The inhibitory power of an organophosphate is dependent on its affinity for the active site of the

acetylcholinesterase in addition to the rate of phosphorylation (Main 1964). The structure of an OP may determine its affinity for the active site. Tetrachlorvinphos is not a registered flystrike preventative but is similar in structure to chlorfenvinphos that is employed in a commercially available dip formulation. Good inhibitors of AChE are normally characterised by a higher pseudo-first-order rate constant (or bimolecular rate constant, K_i value) than a poor inhibitor. Testing more than one inhibitor against insect strains would therefore provide useful information for determining an 'insensitivity profile' for different organophosphates (Moores *et al.* 1988). This profile can ultimately aid in choice of control chemical. It can also provide insight into allelic variation of AChE in field strains (Moores *et al.* 1988) where relevant. This assumes however, that the target site of OPs is indeed the AChEs. There is evidence from human studies to suggest that the acute toxicity of some OP agents may not be due to the overloading of acetylcholine but rather to actions at sites other than AChE (Albuquerque 1984). Newcomb *et al.* (1997) also postulates that the target site of OPs in *L. cuprina* is not AChE but rather another esterase (E_3). An AChE 'insensitivity profile' may therefore be rendered irrelevant.

Influence of environmental factors on resistance

Analysis of variance (ANOVA) (Appendix 2.5.2 (b)) suggests that, if the effect of season is taken into account then the significance of the value of Ki as an indicator of resistance is increased compared with that in the simple linear regression (Figure 2.4.3). The clear distinction in the contribution of region as a factor influencing resistance in *L. sericata* and season with respect to *L. cuprina* arising from this study, differed from that observed from toxicological studies (Chapter 1.1). Toxicological results showed that region had a profound influence on expression of resistance in

both species. It is uncertain exactly why the major influencing factor should differ between species or do these factors interact with the values of total acetylcholinesterase activities or of pseudo-first-order rate constants? A similar discrepancy was observed with GST activities (Chapter 2.3) and may relate to both insecticide usage patterns (see Chapter 1.1) and ecological characteristics of each species, in particular differences in dispersion of the two species. Gleeson & Heath (1997) suggested that tendency for dispersal of *L. cuprina* was low when habitat conditions were favourable and that a key path of migration might be the transport of flystruck sheep by stock vehicles. The dominant habitat requirement for *L. cuprina* is the availability of sheep since they are reliant on a live host for their parasitic lifestyle. In addition to the facultative use of a live host, *L. sericata* is also able to utilise carrion as both a protein source for oocyte development and as an oviposition site. It is less likely that *L. sericata* needs to migrate to more favourable areas. This is because it is not naturally compelled to rely on live sheep for breeding purposes. This arises possibly from a high level of genetic heterogeneity, a consequence of restricted gene flow and localised distinct gene pools. It is this feature of *L. sericata* that may in part, account for the influence of region on level of resistance. In contrast, the *L. cuprina* founding population is unknown but is likely to be small given the findings of Gleeson (1995) which showed a high level of homogeneity even in geographically separated populations. This, coupled with the rapid dispersal of *L. cuprina* throughout New Zealand after its introduction may have reduced the likelihood of selection pressure arising from insecticide use and from leaving a legacy of regionally distinct phenotypes. RAPD PCR of several strains of *L. cuprina* as discussed in a later chapter (3), confirms that there was a high degree of similarity between strains of *L. cuprina* with few area clusterings limited to Blenheim and other

South Island strains. A similar RAPD-PCR study of *L. sericata* is needed to determine whether there are similar differences in this species with respect to area or regional groupings.

Seasonal factors may offer an opportunity for variations to arise based on generational times and numbers and physiological processes in general. *Lucilia cuprina* has traditionally been recognised as inhabiting warm, dry areas of Asia, Africa and Australia compared with its temperate counterpart, *L. sericata* (Zumpt 1965). The temperate New Zealand climate may restrict the length of time suitable for breeding in *L. cuprina* compared with *L. sericata* and therefore the number of generations per season in which genetic drift might permit fixation of some alleles or resistance phenotypes. Reduced opportunity for breeding may be further compounded by differences in climate between distant regions such as Northland and Southland although analysis suggests that regional influence is weak (e.g. p values for region ranged from 0.07 – 0.18 for adult and larval *L. cuprina*) (Appendix 2.4.2). Unfortunately the ANOVA model in which the cholinesterase parameters were included does not provide detailed information about the influence of individual regions on resistance or enzyme levels.

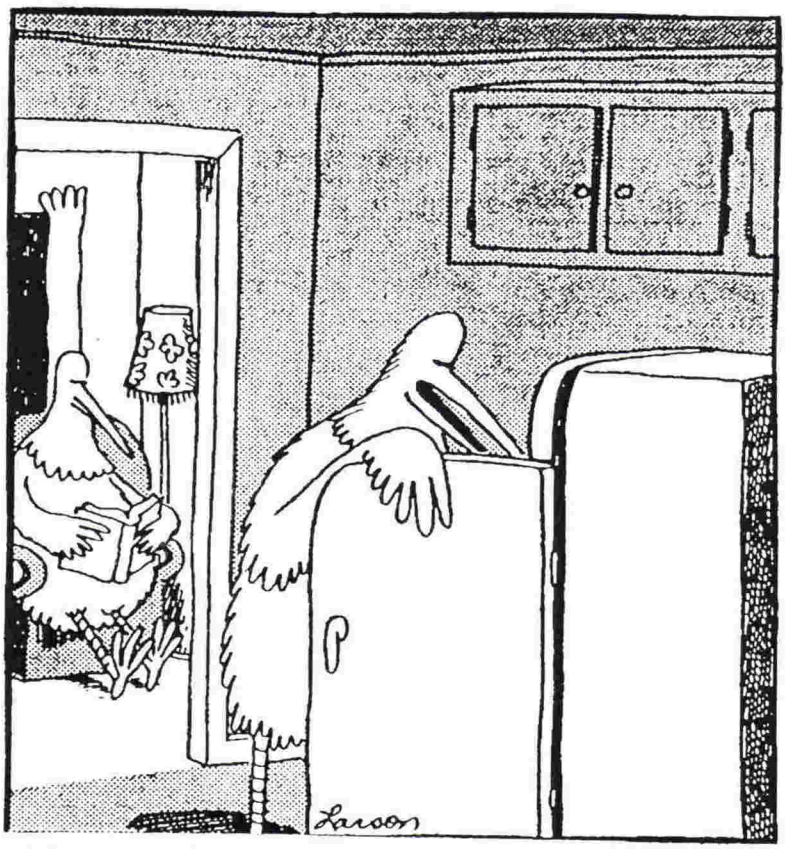
Another striking difference between the two species that might help to explain the influence of season on resistance is the ability of *L. sericata* to enter diapause at both low and high temperatures compared with *L. cuprina* which does not. *Lucilia cuprina* instead undergoes developmental arrest or overwintering which acts as a dormancy mechanism and is typical of a sub-tropical species (Dallwitz & Wardhaugh 1975, Ash & Greenburg 1975). Dieldrin resistant *L. cuprina* were shown to be at a

selective disadvantage during overwintering compared with other times of year (McKenzie 1990). The same was true for diazinon-resistant phenotypes in the absence of a fitness modifier (McKenzie 1994). It is possible that individuals may be vulnerable to environmental selection pressure in the absence of a fitness modifier that could lead to heterogeneity in the population. A fitness modifier has evolved in Australian strains of the species (McKenzie 1994) which counteracts the effects of overwintering and it is likely that the same is true of New Zealand strains. Information on the effect of diapause on resistance in *L. sericata* is not available. Studies on the effect of diapause on deltamethrin and tebufenozide resistance in the codling moth, *Cydia pomonella* suggest however that resistance was stable during diapause (Bouvier *et al* 1998, Sauphanor *et al.* 1999). Despite the foregoing discussion, explanations for differences based on seasonal influence are necessarily speculative in the absence of experimental evidence of the effects of overwintering or diapause on resistance in these species. Both regional and seasonal effects might simply be a function of sample size and reflect the numbers and occurrence of the each species during certain months.

Conclusions

Neither an increase in acetylchloinesterase nor an insensitive AChE in the central nervous system of larvae of *L. cuprina* and larvae or adult *L. sericata* appear to have been selected for in the resistant field strains examined. Surprisingly, there did however appear to be a positive correlation between resistance to diazinon and susceptibility of AChE to inactivation by tetrachlorvinphos in *L. cuprina* adults. The significance of this factor is investigated further by multiple regression with other resistance mechanisms in chapter 2.6.

The Far Side By GARY LARSON



"Dang!...Who ate the heads off the blowflies?"

CHAPTER 2.5

The role of oxidative metabolism in organophosphate resistance in strains of *Lucilia cuprina* and *Lucilia sericata* determined by the synergist piperonyl butoxide

Introduction

The biochemical basis of insecticide resistance has been determined in a number of insect species by the use of synergists (Hughes 1982, Phabhaker *et al.* 1988, Scott *et al.* 1990, Osman *et al.* 1991, Ishaaya 1993). Synergists act by inhibiting specific metabolic pathways possibly involved in detoxification, which may have been altered or amplified in resistant species. The result of the inhibition is a more-than-additive increase in the toxicity of an insecticide when used in combination with the synergist (Price 1991). Certain synergists are known to act on specific metabolic pathways involved in detoxification. For example, suppression of resistance by *S,S,S*-tributylphosphorotrithioate (TBPT or DEF) is an indicator of hydrolytic action by esterases (Plapp & Tong 1966). Synergism by Sesamex® or by piperonyl butoxide (PBO) may indicate the involvement of monooxygenase detoxification enzymes. These enzymes are involved in a multitude of oxidative detoxification reactions (Kulkarni & Hodgson 1984) (some of which are described in the introduction of this section) and elevated activities are thus likely to be associated with the development of resistance. This study examines the effects of the monooxygenase inhibitor, PBO, on the toxicity of diazinon in *L. cuprina* as an approach to establishing the role of these enzymes in resistance in this species.

Materials and Methods

Insects

The OP-susceptible strain and field strains of *Lucilia cuprina* used in this study are the same as those described in Chapter 1.1.

Bioassay

Parallel toxicological tests were carried out on larvae of the same strain. One test acted as a control and provided an indication of resistance of the field population, the other measuring the effect of the synergist, PBO, on resistance.

In the synergist test ten 120 mm x 30 mm strips of Whatmann chromatography paper (3MM) were treated with 1000 ppm PBO (technical grade, 90%, obtained from Aldrich Chemical Company, Inc., USA) in acetone per test. Papers were rolled and placed into 50 mm x 10 mm glass vials and 200-300 first instar larvae applied to each tube. Each tube was provided with 1 ml of fortified (2% yeast extract and 0.5% monobasic potassium orthophosphate) sheep serum and stoppered with non-absorbent cotton wool. Larvae were incubated for 2 hours at 25°C after which larvae were removed by rinsing papers with distilled water onto a filter paper in a Buchner funnel. A vacuum was applied to remove excess water and then larvae were transferred to the toxicological test described by Levot (1990). The toxicological test was the same as that described in Chapter 1.1. As a further control, the toxicity of PBO alone was determined, in both the OP-susceptible laboratory strain and in the most resistant field strain (058, Takapau) as above, using concentrations of 1000, 2000, 5000, 10 000 mg/l of the compound. The

LC_{50} for diazinon was also determined for these two strains in the presence of the above four concentrations of piperonyl butoxide and in its absence.

Statistical Analysis

Dose response was analysed by probit regression (Finney 1971). A probit regression computer program was obtained from the Biological and Chemical Research Institute, Rydalmere, Australia, Sydney. Resistance factors (RFs) were calculated by dividing the LC_{50} (lethal concentration killing 50% of the sampled population) of the field strain by that of the susceptible strain. A synergist ratio was calculated by dividing the LC_{50} of larvae treated only with diazinon by the LC_{50} of larvae pre-treated with PBO. Unweighted linear regression was carried out using the data analysis package in Microsoft Excel™.

Results

Piperonyl butoxide toxicity

The toxicity of PBO alone was tested in the susceptible and the resistant 058 Takapau strains. Mortality was found to be negligible at the concentration that was employed for this study (0 and 3.2 % for susceptible and resistant strains respectively) (Appendix 2.5.1) compared with ten times this concentration (1000 mg/l or 10 mg/ml) where mortality rose to 12.6 and 22.1 % respectively. It was also noted that the concentration of PBO had little effect on the LC_{50} to diazinon in both strains. The mean LC_{50}s for diazinon over the range of PBO concentrations used was 0.13 ± 0.02 mg/l for the OP-susceptible strain and 1.36 ± 0.26 mg/l for the 058 Takapau resistance strain.

Diazinon toxicity

Toxicity data for 29 strains of *L. cuprina* larvae (including one OP-susceptible and two laboratory resistant strains) are presented in table 2.5.1. The LC_{50} for the susceptible strain, which was used in the calculation of all resistance factors, was determined in March 1992 and had been monitored at irregular periods since that time. The mean LC_{50} of the susceptible strain over this time was 0.059 ± 0.011. For the most resistant strain (058, Takapau) the mean LC_{50} determined between April 1995 and October 1997 was 2.35 ± 0.26 ($n = 3$). The toxicological characteristics of these strains, representing the extremes of resistance, remained essentially unchanged throughout this experiment.

Diazinon + piperonyl butoxide

Resistance factors in field strains, calculated from the LC_{50} data for diazinon, in the absence of PBO, ranged from 8.8 to 41.2 in strains from 034 Leeston (18.04.94) and 058 Takapau (04.02.98) respectively. There was a strong correlation between LC_{50} values obtained in the synergised and unsynergised tests ($r = 0.75$, $p < 0.0005$) and a linear regression was carried out between the two sets of data (Figure 2.5.1). The datum from the susceptible strain was excluded from the regression. The line of best fit had a gradient of 0.54 ± 0.12 and intercepted on the vertical axis at a value of 0.59 ± 0.14 mg/l. A significant linear relationship was also obtained between the LC_{99} values obtained in the presence and absence of the synergist.

Table 2.5.1 Effect of piperonyl butoxide on the toxicity of diazinon to first instar larvae from strains of *Lucilia cuprina*.

Location	Sample	Treatment[1]	LC_{50} (95% FL)[2] mg/l	LC_{99} (95% FL) mg/l	Slope(SE)[3]	RF[4]	SR[5]
000 Lab.	00.00.00	DZ	0.05 (0.05 - 0.06)	0.08 (0.07 - 0.09)	4.70 (0.46)	1.0	
000 Lab.	00.00.00	DZ + PBO	0.08 (0.07 - 0.09)	0.15 (0.11 - 0.21)	8.55 (2.05)	1.0	0.6
034 Leeston	18.04.94	DZ	0.44 (0.37 - 0.52)	1.32 (0.95 - 1.86)	4.83 (0.78)	8.8	
034 Leeston	18.04.94	DZ + PBO	0.87 (0.69 - 1.10)	3.43 (1.62 - 7.28)	3.92 (1.06)	10.9	0.5
159 Dorie	20.04.94	DZ	0.46 (0.36 - 0.59)	1.57 (0.99 - 2.49)	4.39 (0.97)	9.2	
159 Dorie	20.04.94	DZ + PBO	0.74 (0.66 - 0.83)	2.44 (1.88 - 3.17)	4.48 (0.50)	9.3	0.6
037 Levin	15.03.94	DZ	0.62 (0.55 - 0.70)	1.42 (1.10 - 1.83)	6.52 (1.05)	12.4	
037 Levin	15.03.94	DZ + PBO	1.14 (1.07 - 1.22)	2.88 (2.49 - 3.34)	5.77 (0.46)	14.3	0.5
038 Marton	20.04.94	DZ	0.66 (0.54 - 0.79)	1.91 (1.25 - 2.92)	5.01 (0.95)	13.2	
038 Marton	20.04.94	DZ + PBO	0.76 (0.61 - 0.95)	2.25 (1.37 - 3.71)	4.93 (1.17)	9.5	0.9
158 Ashburton	17.04.95	DZ	0.66 (0.61 - 0.72)	1.63 (1.36 - 1.94)	5.99 (0.41)	13.2	
158 Ashburton	17.04.95	DZ + PBO	1.01 (0.95 - 1.08)	2.08 (1.81 - 2.39)	7.45 (0.53)	12.6	0.9
035 Levin	11.04.94	DZ	0.68 (0.58 - 0.80)	2.03 (1.37 - 3.02)	4.89 (0.84)	13.6	
035 Levin	11.04.94	DZ + PBO	0.85 (0.71 - 1.02)	2.98 (2.00 - 4.45)	4.27 (0.68)	10.6	0.8
157 Ashburton	25.04.95	DZ	0.80 (0.67 - 0.94)	1.93 (1.28 - 2.90)	6.07 (1.37)	16.0	
157 Ashburton	25.04.95	DZ + PBO	1.11 (0.89 - 1.37)	3.95 (2.28 - 6.82)	4.21 (0.88)	13.9	0.7
027 Lab. L.	00.00.00	DZ	0.83 (0.73 - 0.94)	1.83 (1.33 - 2.52)	6.75 (1.36)	16.6	
027 Lab. L.	00.00.00	DZ + PBO	1.13 (0.98 - 1.30)	2.90 (1.93 - 4.34)	5.68 (1.16)	14.1	0.7
003 Turakina	20.02.95	DZ	0.87 (0.81 - 0.92)	1.96 (1.69 - 2.26)	6.58 (0.62)	17.4	
003 Turakina	20.02.95	DZ + PBO	0.87 (0.69 - 1.08)	4.08 (2.33 - 7.15)	3.46 (0.66)	10.9	1.0
003 Turakina	18.05.95	DZ	0.93 (0.76 - 1.14)	4.66 (2.56 - 8.48)	3.32 (0.60)	18.6	
003 Turakina	18.05.95	DZ + PBO	1.44 (1.27 - 1.63)	3.91 (2.75 - 5.56)	5.34 (0.82)	18.0	0.8
039 Rakaia	14.02.94	DZ	0.93 (0.82 - 1.04)	2.63 (1.94 - 3.58)	5.13 (0.68)	18.6	
039 Rakaia	14.02.94	DZ + PBO	1.12 (1.06 - 1.19)	3.58 (3.08 - 4.15)	4.63 (0.28)	14.0	0.8
003 Turakina	10.05.94	DZ	0.95 (0.77 - 1.19)	4.01 (2.32 - 6.94)	3.74 (0.70)	19.0	
003 Turakina	10.05.94	DZ + PBO	1.08 (1.00 - 1.17)	3.01 (2.54 - 3.57)	5.26 (0.53)	13.5	0.9
004 Bulls	05.04.94	DZ	0.98 (0.91 - 1.06)	2.37 (1.96 - 2.87)	6.10 (0.87)	19.6	
004 Bulls	05.04.94	DZ + PBO	0.84 (0.70 - 1.00)	2.54 (1.71 - 3.78)	4.83 (0.89)	10.5	1.2
012 Hastings	29.12.92	DZ	1.00 (0.93 - 1.07)	2.70 (2.28 - 3.19)	5.39 (0.59)	20.0	
012 Hastings	29.12.92	DZ + PBO	1.08 (1.00 - 1.17)	2.54 (2.11 - 3.05)	6.29 (0.63)	13.5	0.9
008 Kihikihi	07.03.94	DZ	1.02 (0.89 - 1.17)	3.94 (2.80 - 5.55)	3.97 (0.47)	20.4	
008 Kihikihi	07.03.94	DZ + PBO	1.09 (0.93 - 1.27)	3.62 (2.38 - 5.50)	4.46 (0.79)	13.6	0.9
115 Kamo	19.04.95	DZ	1.04 (0.97 - 1.11)	2.98 (2.51 - 3.55)	5.06 (0.45)	20.8	
115 Kamo	19.04.95	DZ + PBO	0.78 (0.63 - 0.97)	3.48 (1.68 - 7.20)	3.59 (0.83)	9.4	1.3
038 Rangiora	04.01.93	DZ	1.11 (1.04 - 1.19)	1.87 (1.59 - 2.20)	10.32 (1.50)	22.2	
038 Rangiora	04.01.93	DZ + PBO	1.82 (1.68 - 1.97)	3.08 (2.55 - 3.72)	10.23 (1.69)	22.8	0.6
016 Lab. K.	00.00.00	DZ	1.15 (1.09 - 1.21)	2.67 (2.36 - 3.02)	6.37 (0.65)	23.0	
016 Lab. K.	00.00.00	DZ + PBO	1.48 (1.30 - 1.68)	3.78 (2.66 - 5.38)	5.69 (1.03)	18.5	0.8

159 Dorie	23.04.95	DZ	1.21 (1.14 - 1.28)	3.55 (3.09 - 4.07)	4.98 (0.34)	24.2	
159 Dorie	23.04.95	DZ + PBO	1.14 (0.89 - 1.45)	3.83 (2.28 - 6.44)	4.41 (0.96)	14.3	1.1
024 Masterton	31.02.94	DZ	1.23 (0.14 - 1.32)	2.38 (1.99 - 2.84)	8.10 (0.98)	24.6	
024 Masterton	31.02.94	DZ + PBO	1.36 (1.17 - 1.58)	2.78 (1.96 - 3.93)	7.48 (1.62)	17.0	0.9
058 Takapau	03.04.95	DZ	1.33 (1.19 - 1.49)	2.87 (2.24 - 3.68)	6.99 (1.00)	26.6	
058 Takapau	03.04.95	DZ + PBO	1.26 (1.10 - 1.44)	2.75 (2.03 - 3.71)	6.85 (1.28)	15.8	1.1
009 Blenheim	19.03.95	DZ	1.54 (1.44 - 1.65)	5.39 (4.68 - 6.21)	4.28 (0.23)	30.8	
009 Blenheim	19.03.95	DZ + PBO	1.16 (1.11 - 1.21)	2.86 (2.60 - 3.15)	5.93 (0.26)	14.5	1.3
016 Kaikohe	18.04.94	DZ	1.55 (1.25 - 1.93)	6.32 (3.64 -10.98)	3.82 (0.72)	31.0	
016 Kaikohe	18.04.94	DZ + PBO	1.38 (0.92 - 2.06)	4.56 (1.62 -12.85)	4.49 (1.93)	17.3	1.3
009 Blenheim	15.04.94	DZ	1.56 (1.46 - 1.66)	4.76 (4.08 - 5.55)	4.81 (0.28)	31.2	
009 Blenheim	15.04.94	DZ + PBO	1.34 (1.25 - 1.43)	4.04 (3.46 - 4.71)	4.86 (0.34)	16.8	1.1
058 Takapau	03.04.95	DZ	1.74 (1.63 - 1.85)	5.15 (4.45 - 5.96)	4.94 (0.26)	34.8	
058 Takapau	03.04.95	DZ + PBO	1.05 (1.00 - 1.11)	2.38 (2.14 - 2.66)	6.55 (0.53)	13.1	1.7
014 Blenheim	25.04.94	DZ	1.82 (1.56 - 2.13)	7.92 (5.27 - 11.9)	3.65 (0.43)	36.4	
014 Blenheim	25.04.94	DZ + PBO	1.58 (1.47 - 1.70)	5.40 (4.54 - 6.43)	4.37 (0.40)	19.8	1.2
014 Blenheim	28.12.92	DZ	2.03 (1.87 - 2.20)	4.41 (3.59 - 5.42)	6.89 (0.86)	40.6	
014 Blenheim	28.12.92	DZ + PBO	2.16 (2.05 - 2.27)	4.36 (3.81 - 4.99)	7.61 (0.83)	27.0	0.9
058 Takapau	04.02.98	DZ	2.06 (1.90 - 8.55)	7.04 (5.80 - 8.55)	5.62 (0.29)	41.2	
058 Takapau	04.02.98	DZ + PBO	1.19 (0.81 - 1.75)	5.97 (2.65-13.48)	4.28 (1.35)	14.9	1.7

[1]Treatment with either diazinon only (DZ) or diazinon with a pretreatment of piperonyl butoxide (DZ + PBO)

[2] FL, 95% fiducial limits

[3] SE, Standard Error

[4] RF, Resistance factor – the ratio of LC_{50} for the resistant strain, in the presence or absence of synergist, to that for the susceptible strain, in the presence or absence of synergist.

[5] SR, Synergist ratio – the ratio of LC_{50} of toxicant alone to that for the LC_{50} of the toxicant in the presence of the synergist.

In order to emphasize trends in the data, the LC_{50} values were recalculated as synergist ratios, as described in the methods. These ratios ranged from 0.5 to 1.7 denoting both antagonistic (<1) and synergistic (>1) responses. The greatest synergistic response (1.7) was shared by the 058 Takapau strains (03.04.95 & 04.02.98) where the LC_{50} for diazinon changed from 1.74 to 1.05 mg/l and 2.06 to 1.19 mg/l respectively when a pre-treatment of PBO was applied. The most pronounced antagonistic response (0.5) was shared by the 034 Leeston (18.04.94) and 037 Levin (15.03.94) strains. The 034 Leeston (18.04.94) strain showed an increase in diazinon LC_{50} from 0.44 to 0.87 mg/l and the 037 Levin strain increased from 0.62 to 1.14 mg/l. A ratio of 0.6 was observed with the OP-susceptible laboratory strain.

Figure 2.5.2 shows that there was a significant, positive correlation between resistance factor and the synergist ratio ($r = 0.75\%$, $p < 0.0001$). This showed that as resistance factors increased, so too did synergism of diazinon toxicity by PBO. However, about 70% of the strains studied responded to PBO by exhibiting an antagonistic response.

Discussion

This study has shown wide variation in the response of field strains of *L. cuprina* larvae towards treatment with piperonyl butoxide and diazinon. The variation observed suggests that the effect of PBO on resistance in *L. cuprina* is complex and that the linear relationship between the toxicity of diazinon in the presence and absence of PBO is not as simple as it first appears. An initial clue to the complexity of the situation is that the regression line does not pass through the origin. This suggests that at least two factors are operating with respect to toxicological response. One factor is responsive to the

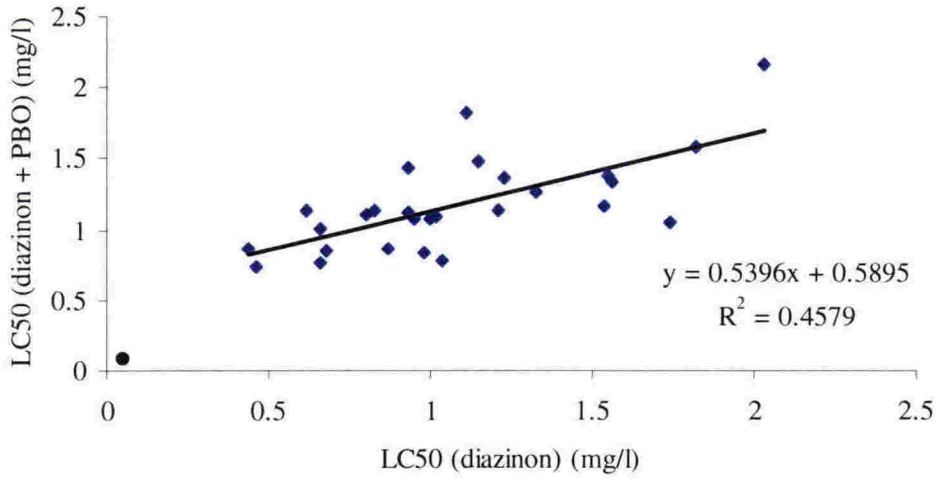


Figure 2.5.1. Relationship between LC_{50} values for diazinon against *Lucilia cuprina*, obtained in the presence and absence of the synergist, piperonyl butoxide. LC_{50} (diazinon) is the LC_{50} value obtained with diazinon alone whereas LC_{50} (diazinon + PBO) is the value obtained after pre-treatment with piperonyl butoxide, as described in methods. The solid line is the line of best fit to data points and is exclusive of the OP-susceptible laboratory strain (•).

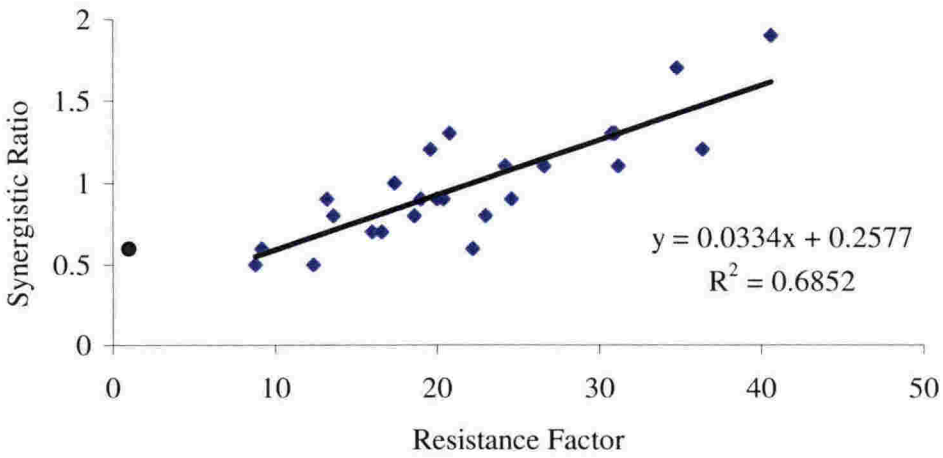


Figure 2.5.2. Relationship between the resistance factor to diazinon by *Lucilia cuprina* larvae, prior to treatment with piperonyl butoxide, and the synergistic ratio calculated by dividing the LC_{50} for larvae treated only with diazinon by the LC_{50} for larvae pre-treated with piperonyl butoxide. The solid line is the line of best fit to data points and is exclusive of the OP-susceptible laboratory strain (•).

synergist (PBO) while the other is apparently unaffected by the synergist. It is thought that these factors correspond to different biochemical mechanisms, one of which is primarily inhibited by the synergist. Clark (personal communication) proposed the following model to describe the variation observed between strains. (The following model is from publication of this experiment (Wilson *et al.* 1999). In the model, two expressions may be formulated for each strain, depending on whether the synergist, at a fixed dose, is present or not. These are, for the *i*th strain:

$$LCi_{50d} = \mathbf{a} + x_i . P_d \qquad (1)$$

$$LCi_{50dpb} = \mathbf{a} + x_i . P_{dpb} \qquad (2)$$

The first term **a** is the synergist-independent term. This term is assumed to represent a basal level of resistance, common to all strains. The basal level of resistance may be attributable to the E_3 esterase described in Chapter 2.2 and in Wilson & Clark (1996). The second term is a variable component of the resistance and is related to the activity (x_i in strain i) of the enzyme(s) involved in the resistance mechanism by a proportionality constant. The value of this constant will be inversely related to the synergist concentration used. In the case of the untreated larvae, the constant is expressed as P_d and as P_{dpb} in the synergised insects. P_{dpb} has a lesser value than P_d, since the effect of a synergist is to reduce the value of the LC_{50}.

$$LC_{50dpb} = \mathbf{a}.(1-P_{dpb}/P_d). + LC_{50d}.(P_{dpb}/P_d) \qquad (3)$$

Equation 3 appears to be compatible with the data shown in figure 2.5.1 where a strong, positive, linear correlation exists between the two sets of data ($p< 0.001$) and the gradient (0.54) and vertical intercept (0.59) (Figure 2.5.1) are positive as required by the equation. The gradient value suggests, in this model, that the enzyme system is inhibited 45% by the synergist. However, calculation of the value **a** suggests that the starting hypothesis is overly simplistic. The value of **a** corresponds to a LC_{50d} of 1.28

mg/l which is higher than 75% of the experimental values, whereas, according to equation 1, it should be the lowest of them all. This conclusion is clearly reinforced by recalculating the data as synergistic ratios and plotting these against the unsynergised RF values (Figure 2.5.2). Synergistic ratios showed that the effect of the synergist was two-fold. For example, for many strains, with resistance factors less than 20, resistance increased when they were pre-treated with PBO (Table 2.5.1) resulting in an antagonistic rather than a synergistic effect. Strains with higher resistance factors exhibited a synergistic response towards diazinon when pre-treated with PBO. There appears then to be a continuous variation between the two extreme responses, depending on the degree of resistance.

Another indication of the complexity of the situation, as mentioned above, is the gradient of the line in figure 2.5.1. The gradient indicates that, if a single enzymatic process is responsible for varying resistance then it is only inhibited by the synergist to the extent of about 50%. If complete inhibition occurred, the line in figure 2.5.1 would have a zero gradient. Tests with varying concentrations of the synergist (Appendix 2.5.1) suggested that the target enzyme of the PBO was completely inhibited, however. This denotes that there is at least one more factor which is increased with increasing resistance, but which is unaffected by the synergist. The work reported in earlier chapters suggests that this factor might involve the glutathione *S*-transferases.

The occurrence of both synergistic and antagonistic effects in this study may reflect the two-fold action of the MOs on phosphorothionate insecticides. Phosphorothionates, such as diazinon, are not potent toxins and must be converted to various metabolic products to become more biologically active. Diazinon is converted to products such as

hydroxydiazinon, diazoxon and hydroxydiazoxon by the action of monooxygenases, of which the latter two are actively toxic (Pisani-Borg *et al.* 1996, Eto 1974, Shishido *et al.* 1972). Subsequent inactivation is likely to be the result of both oxidative and hydrolytic reactions. It is proposed that for strains exhibiting low resistance factors (less than 20) MO activity is dominated by isoforms catalysing the activation of diazinon. The effect of MO inhibition in these low resistance strains was to depress the intoxication process of diazinon, therefore prolonging the stability of the less toxic form of the insecticide. In the more resistant strains, the MO activity is likely to be dominated by isoforms that have been selected for their ability to detoxify the insecticide. The suppression of the MO activity in these more resistant strains therefore, has the effect of slowing the removal of the toxic forms of the insecticide resulting in an enhanced toxicity. The two classes of isoforms of the enzyme may also have differential susceptibility to PBO.

There are numerous reports in the literature to support the above interpretation of these results. For instance, the potency of PBO as a synergist has been shown to vary markedly with insecticide and target species (Welling & De Vries 1985, Silcox *et al.* 1985, Raffia & Priester 1985, Hagler *et al.* 1988, Prabhaker *et al.* 1988). Variation in response to PBO has also been shown within different strains of the same species as in this study (Bagwell & Plapp 1992, Scott *et al.* 1990, Sparks & Byford 1988, Prabhaker *et al.* 1988). Other reports show that rate of metabolism of a substrate is dependent on the qualitative and quantitative balance of isozymes in tissue (Clarke *et al.* 1989). Feyereisen (1983) showed major quantitative differences between resistant and susceptible housefly larvae. Resistant larvae, for example, showed higher levels of cytochrome c reductase and total cytochrome P_{450} isozymes. Cytochrome P_{450} in resistant forms may have high catalytic activity due to mutation; or resistant insects may

have alterations of regulation in the cytochrome P_{450} gene that is expressed differently in susceptible forms (Soderlund & Bloomquist 1990).

The establishment of a possible involvement of the monooxygenases in some New Zealand strains of *L. cuprina* larvae however, lends support to studies of Australian strains (Kotze & Sales 1995, Kotze & Sales 1994, Kotze 1993). These results also show partial support for an earlier synergism study by Hughes (1982). Hughes (1982) investigated the synergistic effect of PBO with four organophosphorus insecticides (paraoxon, coroxon, chlorfenvinphos and fenitro-oxon) against two laboratory strains (Q, a homozygous resistant field strain and LS, an OP-susceptible laboratory strain) of the *L. cuprina*. Pre-treatment of female adults with PBO increased resistance factors (antagonism) of the Q strain to all four OPs but the insecticides were synergised by PBO in the LS strain. Hughes (1982) study is not strictly comparable with the present study as he investigated synergistic effects on oxygen analogues of OPs. It is therefore likely that there will not be suppression of toxicity comparable with diazinon since there is no initial activation phase of the insecticide. Hughes (1982) results do however support this study for low resistance strains. Hughes (1982) achieved greater synergistic activity in the Q strain by using the esterase inhibitor TBPT. Attia *et al.* (1979) also showed a significant synergistic affect with diazinon and DEF pre-treatment on the same strain. Hughes (1982) concluded that resistance in this case was primarily esterase-based rather than MO mediated. Later studies by Kotze (1993) and Kotze & Sales suggested a more considerable contribution of the MOs to resistance in *L. cuprina*. Using three *L. cuprina* strains derived from the field, Kotze & Sales (1994) selected them with either butacarb (Btsel strain), deltamethrin (DLS strain) or diflubenzuron (Dfbsel strain) and maintained selection pressure over 4-6 years. Their bioassay used first instar larvae and

showed that PBO and the carboxyesterase synergist triphenyl phosphate (TPP) synergised the selecting insecticide in all three strains. This showed that MO mechanisms were in part responsible for resistance in these strains, although esterases were still of greatest importance. These strains had been examined earlier for aldrin epoxidase activity (Kotze 1993), and this was shown to increase during larval development and peak just prior to the clear-gut stage (late third instar), after which activity rapidly declined. NAPDH-cytochrome c reductase activity was shown to be similarly high at this stage (Kotze 1993). More pertinent to the present study, an extensive study of aldrin epoxidase activity in 51 field samples of *L. cuprina* also showed a significant correlation with resistance to diazinon (Kotze & Sales 1995). Developmental stage and generation therefore appear to be an important determinant of MO activity in *L. cuprina*. These factors may account for differences in Australian studies as Hughes (1982) investigated adult blowflies rather than larval strains which were the focus of the present study and other Australian studies (Kotze & Sales 1995, Kotze & Sales 1994, Kotze 1993). Hughes (1982) was also restricted in the range of strains tested compared with this and Kotze & Sales' (1995) study.

Clearly this study can only speculate on the possible factors involved in the variation seen within strains towards the synergist PBO. This in part, is due to the limited nature of information that can be gleaned from using a synergist rather than more extensive testing of known MO pathways. One shortcoming of the use of PBO in particular is that not all forms of cytochrome P_{450} are equally susceptible to inhibition by this synergist (Soderlund & Bloomquist 1990). Kotze & Sales (1995) showed that aldrin epoxidase activity in 51 field samples of *L. cuprina* varied over a 74-fold range. This was significantly correlated with diazinon resistance and also tolerance to diflubenzuron

(Kotze & Sales 1995). The synergist is not a specific inhibitor of one MO enzyme reaction or system as these oxidases are involved in a multitude of reactions (Kulkarni & Hodgson 1984). For example, Karoly *et al.* (1996) showed elevated hydroxylation and *N*-demethylation activity in azinphos-methyl resistant fifth instar tufted apple bud moths (*Platynota idaeusalis*) but failed to observe synergism with PBO. This lack of synergism suggested that PBO was not an effective inhibitor of all MO reactions. Comparative studies of resistant and susceptible house fly strains have shown at least six forms of cytochrome P_{450} and qualitative differences in isozyme composition (Ronis *et al.* 1988, Yu & Terriere 1979).

Other factors, such as reduced rate of insecticide penetration due to a synergist, may also play a role. Wahla *et al.* (1976) demonstrated slower penetration of diazinon through the cuticle of white butterfly larvae (*Pieris brassicae*) in the presence of PBO, which lowered the toxicity of the applied diazinon dose resulting in an antagonistic effect. Reduced insecticide penetration due to piperonyl butoxide has also been shown fifth instar tobacco budworm larvae (*Heliothis virescens*) (Martin *et al.* 1997), cotton boll worm, (*Helicoverpa armigera*) (Kennaugh *et al.* 1993) and the Colorado potato beetle, (*Leptinotarsa decemlineata*) (Forgash 1985). Wahla *et al.* (1976) concluded however, that the primary cause of antagonism observed in white butterfly larvae treated with PBO and diazinon was due to the inhibition of diazinon-diazoxon activation.

Conclusions

This study finds evidence compatible with a contribution by monooxygenases to increased levels of organophosphorus insecticide resistance in New Zealand field strains of *L. cuprina* larvae. There appears to be a proportional effect of the mechanism in

relation to level of resistance. Without exact quantification of other resistance mechanisms such as the E_3 esterase, and measurement of specific oxidative pathways the extent of the contribution of this group of enzymes cannot be firmly stated.

CHAPTER 2.6

Main effects of non-oxidative metabolism in organophosphate resistance in strains of *Lucilia cuprina* and *Lucilia sericata* determined by multiple linear regression

Introduction

Results discussed in previous chapters support the role of the mutant aliesterase (E_3) as the principal mechanism of resistance in New Zealand strains of *L. cuprina* as is the case in Australian strains (Hughes & Raftos 1985). Results of the MtB assay (Chapter 2.2) suggest a similar mechanism occurs in resistant strains of *L. sericata*. It appeared to be the sole mechanism of resistance, of those tested in *L. sericata*, although this could not be confirmed by electrophoresis. The exact quantitative contribution of the mutant E_3 to resistance in *L. cuprina* is however unknown. The level of resistance in *L. cuprina* also appeared to be influenced by increased levels of GST activity (Chapter 2.3) in adult and larvae, as well as by MO activity (Chapter 2.5) with respect to larval strains and possibly, by acetylchloinesterases in adults. External variables showing consistent influence on OP-resistance level were season with respect to *L. cuprina* and region for *L. sericata*.

Analysis of variance in these previous chapters did not elucidate the relative importance of each of these mechanisms in contributing to resistance. The purpose of this chapter is therefore to assess the relative significance of the main mechanisms affecting phenotypic expression of resistance in adult and larval forms of *L. cuprina* and *L. sericata* by multiple regression analysis. Once these main effects have been established a predictive equation of explanatory variables can be constructed.

Unfortunately however, due to a too small data set, season and region cannot be used as predictive variables in this model.

Method

Multiple regression analysis was performed on matched data using S-Plus (Everitt 1994). Included in this model were GST conjugating activities towards CDNB and DCNB, non-specific esterase activities using α- and β-naphthyl acetate as substrates and total acetylcholinesterase activities (E_o) and rate of OP inactivation by acetylcholinesterase (Ki). Regional, seasonal and sheep density factors could not be fitted in the model as it created too many levels of explanatory variable by which to analyze a small data set. Multiple regression models are dependent on the order of the predictive variables. Variables were therefore randomized several times to determine true main effects.

Results

Lucilia cuprina

The main effect of resistance in the absence of the mutant E_3 mechanism was shown to be increased levels of GST activity (towards DCNB) ($p = 0.02$) in adult *L. cuprina* (Appendix 2.6.1). This confirms the results in chapter 2.3. Converse to this was the observation that rate of OP-inactivation by AChE (Ki) was the main mechanism of OP-resistance in *L. cuprina* larvae ($p = 0.001$) (Appendix 2.6.1). In this analysis, GST activities did not appear to feature as a mechanism in larvae in contrast with the conclusions drawn in chapter 2.3 (See Appendix 2.6.2 for ANOVA tables which are representative of the effect of randomized variables).

Lucilia sericata

As concluded in previous chapters no other mechanisms showed influence on resistance other than aliesterase activity, in either adult or larval strains of *L. sericata*. Appendix 2.6.1 shows ANOVA tables for larval strains and appendix 2.6.2 show the effect of randomizing order of variables for adult strains of *L. sericata*.

Discussion

Multiple linear regression analysis confirms results of previous chapters with respect to *L. sericata* and *L. cuprina* adults. The suggestion that rate of OP-inactivation by acetylcholinesterases was a principal mechanism of resistance and the lack of a contribution by GSTs with respect to activity towards DCNB in larval *L. cuprina*, was a little surprising however. This may be a result of the small sample size of matched data however (n = 12). It also suggests that differences in statistical methodology can generate contradictory results and need to be treated with caution.

Despite the strong predictive power that multiple regression models have in comparison to the ANOVA models of individual enzyme activities used in previous chapters, there were a number of shortcomings observed in this model. Firstly, there was a small sample number of matched data for each mechanism with respect to some species i.e. *L. cuprina* larvae. Secondly, the addition of region, season and sheep density factors created too many levels of explanatory variable for the model. This is unfortunate as these factors were shown to influence resistance in previous chapters 1.1, 2.2 – 2.4. Removal of these variables may create a greater error and influence the outcome of main effects. Thirdly, small sample size also meant that the

model was unable to give insight into whether mechanisms were acting independently or dependently of each other i.e. interactive effects are lost.

Despite shortcomings of the data, multiple regression has been a useful and necessary exercise for consolidating results from previous chapters. Previous chapters are in effect subsets of this model and anticipate the multiple regression. Also note that to exclude variables is to constrain the model. This and previous chapters have therefore shown that OP-resistance in *L. cuprina* is not simply a function of a single resistance mechanism but rather an array of mechanisms. A theoretical equation of multiple regression is:

$$\underbrace{y = b_o}_{\text{constant}} + \underbrace{b_1x_1 + b_2x_2 + \ldots\ldots\ldots + b_rx_r}_{\text{independent variables}} + \underbrace{e}_{\text{error}}$$

where x represents independent variables and b, the regression coefficients. To use the regression equation as a crude predictive model for *L. cuprina*, we would express it as:

$$LC_{50}\ \text{Diazinon} = \bar{b_0} + \hat{b_1}\,(E_3) + \hat{b_2}\,(\text{DCNB}) + \text{error}$$

A more realistic equation however would include variables that cannot currently be included in the multiple linear regression:

$$LC_{50}\ \text{Diazinon} = \bar{b_0} + \hat{b_1}\,(E_3) + \hat{b_2}\,(\text{DCNB}) + \hat{b_4}\,(\text{MFOs}) + \text{error}$$

These mechanisms may or may not interact with each other, but in aggregate, produce the phenotypic expression of OP-resistance that has lead to the reduced protective period afforded to livestock by organophosphorus insecticides. This

section has only touched the surface of the complexity of the enzyme systems involved but clearly shows that there is an enormous deficiency in knowledge of OP-resistance in *L. cuprina* despite research on this species spanning almost half a century. The multiple resistance mechanisms evident in strains of *L. cuprina* undoubtedly lend this species resistance to organophosphates and, to some degree, cross-resistance with other insecticide groups. Already there is evidence of cross-resistance between diazinon and the IGR, diflubenzuron in New Zealand (N. Haack, unpublished data) and cross-resistance to other IGR insecticides (e.g. triflumuron) may be developing (N. Haack personal communication).

At this point in time it appears that the basis of OP-resistance in New Zealand strains of *L. sericata* is simpler than the multiple mechanisms occurring in *L. cuprina*.

$$LC_{50}\ \text{Diazinon} = \bar{b_0} + \hat{b_1}\ (\text{aliesterase, } E_3') + \text{error}$$

This does not appear to have excluded *L. sericata* from competing strongly with *L. cuprina* as a primary strike fly however (see Introduction, p 27, Table 1). In Australia, *L. sericata* has been displaced by *L. cuprina* as the most important strike fly (G. Levot, personal communication) as a result of its failure to compete. *Lucilia cuprina* was first reported to occur in Australia as early as the 1880s (Graham 1979). It therefore has competed with *L. sericata* for many more years than it has in New Zealand where *L. cuprina* is a more recent arrival. Displacement of *L. sericata* in Australia by *L. cuprina* may be a result of *L. cuprina* developing resistance to OPs more rapidly than *L. sericata*. There are however no known studies of resistance in *L. sericata* in Australia. The preference by *L. cuprina* for a live host compared with

L. sericata may have increased the likelihood of OP-exposure and therefore of selection pressure. *Lucilia sericata* develop on living or dead tissue providing a reduced or intermittent OP-selection pressure. New Zealand strains of *L. sericata* have a 30-year advantage over Australian *L. sericata* for developing of OP-resistance. The 30-year advantage spans the time of first OP-registration in New Zealand and the introduction of the OP-resistant competitor *L. cuprina* and may explain why *L. sericata* has not been displaced by *L. cuprina*.

Conclusions

It appears that *L. cuprina* has multiple mechanisms of OP-resistance compared with *L. sericata*. The underlying mechanism of OP-resistance in *L. cuprina*, and this may also be the case in *L. sericata*, appears to be a mutant aliesterase. Differences between species may relate to life habit and subsequent differences in OP-selection pressure. The work presented in these chapters' only touches the surface of the problem of resistance in both species. It has not explored oxidative resistance in any depth in *L. cuprina* and is absent for *L. sericata*. It is however a useful starting point for investigating resistance in a new species and highlights the need for extensive field sampling both for comparative purposes and for establishing possible interactive effects of resistance mechanisms.

SECTION 3

Intra- and inter-strain variation in *Lucilia cuprina* determined by random amplified polymorphic DNA polymerase chain reaction (RAPD PCR)

Introduction

Welsh & McClelland (1990) and Williams *et al* (1990) described a fast and simple technique for detecting variation within and between species. The method relied on the amplification of random DNA segments, which may be present or absent in individuals, strains or species, by the polymerase chain reaction (PCR). Single short oligonucleotides (≈ 10mer) of arbitrary nucleotide sequence are often used as amplifying primers negating the need for prior sequence information of the target organisms (Raich *et al.* 1993). A high G + C content (60 – 70%) is normally chosen for oligonucleotides to avoid the occurrence of self-complementary ends (Greif *et al.* 1996). Variation in banding patterns or polymorphisms, detected by agarose gel electrophoresis following RAPD (random amplified polymorphic DNA) PCR, may arise from single base changes in the genome or by deletions of a priming site (Clark & Lanigan 1993). Variation may also result from insertions that render a priming site too distant, thus preventing amplification, or insertions that change the size of a DNA segment (Williams *et al.* 1990).

The RAPD PCR method has served as a taxonomic tool for identifying and differentiating between species and subgroups of species that may or may not share phenotypic properties. Wilkerson *et al.* (1995) for example, were able to characterise

four genetically differentiated species of mosquitoes (*Anopheles (Nyssorhynchus)* spp.) by RAPD PCR, one of which was previously undescribed. Stevens & Wall (1995) have also studied the genetic homogeneity of the sheep blowfly *L. sericata* in southern Britain using this technique. The taxonomic advantage of this tool is that it can be used for determining phylogenetic distances (Tibayrenc *et al.* 1993, Williams *et al,* 1990). Population structure issues can also be addressed with respect to parentage, mating success, dispersal and degree of inbreeding and evolutionary history (Tibayrenc *et al.* 1993).

Other workers have used this method to search for identifiers or genetic markers for certain strains of the organism in question. These markers may correlate with factors such as virulence, as in strains of the coccidial parasite, *Eimeria tenella* (Shirley & Bumstead 1994), and pathogencity or nonpathogencity as in strains of the intestinal parasite, *Entamoeba histolytica* (Mackenstedt & Johnson 1995). Other markers include the generation of differentially regulating genes as in the identification of intra- and interspecific genetic polymorphisms in the trypanosomatid parasites of the genus *Leishmania* (Pogue *et al.* 1995).

In previous chapters, a 'field strain' of flies has been defined as a group of individuals collected from a case of flystrike from a particular geographical origin and at a fixed point in time. The aim of this chapter is to characterise intra- and inter-strain variation in *L. cuprina* populations. Key features of this analysis will be the calculation of phylogenetic similarities in an attempt firstly to better define a 'field strain'; secondly the mode of dispersal of the species throughout New Zealand, and thirdly to identify genetic markers of high level insecticide resistance.

Materials and Methods

DNA Extraction

Genomic DNA was obtained from wing muscles of adult *L. cuprina* (3 – 6 days old) to avoid contamination by ingested proteins, parasitoids or eggs. Paired wing muscles were taken from 30 individuals of each of 18 strains (Appendix 3.1) using sterile dissecting equipment, to give a total of 60 samples per strain. Pairs of wing muscles from individuals acted as replicates during PCR as a check on consistency of amplification.

Genomic DNA was extracted using DNA_{zol}™ Reagent. Individual muscles were briefly homogenised in sterile 1.5 ml centrifuge tubes in 100 µl DNA_{zol}™ using fitting, sterile teflon plungers. Tubes were centrifuged at 7 500 rpm for 10 minutes at 4°C to allow insoluble tissue fragments to pellet. The supernatant was removed and transferred to another sterile 1.5 ml centrifuge tube. DNA was precipitated by the addition of 50 µl 100 % Analar ethanol. Tubes were mixed by inversion and stood at room temperature for 3 minutes. Tubes were then centrifuged at 1 000 rpm for 2 minutes. The supernatant was removed and DNA pellets were air-dried for 15 minutes at room temperature. DNA was solubilized by adding 25 µl of 8 m*M* NaOH and pH adjusted to 8.0 by adding 2.9 µl of 0.1 *M* HEPES. Tubes were briefly vortexed and stored at -20°C until required. The yield and quality of DNA was measured by UV absorption at A_{260}/A_{280} nm using a Pharmacia LKB Ultraspec III UV/visible spectrophotometer.

RAPD PCR

Amplifications were performed using a 25 µl reaction volume with a 15 µl mineral oil overlay. The reaction consisted of Stoffel buffer, 0.2 m*M* deoxynucleotide triphosphates (dNTPs), 4 m*M* magnesium chloride, 5m*M* primer, 0.025 units/µl *Taq* polymerase (Stoffel fragment), sterile dH_2O and 20 ng of DNA template. The Stoffel fragment of *Taq* polymerase was used as it has been shown to result in a significant increase in the number of polymorphisms detected per primer compared with other *Taq* (Nicol *et al.* 1997, Sobral & Honeycutt 1993). Magnesium chloride concentration was optimised by a preliminary titration experiment in which 0.5 to 5 m*M* $MgCl_2$ concentrations were used with the primer OPA-3. The $MgCl_2$ concentration showing consistently strong bands (4m*M*) was selected for all amplifications (Appendix 3.2). The DNA template concentration of ≈20 ng was adopted as standard in these amplifications based on Stevens & Wall (1995) optimisation of *L. sericata* template Operon Kit E primers. Twenty –10mer primers (Operon Kit A) (see Appendix 3.3 for primer sequences) were initially screened and three chosen for amplification of *L. cuprina* strains (Appendix 3.4). OPA-3 and OPA-4 were selected based on previous work by Gardiner (1995) with *L. sericata* strains. OPA-7 was selected to provide additional information on polymorphisms in 10 strains of *L. cuprina*. Negative controls were run with no DNA template. A negative control showing no amplification indicated no contamination of reactants.

The reaction mixture was subjected to a cycling profile of 94°C for 5 minutes, 35°C for 1 minute and 72°C for 2 minutes in 45 cycles using a Perkin Elmer DNA thermal cycler 480. These conditions had been previously determined to be optimal by Gardiner (1995).

An aliquot of 10 µl was used for analysis by agarose gel electrophoresis on a 2% agarose gel (molecular biology grade) containing ethidium bromide in TAE buffer (0.04 *M* Tris-acetate buffer containing 0.001 *M* EDTA) at 100V for 2 hours with a 123 bp DNA ladder at opposing ends for comparison of molecular sizes (Sambrook *et al.* 1989). Fluorescent bands were visualised at 302 nm on an UV transilluminator and were photographed for scoring using a Polaroid MP-4 Land camera containing 667 Polaroid film.

Statistical analysis

The numbers of bands occurring for each primer (based on molecular size, in base pairs) were identified for 18 populations of *L. cuprina*. Clear, reproducible DNA bands were then scored as present (1) or absent (0) for each individual in every population. Similarity matrices were calculated from these data by three methods; Jacquard's similarity (Gower 1985), fragment sharing (Hedrick 1985) and Nei & Li's (1979) band sharing using positive frequencies. Three methods were used as a check of clustering relationships in later analyses.

Once within population variation was ascertained, similarities between individuals from the same sample were averaged to reduce between population variation. Similarity matrices were then used to construct dendrograms from a hierarchical cluster analysis with an average link option in the GENSTAT® program (Lawes Agricultural Trust, IACR, Rothamsted, Release 4.1). An additional check of consistency of the data was the analysis of banding from individual primers where clustering of populations in dendrograms was compared with the total data set.

Similarity matrices were further used in a principal co-ordinates analysis (PCA) to illustrate the positioning (or clustering) of individuals of a population and populations in multidimensional space (GENSTAT 5 Committee 1988). Principal co-ordinates analysis looks for orthogonal linear combinations derived from the similarity matrix and maximises variation contained within it (see Glossary for a more thorough explanation of PCA from the GENSTAT 5 reference manual).

Results

Primer OPA-3 generated a total of 17 bands, OPA-4, 18 bands and OPA-7, 19 bands. The total number of bands generated per strain ranged from 5 – 15 for OPA-3, 6 – 13 for OPA-4 and 3 – 13 for OPA-7 (Appendix 3.5, Tables 1 – 3). Primer OPA-3 showed the occurrence of three bands consistently in all strains although not necessarily in each individual. The size of these bands was estimated at 360.0, 288 and 249 base pairs (bp) (Appendix 3.5, Table 1). Primer OPA-4 generated only two bands consistently in all strains. These bands were estimated to be 452 and 151 bp (Appendix 3.5, Table 2). No consistent bands were observed with the OPA-7 primer (Appendix 3.5, Table 3). Appendix 3.6 provides examples of RAPD-PCR profiles generated by individuals of single strains with OPA3, 4 and 7 and visualised on an agarose gel. Appendix 3.7 is a comparison of bands generated by individuals of several strains.

Intra-strain variation

The highest within strain similarity was shown in the OP-susceptible laboratory strain (0.982) (Table 3.1). Of the field strains, 003 Turakina 22.04.96 (L) showed the

highest similarity coefficient of 0.963, and the most dissimilar was a strain from 038 Marton 26.04.96 (J) with a similarity coefficient of 0.656 (Table 3.1).

Table 3.1 Mean self-similarity per strain of *Lucilia cuprina* calculated by using the shared fragments method.

Lucilia cuprina strains				Mean similarity
Code	farm	Area	collection date	
A	009	Blenheim	15.03.94	0.882
B	009	Blenheim	12.02.96	0.913
C	014	Blenheim	12.01.94	0.896
D	027	Greytown	07.01.94	0.946
E	027	Greytown	06.02.92	0.853
F	134	Pokeno	26.02.96	0.872
G	000	Laboratory	00.00.00	0.982
H	066	Dorie	20.04.94	0.927
I	038	Marton	12.04.94	0.863
J	038	Marton	26.04.96	0.656
K	003	Turakina	10.05.94	0.827
L	003	Turakina	22.04.96	0.963
M	004	Bulls	21.12.94	0.955
N	004	Bulls	23.02.96	0.941
O	034	Leeston	18.04.94	0.792
P	008	Kihikihi	07.03.94	0.757
Q	008	Kihikihi	10.01.96	0.949
R	038	Rangiora	04.01.93	0.851

A-L Compared using 3 primers
M-R Compared using 2 primers

Individuals of strains from Blenheim and Marton were selected for further analysis of intra-specific variation based on similarity between strains. A dendrogram representing genetic similarity of all strains amplified with three random primers, showed distinct clustering from within these areas (Figure 3.6). Figure 3.1 represents genetic similarity between individuals and strains from Blenheim. The dendrogram shows that there were strong similarities between individuals from strains A and C, which were both samples from Blenheim in 1994. Individuals from strain B (009 Blenheim 12.02.96) however, only clustered with individuals from the same strain.

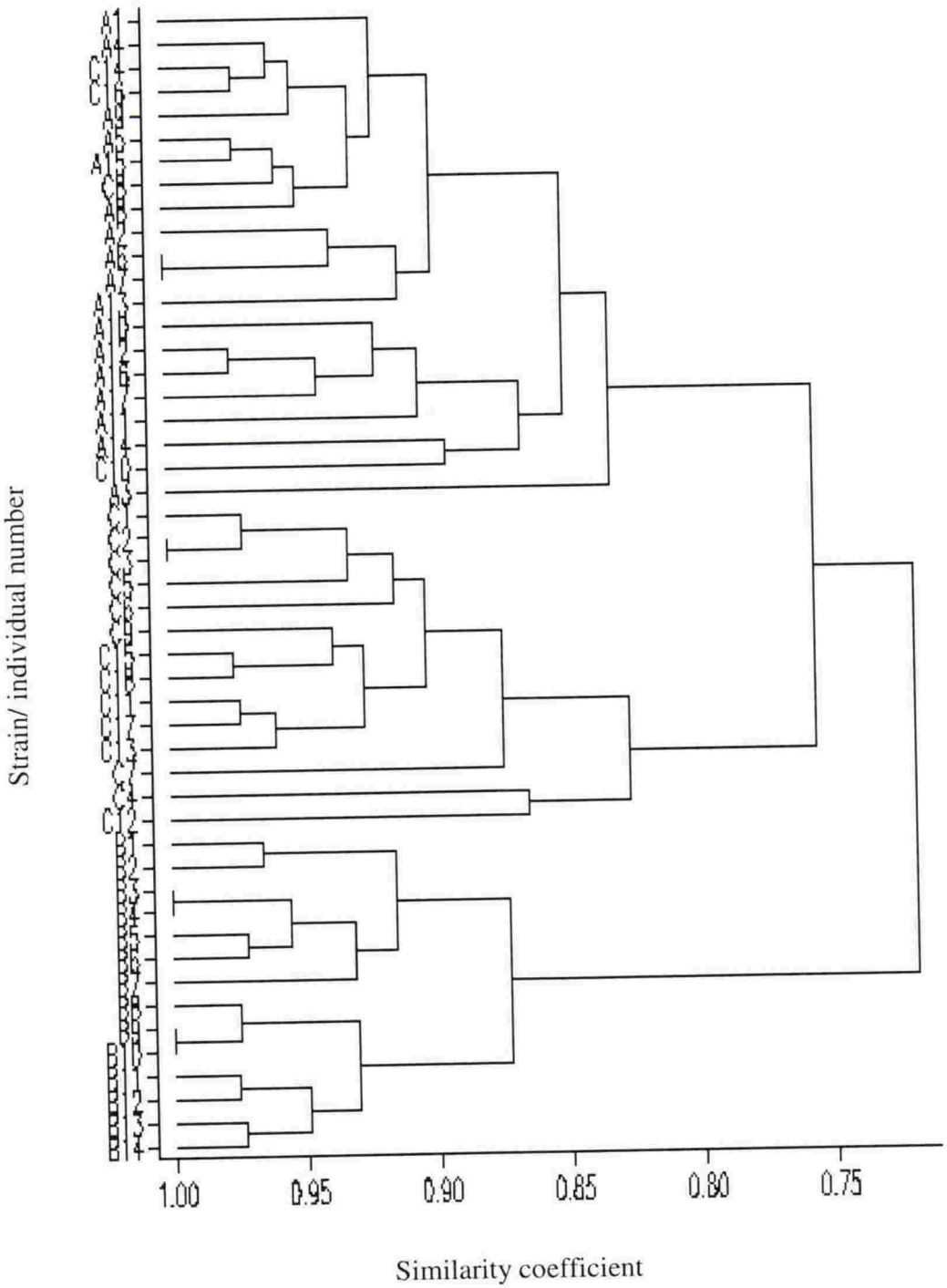


Figure 3.1 Dendrogram representing genetic similarity within three strains of *Lucilia cuprina* from Blenheim (A, B, C). Genetic similarities were calculated by Nei & Li's (1979) method of band sharing using positive frequencies. Dendrograms were constructed using hierarchical cluster analysis with an average link option in the GENSTAT® program.

Principal co-ordinates analysis confirms this separation in spatial dimensions when comparing first and second principal components and highlights the degree of similarity or genetic closeness between all strains from Blenheim in a comparison of second and third principal components (Figure 3.2).

The dendrogram of genetic similarity within and between strains from Marton shows that there was no overlap in similarity between individuals of the two strains. The dendrogram also shows that there is more than one major cluster of individuals within each strain (Figure 3.3). Principal co-ordinates analysis highlights the spread of polymorphisms observed in 038 Marton 26.04.96 (J) compared with the two strong similarity groupings seen in individuals from 038 Marton 12.04.94 (I) in plots of both first and second and second and third principal components (Figure 3.4). Appendix 3.10 provides matrices of similarities within individuals of the three Blenheim and two Marton strains. Numbers of individuals may vary between strains, as only individuals that showed reproducible bands between replicates were included in analysis.

Individuals of strains from Marton (I, J), Turakina (K, L) and Bulls (M, N) were further selected for determining intra-strain variation by frequency histogram plots of genetic distances. Samples L (Turakina), M and N (Bulls) showed histograms strongly skewed to the left (Figure 3.5). This may indicate homogeneity in the population caused by selection of individuals from a single oviposition event. Samples J (Marton) and K (Turakina) both showed a bimodal distribution which may indicate greater heterogeneity in the sample or offspring from two oviposition events (Figure 3.5).

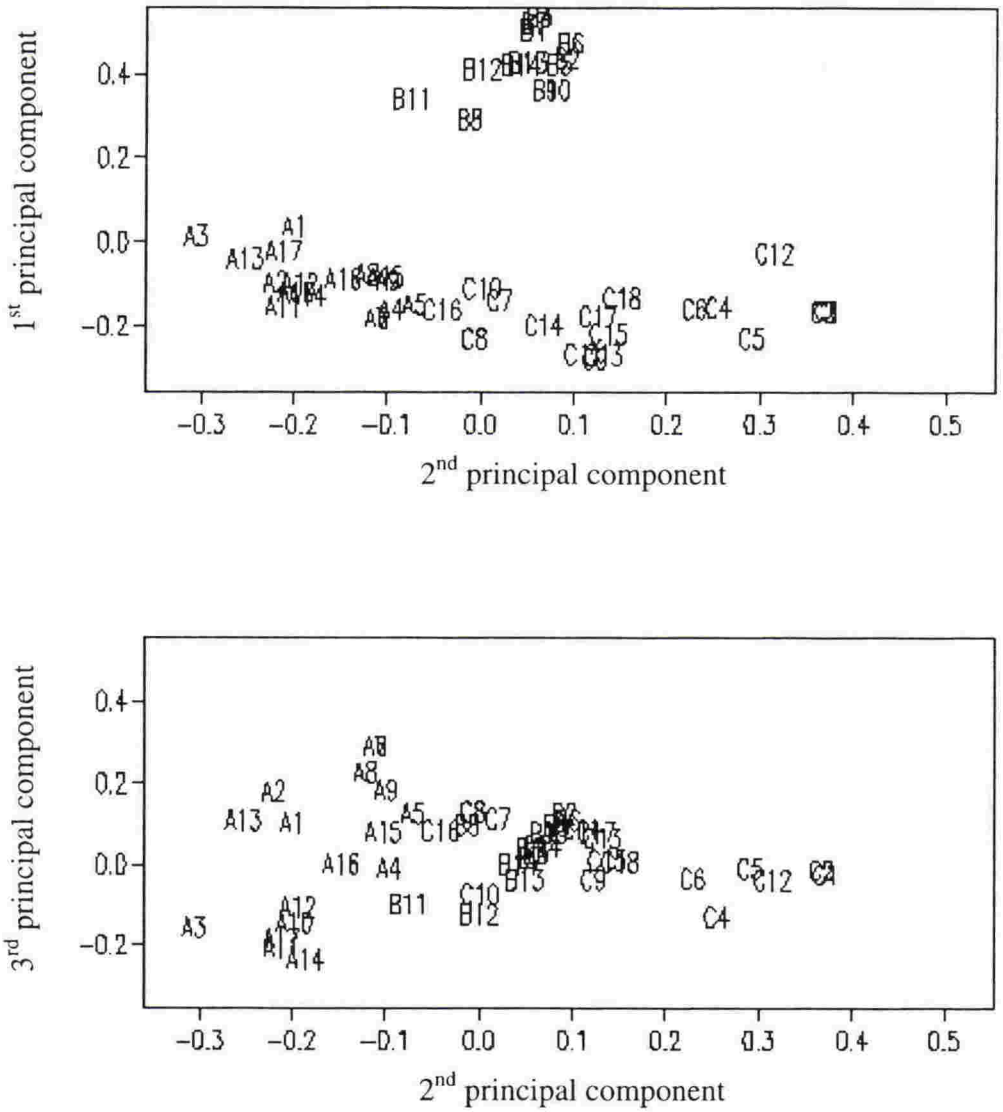


Figure 3.2. Plots of principal co-ordinates analysis illustrating the positioning of *Lucilia cuprina* individuals from Blenheim strains (A,B,C) in multidimensional space.

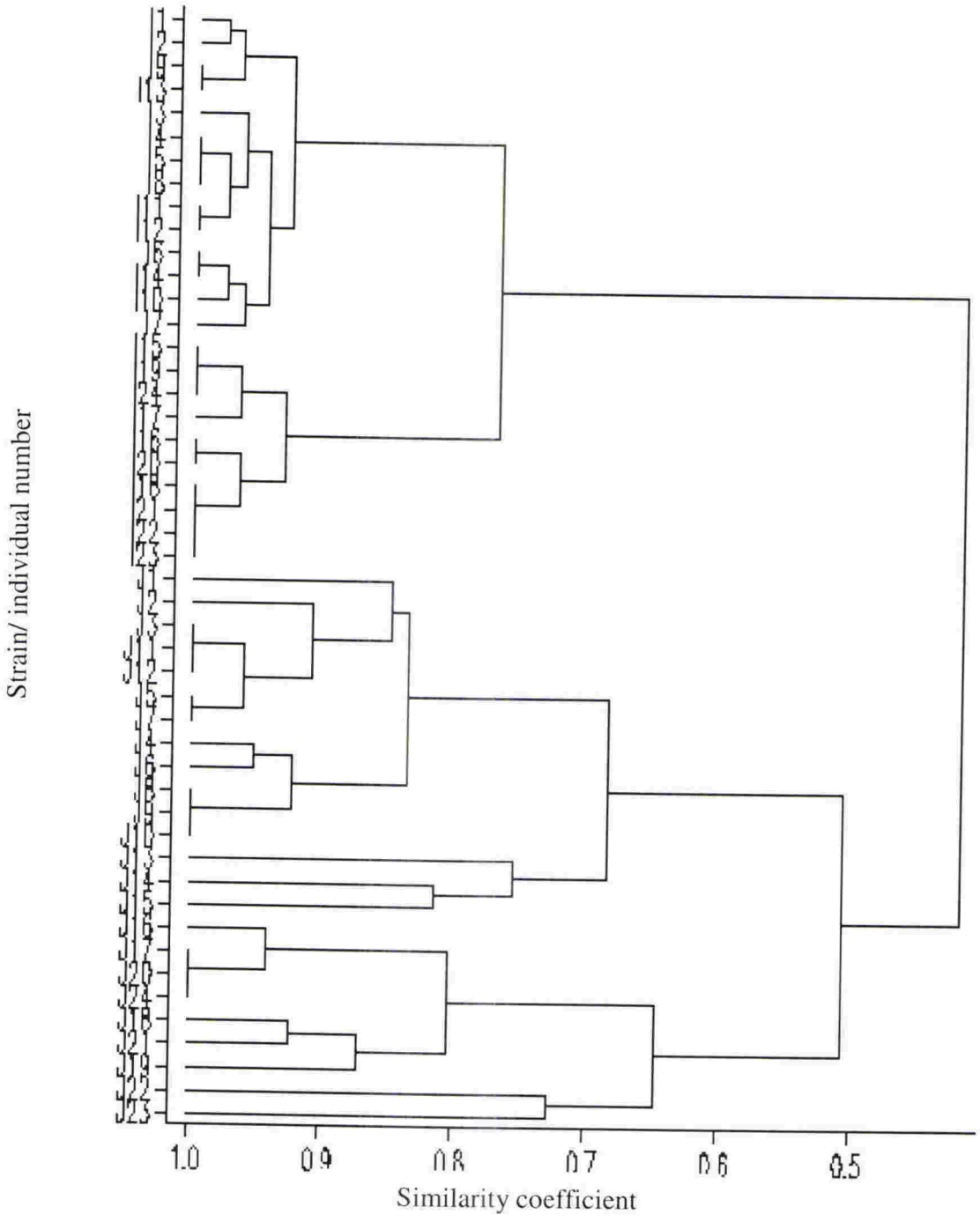


Figure 3.3 Dendrogram representing genetic similarity within two strains of *Lucilia cuprina* from Marton (I, J). Genetic similarities were calculated by Nei & Li's (1979) method of band sharing using positive frequencies. Dendrograms were constructed using hierarchical cluster analysis with an average link option in the GENSTAT® program.

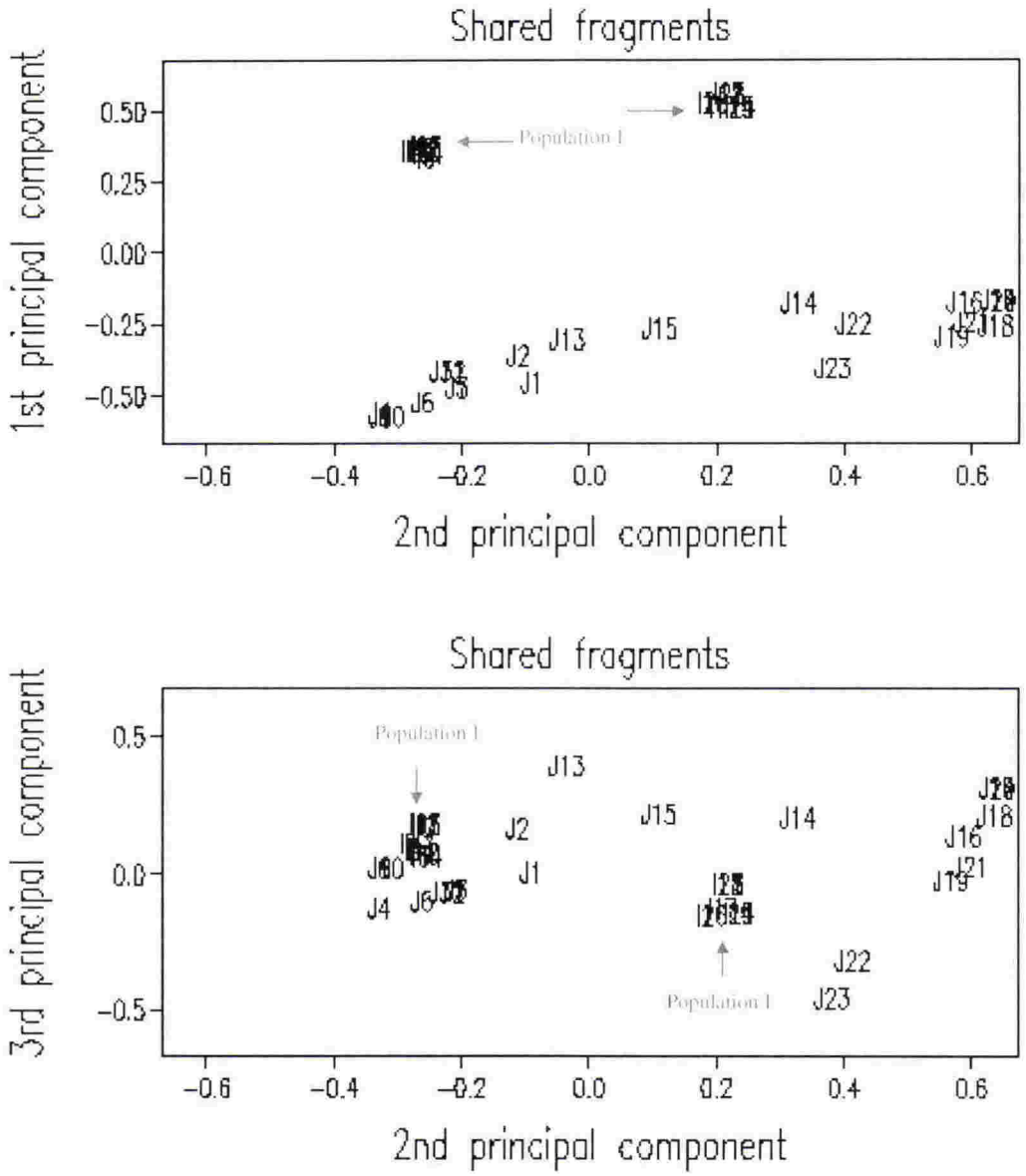


Figure 3.4 Plots of principal co-ordinates analysis illustrating the positioning of *Lucilia cuprina* individuals from Marton (I, J) in multidimensional space.

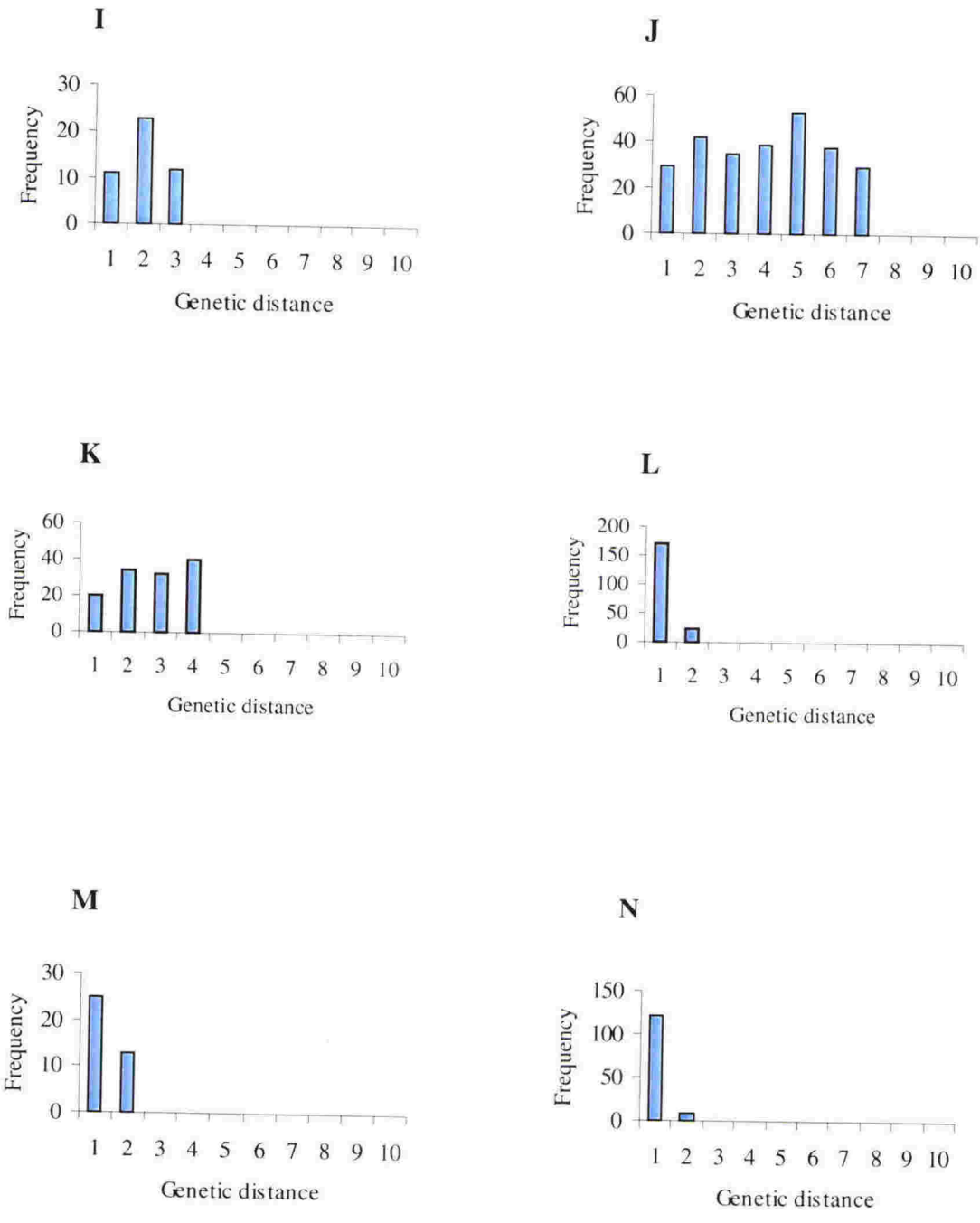


Figure 3.5 Frequency histograms showing distributions of genetically distant individuals from samples **I** – **N** as a measure of intra-strain variation.

Inter-strain variation

The population similarity matrix calculated by the Nei & Li (1979) method using positive frequencies is presented in table 3.2. The most genetically similar strains were (A) 009 Blenheim 15.03.94 and (B) 014 Blenheim 12.01.94 sharing 93.1% (i.e. similarity coefficient = 0.931) of their scored bands (Table 3.2). The least similar were (G) OP-susceptible laboratory strain and (J) 038 Marton 26.04.96 sharing only 37.5% of positive bands (Table 3.2). Twelve strains in the matrix were amplified with three primers and a dendrogram of these samples is presented in Figure 3.6. The dendrogram of these 12 strains shows clustering within areas with respect to Blenheim (A, B, C) and Marton (I, J) suggesting that these populations are stable over time (1994, 1996) (Figure 3.6). Clustering of Greytown and Turakina samples was mixed. Area clusterings became less distinct when the six strains amplified with 2 primers only, were included in the dendrogram (Figure 3.8). Samples from Blenheim remained clustered however and there was a clustering of Mid-Canterbury samples from Dorie, Leeston and Rangiora. Cluster analysis of the presence/absence banding data was not, in general, indicative of level of OP-resistance.

Examples of the Jacquard and shared fragment methods of similarity calculations are presented as dendrograms in Appendix 3.8. The Jacquard similarity index is less commonly used compared with the Nei & Li method as it is prone to bias and is often difficult to interpret (Lamboy 1994). Both the Jacquard and shared fragments method show similar clusters to the Nei & Li (1979) method however, with the exception of branch length determinations.

Table 3.2 Lower triangular similarity matrix generated by Nei & Li's (1979) method of band sharing using positive frequencies for strains of *Lucilia cuprina*, showing inter-strain variation.

Lucilia cuprina strain				A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
A	009	Blenheim	15.03.94	1.000																	
B	009	Blenheim	12.02.96	0.822	1.000																
C	014	Blenheim	12.01.94	0.931	0.787	1.000															
D	027	Greytown	07.01.94	0.774	0.814	0.808	1.000														
E	027	Greytown	06.02.92	0.795	0.726	0.865	0.826	1.000													
F	134	Pokeno	26.02.96	0.687	0.699	0.654	0.708	0.652	1.000												
G	000	Laboratory	00.00.00	0.634	0.557	0.674	0.663	0.733	0.690	1.000											
H	066	Dorie	20.04.94	0.630	0.600	0.727	0.634	0.767	0.628	0.784	1.000										
I	038	Marton	12.04.94	0.697	0.556	0.702	0.685	0.644	0.490	0.570	0.467	1.000									
J	038	Marton	26.04.96	0.494	0.546	0.560	0.591	0.605	0.470	0.375	0.406	0.613	1.000								
K	003	Turakina	10.05.94	0.732	0.711	0.750	0.695	0.845	0.682	0.688	0.764	0.525	0.512	1.000							
L	003	Turakina	22.04.96	0.731	0.720	0.697	0.840	0.693	0.799	0.556	0.538	0.627	0.496	0.692	1.000						
M	004	Bulls	21.12.94	0.779	0.675	0.838	0.750	0.749	0.738	0.694	0.615	0.825	0.650	0.658	0.740	1.000					
N	004	Bulls	23.02.96	0.783	0.784	0.934	0.846	0.911	0.724	0.693	0.751	0.748	0.800	0.724	0.754	0.814	1.000				
O	034	Leeston	18.04.94	0.684	0.579	0.756	0.733	0.802	0.696	0.776	0.797	0.739	0.630	0.725	0.655	0.772	0.782	1.000			
P	008	Kihikihi	07.03.94	0.677	0.667	0.767	0.751	0.810	0.765	0.689	0.790	0.643	0.555	0.810	0.741	0.723	0.791	0.762	1.000		
Q	008	Kihikihi	10.01.96	0.793	0.856	0.804	0.783	0.819	0.710	0.574	0.680	0.622	0.646	0.783	0.861	0.628	0.804	0.633	0.787	1.000	
R	038	Rangiora	04.01.93	0.545	0.550	0.724	0.673	0.791	0.592	0.670	0.773	0.607	0.732	0.665	0.576	0.719	0.818	0.883	0.683	0.589	1.000

A-L Compared using 3 primers
M-R Compared using 2 primers

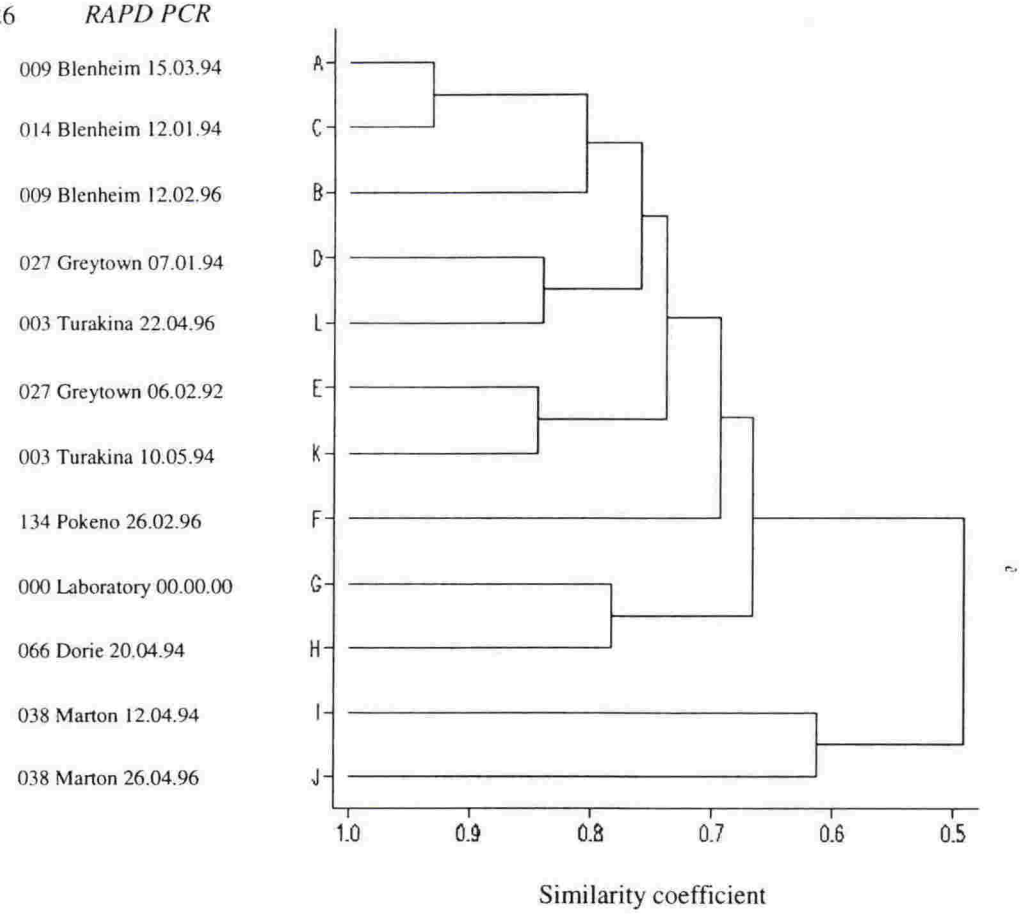


Figure 3.6 Dendrogram representing genetic similarity of *Lucilia cuprina* strains amplified with three random primers (OPA-3, 4 & 7). Genetic similarities were calculated by the Nei & Li (1979) method of band sharing using positive frequencies. Dendrograms were constructed using hierarchical cluster analysis with an average link option in the GENSTAT® program.

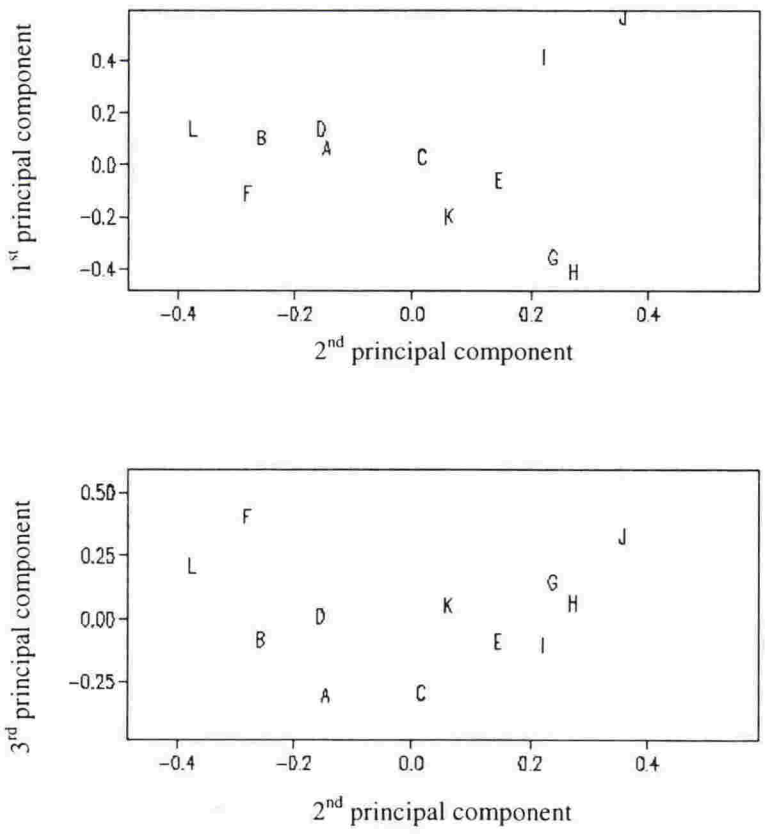


Figure 3.7 Plots of principal co-ordinates analysis illustrating the positioning of *Lucilia cuprina* populations amplified with three random primers (OPA-3, 4 & 7), in multidimensional space.

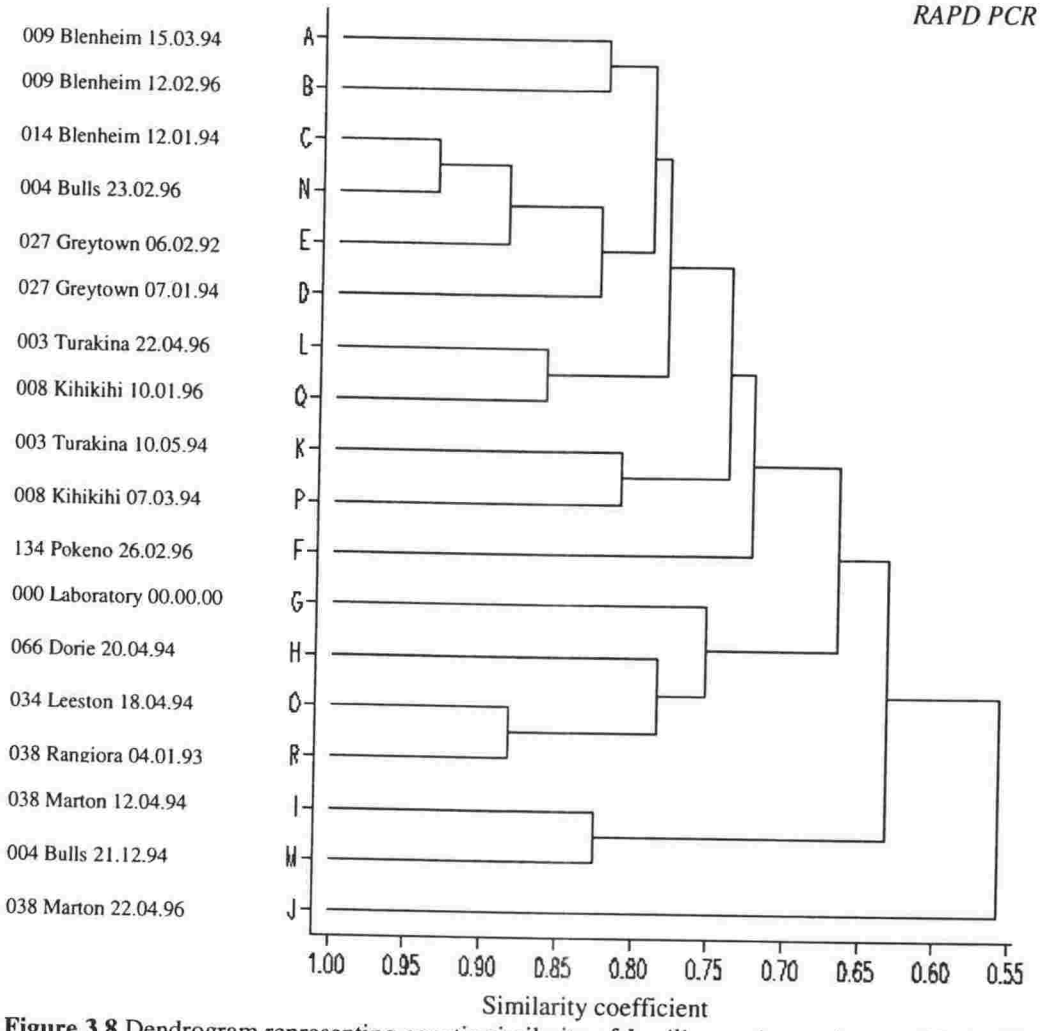
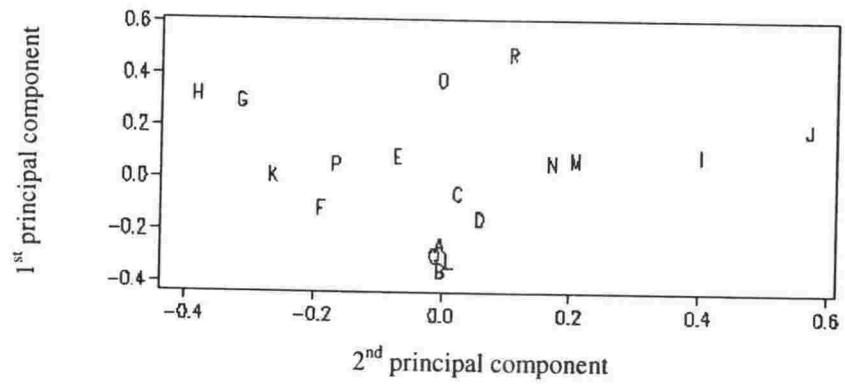


Figure 3.8 Dendrogram representing genetic similarity of *Lucilia cuprina* strains amplified with two random primers (OPA-3 &4). Genetic similarities were calculated by the Nei & Li (1979) method of band sharing using positive frequencies. Dendrograms were constructed using hierarchical cluster analysis with an average link option in the GENSTAT® program.



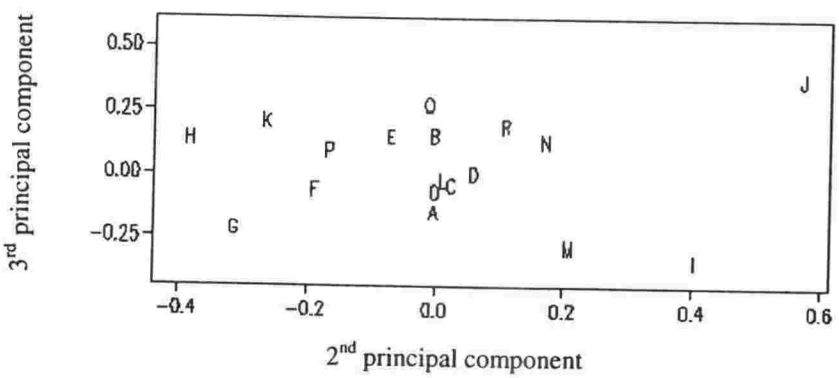


Figure 3.9 Plots of principal co-ordinates analysis illustrating the positioning of *Lucilia cuprina* populations amplified with two random primers (OPA-3 & 4), in multidimensional space.

Data from single primers revealed that clustering relationships were different when compared with the total data set. Clustering appeared to occur randomly for single primers (Appendix 3.9) compared with combined primer data where some 'same' area samples clustered. Lack of consistency of clustering was especially true with respect to primer OPA-3, which generated fewer clusters in common with combined data, than was observed with either OPA-4 or OPA-7. Single primer clustering was therefore assumed to provide poor information regarding population similarity compared with the combined primer data.

Plots of principal co-ordinate analysis are presented in figures 3.7 and 3.9 and show a simpler display of the relationship between populations than that illustrated by the dendrograms. Figure 3.7 is a representation of samples amplified with three primers. The first principal component of this figure (3.7) explains 24.2% of population variation, the second, 18.1% and the third 14.7%. The plot of first and second principal components therefore explains 42.3% of the population variation, and the second and third components explains 32.8% variation (Figure 3.7). Figure 3.9 is a combination of 18 populations amplified with either two primers. The first principal component of figure 3.9 explains 21.0% of population variation, the second 19.2 (1^{st} + 2^{nd} = 40.2%), and the third 12.7% (2^{nd} + 3^{rd} = 29.8%).

Discussion

Lucilia cuprina was first recorded in the North Island of New Zealand in 1988 although it was believed to be present for at least a decade before this. It was not until 1990 that the species was recorded in the South Island and it has continued to invade southwards over successive years. The latest records show its presence as far

south as Southland and southern Otago (Bishop 1999). The RAPD-PCR technique used in this study has helped define *L. cuprina* in New Zealand by determining similarity within and between strains based on banding patterns with 2 – 3 random primers.

Intra-strain variation

The high degree of similarity detected between some individuals may derive from single cases of flystrike (Table 3.1, e.g. 003 Turakina 22.04.96 similarity coefficient = 0.963, Figure 3.5) may have been an indication that individuals were siblings from the same oviposition event. Unfortunately known offspring from a single female were not tested for polymorphic divergence in this study. However, an example of a similarity coefficient from a truly inbred population is that of the OP-susceptible laboratory strain which had a coefficient of 0.982. Despite more than 200 generations in the laboratory this strain still exhibits some polymorphism. If strains were collected from a single oviposition event then it could be assumed that similarity coefficients would be at or near that shown in the OP-susceptible laboratory strain. Seven field strains were shown to have coefficients between 0.9 – 1.0 suggesting that there may have been a limitation with sampling technique. This possible shortcoming of the sampling technique may have been overcome by trapping of flies. Trapping is assumed to yield a more realistic representation of the population especially if it is undertaken over more than one day. Gleeson's (1995) estimate of gene flow between samples derived from incidence of strike and flies caught by trapping showed that there was no bias with sampling method, however. Sampling from cases of strike was the preferred method for this study as *L. cuprina* is less easily trapped than the related *L. sericata* owing to their preference for a live

host (Dymock & Forgie 1993). The relatively low numbers of *L cuprina* compared with *L. sericata* caught in traps by Gleeson & Heath (1997) also supports this preference by *L. cuprina*. Sampling from cases of strike may also have the added advantage that the number of female blowflies contributing to a strike may be determined by RAPD-PCR providing potentially important ecological and behavioural information about the species (Stevens & Wall 1995).

An alternative to the suggestion that the sampling method may have been limiting with respect to some strains is the possibility that these strains were genetically similar due to low migration. Low migration may or may not be a result of geographic isolation or, as mentioned below, may reflect favourable conditions in the habitat.

Strains that showed a low degree of similarity (Table 3.1, e.g. 038 Marton 26.04.96, similarity coefficient = 0.656) are likely to result from populations with a large amount of polymorphic variation between individuals. This in turn may be a reflection of a high rate of migration either into or out of a population within a season.

Inter-strain variation

The banding profiles generated by 2-3 primers in this study indicated that each sample was in some way distinct from others (Table 3.2, Figure 3.8). Many showed a high degree of similarity but area clusterings were limited to Blenheim and other South Island strains. Clusterings of North Island strains was mixed. North Island samples are well established compared with those in the South, where the frontier of

invasion by this species extends each year. The long period of establishment in the North Island is thought to be responsible for moderate to high gene flow, spatially and temporally, between neighbouring regions in the North compared with the South (Gleeson 1995). Gleeson (1995) showed that there was medium to high gene flow in populations of *L. cuprina* in North Island areas where there was a continuum of sheep farming. In contrast to this study however, Gleeson (1995) found that populations from Kihikihi in the Waikato had low gene flow with southerly North Island sites. This was assumed to reflect the lack of sheep production in the central North Island (Gleeson 1995). Kihikihi samples in this study were shown however, to cluster with samples from Turakina in the Wanganui region during the same sample year (Figure 3.8). It can only be assumed that gene flow bypasses the central North Island and finds a route through scattered sheep farms in the predominantly dairying Taranki region or that dispersion is by another means. More recently, Gleeson & Heath (1997) estimated gene flow for the species in the Wanganui, Wellington, Hawkes Bay and Wairarapa regions to be moderate with no indication of genetic isolation of populations. The tendency for dispersal was thought to be low when habitat conditions were favourable and that a key path of migration might be the transport of flystruck sheep by stock vehicles (Gleeson & Heath 1997).

Gleeson (1995) estimated gene flow in South Island areas to be low with the exception of the Harwarden and Waiau areas in North Canterbury. Mid Canterbury samples (Dorie, Leeston and Rangiora) clustered in this study but were separate from Blenheim samples in both cluster analysis (Figure 3.8) and principal co-ordinates plots (Figure 3.9). These clusterings appear to support Gleeson's (1995) findings

although more extensive sampling is needed between Blenheim and Mid-Canterbury to confirm this and to determine the origin of the southward spread of *L. cuprina.*

It is believed that the initial founding population of *L. cuprina* in New Zealand was either large or came from several sources Gleeson (1995). Gleeson (1995) believed this to be the case as New Zealand populations were as allelically diverse as those found in Australia and therefore there was no evidence of a bottleneck to colonisation. The distinct patterns of polymorphism characterised for each sample of *L. cuprina* in this study tends to support this finding.

Markers of insecticide resistance

No relationship was observed with respect to similarity clusters and level of OP-resistance. This is likely to be due to the choice of random primers for the study rather than an indication that RAPD-PCR cannot be used for this purpose. To overcome this problem additional primers targeting known resistance base pair polymorphisms may be needed. These may help to reveal genetic deviations from susceptiblity if this aspect is to be incorporated into a cluster analysis. Williams *et al.* (1990) however point out that one of the drawbacks of the RAPD technique relative to allozyme studies is that RAPD is unable to distinguish between homozygotes (2 copies) and heterozygotes (1 copy) treating all polymorphisms as dominant markers. Differentiation between high, moderate and low resistances may therefore be impossible based on polymorphisms in this study.

Shortcomings of RAPD-PCR

Various workers have described a number of shortcomings of the RAPD-PCR method. These shortcomings include the sensitivity of the method to different DNA quality and concentration and primer sensitivity resulting in a lack of reproducibility of bands (Stevens & Wall 1995). Problems of this nature were addressed, where possible, by standardising quantity (≈20 ng) and quality of DNA (A_{260}/A_{280} nm ratio above 1.6), and by using master mixes of reagents and primers for consistency. RAPD-PCR reactions were replicated by amplifying paired wing muscles from individuals of populations and only bands that appeared in both replicates were scored. Bands were matched between strains by comparison on the same agarose gel. RAPD-PCR has other less controllable problems besides methodological sensitivities, however. One well-documented problem is that of co-migration of non-homologous fragments. Co-migration may occur when bands generated by RAPD-PCR have similar electrophoretic mobility but are from non-homologous fragments. The fragments are different loci but are indistinguishable on an agarose gel. Co-migration problems can be overcome by southern blotting, segregation analysis or analysis with restriction enzymes. Restriction endonucleases are less costly than *Taq* polymerases and have been shown to increase the amount of information from RAPD-PCR in four strains of parasitic Hymenoptera (*Diaeretiella rapae*) (Roehrdanz *et al.* 1993). Dominance of RAPD markers also poses problems for interpretation of this technique. As mentioned earlier RAPD-PCR is unable to distinguish between homozygosity and heterozygosity and results in all markers being dominant (Williams *et al.* 1990). Genotypes may possibly be interpreted by intensity of ethidium bromide staining on agarose gels although this may be highly subjective.

Conclusions

Despite the possible complications of the RAPD-PCR method it has provided fast processing of many samples simultaneously. It has provided information on the geographic and temporal distribution and has reinforced studies by Gleeson (1995) and Gleeson & Heath (1997). RAPD PCR has also provided a measure of gene flow between areas and has indicated that each sample was in some way distinct from others. Many samples showed a high degree of similarity but area clusterings were limited to Blenheim and other South Island strains. Clusterings of North Island strains were, in contrast, mixed. It is probable that North Island populations are well established compared with those in the South, where the frontier of invasion by this species extends each year. The long period of establishment in the North Island is therefore thought to be responsible for mixed clusterings and the subsequent moderate to high gene flow between neighbouring regions (spatially and temporally) compared with the South as observed by Gleeson (1995).

Above all, this study has emphasised the need to treat each sample as a separate strain. Sampling from the same area at different times showed that samples were not identical suggesting there is variable gene flow in and out of areas and populations or that populations are heterogeneous. Variability of gene flow may be dependent on the mobility of flies by stock trucks or by flight.

SECTION 4

Approaches for flystrike control in New Zealand

Introduction

The systematic use of insecticides for the control of blowfly pests of sheep has provided farmers with an effective tool for a number of years. The life of many of these insecticides (e.g. OPs) is declining rapidly however, with the development of resistance by *Lucilia* species documented in the proceeding chapters. This decline is reflected by reduced periods of protection afforded by insecticides for fly control. This often results in increased frequency and dosage of insecticide treatment or changes to a small pool of alternative classes of insecticides (e.g. IGRs). Changes to frequency and dosage of insecticide usage ultimately equates to increased residues in sheep meat, wool and the environment that may directly affect trade between New Zealand and export countries. Changes to alternative insecticides may increase selection pressure on undeveloped resistance mechanisms, accelerating the development of new target-site resistances. The economic importance of resistance in *Lucilia* blowflies and indeed other insect pests is therefore a compounding problem of which solutions must be found. Solutions may not be as simple as changing an active ingredient however, since the cost of developing a new insecticide is high in terms of both money and time (Nari & Hansen 1999). Unfortunately it has been beyond the scope of this thesis to investigate intensively, solutions to the existing resistance problem in *Lucilia*. Some consideration has however been given to the use of potentiating pairs of insecticides as a method of prolonging the life of some existing OP insecticides (Chapter 4.1). Finally, rather than reiterate discussion of resistance mechanisms, the final chapter (4.2) of this

thesis looks towards applying knowledge of resistance mechanisms effectively within the framework of integrated pest management system (IPM). It also discusses areas of weakness in knowledge that need to be addressed if IPM is to succeed at a national level.

The last thing a fly ever sees

CHAPTER 4.1

Potentiation of diazinon toxicity by propetamphos and chlorfenvinphos towards strains of *Lucilia cuprina* larvae

Introduction

The cost of bringing a new pesticide on to the market is estimated to cost approximately $US50 million (Roush & Powles 1996). This is based on the initial discovery, testing, evaluation, and finally manufacturing (Roush & Powles 1996) of a pesticide, but does not include marketing. With this in mind it seems economically logical to prevent resistance developing to currently available pesticides. Two potential methods that have been considered are the addition of a synergist to an existing insecticide (Chapter 2.5) or by combining of potentiating pairs of insecticides.

Khan (1973) described potentiation as the action of one toxic compound increasing the toxicity of another compound. This definition differs slightly, but is not dissimilar from that for synergism which is defined as the combined action of two compounds being greater than their additive effect, the synergist itself often having very low toxicity (O'Brien 1967). (Experiments in synergism are described in chapter 2.5 and have been carried out in order to evaluate the role of the mixed function oxidases in insecticide resistance in *Lucilia cuprina*). A number of advantages can be had from using potentiating mixtures. These include a reduced risk of resistance developing in target insects, reduced residues and environmental contamination, and faster action due to a more potent toxicity (Rohilla & Gupta 1991, Luttrell *et al.* 1991, Roush

1993). Despite these advantages, combinations of insecticides for prevention of flystrike have not been popular for the New Zealand market, being reflected in the limited number of products previously and currently available to sheep farmers. Strike® (Coopers Animal Health New Zealand Ltd), combined the OP diazinon (96 g/l) and the SP cypermethrin (144 g/l) but has been removed from the market, leaving only Flypel® (Ancare Distributors Ltd). Flypel® is a combination of chlorpyrifos (100 g/l) and cypermethrin (10 g/l). The OP component acts as a contact toxin and the SP acts to disrupt oviposition behaviour. The strong odour of the concentrate and increased safety precautions may, in part, have contributed to the lack of popularity of OP/SP mixtures (A. Heath personal communication).

Attempts to achieve potentiation are carried out on the assumption that the toxic components have possibly different routes to resistance, between which there may be mutual disruption. There is also the assumption that insecticides are of equal persistence. As has been discussed in chapter 1.1, individual OPs vary widely in their ability to select for resistance. This suggests that a variety of mechanisms, leading to the total phenomenon of resistance, might interact. The possibility that mutual competition for detoxification mechanisms between organophosphorus insecticides might potentiate their activity is discussed here.

Methods

Toxicities of diazinon, propetamphos and chlorfenvinphos were determined using larvae of strains of *L. cuprina*, using the method of Levot (1990). Briefly, 120 mm x 30 mm strips of Whatman chromatography paper (3MM) were treated with 1 ml dilutions of each insecticide in acetone. Technical grade diazinon (91.5% a.i.),

propetamphos (90.1% a.i.) and chlorfenvinphos (92.2% a.i.) were obtained from Young's Animal Health (New Zealand) Ltd (now Novartis Animal Health). Papers treated with acetone only were used as a control. Papers were rolled and placed into 50 mm x 10 mm glass vials. Between 50 and 150 first-instar larvae, less than 2 hours old, and hatched from eggs laid on ox liver, were placed into the vials. One ml of fortified (2% yeast extract and 0.5% monobasic potassium orthophosphate) sheep serum was added and the vials were plugged with non-absorbent cotton wool. Larvae were incubated for 24 hours at a constant 25°C and photophase. The treated paper strips were unrolled into warm water and larval movement detected against a dark background and mortality scored. The data were analysed by probit regression (Finney 1971) and the LC_{25} (concentration killing 25% of larvae) calculated for each strain for each insecticide together with fiducial limits.

Combinations of diazinon + propetamphos and diazinon + chlorfenvinphos were tested at LC_{25} concentrations in preliminary potentiation experiments replicated five times. Tests using individual insecticides alone were also carried out at the same time. These served as positive controls (expected % mortality) and determined the degree of variability of LC_{25}s. Experimental conditions were as described above.

The degree of potentiation of combinations of insecticides was assessed by the co-toxicity factor described by El-Guindy *et al.* (1979). The co-toxicity factor was estimated by the following equation:

$$\text{Co-toxicity factor} = \frac{\text{observed \% mortality} - \text{expected \% mortality}}{\text{expected \% mortality}} \times 100$$

where a positive co-toxicity factor of 20 or more resulted, the combination was considered to exhibit *potentiation*, a negative of 20 or more was deemed *antagonistic* and between +20 and –20 the combination was considered to indicate an additive effect.

Results

Table 4.1.1 shows the LC_{25}s on which potentiation experiments were based as well as LC_{25}s of control dose-response series run at the same time as combinations. The LC_{25}s for all strains showed some degree of variation between the preliminary determination of LC_{25} and the controls for potentiation experiments (Table 4.1.1). Table 4.1.2 shows percentage mortality of single and combinations of OP insecticides. Propetamphos and chlorfenvinphos were shown not to potentiate the action of diazinon for all four strains of *L. cuprina* (Table 4.1.2). In all cases the effect was antagonistic with respect to expected mortality.

Table 4.1.1 The LC_{25} response of four strains of *Lucilia cuprina* larvae to organophosphorus insecticides. Initial LC_{25} determinations were the standard concentrations used in potentiation experiments whereas control LC_{25}s show the actual LC_{25} response observed during the course of potentiation experiments.

Strain/ Insecticide	Initial LC_{25}[1] determination (F.L.)[2]	Control LC_{25}
000 Susceptibles		
Diazinon	0.05 (0.05 - 0.06)	0.03 (0.02 – 0.06)
Propetamphos	0.13 (0.12 – 0.13)	0.11 (0.09 – 0.14)
Chlorfenvinphos	0.10 (0.09 – 0.10)	0.05 (0.03 – 0.08)
131 Takapau		
Diazinon	2.25 (1.99 – 2.54)	1.14 (1.42 – 0.93)
Propetamphos	0.67 (0.93 – 1.07)	0.41 (0.35 – 0.47)
Chlorfenvinphos	0.71 (0.57 – 0.89)	0.40 (0.30 – 0.52)
009 Blenheim		
Diazinon	0.84 (0.78 – 0.91)	1.27 (1.01 – 1.58)
Propetamphos	0.35 (0.29 – 0.42)	0.51 (0.58 – 0.84)
Chlorfenvinphos	0.24 (0.22 – 0.27)	0.42 (0.35 – 0.52)

Table 4.1.1 continued

059 Wairoa		
Diazinon	1.24 (1.13 – 1.36)	1.19 (0.92 – 1.55)
Propetamphos	0.57 (0.54 – 0.60)	0.35 (0.32 – 0.38)
Chlorfenvinphos	0.37 (0.30 – 0.46)	0.31 (0.28 – 0.35)

[1] LC_{25}, Lethal concentration killing 25% of the population
[2] 95% F.L., 95% fiducial limit

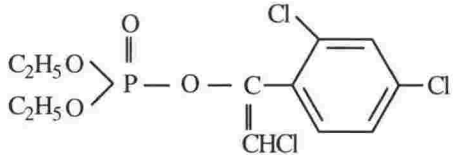
Table 4.1.2 The response of *Lucilia cuprina* larvae to mixtures of organophosphate insecticides.

Strain / Insecticide formulation	**(%) Mortality after 24 hours**	**Standard Deviation**	**Co-toxicity factor**
000 Susceptibles			
Diazinon	32.2	3.1	
Propetamphos	40.2	2.1	
Chlorfenvinphos	69.5	7.2	
Diazinon + Propetamphos	44.5	1.0	-38.5
Diazinon + Chlorfenvinphos	58.7	11.0	-41.3
131 Takapau			
Diazinon	57.7	10.3	
Propetamphos	70.8	14.7	
Chlorfenvinphos	45.0	6.2	
Diazinon + Propetamphos	33.7	6.0	-66.3
Diazinon + Chlorfenvinphos	43.6	17.7	-56.4
009 Blenheim			
Diazinon	3.9	11.1	
Propetamphos	24.3	1.3	
Chlorfenvinphos	7.9	2.1	
Diazinon + Propetamphos	8.6	2.1	-69.5
Diazinon + Chlorfenvinphos	19.1	7.0	-32.3
059 Wairoa			
Diazinon	29.7	5.6	
Propetamphos	87.0	6.6	
Chlorfenvinphos	32.5	16.5	
Diazinon + Propetamphos	51.0	18.2	-49.0
Diazinon + Chlorfenvinphos	40.6	14.6	-59.4

Discussion

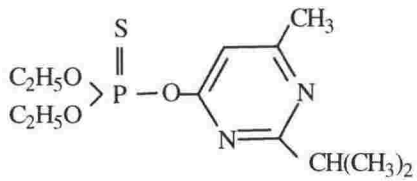
There would seem to be no advantage to be gained from combining diazinon with either propetamphos or chlorfenvinphos in equal proportions (LC_{25}: LC_{25}) in the hope of increasing individual effects. With little or no prior published work on which to

build, this experiment was speculative. It was undertaken with the anticipation that individual structural differences might be exploitable and in light of experiments suggesting that resistance to these three insecticides developed by significantly different routes (Appendix 1.1.4, 1.1.16 or refer to Wilson & Clark 1996). The insecticides selected represent three of twelve possible types of organophosphate insecticide (Chambers & Levi 1992). Chlorfenvinphos is a phosphate, where all four atoms surrounding the phosphorus are oxygen.



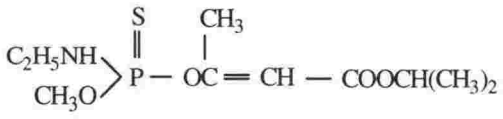
Chlorfenvinphos

Diazinon is a phosphorothionate that contains a sulphur substituent on the phosphorus atom. The metabolic activation of this insecticide requires oxidation of sulphur to oxygen (Yang 1971).



Diazinon

Lastly, propetamphos is a phosphoramidothionate. This is a small group in which one of the substituents linked to the phosphorus is a substituted amine rather than an alcohol. Propetamphos also contains a carboxyl ester group in its side chain and a vinyl group.



Propetamphos

Three mechanisms are suggested which might generate potentiating effects based on these structures.

Mechanism 1

One member of an OP pair may, in its oxon form, inhibit the enzymatic hydrolysis of carboxylester bonds in the leaving group of the other member of the pair and therefore prevent its detoxification if a serine-dependent carboxylesterase is involved. Experiments by Murphy & DuBois (1957) showed this to occur in mammals where EPN (*O*-ethyl-*O*-*p*nitrophenylphenyl phosphonothioate) potentiated the toxicity of malathion (a phosphorothionothiolate). Such a situation could apply to the detoxification of propetamphos, which possesses an isopropyl ester linkage.

Mechanism 2

If one member of an OP pair engages the action of the MFOs then the other OP maybe able to access the site of action more easily. Phosphorothionates such as diazinon are described as latent inhibitors (Corbett 1974), and this is because diazinon must undergo an initial oxidation reaction (or desulfuration) in which the S of the P=S bond is replaced by oxygen to yield an oxon (diazoxon). In contrast, chlorfenvinphos is immediately active but may be detoxified by MFO activity. The activation process of diazinon may thus inhibit the detoxification of the phosphate-based chlorfenvinphos. Mechanisms 1 and 2 both assume that one OP of the potentiating pair compensates for vulnerable structural points in the other.

Mechanism 3

Similarly, in a third possible mechanism, glutathione *S*-transferases tend to detoxify phosphorothionates more effectively than they do phosphates (Dauterman & Hodgson 1978). One might expect therefore, that the detoxification of a thion, such as diazinon by this route would be inhibited by equimolar concentrations of a phosphate, such as chlorfenvinphos.

As an alternative suggestion, the effects of intoxication by OP insecticides may result in the release of pharmacologically active substances within *L. cuprina* larvae. Individual OPs given their differing structures may produce opposing secondary substances that could lead to the inactivation of metabolic enzymes that protect the target site of the OPs. Sternberg (1963) and Dahm (1971) have demonstrated the release of such substances in insects and believe they may originate at the target site itself and therefore, in effect, act as an autointoxication mechanism. No subsequent work supports this theory however.

Other combinations of OPs maybe more appropriate to test despite the lack of potentiation that occurred with selected combinations in this study. The ratio of one OP to another may be critical to successful potentiation. The experiments described here used a ratio of 1:1 in all instances. Rohilla & Gupta (1991) successfully potentiated the action of the OP dimethoate with four other OP insecticides (methyl-parathion, dichlorvos, phosphamidon and malathion) against houseflies. They found the results to be dependent on components, concentration and insecticide proportions (Rohilla & Gupta 1991). Use of a different set of ratios for each contributing OP may have overcome the lack of potentiation in this study.

Success of potentiation studies where alternating ratios of same class of insecticides are used may be explained, in part, by the metabolism of the individual components. For example, Wells *et al.* (1986) found the production of [^{14}C] CO_2 from [^{14}C] propetamphos in houseflies to be dose-dependent. At low doses there was little difference between strains in oxidative metabolism of propetamphos to CO_2. At a threshold (between 16 and 33 ng/ insect) there was an optimal level of the topically applied dose, that was converted to CO_2. Wells *et al.* (1986) speculated that at this point, metabolic pathways became saturated by insecticide and a secondary system such as the glutathione *S*-transferases, was necessary to deal with the overload. Lack of a potentiating response to pairs of OPs in this study may indicate that metabolism of OPs by the mutant E_3 esterase may not have been saturated or that levels of unmetabolised OP remaining were at low enough concentrations to be metabolised by a secondary system such as the GSTs. K_i values for a range of OPs in crude extracts of *L. cuprina* GSTs were determined to be around 1 m*M* (A. Clark personal communication). This indicated that they have a low affinity for OPs, relative to the E_3 esterase, and are likely to be only a secondary detoxification system compared with esterases.

Combinations of an OP with different insecticide classes such as SPs or IGRs may be more likely to succeed than OP/OP pairing. Hughes & Trevethan (1979) found that a 5:1 mixture of chlorfenvinphos:cypermethrin showed potentiation *in vitro* against *L. cuprina*, but the laboratory result was not mirrored in field trails.

Conclusions

Combinations of OP insecticides used in the present study failed to produce a potentiating toxic effect towards larvae of *L. cuprina.* These negative results do not, however, eliminate the possibility that such interactions might be demonstrated by a more intensive study employing differing dose regimes and different combinations. This study also highlighted the need for thorough investigation of insecticide combinations prior to field evaluation and suggested that testing strains of variable resistance level may be useful given that there is likely to be a high degree of strain admixture in the field. Inter-strain variation can only be detected however, by the testing of a greater number of strains and combining this with relevant biochemical and metabolic analyses to determine optimal concentrations of potentiating insecticide pairs.

CHAPTER 4.2

Insecticide resistance management and IPM

Blowfly strike of sheep is a problem of significant economic importance in certain regions of New Zealand and historically has been initiated by *Calliphora stygia*, *Lucilia sericata* and *Chrysomya rufifacies*. The problem has been compounded by the introduction of the Australian sheep blowfly, *Lucilia cuprina* into New Zealand in the last 15 – 20 years and by the development of resistance by blowfly species to certain classes of insecticide. The consequence of these compounding factors has been that farmers are experiencing a reduction in the protection period that organophosphorus (OP) insecticides provide sheep against blowfly strike. Knowledge of insecticide resistance mechanisms and of the dynamics of resistance within populations are two essential elements needed for the effective management of the problem of reduced insecticide efficacy (Georghiou 1993). This thesis has addressed aspects of both of these factors for New Zealand strains of both *L. cuprina* and *L. sericata.* Firstly it has identified widespread and stable resistance to OPs in both *L. cuprina* and *L. sericata* (Chapter 1.1). Secondly, it has established the existence of multiple factors contributing to resistance in *L. cuprina* (Section 2). Evidence has been presented suggesting that *Lucilia cuprina* exploits at least two metabolic detoxification systems i.e. the specific esterase, E_3 (Chapter 2.2), glutathione *S*-transferases (Chapter 2.3), as well as a possible contribution by the mixed function oxidases (Chapter 2.4). The importance of the E_3 mechanism has been confirmed in *L. cuprina.* Extensive Australian research (Newcomb *et al.* 1997, Campbell *et al.* 1997, Parker *et al.* 1996, Newcomb *et al.* 1996, Spackman *et al.* 1994, Parker *et al.* 1991, Hughes & Raftos 1985) has revealed catalytic changes

resulting from amino acid substitutions in the ali-esterase E_3 in resistant strains compared with susceptible ones (Campbell *et al.* 1998). Understanding of additional resistance mechanisms in *L. cuprina* is however largely incomplete, especially with respect to the molecular basis of these mechanisms. Recognition of the importance of the mixed function oxidases and glutathione *S*-transferases in OP resistance in this study has supported Australian studies (Kotze & Rose 1987, Kotze & Rose 1989, Kotze 1993, Kotze & Sales 1994, Kotze & Sales 1995). The picture with respect to *L. sericata* resistance mechanisms is less clear however, although it is likely to involve an esterase-based resistance as seen in *L. cuprina* (Chapter 2.2).

Resistance management

The next logical step, given the knowledge that has been acquired, lies in the practical application of this information for the management of these insecticide resistant blowflies. Before considering how this information can be applied though, one needs to consider the definition of resistance management. Georghiou (1993) defines the concept as "the containment of the frequency of resistance genes below an acceptable limit by means of strategic choice of insecticide, dosage, mode of application, and frequency of use". At present, a common response by farmers faced with reduced protection periods of various insecticides is to try a high-dose approach, or to try one or more different insecticides in an attempt to control the pest. These approaches may or may not work depending on the extent of cross- or side-resistance to the new pesticide although they do act as a starting point for determining possible treatment tactics. Without an understanding of resistance mechanisms as well as of the interaction of genetic, geographical, dispersion and population dynamics of the pest species, insecticide treatment becomes haphazard and the risk of developing or

increasing resistance levels may rise. The choices available to sheep farmers are similar to the types of treatment tactics already in practice for controlling various crop pests and are described below.

(1) Reducing frequency of insecticide treatments

This approach has the advantage of reducing the quantity of insecticide in the environment and if timed with monitored increases in fly populations, can increase the proportion of individuals exposed to insecticide. This is also, in the long term, a cost-effective avenue for farmers. Fluctuations in population numbers of blowflies can be monitored by trapping around sheep grazing areas (a monitor trap design is available free to farmers from the Wallaceville Animal Research Centre, Upper Hutt). Occurrence of intermittent flystrike prior to major fly waves can be treated while prevalence is minimal by various prophylactic alternatives such as Dokko®, containing natural resins, Flystrike Powder® (20 g/ l diazinon), or other available dressings mentioned in Appendix 1.1.17. The effect of reducing the frequency of insecticide treatments is to decrease insecticide residues that act as a selection mechanism for resistance, and therefore to slow the onset of resistance. An added advantage is the reduction of environmental residues that are of growing worldwide concern.

(2) High doses

Roush (1993) predicted that high doses of insecticide can be used effectively only if homozygous resistant individuals are rare in a population and where the estimated resistance allele frequency is less than 10^{-3}. The frequency of homozygote OP-resistant *L. cuprina* individuals in field populations is considered to be high due to

exposure to OPs for up to 50 years in Australia (G. Levot personal communication) and 15 years in New Zealand. The biggest problem associated with using a high dose of an insecticide to eliminate or reduce the frequency of resistant individuals may be in determining an effective dose. Other complicating factors include uneven coverage of an animal or flock with insecticide, resulting in variable exposure to effective doses or variable decay rates of insecticides as well as the increased probability for the selection of highly resistant individuals. Roush (1993) pointed out that if the decay rate of an insecticide is slow, then depending on recruitment of individuals into a population there might be an insecticide residue that selects for resistance. For reasons of cost and safety, high doses of insecticides are not recommended for the control of flystrike as the risks discussed are significant.

(3) Mixtures

Historically the use of insecticides for the control of flystrike in New Zealand has followed a sequential introduction approach. This entails using one insecticide until resistance occurs and then introducing another type or variant. This is not however a criticism of how farmers use insecticide in New Zealand, since until recently they have had little other option. In fact, with the exception of the availability of dieldrin and aldrin in the 1960s, insecticide use has, until the last 10 years been almost entirely restricted to organophosphates.

With a mixture approach to insecticide treatment, individual fly larvae may be exposed to more than one insecticide simultaneously. The rationale for mixture effectiveness is that they would normally act on different target sites within an insect. The previous chapter (4.1) described tests where combinations of the same class of

insecticides were used to test the possibility that mutual competition for detoxification mechanisms between organophosphorus insecticides might potentiate their activity. However, this did not prove to be the case. There are some combinations of different classes of insecticides (OP/SP) available for flystrike control, although these do not appear popular among farmers, the reasons for which are unknown (see Chapter 1.1). In favour of using a mixture approach Curtis (1985) theorised that if resistance to each insecticide was independent, and in addition rare, then development of resistance to the mixture would be likely to be extremely rare. The mixture approach thus aims to slow the development of resistance by killing heterozygote resistant individuals before homozygote resistance can develop. With this aim in mind, the combination of OP/SP mixtures available in New Zealand may already be defeated by the established high level resistance to OPs in *L. cuprina* and to a lesser extent in *L. sericata.* Alternatively agrochemical companies would be better to consider combinations of IGRs and SPs since resistance to these groups is either low or non-existent in blowflies. The use of SPs for blowfly control can not be considered in isolation however, since SP application affects sheep biting lice and is discussed later in this chapter. A useful combination for flystrike control maybe that of diflubenzuron and either cypermethrin or deltamethrin, although it is necessary for each component to have equal persistence so that individuals are never exposed to residues of just one insecticide (Tabashnik 1989). Some populations of *L. cuprina* are beginning to show low-level resistance to diflubenzuron following several years of use of this group in New Zealand (Haack 1997). The addition of a SP may preserve diflubenzuron longer than if it were used alone. For this approach to be effective, the continued sale of products containing only diflubenzuron may need to be re-addressed. Such a combination may not offer a solution to the developing

diflubenzuron resistance however, as it has been pointed out that the success of mixtures requires a small sub-population to act as a susceptibility reservoir (Curtis 1985). This untreated (susceptible) portion is estimated to be between 1 – 20% of individuals in a population (Curtis 1985). A small susceptible sub-population maybe unachievable with a species such as *L. cuprina* that has almost nation-wide distribution, free gene flow between North Island areas despite apparent geographical barriers (Chapter 3) and 3-4 generations per season. There is a chance however, that there can always be sub-populations unexposed to diflubenzuron since not all farmers treat for flystrike at the same time, and with respect to *L. sericata*, not all individuals require sheep as a breeding resource reducing overall exposure to insecticides.

Another advantage of a diflubenzuron/ SP mixture is that it may substantially delay resistance since exposure to diflubenzuron is not widespread and resistance may still be recessive. Low level cross-resistance between diflubenzuron and diazinon (Haack 1997) may however compromise the effectiveness of the combination, as Mani (1985) has shown that even slight deviations in susceptibility may substantially increase the rate of evolution of resistance to a mixture. Complete susceptibility may however be unachievable in a species such as *L. cuprina* that has shown multiple mechanisms of resistance to the one class of insecticide (OPs) tested in this thesis.

(4) Rotations and mosaics

A rotational approach to insecticide application is often capable of extending the life span of an insecticide (Roush 1993). The theory of this method is that individuals resistant to a particular insecticide will decline in frequency while an alternative

insecticide is in use. This only works however if there is a fitness cost associated with resistance and if there is no cross-resistance between rotational insecticides (Georghiou 1983). Unfortunately there does not appear to be a fitness disadvantage to be had with *L. cuprina* in its resistance towards OPs (McKenzie *et al.* 1982, McKenzie & Clarke 1988, Rowland *et al.* 1987, McKenzie 1993) and costs if any, are undetermined with respect to resistance to other classes of insecticide. This aside, studies with highly mobile dipteran and homopteran pests have shown rotations, especially those including mixtures, significantly delay the development of resistance (Georghiou *et al.* 1980, Miyamoto *et al.* 1983, Prabhaker *et al.* 1998, Byford *et al.* 1999). The closest example that can be compared with the ectoparasitic condition of blowfly strike is that of the horn fly, *Haematobia irritans.* Horn flies are obligate bloodsucking ectoparasites of cattle which have developed resistance to synthetic pyrethroid insecticides by a combination of biochemical, physiological (principally active site insensitivity, reduced penetration and increased metabolism) and behavioural mechanisms. Laboratory studies have shown that rotation moderated the development of resistance to non-pyrethroid insecticides. For example, a rotation of permethrin and diazinon delayed tolerance to diazinon by six generations when compared with continuous use of diazinon alone (Byford *et al* 1999). A more significant delay in tolerance was seen however, with rotations of mixtures, permethrin-diazinon and permethrin-ivermectin, when compared with insecticides alone. Tolerance to diazinon applied as a rotation mixture was delayed by 12 generations although development of tolerance was unaffected with ivermectin. The magnitude of tolerance to diazinon and ivermectin was also significantly reduced (Byford *et al* 1999).

Mosaic application of insecticides is similar to rotation of them except that insecticides are alternated over space rather than time (Roush 1993). Curtis *et al.* (1978) suggested that mosaics were limited in efficacy in the absence of the release of susceptible males although this was not the case with the suppression of resistance in horn flies (Byford *et al.* 1999). The mosaic design for horn fly control in the field involved treated separate cattle herds being maintained on different pastures with two different classes of insecticide, namely an OP and a SP (Byford *et al* 1999). Treatment efficacy using the mosaic did not change over three years compared with single class treatments which showed a 10-week decline in protection period for both insecticides in the same trial.

Laboratory studies on horn fly indicated that rotation of mixtures held the most promise for delaying the onset of resistance whereas field studies suggested that a mosaic approach might be more efficacious (Byford *et al.* 1999). The horn fly study highlights the need for similar experimentation for the management of *Lucilia spp.* resistance so farmers may be given informed advice on the best control strategies using insecticides.

(5) Synergists

In view of the multi-factorial nature of resistance and the bi-phasic response (i.e. antagonism/synergism) to the mixed function oxidase inhibitor, piperonyl butoxide (PBO) (Chapter 2.6) it is doubtful that the prospects for using this or other synergists to overcome resistance to OPs in *L. cuprina* in the field are good. For synergists to be more effective, persistent inhibitors of esterases (such as DEF, Hughes 1982, Attia *et al.* 1979) and of glutathione *S*-transferase would need to be considered and trialled. The

use of multiple synergists would no doubt be expensive and may not necessarily guarantee success in the field.

Applying knowledge of resistance mechanisms

Piperonyl butoxide may not be effective at reducing resistance to OPs but it may be exploitable by preserving susceptible individuals in a population. Chapter 2.4 showed that susceptible through to moderately resistant individuals responded to pre-treatment with PBO by increased levels of resistance to diazinon, while those with higher than moderate resistance showed a decrease (biphasic response). This would suggest that highly OP-resistant individuals were at a selective disadvantage compared with susceptible and moderately resistant individuals. As mentioned above, for a mixture approach of insecticide application to succeed there needs to be a reservoir of susceptibility. The preference for a live host, namely sheep, by *L. cuprina* may limit such a reservoir. This can be evidenced by the well-established expression of OP-resistance in this species compared with *L. sericata*, which does not require such exclusivity of host (Chapter 1.1 & 1.2).

The major theme for *L. cuprina* control and management of resistance should therefore be the preservation of a susceptibility reservoir. The widespread establishment of the mutant E_3 esterase as the principal mechanism of OP resistance in *L. cuprina* in New Zealand (Chapter 2.2) as it is in Australia suggests that it is too late for preserving susceptibility with respect to OP insecticides. In the absence of a major resistance mechanism to IGRs and SPs there is still a chance with these alternatives.

This thesis has also highlighted the strong association of glutathione *S*-transferase (GST) activity with resistance. They may be seen as a mechanism to watch for in the future. Elevated levels of GSTs have been shown to be a major mechanism of OP resistance in other insects (Yu 1996) and it is possible that this mechanism could develop a more significant role in *L. cuprina* OP-resistance and to other insecticide groups over time. Monitoring of enzyme levels in relation to resistance is information that is necessary for the fine-tuning of any IPM system.

Role of Industry

Agrochemical companies invest large amounts of money in the development of new insecticides. Roush & Powles (1996) suggested that this figure is in excess of US $50 million for development of a single compound. It is therefore in the best interests of these companies, as well as of the agricultural community at large, to promote resistance management strategies that will maintain efficacy of both existing and new insecticidal compounds. Such strategies are difficult to implement however, especially where there are multiple competing companies and products. Competition for market share is likely to render resistance management strategies and co-operation between competitors unrealistic. In the absence of co-operation between companies, advertisement of any resistance management strategies by a single company may provide a market edge if information is presented to farmers in a reliable, trustworthy fashion, supported by reputable public sector research. Economic benefits may not be immediately clear to agrochemical companies, but if the cost in time and money for the development of new chemicals could be delayed by enhanced sustainability of currently available products, the benefits to them would be enormous.

Resistance management and IPM

Although insecticide-use tactics can make a huge impact on managing resistance of a target insect, there is need to reduce reliance on insecticides. Reducing insecticide residues in the environment is of global concern and is of paramount importance for New Zealand where market eligibility of sheep meat and wool products needs to be maintained for export. Management of resistance is therefore only a part of a larger framework of integrated pest management (IPM) in which reduction in insecticide usage is an integral component. IPM is a knowledge-based system that relies on a multitude of tactics to combat a pest species. In addition IPM aims to promote environmental quality. Therefore, complementary to insecticide usage, there needs to be consideration of biological and cultural methods of blowfly control. Some of these methods have been described in the overall introduction and include reducing the attractiveness of sheep to strike by shearing, crutching, docking, and treatment of endoparasites to reduce scouring. Farmers routinely undertake these management practices and other methods need to be encouraged. These include reducing the overall fly populations at critical times by bait-bin trapping, and monitoring fly activity to allow farmers to time insecticide applications effectively (Heath 1998, Cole & Heath 1999). Progress is being made in educating farmers in alternative strategies as well as encouraging the correct application of insecticide (Cole & Heath 1999) but there are still a number of untapped resources that need to be investigated. One in particular is that of biological control agents, namely endemic and introduced parasitoid wasps that have a largely undetermined but nonetheless real impact on fly populations. Unfortunately chemical use has little consideration for beneficial biological control agents that in most instances have little or no resistance to insecticides. Determining the susceptibility of parasitoid wasps to insecticides and

various application strategies is an important consideration of an IPM system. This has in part been determined in *Tachinaephagus zealandicus* and *Aphaereta aotea* with diazinon and cyromazine. *Tachinaephagus zealandicus* was shown to be more tolerant of diazinon than *A. aotea* but both were as susceptible to cyromazine as *L. cuprina* (Bishop 1999). Of even greater advantage may be the development of insecticide-resistant wasps. Studies have shown that in many parasitoid species resistance can be difficult to develop and maintain (e.g. leafminer parasitoid, *Diglyphis begini*, Spollen *et al.* 1995) while others have shown greater resistance and stability than their host (e.g. *Anisopteromalus calandrae*, a parasitoid of *Sitophilus oryzae,* Baker 1995). If insecticide-resistant populations of parasitoids can be developed there maybe a niche for boosting population numbers in deficient regions of New Zealand.

Prospects for the future

The above control measures aside, there have been many new developments in both Australia and New Zealand for control of blowflies. In particular, more effective baits (Ashworth & Wall 1994, Morris *et al.* 1997) and trap design for bulk trapping have been investigated (Anderson *et al.* 1990). In addition, development towards a flystrike vaccine (Bowles *et al.* 1996, Casu *et al.* 1997, Willadsen *et al.* 1997) as well as more environmentally-friendly pesticides such as the bacterial pathogen *Bacillus thuringiensis* (Bt) continue (A. Heath personal communication). Consideration needs to be given however, to how long it will take before blowflies may develop resistance to Bt, and the period of protection a vaccine may provide. More than a dozen species of pest insects have been documented as showing resistance to Bt already (Huang *et al.* 1999, Tabashnik *et al.* 1998, Cardoen *et al.* 1990) although few

have evolved resistance in the field (Tabashnik *et al.* 1998, Bauer 1995). Insects have been shown to develop resistance to a Bt crystal protein by changing binding characteristics of a mid-gut receptor (McGaughey *et al.* 1996, Cardoen *et al.* 1990). Modern genetic recombination technology may provide an opportunity however, to design the Bt genome such that insect resistance mechanisms can be circumvented as the need arises.

Developments have been made in grazing management. Crops such as birdsfoot trefoil, *Lotus corniculatus*, *L. pedunculatus*, sulla and *Hedysarum coronarium* contain tannin compounds that have been shown to increase the absorption of protein into the sheep diet, boosting growth, reducing dagginess and consequently flystrike (Leathwick & Aitkinson 1995, Robertson *et al.* 1995, Leathwick & Aitkinson 1996, Niezen *et al.* 1996, Waghorn *et al* 1999).

Control of *Dermatophilosis* and fleece rot as pre-disposing factors of flystrike, are also areas of ongoing research in New Zealand and Australia (A. Heath personal communication, Chin & Gogolewski 1991). *Dermatophilosis* (lumpy wool or mycotic dermatitis) results from a bacterial infection of fleece triggered by high rainfall and subsequent invasion by motile zoospores (Roberts 1967). An acute inflammatory response occurs at the skin level resulting in the formation of thick scabs of dried purulent exudate. Fleece rot is similarly a type of dermatitis induced by moisture and bacterial growth of *Pseudomonas spp.* The fleece becomes stained as the result of skin exudates. Both conditions provide an attractive environment for gravid female blowflies as well as providing moisture for hatching eggs and a source of protein for initial larval development. Research typically involves control of these

bacterial infections and selective breeding for sheep resistant to these conditions and flystrike (Raadsma 1987, Chin & Gogolewski 1991).

Genetic control measures involving sterile or defective males have so far not been implemented in either Australia or New Zealand despite considerable research into its feasibility. This is in part due to the limited degree of success achieved in small-scale trials in Australia (Davidson 1989, Whitten *et al.* 1977) as well as the logistics and expense of large-scale releases.

Overall, the adaptive capabilities of blowflies should not be underestimated and it is these adaptive features that highlight the need for a constant search for alternative strategies. A promising approach for future blowfly control lies in the exploitation of 'environmentally friendly' biological pesticides that have unique sites of action within insects and that maybe specific to the target species. There are also often fewer regulatory requirements for biological pesticides resulting in faster registration (Froyd 1997). One such group of biological insecticides in which industry is investing is the baculoviruses that can be genetically modified to kill insects more effectively than wild-type viruses. These viruses are often host specific, and so safe for beneficial insects, mammals and the environment (Froyd 1997). The disadvantage of this host specificity is however, that very few new developments are made with respect to parasites in comparison with crop pests, treatments for which have a much larger market share. Due to this economic scale difference, control of ectoparasites has so far relied upon reformulation of compounds used for pest control of crops (Londershausen 1996). It is likely then that any new developments for ectoparasite control have to come from the public sector and such products can later

be commercialised by industry. Movement by the public sector is gaining momentum with respect to *in vivo* trials of various Bt strains as well as with plant and microbial toxins. In particular, a range of native New Zealand plant extracts are being screened for toxicity (Gerard *et al.* 1997) and synergistic activities with OPs against *L. cuprina* larvae (A. Clark & J. Wilson unpublished data). The ecdysteroid receptor (LcEcR) from *L. cuprina* has been cloned so that these plant toxins can be screened *in vitro* for their potential use as insect endocrine system inhibitors (Huang *et al* 1996). Complementary to the expression of the LcEcR is the production of antibodies and antisense RNA to the receptor that have the potential to be used to block the ecdysteroid signal transduction mechanism (Huang *et al.* 1996). The momentum of the public sector is principally dependent however, upon the uncertainty of public good science funding rather than industry funding, or a mix of both.

Other considerations for blowfly IPM

The above discussion has focussed only on management of insecticide resistance and IPM strategies for blowflies, and has neglected other problems interactive in sheep production. Among these problems are gastrointestinal nematode parasites and the ectoparasite, *Bovicola ovis* (sheep biting louse). Anthelmintic resistance by sheep nematodes is a significant economic problem and is estimated to cost the New Zealand sheep industry $270 million/ year (Vlassoff & McKenna 1994, Leathwick *et al.* 1996, Waghorn 1999). Management of this problem is interrelated with blowfly control since the occurrence and abundance of nematodes can directly affect dagginess and scouring, both of which can predispose sheep to flystrike. The already low to moderate SP resistance in the sheep biting louse (Wilson *et al.* 1997) also

needs to be considered when determining blowfly insecticide control strategies. This is because lice are exposed to the range of insecticide treatments applied for fly control and therefore similar selection pressures. An insecticidal product that can be used to treat lice and flies at the same time is the ideal and there are such products available for combined fly and louse control. However, an obstacle occurs because of the partial seasonality of the two parasites. Flystrike peaks in warm conditions while louse populations often increase during cooler periods although differential wool growth, opportunity for dipping and age group susceptibility of sheep complicate strict seasonality patterns. Current practice that employs separate treatments for fly and louse control run the risk of increasing levels of resistance in either pest due to the occurrence of overlapping residues, but is resistance in one ectoparasite a cost we can afford? The simple answer is no, since the cost of damage to sheep by lice is significant although less than that of flystrike ($10 – 15 million versus $30 – 40 million for flystrike and its control, Heath 1990). Lice are responsible for a condition known as cockle, which is a sheep pelt defect that appears as nodules up to 1cm in diameter on the pickled pelt. These nodules fail to take up dye evenly during the tanning process resulting in downgrading of the leather. Immunological and parasitological studies indicate that cockle is due to an allergic response associated with infestation of sheep by the biting louse (Bany *et al.* 1995, Heath *et al.* 1995). The consequences of neglecting consideration of louse control in an IPM system for blowflies (Cole & Heath 1999) may be dire with respect to insecticide resistance, and the future of the New Zealand leather industry. Alternative methods to insecticide treatment of lice are currently being researched and include vaccine and immunomodulation techniques that aim to alter the allergic response caused by lice (A. Pfeffer personal communication).

Conclusion

Despite the evidently multi-factorial resistance in *L. cuprina* and the suspected esterase-based resistance in *L. sericata*, improved control of blowfly strike and management of the existing OP-insecticide resistance is feasible. Understanding of mechanisms of resistance allows research to focus on a multitude of alternative pathways to be targeted by novel control measures (Figure 4.2.1). The biggest problem may now lie in educating farmers to use an integrated pest management system in a co-ordinated effort with scientists and agrochemical companies in order to maximise profitability and sustainability of IPM practises for blowfly control. If only the evolution of mankind's thinking evolved at the same rate as insecticide resistance our problems of pest control would be solved, *or would it (Figure 4.2.2)?*

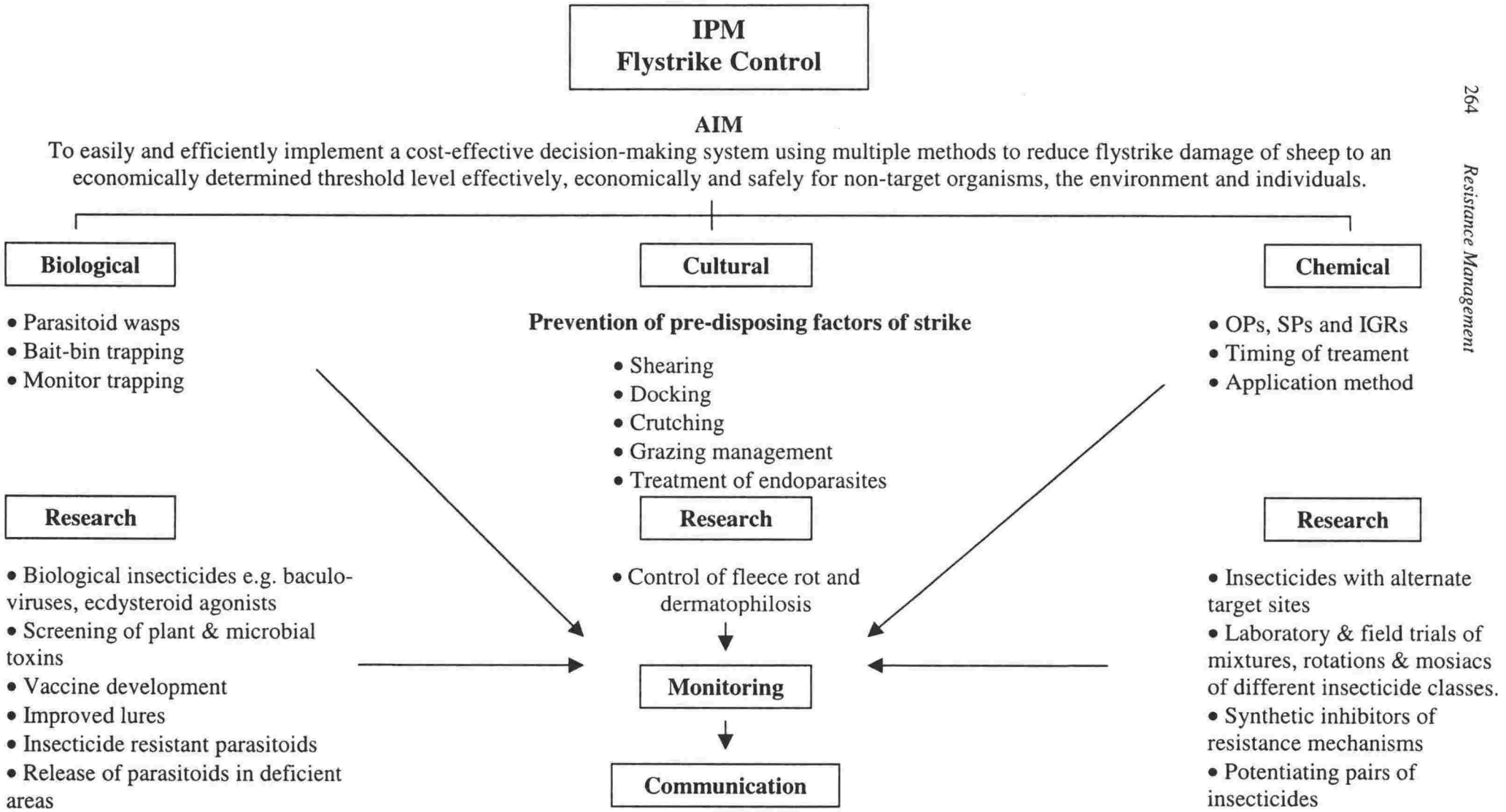


Figure 4.2.1 Summary of IPM for Flystrike Control

"Ninety-seven percent of all species are insects, pal. You're the freak."

Figure 4.2.2 from 'American Scientist, Volume 83, page 268'.

GLOSSARY

Coefficient estimates detect outliers by plotting the effect of removing individual points from the data set one at a time. Outliers are recognised by large peaks or troughs matched to observations in the coefficient estimate graph.

Cross-resistance describes the relationship between resistances to insecticides of different classes. For example, it has been reported that cross-resistance exists between diazinon and diflubenzuron in *L. cuprina* (Levot & Shipp 1983, 1984, Hughes & Levot 1987). Diazinon is an organophosphate and diflubenzuron is a benzoyl urea.

Discriminating dose – a concentration of insecticide killing 99.9% of the susceptible population (ffrench-Constant & Roush 1990).

Fiducial limits – confidence limits (Finney 1971).

Formulation - the form in which a pesticide is supplied by the manufacturer for use.

Hat matrix values measure leverage. They identify high leverage points that are outliers among independent variables. Ideally observations are clustered close to zero.

Insecticide is an agent that is intended to control or kill insects.

Normal equivalent deviate (NED) – transformation of percentage mortality using an inverse function of the normal probability integral (Finney 1971). NED of the percentage mortality gives a straight line when plotted against the log concentration of the applied insecticide (Finney 1971).

Persistence is the tendency of a pesticide to remain active after it is applied.

Principal co-ordinates analysis is described in the GENSTAT 5 reference manual (GENSTAT 5 Committee 1988) as using data from a symmetric matrix of associations. It attempts to find a set of points for the n units in a multidimensional space so that the squared distance between ith and jth points is given by

$$d_{ij} = a_{ii} + a_{jj} - 2a_{ij}$$

e.g. If A is the similarity matrix then a_{ii} and a_{jj} are both equal to 1, and so this is equivalent to

$$d_{ij} = 2 \times (1 - a_{ij})$$

The effect of this is that similar units are placed close together and dissimilar ones further apart. Co-ordinates of the points are arranged so that their mean position is at the origin. They are also arranged relative to their principal axes. This means that the first dimension of the solution gives the best one-dimensional fit to the full data set, the first two dimensions give the best two-dimensional fit, and so on. This type of analysis produces distances of points from their origin as well as latent roots (i.e. sum of squares of the co-ordinates of all points in the dimension). See Appendix

3.11 for an example of output from principal co-ordinates analysis of *Lucilia cuprina* populations using Nei & Li's (1979) method of band sharing.

Probit transformation - Division of the interval between 0.01 and 99.99% mortality into units of normal deviation called probits. It is also a modification of the normal equivalent deviate (NED) where the NED is increased by 5 to avoid negative values. A mortality of 50% is equivalent to 5 probits (Finney 1971).

Regression diagnostics assess whether residuals are random and normally distributed from the regression model. They are useful for identifying outliers or unusual observations that significantly influence regression results. Regression diagnostics used in this thesis were standardised residuals, hat matrices and coefficient estimates (Sokal & Rohlf 1981, B. Dawkins personal communication).

Resistance is defined as a reduction in the sensitivity of a population to an insecticide and results from the genetic selection for traits that favour survival of either direct treatment by insecticides or their residues. It is observed as a reduced ability of an insecticide product to achieve an expected level of control for a pest species when used in accordance to label recommendations. It is dependent on the assumptions that no problems of product storage, application and unusual climatic or environmental conditions exist. The developmental time of resistance of a population is dependent on several factors. These factors include how quickly the insects reproduce, the migration and host range of the pest, the insecticide's persistence and specificity, and the rate, timing and number of applications made.

Resistance management Georghiou (1993) defines the concept as "the containment of the frequency of resistance genes below an acceptable limit by means of strategic choice of insecticide, dosage, mode of application, and frequency of use". It must also be pointed out however, that management of resistance implies that it can be controlled. Hoy (1999) considers this is unobtainable and that we can only attempt to delay its onset and therefore re-terms it **resistance mitigation**.

Side-resistance describes the relationship between resistances to insecticides of the same class. For example, this study shows a relationship between diazinon and chlorpyrifos resistance in larval *L. cuprina.* Both diazinon and chlorpyrifos are organophosphorus compounds (G. Levot personal communication).

Standardised residuals are used to measure the model error introduced by particular observations when the residual has constant variance. A graph of standardised residuals for normal data would show a random scatter of points close to zero (Sokal & Rohlf 1981).

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Appendix A. Materials and Suppliers

Reagent	**Source**
[^{14}C] Diazinon	International Isotopes, Munich, Germany
[^{14}C] Lindane	Amersham, Braunschweig, Germany
[^{14}C] Methyl parathion	Amersham, Braunschweig, Germany
1,2-dichloro-4-nitrobenzene	BDH Laboratory Supplies, Poole, England
10x Stoffel buffer	Perkin-Elmer, Australia
1-chloro-2,4-dinitrobenzene	BDH Laboratory Supplies, Poole, England
α-naphthyl acetate	Ajax Chemicals Ltd, Sydney, Australia
β-naphthyl acetate	Sigma Chemical Co., St Louis, USA
Acetic acid (AnalaR)	Ajax Chemicals Ltd, Sydney, Australia
Acetone	Ajax Chemicals Ltd, Sydney, Australia
Acetylthiocholine iodide	BDH Laboratory Supplies, Poole, England
Acrylamide	Sigma Chemical Co., St Louis, USA
Agarose (molecular biology grade)	Gibco BRL, Life Technologies, Auckland, NZ
Ammonium sulphate	BDH Laboratory Supplies, Poole, England
Ampholytes (Pharmalytes)	Sigma Chemical Co., St Louis, USA
AmpliTaq DNA Polymerase Stoffel Fragment	Perkin-Elmer, Australia
Barbitone	BDH Laboratory Supplies, Poole, England
Bovine serum albumin	Sigma Chemical Co., St Louis, USA
Brentamine fast blue BB salt	BDH Laboratory Supplies, Poole, England
Bromophenol Blue	Sigma Chemical Co., St Louis, USA
Chlorfenvinphos (81.7%)	Pitman Moore (NZ) Ltd, Upper Hutt, NZ
Chloroform	BDH Laboratory Supplies, Poole, England
Chlorpyrifos (93%)	Ancare Distributors Ltd, Auckland, NZ
Cysteine	Sigma Chemical Co., St Louis, USA
Diazinon (91.5%)	Young's Animal Health (NZ) Ltd, Upper Hutt, NZ now Novartis New Zealand Ltd
Dichlofenthion (99.4%)	Pitman Moore (NZ) Ltd, Upper Hutt, NZ now Schering-Plough Animal Health
Dithiobisnitrobenzoic acid	Sigma Chemical Co., St Louis, USA
Dithiothreitol	Sigma Chemical Co., St Louis, USA
DNAzol Reagent, Genomic isolation kit	Gibco BRL, Life Technologies, Auckland, NZ
dNTP set (100 mM)	Gibco BRL, Life Technologies, Auckland, NZ
Ethanol (AnalaR)	BDH Laboratory Supplies, Poole, England
Ethanolamine	BDH Laboratory Supplies, Poole, England
Ethidium bromide	Gibco BRL, Life Technologies, Auckland, NZ
Ethyl acetate (AnalaR)	BDH Laboratory Supplies, Poole, England

Ethylenediaminetetra acetic acid	Sigma Chemical Co., St Louis, USA
Glutathione	Sigma Chemical Co., St Louis, USA
Glycerol	BDH Laboratory Supplies, Poole, England
Hyamine 10-X hydroxide solution	BDH Chemicals Ltd, Palmerston North, NZ
HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid)	Sigma Chemical Co., St Louis, USA
Hydrochloric acid (AnalaR)	Ajax Chemicals Ltd, Sydney, Australia
Methylthiobutyrate	Sigma Chemical Co., St Louis, USA
Mineral Oil	Gibco BRL, Life Technologies, Auckland, NZ
Optiphase 'Hisafe' II Scintillation fluid	Pharmacia-Wallac (UK) Ltd, Milton Keynes, United Kingdom.
Phenyl thiourea	BDH Laboratory Supplies, Poole, England
Phenylmethylsufonyl fluoride	Sigma Chemical Co., St Louis, USA
Polyvinylpolypyrrolidone	Sigma Chemical Co., St Louis, USA
Propetamphos (90.1%)	Young's Animal Health (NZ) Ltd, Upper Hutt, NZ Now Novartis New Zealand Ltd
Sephadex G-25	Pharmacia Fine Chemicals, Uppsala, Sweden
Sodium chloride	BDH Laboratory Supplies, Poole, England
Sodium hydroxide	BDH Laboratory Supplies, Poole, England
Sodium phosphate	Sigma Chemical Co., St Louis, USA
Sucrose	BDH Laboratory Supplies, Poole, England
Sulphobromophthalein	Sigma Chemical Co., St Louis, USA
Sulphuric acid (AnalaR)	BDH Laboratory Supplies, Poole, England
Tetrachlorvinphos	Gift from A. G. Clark, Victoria University
Tris (hydrixymethyl) aminomethane	Sigma Chemical Co., St Louis, USA
Triton X-100	J T Baker

Appendix B

Notes on the function of various homogenate buffer components (Walker 1980)

BSA (bovine serum albumin)

Inhibits diphenyloxidases. Diphenyloxidases convert phenolics to quinones and quinones cross-link proteins which result in a blackening reaction. BSA also acts as an alternative substrate for proteases.

DTT (dithiothreitol) A reducing agent of disulphide bonds

EDTA (ethylenediaminetetraacetic acid)

Metal chelater. EDTA is a versatile chelating agent. It can form four or six bonds with a metal ion, and it chelates with both transition-metal ions and main-group ions.

PMSF (phenylmethylsulfonyl fluoride)

$C_7H_7FO_2S$. Protease inhibitor

PTU (*1*-phenyl-*2*-thiourea)

Inhibits diphenyloxidases and tyronase and enhances epoxidase activity.

PVP (polyvinylpyrrolidone)

Soluble form of PVPP.

PVPP (polyvinylpolypyrrolidone)

Absorbs phenolics and quinones.

Appendix C. Methods of protein quantitation

(1) Bradford Method

Quantitation of protein (used to calculate enzyme activity per quantity of protein) for glutathione *S*-transferase assays and non-specific esterase assays was by an adaptation of an assay described by Bradford (1976). The protein solution consisted of 10 µl of homogenate protein, 1.49 ml 0.9 % NaCl and 1.5 ml 0.06 % w/v Coomassie® Brilliant Blue G250 (in 3.5 % perchloric acid). The solution was inverted in a cuvette to mix and absorbance was measured after 10 minutes at 610 nm. A reagent blank consisting of 1.5 ml 0.9 % NaCl and 1.5 ml 0.06% Coomassie® Brilliant Blue G250 (in 3.5 % perchloric acid) was used to 'zero' a Cary® spectrophotometer between each sample.

A series of protein solutions containing 5 µl - 100 µl bovine serum albumin (BSA) were used to produce a calibration curve. The calibration curve plotted concentration of BSA against the corresponding absorbance.

(2) Lowry Method

Stock Reagents

1. Copper tartrate carbonate (CTC)

   0.1% copper sulphate

   0.2% potassium tartrate in 50 ml dH_2O

   (50 ml of 20% sodium carbonate was added to give final volume of 100 ml)

2. 10 % Sodium dodecyl sulphate (SDS)

3. 0.8 M Sodium hydroxide

4. Folin-Ciocalteu phenol reagent.

   1 volume of folin was added to 5 volumes of dH_2O immediately before use.

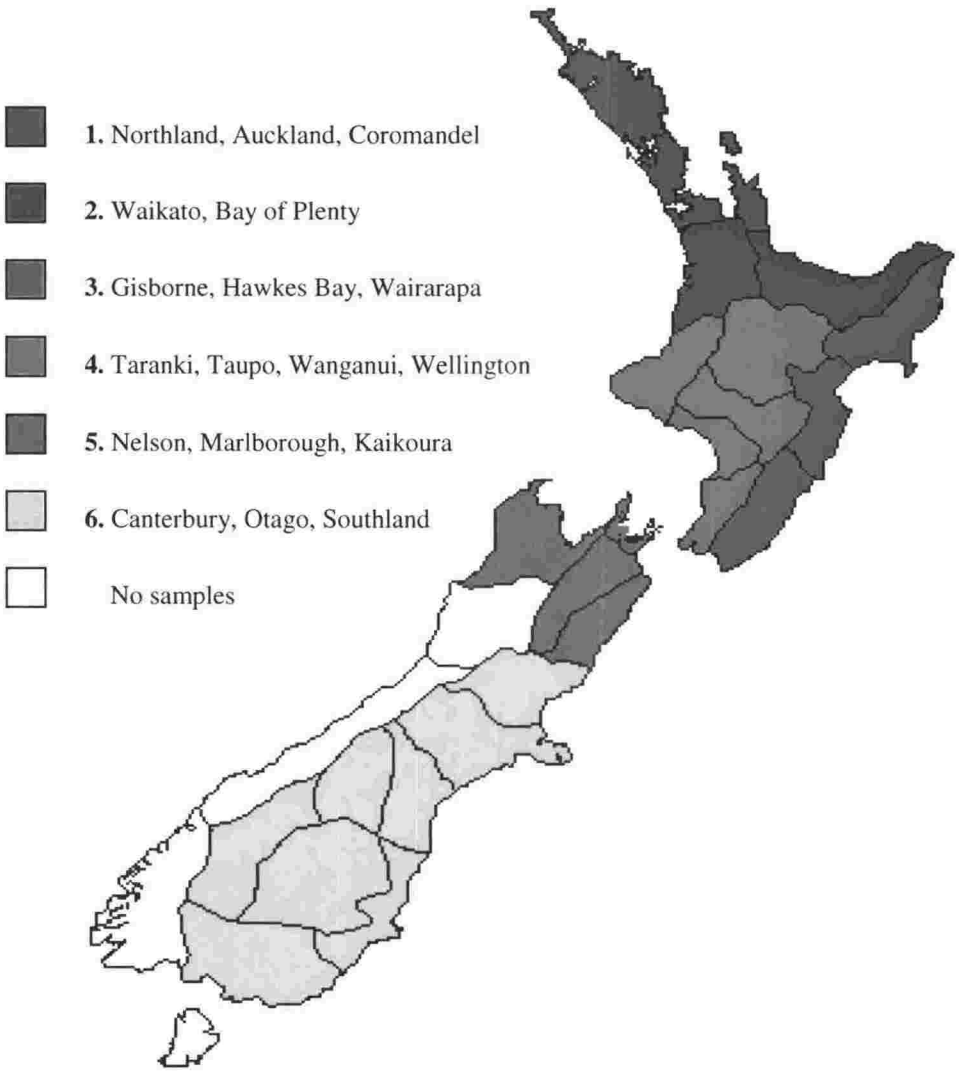
Working Solutions

1. 0.15% Sodium deoxycholate (DOC)
2. 72% Trichoroacetic acid (TCA)
3. Bovine serum albumin (BSA) 0.5 mg/ml in 0.9% NaCl
4. **Reagent A** consisted of equal parts of stock CTC, NaOH, SDS and dH_2O

Assay

The sample containing between 2.5 - 50 μg of protein was brought to a total volume of 0.5 ml with dH_2O. Fifty μl of 15 % DOC was then added and the sample and was allowed to stand for 10 minutes at room temperature. Fifty μl of 72% TCA was then added and the sample was centrifuged at 3000g for 15 minutes. The supernatant was discarded and 0.5 ml of dH_2O was added. The sample was mixed with 0.5 ml of reagent A and allowed to stand for 10 minutes at room temperature. The sample stood for a further 30 minutes after the addition of 0.25 ml of diluted Folin Ciocalteu reagent. Absorbance was read at 750 nm.

Appendix 1.1.1 Regional designations for toxicological and enzyme analysis of variance

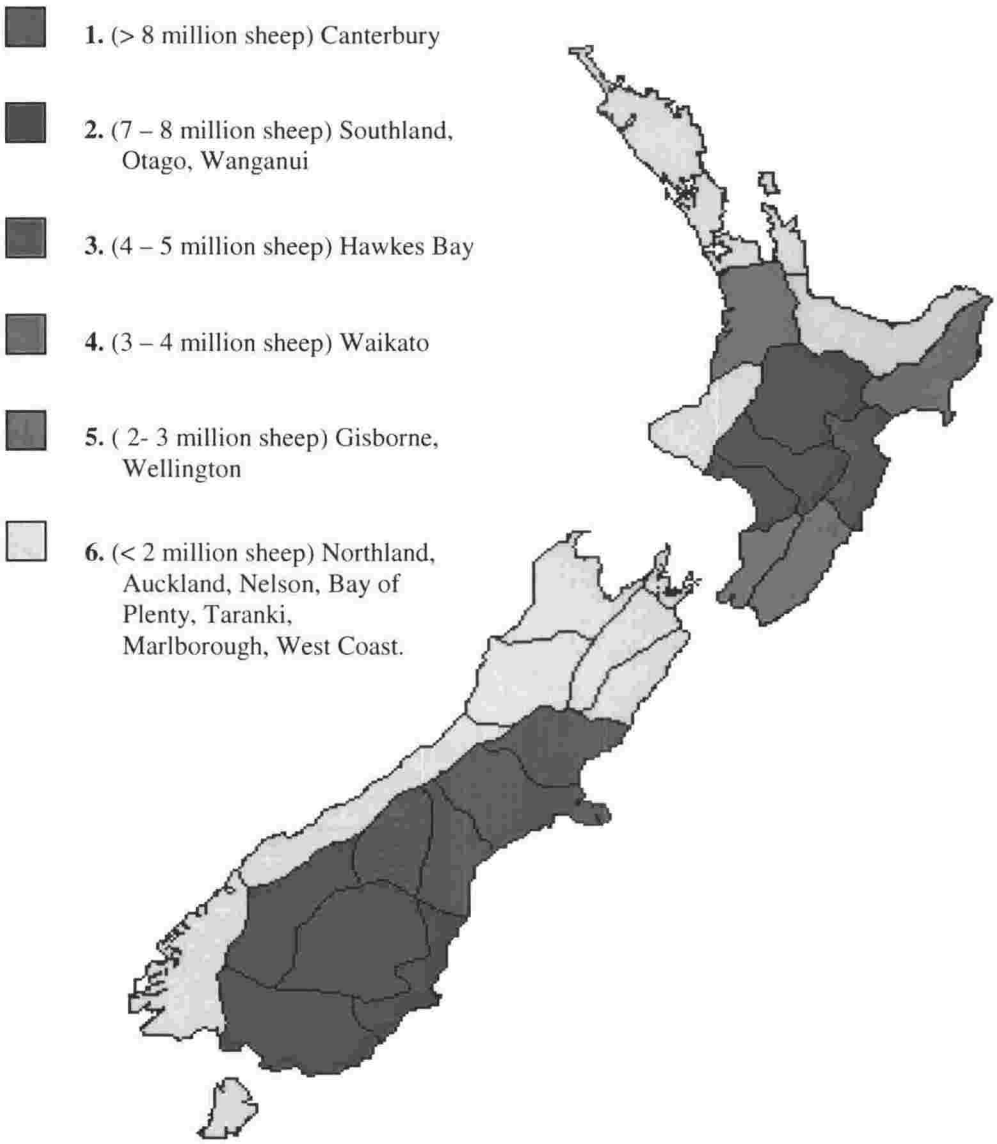


Appendix 1.1.2

Seasonal designations for toxicological and enzyme analysis of variance

Number designation	Season	Month
1	Summer	December, January, February
2	Autumn	March, April, May
3	Winter	June, July, August
4	Spring	September, October, November

Appendix 1.1.3 Sheep density designations for toxicological and enzyme analysis of variance



Appendix 1.1.4

Analysis of variance tables showing the relationship between insecticides and environmental factors

(a) Lucilia cuprina - Diazinon

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Region	5	10.1595	2.0319	12.304	0.0000
Season	2	0.5273	0.2636	1.5964	0.2041
Year	1	0.5921	0.5921	3.5855	0.0591
Sheep density	5	2.9833	0.5967	3.6131	0.0034
Residuals	344	56.8088	0.1651		

(b) Lucilia cuprina - Chlorfenvinphos

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Region	5	0.0457	0.0091	0.4781	0.7886
Season	1	0.0152	0.0152	0.7970	0.3816
Year	1	0.0368	0.0361	1.8891	0.1831
Residuals	22	0.4202	0.0191		

(c) Lucilia cuprina - Chlorpyrifos

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Region	5	0.9709	0.1942	3.6619	0.0074
Season	2	0.8231	0.4116	7.7615	0.0013
Year	1	0.8728	0.8728	16.4586	0.0002
Residuals	44	2.3332	0.0530		

(d) Lucilia cuprina - Dichlofenthion

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Region	5	18.0114	3.6022	8.2000	0.0000
Season	2	9.5187	4.7594	10.8340	0.0001
Year	1	7.7853	7.7853	17.7221	0.0001
Residuals	48	21.0864	0.4393		

(e) Lucilia cuprina - Propetamphos

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Region	5	0.0424	0.0085	0.4531	0.8091
Season	2	0.0221	0.0111	0.5905	0.5577
Year	1	0.0059	0.0060	0.3181	0.5752
Residuals	52	0.9734	0.0187		

(f) Lucilia cuprina - Diflubenzuron

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Region	3	8.5568	2.8523	4.1841	0.0412
Season	1	1.2887	1.2887	1.8904	0.2024
Year	1	0.0029	0.0029	0.0042	0.9496
Residuals	9	6.1351	0.6817		

(g) Lucilia cuprina - Deltamethrin

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Region	5	1.0743	0.2149	0.6400	0.6690
Residuals	25	6.6763	0.3338		

(h) Lucilia sericata - Diazinon

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Region	4	2.3584	0.5896	9.1013	0.0000
Season	2	0.1245	0.0623	0.9613	0.3862
Year	1	0.3093	0.3093	4.7746	0.0315
Residuals	91	5.8950	0.0648		

Appendix 1.1.5

Toxicological response of *Lucilia cuprina* larvae to diazinon

Farm	Location	Sample date	LC_{50} (95% F.L.)[1] ppm	LC_{99} (95% F.L.) ppm	Slope (SE)[2]	RF[3]
000	Laboratory	00.00.00	0.05 (.048 - .054)	0.09 (.088 - 1.00)	8.21 (0.33)	1.0
Northland						
025	Dargaville	16.01.91	0.85 (0.78 - 0.92)	4.14 (3.37 - 5.10)	3.38 (0.15)	17.0
025	Dargaville	17.01.91	0.87 (0.81 - 0.92)	2.85 (2.48 - 3.27)	4.50 (0.29)	17.4
016	Kaikohe	01.04.91	1.58 (1.43 - 1.75)	6.76 (5.04 - 9.07)	3.69 (0.34)	31.6
115	Kamo	16.04.91	1.17 (1.03 - 1.34)	4.40 (3.18 - 6.11)	4.05 (0.46)	23.4
115	Kamo	16.04.91	1.20 (1.05 - 1.36)	4.64 (3.37 - 6.37)	3.96 (0.58)	24.0
025	Dargaville	04.12.91	1.02 (0.86 - 1.21)	2.34 (1.59 - 3.46)	6.44 (1.46)	21.4
016	Kaikohe	28.02.92	1.90 (1.75 - 2.07)	6.16 (4.95 - 7.67)	4.56 (0.40)	38.0
016	Kaikohe	18.04.94	1.51 (1.30 - 1.77)	4.30 (2.97 - 6.23)	4.07 (0.79)	30.2
036	Kaiwaka	14.03.94	0.94 (0.81 - 1.08)	3.77 (2.60 - 5.46)	3.85 (0.47)	18.8
016	Kaikohe	21.12.94	1.44 (1.34 - 1.56)	3.94 (3.29 - 4.73)	5.33 (0.25)	28.8
115	Kamo	07.12.95	0.63 (0.55 - 0.73)	1.53 (1.08 - 2.16)	6.09 (1.18)	12.6
003	Dargaville	11.01.96	1.11 (1.03 - 1.18)	2.78 (2.38 - 3.26)	5.80 (0.54)	22.2
115	Kamo	09.02.96	0.92 (0.86 - 0.97)	2.06 (1.82 - 2.33)	6.62 (0.45)	18.4
115	Kano	02.01.97	1.38 (1.16 - 1.65)	5.26 (3.35 - 8.26)	4.01 (0.63)	27.6
071	Waimaukau	19.01.97	1.19 (1.11 - 1.27)	3.18 (2.79 - 3.63)	5.43 (0.32)	23.8
Auckland						
094	Paerata	09.04.91	1.34 (1.24 - 1.44)	3.80 (3.23 - 4.48)	5.13 (0.50)	26.8
069	Mangere	15.01.91	0.89 (0.81 - 0.97)	1.91 (1.57 - 2.32)	6.97 (0.78)	17.8
082	Bombay	07.02.91	0.92 (0.82 - 1.03)	2.88 (2.18 - 3.80)	4.70 (0.51)	18.4
104	Pokeno	09.01.91	0.85 (0.76 - 0.95)	2.73 (2.12 - 5.10)	4.62 (0.47)	17.0
071	Waimauku	22.01.96	0.79 (0.74 - 0.85)	2.48 (2.13 - 2.89)	4.71 (0.34)	15.8
134	Pokeno	26.02.96	0.75 (0.60 - 0.95)	2.54 (1.55 - 4.17)	4.40 (0.89)	15.0
109	Waimana	11.03.96	1.00 (0.66 - 1.50)	2.39)0.91 - 6.27)	6.12 (3.17)	20.0
109	Waimana	10.01.97	0.92 (0.88 - 0.97)	1.86 (1.64 - 2.11)	7.62 (0.50)	18.4
Waikato						
008	Kihikihi	22.01.91	0.88 (0.85 - 0.91)	2.20 (2.02 - 2.41)	5.85 (0.34)	17.6
008	Kihikihi	22.01.91	0.92 (0.87 - 0.97)	2.95 (2.60 - 3.35)	4.60 (0.23)	18.4
008	Kihikihi	26.02.91	0.52 (0.41 - 0.66)	1.64 (1.00 - 2.68)	4.67 (1.09)	10.4
008	Kihikihi	26.02.91	0.47 (0.45 - 0.49)	0.84 (0.77 - 0.92)	9.38 (0.62)	9.4
043	Waingaro	24.02.91	0.79 (0.71 - 0.89)	1.83 (1.41 - 2.37)	6.43 (0.89)	15.8
070	Te Kuiti	28.02.91	0.89 (0.81 - 0.98)	2.42 (1.96 - 2.98)	5.37 (0.53)	17.8
121	Ngaruawhia	01.03.91	1.48 (1.42 - 1.55)	4.78 (4.28 - 5.35)	4.58 (0.25)	29.6
043	Waingaro	31.03.91	1.67 (1.47 - 1.90)	7.89 (5.25-11.87)	3.45 (0.42)	33.4
043	Waingaro	21.04.91	0.79 (0.72 - 0.88)	2.14 (1.69 - 2.70)	5.42 (0.64)	15.8
090	Mokau	15.04.91	1.21 (1.09 - 1.35)	4.22 (3.23 - 5.52)	4.29 (0.43)	24.2
022	Te Kuiti	01.05.91	1.21 (1.12 - 1.30)	3.82 (3.19 - 4.58)	4.65 (0.46)	24.2
105	Manunui	26.10.91	0.96 (0.88 - 1.06)	1.95 (1.57 - 2.41)	7.63 (1.11)	19.2
081	Raglan	28.01.92	1.21 (1.15 - 1.28)	2.37 (2.11 - 2.66)	8.01 (0.68)	24.2
010	Te Kuiti	15.01.92	0.79 (0.59 - 1.07)	2.42 (1.18 - 4.96)	4.80 (1.54)	15.8
022	Te Kuiti	23.01.92	1.24 (1.04 - 1.48)	3.22 (2.15 - 4.82)	5.61 (1.14)	24.8
010	Te Kuiti	29.01.92	1.27 (1.21 - 1.33)	3.17 (2.82 - 3.56)	5.84 (0.40)	25.4
010	Te Kuiti	16.02.92	1.50 (1.42 - 1.58)	3.30 (2.93 - 3.71)	6.80 (0.63)	30.0
008	Kihikihi	21.04.92	1.05 (1.05 - 1.15)	2.42 (2.17 - 2.70)	6.81 (0.64)	21.0
008	Kihikihi	16.01.93	1.43 (1.26 - 1.63)	3.79 (2.73 - 5.26)	4.14 (0.87)	28.6
043	Waingaro	06.02.93	1.58 (1.48 - 1.68)	5.23 (4.46 - 6.13)	4.47 (0.28)	26.3
049	Wairoa	10.02.93	1.11 (1.00 - 1.22)	2.14 (1.67 - 2.75)	8.12 (1.42)	22.2
008	Kihikihi	07.03.94	0.59 (0.54 - 0.65)	1.52 (1.27 - 1.82)	5.68 (0.80)	11.8
043	Waingaro	31.01.94	1.20 (1.07 - 1.36)	2.63 (1.96 - 3.54)	6.85 (1.17)	24.0
140	Te Awamutu	09.01.95	0.92 (0.85 - 1.00)	1.82 (1.51 - 2.19)	7.88 (1.31)	18.4
140	Te Awamutu	09.01.95	0.92 (0.85 - 1.00)	1.82 (1.51 - 2.19)	7.88 (1.31)	18.4
043	Waingaro	30.01.95	0.81 (0.68 - 0.96)	1.79 (1.18 - 2.71)	6.73 (1.67)	16.2

008	Kihikihi	08.03.95	0.84 (0.70 - 1.00)	2.42 (1.45 - 4.06)	5.03 (1.15)	16.8
161	Mohoenui	16.05.95	1.54 (1.35 - 1.76)	4.74 (3.53 - 6.38)	4.76 (0.59)	30.8
008	Kihikihi	10.01.96	0.70 (0.63 - 0.78)	1.30 (1.00 - 1.69)	8.71 (1.74)	14.0
010	Te Kuiti	22.01.96	1.49 (1.43 - 4.55)	4.45 (3.99 - 4.97)	4.89 (0.20)	29.8
043	Waingaro	30.01.97	1.36 (1.25 - 1.47)	3.84 (3.18 - 4.63)	5.16 (0.42)	20.0
043	Waingaro	04.02.96	0.98 (0.75 - 1.28)	4.67 (3.25 - 6.70)	4.28 (0.62)	19.4
043	Waingaro	04.02.96	0.97 (0.88 - 1.07)	2.85 (2.35 - 3.47)	4.97 (0.58)	19.4
Coromandel						
074	Waihi	10.04.94	0.63 (0.54 - 0.73)	2.17 (1.55 - 3.02)	4.33 (0.65)	12.6
Bay of Plenty						
138	Kati Kati	09.12.91	1.11 (0.97 - 1.28)	3.61 (2.55 - 5.10)	4.56 (0.64)	22.2
135	Tauranga	12.12.91	1.08 (0.96 - 1.21)	2.60 (2.02 - 3.36)	6.09 (0.78)	22.0
017	Tauranga	10.01.92	1.36 (1.23 - 1.50)	3.60 (2.83 - 4.57)	5.49 (0.65)	27.2
017	Tauranga	03.02.92	1.20 (0.99 - 1.45)	2.59 (1.69 - 3.98)	6.95 (1.75)	24.0
014	Rotorua	16.02.92	1.08 (0.98 - 1.18)	2.95 (2.36 - 3.68)	5.32 (0.55)	21.6
135	Tauranga	20.01.94	0.92 (0.80 - 1.05)	2.20 (1.61 - 3.02)	6.11 (1.07)	18.4
108	Waimana	24.01.95	1.40 (1.32 - 1.48)	3.00 (2.63 - 3.42)	7.03 (0.55)	28.0
014	Rotorua	07.12.94	0.39 (0.34 - 0.44)	0.91 (0.75 - 1.11)	6.29 (0.33)	7.8
Gisborne						
067	Manutuke	04.03.91	1.54 (1.31 - 1.81)	5.93 (3.87 - 9.08)	3.97 (0.58)	30.8
067	Manutuke	08.04.91	1.34 (1.23 - 1.45)	4.76 (3.93 - 5.75)	4.23 (0.30)	26.8
006	Wairoa	20.02.91	0.90 (0.76 - 1.07)	2.57 (1.65 - 3.99)	5.13 (1.06)	18.0
117	Wairoa	21.10.91	1.42 (1.33 - 1.51)	4.69 (4.03 - 5.45)	4.48 (0.27)	28.4
011	Gisborne	03.02.92	1.06 (0.95 - 1.19)	3.09 (2.36 - 4.05)	5.03 (0.66)	21.2
044	Wairoa	12.02.92	1.35 (1.26 - 1.44)	4.19 (3.54 - 4.96)	4.72 (0.31)	27.0
011	Gisborne	16.02.92	2.05 (1.96 - 2.14)	4.26 (3.84 - 4.73)	7.31 (0.43)	41.0
006	Wairoa	18.02.92	2.07 (1.77 - 2.43)	7.67 (4.92-11.86)	4.11 (0.66)	41.4
011	Gisborne	19.03.92	1.71 (1.63 - 1.78)	3.77 (3.41 - 4.17)	6.75 (0.44)	34.2
095	Waiomatatini	13.01.93	1.42 (1.29 - 1.55)	2.94 (2.36 - 3.66)	7.34 (1.01)	28.4
077	Wairoa	11.01.93	1.61 (1.42 - 1.83)	3.69 (2.73 - 5.00)	6.46 (1.10)	32.2
112	Gisborne	11.01.93	1.96 (1.67 - 2.30)	4.39 (2.91 - 6.62)	6.63 (1.52)	39.2
112	Gisborne	08.01.93	1.52 (1.45 - 1.58)	3.23 (2.89 - 3.60)	7.10 (0.70)	30.4
012	Hastings	30.12.93	1.22 (1.15 - 1.30)	2.60 (2.26 - 2.99)	7.10 (0.28)	24.4
049	Wairoa	08.03.94	0.62 (0.47 - 0.82)	3.32 (1.65 - 6.68)	3.19 (0.71)	12.4
067	Gisborne	23.11.94	1.34 (1.14 - 1.59)	3.57 (2.39 - 5.35)	5.47 (1.05)	26.8
067	Gisborne	21.12.94	1.10 (0.72 - 1.69)	3.97 (0.98 -16.14)	4.18 (2.04)	22.0
067	Gisborne	23.11.94	1.34 (1.14 - 1.59)	3.57 (2.39 - 5.35)	5.47 (1.05)	26.8
067	Gisborne	21.12.94	1.10 (0.72 - 1.69)	3.97 (0.98 -16.14)	4.18 (2.04)	22.0
059	Wairoa	20.02.95	1.22 (1.14 - 1.30)	2.65 (2.28 - 3.08)	6.90 (0.82)	24.4
006	Wairoa	20.02.95	0.94 (0.79 - 1.11)	3.91 (2.51 - 6.08)	3.76 (0.56)	18.8
112	Gisborne	20.11.95	0.93 (0.82 - 1.07)	3.17 (2.32 - 4.32)	4.38 (0.54)	18.6
112	Gisborne	23.11.95	1.25 (1.78 - 1.33)	2.83 (2.49 - 3.22)	6.56 (0.22)	25.0
006	Wairoa	03.12.95	2.19 (1.87 - 2.57)	6.36 (4.31 - 9.37)	5.03 (0.83)	43.8
059	Wairoa	20.03.96	0.66 (0.57 - 0.76)	2.08 (1.43 - 3.01)	4.69 (0.75)	13.2
059	Wairoa	08.01.97	1.67 (1.47 - 1.89)	4.70 (3.50 - 6.31)	5.18 (0.69)	33.4
Taranaki						
080	Okato	07.02.91	0.90 (0.82 - 0.99)	2.45 (1.98 - 3.02)	5.36 (0.82)	18.0
Hawkes Bay						
012	Hastings	16.12.90	1.37 (1.23 - 1.54)	3.54 (2.74 - 4.50)	5.65 (0.74)	27.4
102	Hastings	19.12.90	1.13 (0.99 - 1.29)	3.73 (2.73 - 5.12)	4.49 (0.55)	22.6
118	Hastings	15.04.91	0.59 (0.33 - 1.08)	5.59 (1.41-22.17)	2.39 (0.88)	11.8
078	Hastings	14.01.91	1.24 (1.14 - 1.35)	4.34 (3.49 - 5.41)	4.28 (0.50)	24.8
131	Takapau	18.02.91	0.65 (0.55 - 0.77)	1.54 (1.06 - 2.23)	6.23 (1.31)	13.0
131	Takapau	18.02.91	0.64 (0.61 - 0.67)	1.32 (1.18 - 1.48)	7.37 (0.70)	12.8
131	Takapau	21.04.91	0.62 (0.59 - 0.65)	1.40 (1.27 - 1.56)	6.59 (0.54)	12.4
018	Waipawa	21.04.91	1.57 (1.44 - 1.71)	4.27 (3.43 - 5.32)	5.34 (0.52)	31.4
012	Hastings	30.10.91	1.36 (1.18 - 1.57)	3.85 (2.63 - 5.66)	5.14 (0.88)	27.2
012	Hastings	14.01.92	1.77 (1.66 - 1.90)	5.07 (4.26 - 6.03)	5.11 (0.38)	35.4

020	Hastings	21.01.92	1.19 (1.13 - 1.27)	3.40 (2.96 - 3.90)	5.12 (0.41)	23.8
020	Hastings	21.01.92	1.43 (1.31 - 1.57)	3.25 (2.56 - 4.13)	6.53 (0.89)	28.6
131	Takapau	21.03.92	1.35 (1.23 - 1.49)	3.92 (3.05 - 5.05)	5.02 (0.55)	27.0
012	Hastings	16.03.92	1.37 (1.32 - 1.43)	2.78 (2.54 - 3.07)	7.55 (0.43)	27.4
012	Hastings	29.12.92	1.00 (0.93 - 1.07)	2.70 (2.28 - 3.19)	5.39 (0.59)	20.0
012	Hastings	18.01.93	1.72 (1.58 - 1.87)	6.02 (4.85 - 7.47)	4.28 (0.33)	34.4
018	Waipawa	11.01.93	0.80 (0.70 - 0.92)	1.49 (1.09 - 2.03)	8.68 (2.11)	16.0
018	Waipawa	08.02.93	1.38 (1.31 - 1.46)	2.99 (2.62 - 3.42)	6.92 (0.58)	27.6
018	Waipawa	30.12.93	0.81 (0.69 - 0.95)	3.67 (2.48 - 5.43)	3.55 (0.48)	16.2
012	Hastings	08.04.94	1.20 (1.10 - 1.30)	3.12 (2.57 - 3.78)	5.60 (0.48)	24.0
018	Waipawa	20.01.94	0.67 (0.59 - 0.76)	1.80 (1.36 - 2.38)	5.44 (0.82)	13.4
018	Waipawa	31.03.94	0.65 (0.58 - 0.71)	1.60 (1.27 - 2.02)	5.89 (0.92)	13.0
012	Hastings	06.12.94	1.18 (1.07 - 1.30)	3.70 (2.94 - 4.66)	4.67 (0.45)	23.6
018	Waipawa	19.12.94	0.86 (0.81 - 0.92)	2.32 (1.99 - 2.70)	5.40 (0.36)	17.2
058	Hawkes Bay	03.04.95	2.13 (1.68 - 2.71)	6.99 (3.57-13.66)	4.52 (1.01)	42.6
012	Hastings	04.11.95	1.74 (1.62 - 1.87)	4.88 (4.21 - 5.66)	5.19 (0.32)	34.8
018	Waipawa	13.12.95	0.97 (0.92 - 1.02)	2.20 (1.94 - 2.50)	6.51 (0.43)	19.4
018	Waipawa	26.11.96	0.99 (0.83 - 1.19)	3.48 (2.29 - 5.30)	4.26 (0.71)	19.8
091	Waipukurau	14.04.97	2.05 (1.75 - 2.40)	7.08 (4.68-10.71)	4.32 (0.65)	41.0
097	Waipawa	09.05.97	1.66 (1.44 - 1.91)	5.88 (4.02 - 8.59)	4.23 (0.62)	33.2
018	Waipawa	26.11.96	0.99 (0.83 - 1.18)	3.48 (2.29 - 5.30)	4.25 (0.71)	19.8
012	Hastings	15.01.97	1.22 (1.14 - 1.30)	2.97 (2.59 - 3.41)	6.00 (0.22)	24.4
Wanganui						
004	Bulls	27.11.90	1.59 (1.44 - 1.75)	5.33 (4.08 - 6.98)	4.42 (0.42)	31.8
072	Bulls	03.01.91	0.92 (0.88 - 0.96)	2.19 (1.98 - 2.42)	6.16 (0.12)	18.4
072	Bulls	03.01.91	0.91 (0.78 - 1.06)	2.59 (1.88 - 3.58)	5.11 (0.76)	18.2
004	Bulls	31.01.91	1.28 (1.17 - 1.41)	2.83 (2.25 - 3.57)	6.75 (0.92)	25.6
004	Bulls	05.02.91	1.31 (1.24 - 1.38)	3.49 (3.10 - 3.93)	5.47 (0.39)	26.2
004	Bulls	08.02.91	1.73 (1.66 - 1.81)	4.93 (4.35 - 5.59)	5.13 (0.20)	34.6
072	Bulls	11.02.91	1.68 (1.55 - 1.81)	5.74 (4.69 - 7.03)	4.36 (0.31)	33.6
072	Bulls	11.02.91	1.55 (1.38 - 1.75)	3.57 (2.67 - 4.76)	6.45 (1.01)	31.0
045	Feilding	07.03.91	1.01 (0.89 - 1.14)	2.52 (1.86 - 3.41)	5.84 (0.90)	20.2
004	Bulls	08.03.91	1.48 (1.41 - 1.57)	5.49 (4.79 - 6.30)	4.10 (0.17)	29.6
004	Bulls	11.03.91	1.26 (1.16 - 1.38)	3.18 (2.56 - 3.94)	5.81 (0.61)	25.2
004	Bulls	25.03.91	1.57 (1.36 - 1.81)	4.72 (3.26 - 6.83)	4.87 (0.79)	31.4
093	Feilding	08.04.91	1.27 (1.11 - 1.44)	3.75 (2.76 - 5.10)	4.93 (0.68)	25.4
004	Bulls	09.04.91	1.41 (1.24 - 1.60)	3.51 (2.61 - 4.73)	5.86 (0.94)	28.2
124	Waverley	12.04.91	1.59 (1.49 - 1.69)	5.01 (4.25 - 5.89)	4.66 (0.28)	31.8
004	Bulls	15.04.91	1.53 (1.47 - 1.59)	3.39 (3.10 - 3.69)	6.73 (0.35)	30.6
004	Bulls	15.04.91	1.42 (1.27 - 1.60)	3.68 (2.83 - 4.78)	5.64 (0.74)	28.4
004	Bulls	24.04.91	1.81 (1.62 - 2.01)	4.41 (3.34 - 5.83)	6.01 (0.83)	36.2
004	Bulls	24.04.91	1.90 (1.80 - 2.01)	5.01 (4.37 - 5.75)	5.53 (0.32)	38.0
004	Bulls	29.04.91	1.21 (0.99 - 1.48)	5.06 (3.01 - 8.48)	3.76 (0.66)	24.2
004	Bulls	29.04.91	1.30 (1.17 - 1.45)	3.60 (2.79 - 4.65)	5.26 (0.63)	26.0
003	Wanganui	14.12.91	1.04 (0.94 - 1.14)	3.71 (2.90 - 4.73)	4.21 (0.38)	20.8
004	Bulls	08.01.92	2.24 (2.15 - 2.33)	5.60 (5.03 - 6.22)	5.85 (0.24)	44.8
003	Wanganui	10.01.92	2.06 (1.96 - 2.15)	4.65 (4.13 - 5.25)	6.56 (0.38)	41.0
004	Bulls	13.01.92	2.14 (1.55 - 2.95)	6.89 (3.16-15.01)	4.58 (1.46)	42.8
004	Bulls	03.02.92	2.76 (2.53 - 3.02)	8.11 (6.35-10.35)	4.97 (0.76)	55.2
004	Bulls	12.02.92	1.69 (1.49 - 1.90)	4.42 (3.25 - 5.99)	5.57 (0.81)	33.8
004	Bulls	14.02.92	1.67 (1.59 - 1.76)	3.59 (3.17 - 4.05)	7.02 (0.64)	33.4
003	Wanganui	07.03.92	1.11 (1.00 - 1.24)	2.70 (2.07 - 3.52)	6.06 (0.87)	22.2
004	Bulls	11.12.92	1.28 (1.11 - 1.48)	3.31 (2.28 - 4.81)	5.66 (0.79)	25.6
004	Bulls	21.12.92	1.23 (1.12 - 1.35)	2.34 (1.83 - 2.99)	8.34 (1.41)	24.6
004	Bulls	06.01.93	1.31 (1.25 - 1.37)	2.99 (2.66 - 3.35)	6.49 (0.38)	26.2
004	Bulls	27.01.93	1.48 (1.33 - 1.64)	3.64 (2.83 - 4.69)	5.93 (0.79)	29.6
004	Bulls	19.01.93	2.27 (2.15 - 2.39)	6.50 (5.67 - 7.46)	5.09 (0.43)	45.4
004	Bulls	08.02.93	1.36 (1.29 - 1.43)	3.89 (2.99 - 3.83)	5.87 (0.46)	27.2
003	Wanganui	27.01.93	1.46 (1.40 - 1.53)	2.70 (2.44 - 2.98)	8.79 (0.64)	29.2
003	Turakina	30.01.94	1.78 (1.40 - 2.26)	5.56 (3.09 - 10.0)	4.71 (1.12)	35.6
003	Turakina	10.05.94	0.94 (0.85 - 1.02)	1.75 (1.42 - 2.16)	8.57 (1.36)	18.8
003	Turakina	10.05.94	1.06 (0.89 - 1.27)	2.95 (1.95 - 4.47)	4.87 (1.02)	21.2

004	Bulls	05.04.94	0.95 (0.89 - 1.02)	2.39 (2.06 - 2.76)	5.82 (0.58)	11.6
004	Bulls	21.02.94	2.04 (1.87 - 2.22)	6.56 (5.37 - 8.01)	4.58 (0.36)	40.8
045	Feilding	13.04.94	2.24 (1.76 - 2.86)	7.65 (4.31 -13.57)	4.37 (0.95)	44.8
075	Feilding	28.03.94	0.89 (0.82 - 0.97)	2.01 (1.63 - 2.48)	6.61 (1.10)	17.8
038	Marton	12.04.94	1.07 (0.98 - 1.17)	2.02 (1.65 - 2.47)	8.43 (1.24)	21.4
038	Marton	09.05.94	0.59 (0.54 - 0.64)	1.88 (1.57 - 2.25)	4.62 (0.51)	11.8
072	Bulls	12.01.95	1.04 (0.96 - 1.12)	2.19 (1.81 - 2.66)	7.14 (0.72)	20.8
106	Marton	17.01.95	0.86 (0.80 - 0.93)	1.85 (1.57 - 2.19)	7.03 (0.78)	17.2
098	Marton	24.01.95	1.41 (1.20 - 1.66)	3.78 (2.64 - 5.42)	5.42 (0.94)	28.2
004	Bulls	06.02.95	1.94 (1.82 - 2.06)	4.06 (3.52 - 4.68)	7.26 (0.50)	38.8
045	Feilding	01.02.95	1.43 (1.26 - 1.61)	6.62 (4.89 - 8.96)	3.50 (0.33)	28.6
004	Bulls	06.03.95	1.22 (1.15 - 1.31)	2.81 (2.44 - 3.23)	6.46 (0.55)	24.4
153	Levin	01.04.95	0.99 (0.87 - 1.13)	2.61 (1.85 - 3.69)	5.53 (0.90)	19.8
003	Turakina	04.04.95	1.43 (1.28 - 1.60)	3.80 (2.89 - 5.01)	5.48 (0.71)	28.6
098	Marton	21.04.95	1.75 (1.61 - 1.91)	5.37 (4.45 - 6.48)	4.78 (0.32)	35.0
106	Marton	23.04.95	1.16 (1.02 - 1.32)	3.52 (2.64 - 4.70)	4.82 (0.60)	23.2
075	Feilding	17.04.95	1.07 (0.99 - 1.15)	2.70 (2.21 - 3.30)	5.76 (0.78)	21.4
072	Bulls	04.05.95	1.19 (1.34 - 1.24)	2.84 (2.53 - 3.18)	6.16 (0.50)	23.8
106	Marton	09.05.95	0.61 (0.56 - 0.67)	1.72 (1.39 - 2.12)	5.18 (0.75)	12.2
003	Turakina	18.05.95	0.86 (0.78 - 0.94)	2.90 (2.38 - 3.53)	4.40 (0.39)	17.2
072	Bulls	02.05.95	1.32 (1.09 - 1.60)	4.48 (2.88 - 6.95)	4.38 (0.75)	26.4
072	Bulls	20.12.95	0.94 (0.89 - 1.00)	1.95 (1.71 - 2.22)	7.37 (0.83)	18.8
072	Bulls	12.01.96	1.74 (1.65 - 1.84)	4.42 (3.94 - 4.96)	5.76 (0.24)	34.8
098	Marton	27.01.96	1.31 (1.18 - 1.46)	3.76 (2.97 - 4.78)	5.08 (0.55)	26.2
038	Marton	29.01.96	1.05 (0.95 - 1.15)	2.36 (1.86 - 3.00)	6.58 (0.90)	21.0
003	Turakina	22.01.96	1.12 (0.97 - 1.30)	3.45 (2.41 - 4.92)	4.78 (0.69)	22.4
045	Feilding	17.02.96	0.66 (0.60 - 0.72)	1.58 (1.26 - 1.98)	6.11 (0.73)	13.2
045	Feilding	05.03.96	1.14 (1.08 - 1.21)	2.59 (2.28 - 2.95)	6.56 (0.43)	22.8
004	Bulls	23.02.96	2.05 (1.70 - 2.48)	6.19 (4.07 - 9.41)	4.85 (0.88)	41.0
072	Bulls	07.03.96	1.24 (1.14 - 1.36)	3.32 (2.72 - 4.05)	5.45 (0.53)	24.8
003	Turakina	28.02.96	1.44 (1.36 - 1.52)	3.56 (3.14 - 4.05)	5.90 (0.38)	28.8
040	Okoia	25.03.96	0.73 (0.63 - 0.85)	2.33 (1.73 - 3.14)	4.62 (0.66)	14.6
072	Bulls	14.03.96	0.96 (0.92 - 1.00)	2.14 (1.93 - 2.37)	6.66 (0.14)	19.2
072	Bulls	17.03.96	0.87 (0.77 - 0.98)	2.55 (1.92 - 3.40)	4.97 (0.64)	17.4
072	Bulls	31.03.96	0.80 (0.73 - 0.89)	2.36 (1.87 - 2.97)	4.96 (0.54)	16.0
040	Okoia	20.02.96	1.59 (1.42 - 1.79)	5.93 (4.52 - 7.77)	4.08 (0.40)	31.8
040	Okoia	28.02.96	1.60 (1.53 - 1.67)	3.20 (2.88 - 3.54)	7.74 (0.43)	32.0
038	Marton	08.04.96	1.37 (1.29 - 1.46)	3.54 (3.08 - 4.08)	5.65 (0.60)	27.4
040	Okoia	25.03.96	0.73 (0.63 - 0.85)	2.33 (1.73 - 3.14)	4.62 (0.66)	14.6
098	Marton	02.04.96	1.08 (0.97 - 1.21)	2.52 (1.95 - 3.24)	6.36 (0.89)	21.6
098	Marton	09.04.96	1.11 (0.98 - 1.26)	3.06 (2.21 - 4.22)	5.29 (0.77)	22.2
038	Marton	26.04.96	1.19 (1.13 - 1.25)	2.68 (2.39 - 3.01)	6.60 (0.21)	23.8
038	Marton	05.04.97	0.92 (0.81 - 1.04)	3.19 (2.43 - 4.19)	4.29 (0.49)	18.4
038	Marton	29.03.97	1.38 (1.26 - 1.51)	5.20 (4.15 - 6.52)	4.04 (0.35)	27.6
Wairarapa						
027	Greytown	01.12.90	1.10 (1.03 - 1.17)	2.47 (2.10 - 2.91)	6.59 (0.48)	22.0
030	Kaitoke	23.11.90	1.45 (1.38 - 1.54)	4.55 (3.92 - 5.27)	4.70 (0.29)	29.0
027	Greytown	24.12.90	1.50 (1.37 - 1.65)	3.58 (2.82 - 4.53)	6.19 (0.77)	30.0
027	Greytown	11.01.91	1.36 (1.24 - 1.50)	3.00 (2.38 - 3.78)	6.80 (0.86)	27.2
024	Masterton	26.01.91	0.77 (0.74 - 0.81)	1.40 (1.26 - 1.56)	8.99 (1.02)	15.4
111	Masterton	10.02.91	1.14 (1.04 - 1.24)	2.75 (2.26 - 3.34)	6.07 (0.64)	22.8
111	Masterton	09.03.91	0.82 (0.73 - 0.92)	2.87 (2.17 - 3.81)	4.27 (0.45)	16.4
111	Masterton	18.03.91	1.31 (1.23 - 1.38)	3.93 (3.42 - 4.51)	4.87 (0.36)	26.2
111	Masterton	03.04.91	1.28 (1.20 - 1.38)	3.09 (2.61 - 3.66)	6.10 (0.54)	25.6
023	Masterton	05.05.91	0.72 (0.68 - 0.75)	2.01 (1.79 - 2.27)	5.18 (0.23)	14.4
024	Masterton	15.12.94	1.55 (1.48 - 1.62)	3.77 (3.38 - 4.20)	6.02 (0.47)	31.0
061	Masterton	09.12.94	0.48 (0.39 - 0.58)	1.61 (1.04 - 2.49)	4.39 (0.87)	9.6
024	Masterton	15.12.94	1.55 (1.48 - 1.62)	3.77 (3.38 - 4.20)	6.02 (0.47)	31.0
056	Carterton	22.02.95	1.21 (1.10 - 1.32)	2.72 (2.23 - 3.32)	6.58 (0.77)	24.1
053	Martinborough	10.02.95	0.49 (0.38 - 0.64)	1.74 (1.01 - 3.00)	4.24 (0.93)	9.8
056	Carterton	22.02.95	1.21 (1.10 - 1.32)	2.72 (2.23 - 3.32)	6.58 (0.77)	24.1
111	Masterton	26.03.95	1.27 (1.05 - 1.54)	5.46 (3.47 - 8.59)	3.68 (0.54)	25.4

154	Carterton	04.04.95	1.47 (1.40 - 1.54)	3.95 (3.54 - 4.41)	5.43 (0.32)	29.4
053	Martinbor.	13.12.95	1.59 (1.51 - 1.67)	3.50 (3.08 - 3.97)	6.81 (0.68)	31.8
111	Masterton	07.01.96	1.13 (1.07 - 1.19)	3.28 (2.90 - 3.71)	5.02 (0.33)	22.6
120	Masterton	31.01.96	0.68 (0.65 - 0.72)	1.68 (1.48 - 1.91)	5.91 (0.31)	13.6
024	Masterton	04.02.96	0.77 (0.73 - 0.82)	1.38 (1.20 - 1.58)	9.24 (0.43)	15.4
027	Greytown	09.02.96	0.83 (0.78 - 0.89)	1.80 (1.55 - 2.10)	6.95 (0.51)	16.6
056	Carterton	08.04.96	0.91 (0.87 - 0.95)	1.72 (1.56 - 1.91)	8.41 (0.35)	18.2
056	Carterton	07.05.96	1.42 (1.32 - 1.54)	3.32 (2.74 - 4.02)	6.33 (0.64)	28.4
027	Greytown	27.01.97	1.51 (1.40 - 1.63)	3.78 (3.19 - 4.48)	5.85 (0.50)	30.2
056	Carterton	21.07.97	1.19 (1.12 - 1.26)	3.54 (3.13 - 4.01)	4.91 (0.32)	38.0
056	Carterton	16.04.97	0.85 (0.68 - 1.06)	2.51 (1.60 - 3.94)	4.95 (1.01)	17.0
111	Masterton	10.01.97	0.90 (0.74 - 1.10)	2.76 (1.92 - 3.95)	4.79 (0.88)	18.0
056	Carterton	18.05.97	1.06 (0.94 - 1.19)	2.79 (2.00 - 3.64)	5.72 (0.86)	21.2
061	Masterton	15.01.97	1.15 (1.07 - 1.24)	3.04 (2.55 - 3.62)	5.52 (0.73)	23.0
056	Carterton	15.01.97	0.90 (0.69 - 1.17)	2.67 (1.51 - 4.71)	4.94 (1.18)	18.0
024	Masterton	23.01.97	1.10 (0.99 - 1.23)	2.55 (2.05 - 3.17)	6.39 (0.82)	22.0
027	Greytown	27.01.97	1.51 (1.40 - 1.63)	3.78 (3.18 - 4.48)	5.85 (0.50)	30.2
056	Carterton	24.03.97	1.08 (0.98 - 1.19)	3.20 (2.63 - 3.90)	4.94 (0.44)	21.6
Wellington						
037	Levin	11.12.90	0.67 (0.61 - 0.73)	1.90 (1.53 - 2.37)	5.12 (0.51)	13.4
050	Levin	23.01.91	1.11 (1.01 - 1.23)	3.08 (2.47 - 3.84)	5.27 (0.56)	22.2
037	Levin	04.02.91	1.00 (0.91 - 1.10)	2.71 (2.16 - 3.40)	5.36 (0.55)	20.0
037	Levin	04.02.91	1.06 (1.01 - 1.12)	2.59 (2.30 - 2.92)	6.01 (0.25)	21.2
119	Levin	12.02.91	1.34 (1.29 - 1.39)	2.91 (2.65 - 3.20)	6.90 (0.30)	26.8
050	Levin	27.02.91	1.02 (0.92 - 1.14)	2.53 (1.99 - 3.23)	5.91 (0.73)	20.4
119	Levin	06.03.91	1.00 (0.92 - 1.10)	2.22 (1.80 - 2.74)	6.76 (0.83)	20.0
119	Levin	27.03.91	1.22 (1.03 - 1.45)	5.63 (3.61 - 8.78)	3.51 (0.49)	24.4
050	Levin	16.04.91	0.58 (0.46 - 0.72)	2.00 (1.16 - 3.44)	4.34 (0.95)	11.6
068	Levin	20.11.91	1.33 (1.16 - 1.54)	4.67 (3.25 - 6.70)	4.28 (0.62)	26.6
026	Foxton	27.01.92	1.73 (1.64 - 1.83)	5.02 (4.38 - 5.75)	5.03 (0.33)	34.6
037	Levin	27.03.92	1.12 (1.07 - 1.18)	2.71 (2.42 - 3.03)	6.08 (0.49)	22.4
101	Silverstream	23.04.92	1.39 (1.32 - 1.46)	4.11 (3.60 - 4.70)	4.93 (0.35)	27.8
035	Levin	14.01.93	1.25 (1.17 - 1.35)	3.02 (2.53 - 3.62)	6.09 (0.57)	25.0
037	Levin	15.03.94	0.80 (0.70 - 0.91)	2.50 (1.79 - 3.49)	4.70 (0.71)	16.0
035	Levin	11.04.94	0.71 (0.60 - 0.38)	1.78 (1.13 - 2.80)	5.79 (0.83)	14.2
035	Levin	09.12.94	0.48 (0.39 - 0.58)	1.61 (1.04 - 2.49)	4.39 (0.87)	9.6
035	Levin	26.02.95	0.52 (0.47 - 0.59)	1.92 (1.46 - 2.54)	4.12 (0.25)	10.4
007	Upper Hutt	27.02.95	1.12 (1.05 - 1.19)	2.30 (2.00 - 2.64)	7.45 (0.74)	22.4
037	Levin	15.03.95	1.25 (1.07 - 1.46)	5.08 (3.40 - 7.57)	3.82 (0.47)	25.0
035	Levin	22.01.96	1.12 (1.06 - 1.19)	2.86 (2.51 - 3.25)	5.73 (0.40)	22.4
037	Levin	06.02.96	0.71 (0.66 - 0.76)	1.19 (1.02 - 1.39)	10.29 (0.24)	14.2
007	Upper Hutt	13.03.96	0.67 (0.58 - 0.77)	2.75 (1.98 - 3.81)	3.79 (0.46)	13.4
037	Levin	20.01.97	0.89 (0.76 - 1.04)	1.46 (0.97 - 2.20)	10.7(3.85)	17.8
Marlborough						
130	Blenheim	20.02.91	1.61 (1.50 - 1.73)	3.74 (3.17 - 4.43)	6.36 (0.57)	32.2
130	Blenheim	16.03.91	1.58 (1.51 - 1.65)	4.17 (3.75 - 4.63)	5.51 (0.27)	31.6
014	Blenheim	07.12.91	2.08 (1.87 - 2.32)	5.37 (4.05 - 7.13)	5.66 (0.75)	41.6
009	Blenheim	14.12.91	1.69 (1.54 - 1.86)	5.35 (4.18 - 6.84)	4.66 (0.47)	33.8
014	Blenheim	15.12.91	2.25 (1.99 - 2.54)	6.04 (4.36 - 8.38)	5.42 (0.82)	45.0
014	Blenheim	26.01.92	2.33 (2.08 - 2.62)	7.79 (5.78 -10.51)	4.45 (0.58)	46.6
090	Blenheim	17.02.92	2.52 (2.27 - 2.79)	5.86 (4.51 - 7.61)	6.34 (0.92)	50.4
014	Blenheim	24.02.92	1.22 (1.14 - 1.31)	2.98 (2.49 - 3.57)	6.02 (0.72)	24.4
014	Blenheim	24.02.92	1.01 (0.88 - 1.17)	2.10 (1.49 - 2.95)	7.37 (1.62)	20.2
002	Blenheim	25.02.92	2.10 (1.91 - 2.31)	5.64 (4.38 - 7.26)	5.43 (0.65)	42.0
009	Blenheim	21.04.92	2.48 (2.23 - 2.77)	5.64 (4.33 - 7.35)	6.53 (0.98)	49.6
014	Blenheim	28.12.92	2.03 (1.87 - 2.20)	4.42 (3.60 - 5.42)	6.89 (0.86)	40.6
014	Blenheim	03.02.93	1.82 (1.57 - 2.12)	6.83 (4.48 -10.41)	4.06 (0.62)	36.4
014	Blenheim	24.02.93	1.66 (1.57 - 1.77)	4.33 (3.73 - 5.03)	5.59 (0.52)	33.2
014	Blenheim	12.01.94	1.29 (1.21 - 1.38)	4.06 (3.45 - 4.78)	4.68 (0.35)	25.8
009	Blenheim	15.03.94	1.69 (1.59 - 1.81)	3.72 (3.20 - 4.32)	6.81 (0.73)	33.8
032	Blenheim	13.01.95	1.40 (1.29 - 1.51)	4.02 (3.32 - 4.86)	5.07 (0.41)	28.0

009	Blenheim	25.02.95	1.48 (1.15 - 1.90)	5.64 (2.58-12.33)	4.00 (1.08)	29.6
009	Blenheim	12.02.95	2.74 (2.57 - 2.91)	7.42 (6.32 - 8.71)	5.37 (0.23)	54.8
009	Blenheim	05.03.96	2.18 (1.87 - 2.55)	6.93 (2.58-10.50)	4.63 (0.75)	43.6
009	Blenheim	19.03.95	1.53 (1.43 - 1.63)	4.53 (3.90 - 5.27)	4.92 (0.31)	30.6
009	Blenheim	10.04.96	1.66 (1.57 - 1.75)	5.63 (4.86 - 6.52)	4.39 (0.29)	33.2
009	Blenheim	10.04.96	2.18 (1.99 - 2.38)	5.76 (4.61 - 7.20)	5.50 (0.58)	43.6
126	Marlborough	03.04.96	1.31 (1.18 - 1.46)	3.76 (2.87 - 4.92)	5.08 (0.57)	26.2
009	Blenheim	21.04.96	1.87 (1.75 - 2.00)	6.84 (5.86 - 7.99)	4.13 (0.31)	37.2
009	Blenheim	21.05.96	2.09 (1.77 - 2.47)	8.23 (5.21-12.98)	3.91 (0.60)	41.8
050	Blenheim	23.01.97	2.18 (1.88 - 2.55)	6.86 (4.75 - 9.89)	4.68 (0.68)	43.6
014	Blenheim	12.01.97	1.06 (0.92 - 1.23)	2.92 (2.08 - 4.10)	5.29 (0.81)	21.2
099	Havelock	21.02.97	2.05 (1.85 - 2.27)	7.88 (5.99-10.38)	3.98 (0.37)	41.0
009	Blenheim	14.04.97	1.94 (1.53 - 2.45)	9.73 (5.46-17.32)	3.32 (0.52)	38.8
009	Blenheim	07.01.97	1.48 (1.28 - 1.71)	3.78 (2.57 - 5.54)	5.73 (1.09)	29.6
014	Blenheim	12.01.97	1.06 (0.92 - 1.22)	2.92 (2.08 - 4.10)	5.29 (0.81)	21.2
032	Blenheim	26.01.97	2.07 (1.98 - 2.18)	5.90 (5.26 - 6.63)	5.12 (0.34)	41.4
050	Blenheim	27.01.97	2.18 (1.88 - 2.53)	6.86 (4.75 - 9.89)	4.68 (0.68)	43.6
137	Picton	13.03.97	0.83 (0.74 - 0.95)	2.17 (1.64 - 2.87)	5.61 (0.78)	16.6
Nelson						
015	Wakefield	09.01.91	0.88 (0.75 - 1.02)	2.08 (1.47 - 2.94)	6.19 (1.14)	17.6
104	Richmond	10.01.91	0.91 (0.82 - 1.02)	2.27 (1.77 - 2.90)	5.90 (0.82)	18.2
019	Wakefield	20.01.92	1.57 (1.26 - 1.95)	4.21 (2.52 - 7.03)	5.43 (1.30)	31.4
110	Takaka	10.02.92	1.87 (1.70 - 2.06)	5.08 (4.04 - 6.40)	5.36 (0.57)	37.4
019	Wakefield	17.02.92	1.54 (1.44 - 1.64)	4.38 (3.78 - 5.07)	5.12 (0.38)	30.8
015	Nelson	05.03.92	1.19 (1.14 - 1.25)	2.51 (2.24 - 2.81)	7.22 (0.62)	23.8
063	Wakefield	26.03.97	0.71 (0.66 - 0.76)	2.33 (1.98 - 2.75)	4.50 (0.44)	14.2
Kaikoura						
136	Kaikoura	30.01.91	0.87 (0.79 - 0.96)	1.94 (1.55 - 2.43)	6.69 (0.89)	17.4
136	Kaikoura	07.02.92	1.20 (1.14 - 1.26)	2.33 (2.05 - 2.65)	8.06 (0.78)	24.0
North Canterbury						
133	Cheviot	16.03.91	0.77 (0.73 - 0.81)	2.35 (2.06 - 2.67)	4.79 (0.31)	15.4
005	Waiau	10.01.92	1.84 (1.65 - 2.05)	5.12 (3.89 - 6.73)	5.24 (0.67)	36.8
005	Waiau	18.01.92	1.04 (0.98 - 1.11)	3.16 (2.72 - 3.68)	4.83 (0.40)	20.8
005	Waiau	18.01.92	1.25 (1.09 - 1.42)	3.06 (2.30 - 4.07)	5.98 (0.93)	25.0
076	Seddon	17.02.92	2.74 (2.59 - 2.89)	6.41 (5.55 - 7.41)	6.29 (0.61)	54.8
084	Rangiora	14.02.92	1.39 (1.30 - 1.48)	3.73 (3.24 - 4.30)	5.41 (0.44)	27.8
028	Cheviot	19.03.92	1.87 (1.78 - 1.96)	4.96 (4.40 - 5.60)	5.48 (0.38)	37.4
038	Rangiora	04.01.93	1.11 (1.04 - 1.19)	1.87 (1.59 - 2.20)	10.32 (1.49)	22.2
086	Waiau	02.02.93	1.60 (1.54 - 1.66)	2.75 (2.49 - 3.04)	9.84 (1.17)	32.0
038	Rangiora	15.02.94	0.82 (0.76 - 0.88)	2.25 (1.83 - 2.77)	5.31 (0.68)	16.4
042	Waikari	16.02.94	0.99 (0.93 - 1.05)	2.03 (1.77 - 2.32)	7.46 (0.59)	19.8
021	Amberley	10.01.95	1.11 (1.04 - 1.18)	2.47 (2.11 - 2.90)	6.67 (0.75)	22.2
127	Waiau	31.01.95	1.09 (1.03 - 1.16)	3.08 (2.68 - 3.54)	5.16 (0.31)	21.8
103	Nth Canty	21.02.95	0.97 (0.87 - 1.09)	2.72 (2.07 - 3.59)	5.20 (0.68)	19.4
021	Amberley	10.01.95	1.11 (1.04 - 1.18)	2.47 (2.11 - 2.90)	6.67 (0.75)	22.2
127	Waiau	31.01.95	1.09 (1.03 - 1.16)	3.08 (2.68 - 3.54)	5.16 (0.31)	21.8
031	Amberley	15.02.96	1.29 (1.20 - 1.38)	3.17 (2.67 - 3.77)	5.95 (0.51)	25.8
073	Rangiora	04.04.97	1.35 (1.27 - 1.43)	4.04 (3.51 - 4.64)	4.89 (0.41)	27.0
060	Waiau	27.01.97	1.04 (0.92 - 1.17)	3.58 (2.78 - 4.62)	4.33 (0.44)	20.8
Mid Canterbury						
034	Leeston	18.04.94	0.40 (0.35 - 0.46)	1.19 (0.94 - 1.52)	4.94 (0.60)	8.0
034	Leeston	18.04.94	0.41 (0.36 - 0.47)	1.20 (0.90 - 1.61)	4.98 (0.70)	8.2
039	Rakaia	14.02.94	0.92 (0.76 - 1.10)	3.42 (2.17 - 5.40)	4.07 (0.74)	18.4
066	Dorie	20.04.94	1.01 (0.78 - 1.31)	6.59 (1.53 - 6.59)	4.79 (1.40)	20.2
066	Dorie	20.04.94	0.59 (0.54 - 0.64)	1.78 (1.51 - 2.11)	4.82 (0.53)	11.8
064	Ashburton	16.02.95	0.60 (0.55 - 0.65)	1.87 (1.57 - 2.23)	4.71 (0.35)	12.0
108	Christchurch	27.02.95	0.66 (0.57 - 0.77)	2.59 (1.86 - 3.62)	3.93 (0.48)	13.2
065	Ashburton	27.03.95	1.00 (0.85 - 1.16)	2.63 (1.79 - 3.86)	5.52 (0.93)	20.0
100	Lincoln	30.03.95	1.39 (1.28 - 1.50)	4.41 (3.61 - 5.38)	4.63 (0.41)	27.8

100	Lincoln	03.04.95	1.49 (1.20 - 1.84)	6.10 (3.88 - 9.60)	3.79 (0.63)	29.8
160	Ashburton	12.04.95	0.92 (0.88 - 0.95)	1.81 (1.63 - 2.00)	7.87 (0.52)	18.4
158	Ashburton	17.04.95	0.95 (0.89 - 1.00)	1.96 (1.70 - 2.26)	7.35 (0.82)	19.0
159	Dorie	23.04.95	1.26 (1.17 - 1.36)	3.35 (2.76 - 4.06)	5.48 (0.49)	25.2
157	Ashburton	26.04.95	0.59 (0.52 - 0.67)	1.61 (1.26 - 2.04)	5.38 (0.71)	11.8
155	Darfield	04.05.95	1.30 (1.18 - 1.43)	4.63 (3.72 - 5.75)	4.22 (0.35)	26.0
156	Ashburton	01.05.95	0.74 (0.62 - 0.89)	1.97 (1.35 - 2.85)	5.52 (1.08)	12.4
114	Ashburton	09.01.96	1.12 (0.94 - 1.35)	3.17 (2.27 - 4.42)	5.17 (0.83)	22.4
129	Ashburton	16.01.96	1.13 (1.34 - 1.53)	4.22 (3.65 - 4.87)	4.95 (0.29)	22.6
047	Ashburton	09.01.96	1.13 (1.03 - 1.23)	2.43 (2.01 - 2.94)	6.97 (0.73)	22.6
123	Ashburton	19.02.96	0.80 (0.75 - 0.85)	1.49 (1.31 - 1.71)	8.54 (0.35)	16.0
065	Ashburton	24.02.96	1.13 (0.99 - 1.29)	3.32 (2.33 - 4.73)	4.96 (0.78)	22.6
107	Ashburton	22.03.96	0.69 (0.58 - 0.82)	2.27 (1.53 - 3.35)	4.51 (0.75)	13.8
055	Ashburton	22.03.96	2.07 (1.83 - 2.35)	5.71 (4.15 - 7.84)	5.29 (0.77)	41.4
064	Ashburton	06.04.96	1.28 (1.20 - 1.36)	2.88 (2.50 - 3.32)	6.61 (0.57)	25.6
065	Ashburton	17.04.96	0.94 (0.87 - 1.03)	3.34 (2.72 - 4.10)	4.24 (0.27)	18.8
065	Ashburton	08.05.96	1.91 (1.70 - 2.13)	5.45 (4.15 - 7.17)	5.09 (0.60)	38.2
055	Ashburton	18.01.97	2.13 (1.87 - 2.42)	6.96 (5.10 - 9.49)	4.53 (0.55)	42.6
122	Darfield	01.03.97	1.42 (1.29 - 1.55)	4.05 (3.27 - 5.03)	5.09 (0.49)	28.4
055	Ashburton	18.01.97	2.13 (1.87 - 2.42)	6.96 (5.10 - 9.49)	4.53 (0.55)	42.6
064	Ashburton	23.01.97	1.34 (1.22 - 1.47)	3.02 (2.45 - 3.73)	6.60 (0.81)	26.4
065	Ashburton	18.01.97	1.14 (1.07 - 1.22)	2.90 (2.45 - 3.42)	5.76 (0.56)	22.8
079	Darfield	07.02.97	1.37 (1.18 - 1.58)	3.95 (2.74 - 5.70)	5.05 (0.79)	27.4
South Canterbury						
003	Rangitata	15.03.95	0.98 (0.76 - 1.25)	3.77 (1.96 - 7.23)	3.98 (0.95)	19.6
003	Rangitata	02.04.95	1.59 (1.48 - 1.70)	3.79 (3.24 - 4.43)	6.16 (0.52)	31.8
125	Timaru	25.04.95	1.30 (1.11 - 1.51)	3.36 (2.26 - 5.01)	5.62 (1.03)	26.0
125	Timaru	14.05.95	1.68 (1.48 - 1.92)	5.97 (4.40 - 8.11)	4.23 (0.51)	33.6
132	Geraldine	24.01.96	0.91 (0.87 - 0.95)	1.76 (1.57 - 1.99)	8.14 (0.79)	18.2
125	Timaru	20.02.96	0.47 (0.40 - 0.54)	1.69 (1.24 - 2.31)	4.15 (0.50)	9.4
125	Timaru	27.02.96	0.72 (0.67 - 0.77)	2.39 (2.03 - 2.83)	4.47 (0.29)	14.4
139	Timaru	20.03.97	0.93 (0.78 - 1.11)	2.61 (1.66 - 4.10)	5.19 (1.04)	18.6
085	Timaru	20.03.97	1.49 (1.40 - 1.59)	2.99 (2.62 - 3.41)	7.72 (0.57)	29.8
088	Waimate	25.02.97	0.91 (0.65 - 1.27)	2.45 (1.16 - 3.16)	5.39 (1.97)	18.2
132	Geraldine	08.02.97	0.94 (0.80 - 1.09)	3.07 (2.23 - 4.22)	4.52 (0.65)	18.8
141	Waimate	29.01.97	1.13 (1.07 - 1.19)	2.52 (2.24 - 2.83)	6.67 (0.37)	22.6
139	Timaru	20.03.97	0.93 (0.78 - 1.11)	2.61 (1.66 - 4.10)	5.19 (1.05)	18.6
Dunedin						
116	Oamaru	06.03.97	0.85 (0.69 - 1.04)	2.80 (1.70 - 4.60)	4.49 (0.91)	17.0
096	Oamaru	07.04.97	0.76 (0.60 - 0.95)	2.29 (1.41 - 3.70)	4.85 (1.14)	15.2
005	Oamaru	19.03.97	1.07 (0.93 - 1.24)	5.13 (3.55 - 7.43)	3.42 (0.39)	21.4
083	Hampden	10.04.97	0.74 (0.63 - 0.87)	2.80 (1.98 - 3.95)	4.03 (0.54)	14.8

[1] Fiducial limits
[2] Standard Error
[3] Resistance Factor

Appendix 1.1.6

Toxicological response of *Lucilia cuprina* larvae to chlorfenvinphos

Farm	Location	Sample Date	LC_{50} (95% F.L.) ppm	LC_{99} (95% F.L.) ppm	Slope (SE)	RF
000	Lab	00.00.00	0.11 (0.11 - 0.12)	0.21 (0.20 - 0.23)	8.72 (0.37)	1.0
Northland						
016	Kaikohe	21.04.92	0.34 (0.29 - 0.39)	1.06 (0.83 - 1.36)	4.70 (0.50)	3.1
036	Kaiwaka	14.03.94	0.57 (0.48 - 0.69)	2.04 (1.17 - 3.57)	4.21 (0.81)	5.2
Waikato						
008	Kihikihi	16.01.93	0.42 (0.38 - 0.45)	0.82 (0.66 - 1.01)	7.92 (1.13)	3.8
043	Waingaro	06.02.93	0.63 (0.53 - 0.75)	3.68 (2.20 - 6.17)	3.04 (0.41)	5.7
Coromandel						
074	Waihi	10.04.94	0.56 (0.53 - 0.60)	1.11 (0.95 - 1.31)	7.78 (0.32)	5.1
Bay of Plenty						
135	Tauranga	20.01.94	0.56 (0.46 - 0.67)	1.81 (1.19 - 2.76)	4.54 (0.80)	5.1
Gisborne						
006	Wairoa	18.02.92	0.53 (0.45 - 0.61)	1.96 (1.41 - 2.73)	4.07 (0.54)	4.8
077	Wairoa	11.01.93	0.64 (0.61 - 0.67)	1.57 (1.36 - 1.81)	5.98 (0.55)	5.8
095	Waiomatatini	13.01.93	0.67 (0.63 - 0.71)	1.69 (1.47 - 1.95)	5.78 (0.44)	6.1
Hawkes Bay						
012	Hastings	29.12.92	0.58 (0.54 - 0.61)	1.56 (1.32 - 1.83)	5.39 (0.12)	5.3
012	Hastings	18.01.93	0.76 (0.70 - 0.83)	1.37 (1.08 - 1.75)	9.15 (1.59)	6.9
018	Waipawa	31.03.94	0.43 (0.34 - 0.56)	1.27 (0.68 - 2.38)	4.98 (1.29)	3.9
012	Hastings	08.04.94	0.39 (0.36 - 0.43)	0.95 (0.77 - 1.16)	6.09 (0.61)	3.5
Wanganui						
004	Bulls	11.12.92	0.64 (0.50 - 0.80)	2.28 (1.29 - 4.03)	4.20 (0.88)	5.8
004	Bulls	21.12.92	0.48 (0.44 - 0.51)	1.38 (1.15 - 1.67)	5.02 (0.64)	4.4
003	Wanganui	27.01.93	0.40 (0.38 - 0.43)	1.04 (0.92 - 1.18)	5.66 (0.24)	3.6
004	Bulls	21.02.94	0.59 (0.46 - 0.75)	1.66 (0.89 - 3.07)	5.17 (1.38)	5.4
045	Feilding	13.04.94	0.39 (0.30 - 0.49)	1.62 (1.03 - 2.56)	3.74 (0.68)	3.5
Wairarapa						
027	Greytown	07.01.94	0.43 (0.41 - 0.46)	1.15 (1.00 - 1.32)	5.47 (0.15)	3.9
Wellington						
035	Levin	14.01.93	0.34 (0.34 - 0.38)	0.81 (0.62 - 1.07)	6.25 (1.05)	3.1
035	Levin	11.04.94	0.30 (0.24 - 0.30)	1.06 (0.84 - 1.34)	3.89 (0.34)	2.7
Marlborough						
014	Blenheim	24.02.93	0.28 (0.21 - 0.37)	0.76 (0.48 - 1.22)	5.34 (1.63)	2.5
009	Blenheim	15.03.94	0.81 (0.73 - 0.90)	3.45 (2.63 - 4.52)	3.71 (0.37)	7.4
Kaikoura						
136	Kaikoura	07.02.92	0.38 (0.36 - 0.40)	0.69 (0.63 - 0.75)	8.79 (1.01)	3.4

North Canterbury						
028	Cheviot	19.03.92	0.42 (0.39 - 0.45)	1.16 (1.01 - 1.32)	5.30 (0.34)	3.8
038	Rangiora	04.01.93	0.46 (0.39 - 0.53)	0.81 (0.56 - 1.18)	9.27 (2.83)	4.2
086	Waiau	02.02.93	0.42 (0.39 - 0.46)	0.85 (0.72 - 1.00)	7.67 (1.30)	3.8
038	Rangiora	15.02.94	0.60 (0.53 - 0.68)	1.50 (1.16 - 1.94)	5.87 (1.10)	5.4
042	Waikari	16.02.94	0.52 (0.48 - 0.57)	1.43 (1.17 - 1.75)	5.31 (0.54)	4.7
Mid Canterbury						
039	Rakaia	14.02.94	0.61 (0.53 - 0.71)	2.95 (2.07 - 4.22)	3.41 (0.41)	5.5

Appendix 1.1.7

Toxicological response of *Lucilia cuprina* larvae to chlorpyrifos

Farm	Location	Sample date	LC_{50} (95% F.L.) ppm	LC_{99} (95% F.L.) ppm	Slope (SE)	RF
000	Laboratory	00.00.00	0.15 (0.13 - 0.16)	0.52 (0.37 - 0.73)	4.20 (0.51)	1.0
Northland						
025	Dargaville	04.12.91	0.62 (0.53 - 0.72)	2.24 (1.58 - 3.17)	4.18 (0.60)	4.1
036	Kaiwaka	14.03.94	0.46 (0.31 - 0.69)	3.07 (1.29 - 7.30)	2.84 (0.69)	3.1
016	Kaikohe	18.04.94	0.75 (0.58 - 0.98)	4.89 (2.60 - 9.22)	5.36 (0.51)	5.0
Auckland						
008	Kihikihi	20.11.91	0.67 (0.61 - 0.74)	1.63 (1.28 - 2.06)	6.06 (0.82)	4.5
022	Te Kuiti	11.12.91	0.72 (0.60 - 0.86)	2.09 (1.41 - 3.09)	5.03 (0.96)	4.8
022	Te Kuiti	23.01.92	0.92 (0.86 - 0.97)	2.56 (2.21 - 2.96)	5.21 (0.42)	6.1
008	Kihikihi	21.04.92	0.67 (0.61 - 0.73)	1.62 (1.32 - 1.99)	6.09 (0.74)	4.5
008	Kihikihi	07.03.94	0.61 (0.50 - 0.74)	2.51 (1.64 - 3.85)	3.77 (0.54)	4.1
Coromandel						
074	Waihi	10.04.94	0.68 (0.55 - 0.86)	2.68 (1.60 - 4.47)	3.93 (0.94)	4.5
Bay of Plenty						
135	Tauranga	12.12.91	0.56 (0.44 - 0.71)	2.19 (1.34 - 3.60)	3.91 (0.81)	3.7
017	Tauranga	03.02.92	0.89 (0.79 - 1.00)	1.75 (1.37 - 2.24)	7.87 (1.44)	5.9
135	Tauranga	20.01.94	0.44 (0.38 - 0.51)	1.14 (0.83 - 1.57)	5.63 (0.79)	2.9
Gisborne						
006	Wairoa	18.02.92	1.56 (1.47 - 1.64)	3.92 (3.43 - 4.49)	5.79 (0.46)	10.4
011	Gisborne	19.03.92	1.36 (1.29 - 1.42)	2.83 (2.51 - 3.19)	7.29 (0.46)	9.1
095	Waiomatatini	13.01.93	0.74 (0.68 - 0.80)	1.51 (1.25 - 1.82)	7.53 (0.95)	4.9
Hawkes Bay						
012	Hastings	14.01.91	1.12 (1.01 - 1.25)	3.22 (2.49 - 4.17)	5.08 (0.60)	7.5
012	Hastings	27.11.91	2.02 (1.76 - 2.31)	5.87 (4.07 - 8.47)	5.02 (0.80)	13.5
012	Hastings	29.12.92	0.99 (0.89 - 1.10)	2.06 (1.61 - 2.63)	7.32 (1.12)	6.6
012	Hastings	18.01.93	0.91 (0.81 - 1.02)	2.31 (1.73 - 3.08)	5.74 (0.86)	6.1
018	Waipawa	31.12.93	0.41 (0.26 - 0.64)	3.73 (1.36 - 10.2)	2.42 (0.63)	2.7
018	Waipawa	31.03.94	0.65 (0.59 - 0.71)	1.43 (1.13 - 1.80)	6.79 (0.74)	4.3
012	Hastings	08.04.94	0.66 (0.59 - 0.74)	1.95 (1.50 - 2.54)	4.92 (0.43)	4.4
Wanganui						
003	Wanganui	14.12.91	0.72 (0.63 - 0.82)	2.93 (2.20 - 3.91)	3.82 (0.42)	4.8
003	Wanganui	10.01.92	1.39 (1.22 - 1.59)	3.23 (2.36 - 4.42)	6.38 (1.10)	9.3
004	Bulls	13.01.92	1.27 (1.21 - 1.34)	2.83 (2.49 - 3.21)	6.71 (0.70)	8.5
004	Bulls	03.02.92	0.95 (0.89 – 1.00)	2.78 (2.42 - 3.20)	4.97 (0.43)	6.3
004	Bulls	11.12.92	0.74 (0.71 - 0.77)	1.17 (1.06 - 1.30)	11.55 (0.36)	4.9
004	Bulls	21.12.92	0.76 (0.70 - 0.83)	1.30 (1.05 - 1.60)	10.12 (2.10)	5.1
004	Bulls	06.01.93	0.84 (0.78 - 0.91)	1.59 (1.32 - 1.92)	8.40 (1.17)	5.6
004	Bulls	21.02.94	0.91 (0.80 - 1.03)	1.84 (1.35 - 2.51)	7.59 (1.44)	6.1
004	Bulls	05.04.94	0.54 (0.48 - 0.60)	1.59 (1.18 - 2.11)	4.97 (0.60)	3.6
045	Feilding	13.04.94	0.60 (0.42 - 0.85)	3.88 (1.88 - 7.98)	2.87 (0.62)	4.0
003	Turakina	10.05.94	0.68 (0.49 - 0.95)	3.89 (1.77 - 8.54)	3.08 (0.69)	4.5

Wairarapa						
023	Masterton	07.01.91	1.37 (1.24 - 1.52)	3.49 (2.72 - 4.48)	5.75 (0.71)	9.1
024	Masterton	29.12.91	0.92 (0.80 - 1.05)	4.32 (3.22 - 5.80)	3.46 (0.34)	6.1
027	Greytown	16.02.92	0.93 (0.86 - 1.00)	1.89 (1.59 - 2.26)	7.5 (0.84)	6.2
027	Greytown	07.01.94	0.80 (0.62 - 1.03)	2.34 (1.26 - 4.34)	4.99 (1.34)	5.3
024	Masterton	31.02.94	0.66 (0.58 - 0.76)	3.09 (2.20 - 4.34)	3.49 (0.48)	4.4
Wellington						
068	Levin	20.11.91	1.04 (0.98 - 1.09)	2.82 (2.46 - 3.23)	5.37 (0.43)	6.9
037	Levin	15.03.94	0.62 (0.46 - 0.82)	2.64 (1.34 - 5.21)	3.69 (0.84)	4.1
035	Levin	11.04.94	0.31 (0.21 - 0.46)	2.27 (1.08 - 4.76)	2.71 (0.60)	2.1
Marlborough						
014	Blenheim	07.12.91	1.22 (1.16 - 1.29)	3.60 (3.15 - 4.1)	4.96 (0.30)	8.1
014	Blenheim	26.01.92	1.19 (1.10 - 1.30)	3.02 (4.46 - 3.71)	5.77 (0.60)	7.9
009	Blenheim	15.03.94	0.95 (0.85 - 1.07)	2.19 (1.70 - 2.83)	6.44 (0.74)	6.3
Nelson						
019	Wakefield	20.01.92	0.83 (0.73 - 0.95)	1.59 (1.17 - 2.16)	8.28 (1.83)	5.5
110	Takaka	10.02.92	1.09 (1.01 - 1.17)	3.52 (2.99 - 4.16)	4.56 (0.50)	7.3
015	Nelson	05.03.92	0.74 (0.69 - 0.81)	1.71 (1.40 - 2.08)	6.45 (0.77)	4.9
Kaikoura						
136	Kaikoura	07.02.92	0.91 (0.85 - 0.98)	2.98 (2.51 - 3.54)	4.53 (0.36)	6.1
North Canterbury						
038	Rangiora	04.01.93	0.85 (0.82 - 0.89)	1.34 (1.23 - 1.45)	11.94 (0.96)	5.7
042	Waikari	16.02.94	0.72 (0.57 - 0.91)	2.76 (1.65 - 4.62)	3.99 (0.74)	4.8
Mid Canterbury						
039	Rakaia	14.02.94	0.75 (0.47 - 1.21)	4.17 (1.23 - 14.1)	3.13 (1.14)	5.0
034	Leeston	18.04.94	0.59 (0.36 - 0.95)	1.67 (0.51 - 5.45)	5.12 (2.67)	3.9
066	Dorie	20.04.94	0.95 (0.72 - 1.24)	4.68 (2.43 - 8.99)	5.08 (0.70)	6.3

Appendix 1.1.8

Toxicological response of *Lucilia cuprina* larvae to dichlofenthion

Farm	Location	Sample date	LC_{50} (95% F.L.) ppm	LC_{99} (95% F.L.) ppm	Slope (S.E.)	RF
000	Laboratory	00.00.00	0.31 (0.30 - 0.32)	0.53 (0.49 - 0.57)	9.90 (1.33)	1.0
Northland						
025	Dargaville	04.12.91	2.03 (1.94 - 2.13)	4.92 (4.37 - 5.53)	6.05 (0.34)	6.5
036	Kaiwaka	14.03.94	0.90 (0.74 - 1.11)	4.33 (2.83 - 6.63)	5.15 (0.47)	2.9
016	Kaikohe	18.04.94	1.90 (1.55 - 2.32)	10.3(6.47 -16.44)	4.12 (0.46)	6.1
Coromandel						
074	Waihi	10.04.94	1.27 (1.16 - 1.40)	3.87 (3.18 - 4.70)	4.82 (0.30)	4.1
Bay of Plenty						
135	Tauranga	12.12.91	2.29 (2.02 - 2.59)	5.51 (4.03 - 7.53)	6.10 (0.98)	7.4
014	Rotorua	16.02.92	2.79 (2.66 - 2.92)	5.47 (4.85 - 6.16)	7.95 (0.45)	9.0
135	Tauranga	20.01.94	1.72 (1.63 - 1.80)	3.32 (2.94 - 3.75)	8.13 (0.17)	5.5
Waikato						
008	Kihikihi	20.11.91	1.39 (1.24 - 1.55)	7.00 (5.32 - 9.22)	3.31 (0.29)	4.5
022	Te Kuiti	11.12.91	1.24 (1.13 - 1.37)	3.94 (3.05 - 5.08)	4.64 (0.49)	4.0
022	Te Kuiti	23.01.92	2.74 (2.27 - 3.32)	7.22 (4.19 -12.43)	5.54 (1.39)	8.8
008	Kihikihi	21.04.92	1.64 (1.56 - 1.72)	4.20 (3.70 - 4.76)	5.69 (0.39)	5.3
008	Kihikihi	07.03.94	1.04 (0.89 - 1.21)	3.46 (2.54 - 4.72)	4.45 (0.62)	3.3
Gisborne						
117	Wairoa	21.10.91	1.65 (1.56 - 1.74)	4.83 (4.24 - 5.50)	4.98 (0.32)	5.3
011	Gisborne	16.02.92	3.66 (3.19 - 4.20)	8.89 (5.94 -13.29)	6.04 (1.12)	11.8
011	Gisborne	19.03.92	4.30 (3.82 - 4.85)	9.94 (7.11 -13.91)	6.40 (1.06)	13.9
095	Waiomatatini	13.01.93	2.76 (2.46 - 3.10)	8.83 (6.54 -11.93)	4.61 (0.54)	8.9
Hawkes Bay						
012	Hastings	27.11.91	3.85 (3.67 - 4.04)	9.55 (8.47 -10.76)	5.90 (0.53)	12.4
118	Hastings	21.10.91	1.59 (1.39 - 1.82)	4.23 (3.09 - 5.77)	5.48 (0.74)	5.1
012	Hastings	14.01.92	3.46 (3.22 - 3.71)	8.68 (7.11-10.60)	5.82 (0.54)	11.2
012	Hastings	29.12.92	2.67 (2.46 - 2.89)	7.35 (6.11 - 8.85)	5.29 (0.44)	8.6
012	Hastings	18.01.93	2.88 (2.66 - 3.11)	7.68 (6.35 - 9.30)	5.46 (0.47)	9.3
012	Hastings	08.04.94	1.79 (1.65 - 1.95)	4.57 (3.76 - 5.54)	5.74 (0.86)	5.8
018	Waipawa	31.03.94	1.50 (1.23 - 1.83)	5.01 (3.21 - 7.84)	4.44 (0.78)	4.8
Wanganui						
003	Wanganui	14.12.91	2.43 (2.20 - 2.68)	6.95 (5.35 - 9.03)	5.09 (0.56)	7.8
003	Wanganui	10.01.92	3.35 (3.07 - 3.66)	8.19 (6.36-10.55)	6.00 (0.76)	10.8
004	Bulls	13.01.92	3.69 (3.36 - 4.06)	9.44 (7.18-12.41)	5.71 (0.71)	11.9
026	Foxton	27.01.92	2.82 (2.67 - 2.97)	7.21 (6.19 - 8.40)	5.70 (0.57)	9.1
003	Wanganui	07.03.92	2.38 (2.27 - 2.51)	6.26 (5.47 - 7.16)	5.56 (0.51)	7.7
004	Bulls	11.12.92	2.05 (1.97 - 2.14)	3.57 (3.25 - 3.91)	9.71 (0.40)	6.6
004	Bulls	21.12.92	2.50 (2.27 - 2.75)	5.57 (4.45 - 6.98)	6.68 (0.85)	8.1
004	Bulls	06.01.93	2.54 (2.32 - 2.77)	5.16 (4.09 - 6.52)	7.53 (1.08)	8.2
004	Bulls	21.02.94	1.45 (1.26 - 1.67)	5.12 (3.75 - 6.99)	4.25 (0.52)	4.7
004	Bulls	05.04.94	1.23 (1.15 - 1.32)	3.76 (3.20 - 4.42)	4.79 (0.23)	4.0
045	Feilding	13.04.94	0.93 (0.80 - 1.09)	4.41 (3.08 - 6.31)	3.44 (0.55)	3.0
003	Turakina	10.05.94	1.46 (1.23 - 1.73)	3.99 (2.65 - 6.01)	5.32 (1.08)	4.7
Wairarapa						
024	Masterton	29.12.91	2.94 (2.65 - 3.27)	8.70 (6.42-12.33)	4.84 (0.63)	9.5
023	Masterton	07.01.92	3.47 (3.19 - 3.78)	7.78 (6.13 - 9.89)	6.64 (0.86)	11.2
027	Greytown	07.01.94	1.83 (1.45 - 2.31)	5.38 (2.79 -10.35)	4.97 (1.44)	5.9
024	Masterton	31.02.94	1.20 (0.92 - 1.56)	4.95 (2.50 - 9.79)	3.79 (0.95)	3.9

024	Masterton	31.02.94	1.39 (1.24 - 1.55)	4.91 (3.87 - 6.24)	4.24 (0.56)	4.5
Wellington						
068	Levin	20.11.91	3.12 (2.90 - 3.33)	14.7 (11.96-18.2)	3.44 (0.20)	10.1
037	Levin	15.03.94	1.45 (1.27 - 1.67)	4.03 (2.94 - 5.53)	5.25 (0.83)	4.7
035	Levin	11.04.94	1.03 (0.94 - 1.14)	2.18 (1.79 - 2.66)	7.19 (0.91)	3.3
Marlborough						
014	Blenheim	07.12.91	3.85 (3.66 - 4.06)	9.26 (8.00 -10.72)	6.11 (0.37)	12.4
009	Blenheim	14.12.91	3.35 (2.93 - 3.84)	9.60 (6.51 -14.14)	5.09 (0.81)	10.8
014	Blenheim	15.12.91	4.07 (3.81 - 4.34)	11.0 (9.21 -13.13)	5.39 (0.36)	13.1
014	Blenheim	26.01.92	5.45 (5.08 - 5.85)	16.4 (13.42-19.94)	4.88 (0.48)	17.6
009	Blenheim	15.03.94	2.72 (2.45 - 3.03)	6.76 (5.32 - 8.60)	5.90 (0.72)	8.8
009	Blenheim	15.03.94	3.15 (2.85 - 3.48)	12.6 (9.80 -16.16)	3.87 (0.39)	10.2
Nelson						
019	Wakefield	20.01.92	3.38 (3.06 - 3.75)	8.81 (6.60 -11.77)	5.60 (0.72)	10.9
015	Nelson	05.03.92	2.20 (1.96 - 2.47)	4.55 (3.36 - 6.16)	7.36 (1.41)	7.1
Kaikoura						
136	Kaikoura	07.02.92	2.77 (2.47 - 3.10)	8.41 (6.12 -11.55)	4.82 (0.64)	8.9
North Canterbury						
005	Waiau	18.01.92	2.74 (2.43 - 3.08)	7.29 (5.22-10.19)	5.47 (0.85)	8.8
038	Rangiora	04.01.93	2.67 (2.46 - 2.89)	5.38 (4.38 - 6.60)	7.64 (1.03)	8.6
038	Rangiora	15.02.94	1.46 (1.34 - 1.60)	2.45 (1.93 - 3.11)	10.4 (1.21)	4.7
042	Waikari	16.02.94	2.66 (2.40 - 2.95)	6.42 (5.11 - 8.07)	6.07 (0.75)	8.6
039	Rakaia	14.02.94	2.88 (2.27 - 3.66)	14.3 (7.69-26.78)	3.34 (0.61)	9.3
034	Leeston	18.04.94	0.98 (0.79 - 1.21)	4.94 (2.93 - 8.32)	3.31 (0.55)	3.2
Mid Canterbury						
066	Dorie	20.04.94	1.29 (1.06 - 1.50)	4.24 (3.05 - 5.88)	4.51 (0.72)	4.2

Appendix 1.1.9

Toxicological response of *Lucilia cuprina* larvae to propetamphos

Farm	Location	Sample date	LC_{50} (95% F.L.) ppm	LC_{99} (95% F.L.) ppm	Slope (SE)	RF
000	Laboratory	00.00.00	0.15 (0.146-0.154)	0.26 (0.247-0.283)	9.4 (0.7)	1.0
Northland						
025	Dargaville	04.12.91	0.53 (0.46 - 0.62)	1.42 (0.95 - 2.11)	5.48 (1.02)	3.5
036	Kaiwaka	14.03.94	0.45 (0.42 - 0.47)	0.83 (0.72 - 0.95)	8.68 (0.84)	3.0
Waikato						
010	Te Kuiti	15.01.92	0.33 (0.31 - 0.36)	0.89 (0.77 - 1.04)	5.41 (0.43)	2.2
022	Te Kuiti	29.01.92	0.55 (0.51 - 0.59)	0.84 (0.69 - 1.02)	12.6 (2.63)	3.7
008	Kihikihi	16.01.93	0.41 (0.39 - 0.42)	0.81 (0.73 - 0.89)	7.85 (0.80)	2.7
Coromandel						
074	Waihi	10.04.94	0.56 (0.53 - 0.60)	1.15 (0.99 - 1.33)	7.52 (0.52)	3.7
Bay of Plenty						
135	Tauranga	12.12.91	0.52 (0.38 - 0.69)	1.43 (0.70 - 2.93)	5.27 (1.73)	3.5
017	Tauranga	10.01.92	0.75 (0.65 - 0.87)	1.44 (0.98 - 2.11)	8.24 (2.22)	5.0
017	Tauranga	03.02.92	0.53 (0.50 - 0.56)	0.96 (0.87 - 1.07)	8.90 (0.20)	3.5
135	Tauranga	20.01.94	0.70 (0.40 - 1.22)	3.91 (0.97 - 15.8)	3.11 (1.18)	4.7
Gisborne						
011	Gisborne	03.02.92	0.50 (0.45 - 0.56)	1.01 (0.77 - 1.33)	7.65 (1.46)	3.3
077	Wairoa	11.01.93	0.60 (0.54 - 0.68)	1.14 (0.84 - 1.56)	8.35 (1.74)	4.0
095	Waiomatatini	13.01.93	0.66 (0.63 - 0.68)	0.99 (0.91 - 1.08)	13.1 (1.19)	4.4
049	Wairoa	03.04.94	0.42 (0.39 - 0.45)	0.95 (0.81 - 1.12)	6.53 (0.67)	2.8
Hawkes Bay						
012	Hastings	27.11.91	0.71 (0.66 - 0.77)	2.22 (1.78 - 2.78)	4.71 (0.59)	4.7
018	Waipawa	06.01.92	0.83 (0.74 - 0.92)	1.70 (1.30 - 2.22)	7.42 (1.27)	5.5
012	Hastings	14.01.92	0.48 (0.43 - 0.52)	1.33 (1.06 - 1.66)	5.23 (0.58)	3.2
020	Hastings	21.01.92	0.53 (0.47 - 0.61)	1.84 (1.32 - 2.55)	4.34 (0.57)	3.5
012	Hastings	16.03.92	0.87 (0.81 - 0.94)	1.83 (1.55 - 2.16)	7.20 (0.77)	5.8
012	Hastings	29.12.92	0.63 (0.60 - 0.65)	1.44 (1.28 - 1.61)	6.46 (0.39)	4.2
012	Hastings	18.01.93	0.76 (0.68 - 0.85)	1.67 (1.26 - 2.20)	6.85 (1.12)	5.1
012	Hastings	30.12.93	0.42 (0.40 - 0.44)	0.70 (0.63 - 0.77)	10.5 (0.70)	2.8
018	Waipawa	31.12.93	0.45 (0.42 - 0.47)	0.60 (0.54 - 0.67)	18.1 (0.18)	3.0
018	Waipawa	31.03.94	0.42 (0.36 - 0.49)	1.30 (0.92 - 1.82)	4.78 (0.70)	2.8
012	Hastings	08.04.94	0.66 (0.62 - 0.71)	1.13 (0.98 - 1.31)	10.1 (0.34)	4.4
Wanganui						
003	Wanganui	14.12.91	0.41 (0.39 - 0.43)	0.88 (0.79 - 0.97)	7.09 (0.49)	2.7
004	Bulls	13.01.92	0.69 (0.66 - 0.72)	1.27 (1.15 - 1.41)	8.75 (0.43)	4.6
004	Bulls	03.02.92	0.70 (0.64 - 0.77)	1.48 (1.18 - 1.84)	7.22 (1.05)	4.7
004	Bulls	11.12.92	0.57 (0.53 - 0.60)	0.95 (0.80 - 1.14)	10.2 (1.75)	4.7
004	Bulls	21.12.92	0.52 (0.41 - 0.65)	0.91 (0.54 - 1.56)	9.45 (4.92)	3.5
004	Bulls	06.01.93	0.62 (0.60 - 0.66)	1.23 (1.10 - 1.38)	7.86 (0.26)	4.1
003	Wanganui	30.01.94	0.62 (0.55 - 0.69)	1.33 (1.07 - 1.65)	6.99 (0.40)	4.1
004	Bulls	21.02.94	0.74 (0.69 - 1.89)	1.89 (1.58 - 2.26)	5.75 (0.52)	4.9
045	Feilding	13.04.94	0.59 (0.54 - 0.64)	1.21 (0.99 - 1.48)	7.39 (0.36)	3.9

Wairarapa						
024	Masterton	29.12.91	0.45 (0.42 - 0.48)	0.96 (0.82 - 1.12)	7.07 (0.68)	3.0
023	Masterton	07.01.92	0.89 (0.92 - 1.52)	4.60 (1.88 - 8.55)	4.41 (1.23)	5.9
027	Greytown	07.01.94	0.53 (0.38 - 0.72)	1.99 (0.98 - 4.04)	4.02 (1.10)	3.5
024	Masterton	31.02.94	0.38 (0.32 - 0.45)	0.71 (0.45 - 1.14)	8.48 (2.84)	2.5
Wellington						
026	Foxton	27.01.92	0.69 (0.65 - 0.73)	1.72 (1.52 - 1.95)	5.87 (0.33)	4.6
101	Silverstream	23.04.92	0.44 (0.42 - 0.46)	1.09 (0.98 - 1.22)	5.87 (0.41)	2.9
037	Levin	15.03.94	0.37 (0.32 - 0.42)	0.89 (0.66 - 1.20)	6.06 (1.11)	2.5
035	Levin	11.04.94	0.42 (0.38 - 0.46)	0.87 (0.69 - 1.10)	7.31 (1.11)	2.8
Marlborough						
014	Blenheim	07.12.91	0.58 (0.52 - 0.66)	1.27 (0.94 - 1.71)	6.91 (1.23)	3.9
009	Blenheim	14.12.91	0.61 (0.57 - 0.65)	1.23 (1.05 - 1.43)	7.70 (0.80)	4.1
014	Blenheim	15.12.91	0.42 (0.36 - 0.49)	1.06 (0.74 - 1.53)	5.83 (1.12)	2.8
014	Blenheim	26.01.92	0.65 (0.60 - 0.71)	1.62 (1.29 - 2.03)	5.86 (0.65)	4.3
002	Blenheim	25.02.92	0.78 (0.72 - 0.85)	1.54 (1.28 - 1.85)	7.92 (1.02)	5.2
014	Blenheim	12.01.94	0.49 (0.45 - 0.53)	0.80 (0.68 - 0.95)	10.8 (1.08)	3.3
009	Blenheim	15.03.94	0.76 (0.72 - 0.79)	1.53 (1.34 - 1.75)	7.62 (0.99)	5.1
Nelson						
019	Wakefield	20.01.92	0.56 (0.54 - 0.59)	1.22 (1.1 - 1.36)	6.87 (0.50)	3.7
North Canterbury						
038	Rangiora	04.01.93	0.44 (0.43 - 0.45)	0.60 (0.57 - 0.64)	17.41 (0.21)	2.9
042	Waikari	12.01.94	0.48 (0.43 - 0.52)	0.88 (0.71 - 1.08)	8.73 (1.40)	3.2
038	Rangiora	15.02.94	0.63 (0.60 - 0.66)	0.93 (0.82 - 1.06)	13.6 (0.60)	4.2
Mid Canterbury						
066	Dorie	20.04.94	0.43 (0.25 - 0.74)	2.59 (0.65 -10.31)	3.00 (1.24)	2.9
039	Rakaia	14.02.94	0.66 (0.60 - 0.72)	1.31 (1.07 - 1.60)	7.77 (1.16)	4.4

Appendix 1.1.10

Toxicological response of *Lucilia cuprina* larvae to deltamethrin.

Farm	Location	Sample date	LC_{50} (95% F.L.) ppm	LC_{99} (95% F.L.) ppm	Slope (S.E.)
Northland					
025	Dargaville	04.12.91	2.29 (1.96 - 2.67)	18.3 (11.21-29.72)	2.58 (0.27)
Bay of Plenty					
135	Tauranga	12.12.91	1.86 (1.60 - 2.16)	9.49 (6.20 -14.52)	3.29 (0.40)
Gisborne					
117	Wairoa	21.10.91	2.21 (1.81 - 2.69)	6.45 (4.06 -10.23)	5.00 (0.98)
095	Waiomatatini	13.01.93	1.77 (1.66 - 1.89)	8.40 (6.89 -10.10)	3.44 (0.24)
112	Gisborne	08.01.93	1.88 (1.70 - 2.08)	7.13 (5.44 - 9.35)	4.02 (0.37)
Waikato					
008	Kihikihi	20.11.91	1.26 (1.13 - 1.41)	8.60 (6.27 -11.80)	2.79 (0.27)
008	Kihikihi	20.11.91	1.13 (0.84 - 1.52)	11.23(5.15 -24.45)	2.33 (0.35)
022	Te Kuiti	11.12.91	1.38 (1.22 - 1.56)	7.41 (4.99 -11.01)	3.19 (0.42)
Wanganui					
004	Bulls	11.12.92	1.11 (0.94 - 1.30)	5.17 (3.49 - 7.67)	3.47 (0.43)
004	Bulls	21.12.92	0.87 (0.74 - 1.03)	3.13 (2.05 - 4.78)	4.20 (0.68)
003	Wanganui	10.01.92	2.60 (2.34 - 2.90)	9.33 (6.99 -12.46)	4.20 (0.44)
026	Foxton	27.01.92	1.34 (1.12 - 1.60)	4.56 (3.00 - 6.93)	4.38 (0.75)
004	Bulls	13.01.92	1.55 (1.38 - 1.73)	4.55 (3.51 - 5.89)	4.97 (0.55)
004	Bulls	03.02.92	1.66 (1.56 - 1.78)	6.87 (5.77 - 8.19)	3.78 (0.29)
004	Bulls	06.01.93	1.54 (1.39 - 1.71)	8.40 (6.98 - 10.1)	3.44 (0.24)
003	Wanganui	27.01.93	2.75 (2.61 - 2.90)	7.43 (6.37 - 8.66)	5.40 (0.57)
Hawkes Bay					
118	Hastings	21.10.91	0.92 (0.78 - 1.08)	5.90 (4.01 - 8.69)	2.88 (0.30)
012	Hastings	14.01.92	1.15 (1.04 - 1.27)	6.31 (4.78 - 8.31)	3.15 (0.24)
012	Hastings	29.12.92	1.03 (0.90 - 1.18)	3.40 (2.44 - 4.73)	4.50 (0.62)
012	Hastings	18.01.93	1.91 (1.82 - 2.03)	4.73 (1.81 - 2.03)	4.73 (0.30)
Wellington					
068	Levin	20.11.91	0.99 (0.68 - 1.43)	7.11 (2.69 -18.80)	2.72 (0.66)
Wairarapa					
024	Masterton	29.12.91	1.44 (1.34 - 1.55)	10.2 (8.27 -12.58)	2.73 (0.17)
111	Masterton	30.12.91	0.33 (0.30 - 0.36)	2.45 (2.01 - 2.98)	2.68 (0.17)
023	Masterton	07.01.92	1.19 (1.12 - 1.25)	4.03 (3.50 - 4.64)	4.38 (0.30)
Marlborough					
014	Blenheim	07.12.91	1.64 (1.46 - 1.85)	6.55 (4.82 - 8.91)	3.88 (0.40)
014	Blenheim	15.12.91	1.88 (1.70 - 2.08)	9.00 (6.80 -11.91)	3.42 (0.27)
North Canterbury					
038	Rangiora	04.01.93	1.91 (1.67 - 2.18)	7.42 (5.13 -10.73)	3.94 (0.49)

Appendix 1.1.11

Toxicological response of *Lucilia cuprina* larvae to diflubenzuron

Farm	Location	Sample date	LC_{50} (95% F.L.) ppm	LC_{99} (95% F.L.) ppm	Slope (SE)	RF
046	Laboratory	00.00.00	0.11 (0.09 - 0.13)	0.34 (0.21 - 0.55)	4.60 (0.83)	1.0
058	Takapau	03.04.95	2.23 (2.03 - 2.46)	11.1 (8.77 -14.04)	3.35 (0.21)	20.3
009	Blenheim	05.03.96	0.88 (0.76 - 1.01)	7.95 (4.82 -13.13)	2.43 (0.32)	8.0
055	Ashburton	18.01.97	0.51 (0.30 - 0.86)	12.2 (1.19 -124.3)	1.68 (0.53)	4.6
060	Waiau	27.01.97	0.15 (0.09 - 0.22)	1.11 (0.42 - 2.98)	2.65 (0.71)	1.4
027	Greytown	23.01.97	0.17 (0.15 - 0.20)	0.53 (0.38 - 0.76)	4.79 (0.60)	1.5
072	Bulls	13.02.97	0.37 (0.31 - 0.42)	3.04 (1.77 - 5.21)	2.53 (0.35)	3.4
122	Darfield	01.03.97	0.26 (0.23 - 0.29)	0.82 (0.61 - 1.10)	4.61 (0.56)	2.4
116	Timaru	06.03.97	0.20 (0.16 - 0.23)	0.55 (0.37 - 0.82)	5.17 (0.99)	1.8
085	Timaru	20.03.97	0.15 (0.14 - 0.17)	0.51 (0.41 - 0.63)	4.51 (0.58)	1.4
038	Marton	05.04.97	0.42 (0.39 - 0.46)	1.39 (1.13 - 1.72)	4.49 (0.50)	3.8
096	Timaru	07.04.97	0.32 (0.26 - 0.39)	4.37 (1.86 -10.27)	2.04 (0.32)	2.9
009	Blenheim	14.04.97	0.85 (0.67 - 1.06)	6.93 (3.35 -14.33)	2.55 (0.46)	7.7
091	Waipukurau	14.04.97	3.98 (3.57 - 4.42)	25.2 (17.7 -35.91)	2.9 (0.30)	36.2
097	Waipukurau	09.05.97	1.95 (1.73 - 2.19)	8.95 (6.84 -11.71)	3.51 (0.30)	17.7
003	Turakina	11.05.97	0.62 (0.56 - 0.68)	2.17 (1.64 - 2.86)	4.28 (0.22)	5.6

Appendix 1.1.12

Toxicological response of *Lucilia sericata* larvae to diazinon

Farm	Location	Sample date	LC_{50} (95% F.L.) ppm	LC_{99} (95% F.L.) ppm	Slope (S.E.)	RF
001	Laboratory	00.00.00	0.06 (0.06 - 0.07)	0.11 (0.09 - 0.11)	10.1 (0.56)	1.0
Waikato						
043	Waingaro	31.01.94	0.30 (0.17 - 0.53)	1.76 (0.48 - 6.40)	3.04 (1.26)	5.0
008	Kihikihi	13.02.95	0.57 (0.48 - 0.66)	0.92 (0.59 - 1.41)	11.1 (0.41)	9.5
Gisborne						
112	Gisborne	17.11.91	0.51 (0.46 - 0.57)	1.18 (0.93 - 1.48)	6.45 (1.18)	8.5
112	Gisborne	21.01.93	0.36 (0.3 - 0.43)	0.96 (0.72 - 1.28)	5.47 (1.02)	6.0
151	Gisborne	24.11.93	0.28 (0.22 - 0.36)	1.03 (0.60 - 1.78)	4.14 (0.94)	4.7
044	Gisborne	23.02.94	0.33 (0.28 - 0.39)	0.88 (0.62 - 1.26)	5.47 (0.92)	5.5
059	Wairoa	23.11.94	0.83 (0.72 - 0.96)	2.53 (1.77 - 3.62)	4.81 (1.00)	13.8
067	Gisborne	31.11.94	0.43 (0.35 - 0.52)	2.22 (1.28 - 3.86)	3.25 (0.77)	7.2
112	Gisborne	20.11.95	0.49 (0.42 - 0.56)	1.31 (0.88 - 1.95)	5.39 (0.63)	8.1
142	Wairoa	25.10.95	0.71 (0.58 - 0.87)	2.39 (1.38 - 4.13)	4.42 (0.98)	11.8
049	Wairoa	10.01.96	0.76 (0.62 - 0.94)	1.96 (1.19 - 3.22)	5.68 (1.36)	12.7
059	Wairoa	12.03.96	0.53 (0.44 - 0.64)	2.54 (1.51 - 4.25)	3.42 (0.56)	8.8
059	Wairoa	08.01.97	0.73 (0.66 - 0.80)	1.67 (1.33 - 2.08)	6.46 (0.97)	12.2
Hawkes Bay						
033	Waipukurau	03.01.92	0.73 (0.69 - 0.76)	1.44 (1.27 - 1.62)	7.88 (0.77)	12.2
018	Waipawa	06.01.92	0.66 (0.61 - 0.70)	1.66 (1.43 - 1.92)	5.78 (0.62)	11.0
020	Hastings	07.04.94	0.34 (0.28 - 0.41)	0.99 (0.62 - 1.58)	5.05 (0.76)	5.7
012	Hastings	08.04.94	0.45 (0.36 - 0.56)	3.83 (1.06 - 3.54)	3.66 (0.71)	7.5
012	Hastings	15.02.95	1.11 (1.02 - 1.20)	1.91 (1.59 - 2.29)	9.84 (0.70)	18.5
012	Hastings	04.11.95	0.64 (0.57 - 0.72)	1.50 (1.16 - 1.93)	6.34 (0.98)	10.7
018	Waipawa	28.12.95	0.59 (0.55 - 0.64)	1.41 (1.16 - 1.72)	6.16 (0.44)	9.8
012	Hastings	18.04.97	1.04 (0.96 - 1.13)	2.92 (2.35 - 3.61)	5.21 (0.68)	17.3
Wanganui						
003	Wanganui	14.12.91	0.55 (0.51 - 0.59)	1.58 (1.31 - 1.89)	5.78 (0.62)	9.2
040	Wanganui	03.01.94	0.49 (0.43 - 0.56)	1.32 (0.97 - 1.78)	5.42 (1.12)	8.2
040	Wanganui	25.03.94	0.36 (0.28 - 0.47)	1.02 (0.59 - 1.75)	5.22 (1.51)	6.0
143	Kimbolton	20.02.95	0.73 (0.64 - 0.83)	1.94 (1.42 - 2.65)	5.49 (1.03)	12.2
143	Kimbolton	18.01.96	0.67 (0.63 - 0.71)	1.22 (1.11 - 1.35)	6.26 (0.36)	11.2
003	Wanganui	22.01.96	1.25 (1.12 - 1.39)	2.84 (2.32 - 3.62)	6.51 (0.41)	20.8
045	Feilding	05.03.96	0.53 (0.48 - 0.58)	1.78 (1.42 - 2.25)	4.39 (0.53)	8.8
040	Wanganui	25.03.96	0.67 (0.60 - 0.75)	1.88 (1.39 - 2.53)	5.19 (0.66)	11.2
040	Wanganui	14.11.96	0.72 (0.66 - 0.79)	1.60 (1.30 - 1.95)	6.79 (1.07)	12.0
Wairarapa						
111	Masterton	30.12.91	0.43 (0.30 - 0.60)	1.50 (0.69 - 3.25)	4.27 (1.37)	7.2
056	Carterton	19.11.95	0.84 (0.77 - 0.92)	1.49 (1.22 - 1.82)	9.42 (1.01)	14.0
024	Masterton	10.01.96	0.61 (0.56 - 0.66)	1.62 (1.32 - 1.99)	5.45 (0.44)	10.2
053	Martinborough	17.01.96	1.09 (1.01 - 1.18)	2.17 (1.84 - 2.56)	7.79 (1.09)	18.2
056	Carterton	19.01.96	0.73 (0.67 - 0.80)	1.90 (1.54 - 2.34)	5.62 (0.57)	12.2
024	Masterton	24.02.96	0.78 (0.71 - 0.85)	2.62 (2.11 - 3.26)	4.41 (0.26)	13.0
111	Masterton	14.03.96	0.98 (0.87 - 1.10)	2.43 (1.84 - 3.21)	5.89 (0.84)	16.3

056	Carterton	07.05.96	0.84 (0.76 - 0.93)	1.85 (1.46 - 2.36)	6.78 (1.02)	14.0
061	Masterton	15.01.97	0.56 (0.51 - 0.62)	1.52 (1.28 - 1.80)	5.40 (0.61)	9.3
056	Carterton	15.01.97	0.68 (0.61 - 0.77)	2.03 (1.59 - 2.59)	4.92 (0.33)	11.3
024	Masterton	17.03.97	0.97 (0.89 - 1.05)	2.10 (1.77 - 2.49)	6.94 (0.63)	16.2
Wellington						
050	Levin	09.02.94	0.38 (0.34 - 0.42)	1.31 (1.03 - 1.67)	4.31 (0.33)	6.3
098	Marton	09.04.96	0.75 (0.70 - 0.80)	2.41 (2.05 - 2.83)	4.61 (0.22)	6.3
038	Marton	09.04.96	1.18 (1.03 - 1.34)	3.03 (2.17 - 4.22)	5.68 (0.93)	19.7
Marlborough						
032	Blenheim	28.02.91	0.32 (0.24 - 0.43)	0.95 (0.55 - 1.65)	4.88 (2.01)	5.3
032	Blenheim	13.12.91	0.58 (0.52 - 0.64)	1.59 (1.22 - 2.08)	5.28 (0.76)	9.7
032	Blenheim	20.01.92	0.75 (0.68 - 0.84)	1.55 (1.21 - 2.08)	7.43 (0.93)	12.3
009	Blenheim	07.12.92	1.29 (1.15 - 1.45)	3.28 (2.42 - 4.44)	5.76 (0.81)	21.3
009	Blenheim	07.12.92	1.29 (1.15 - 1.45)	3.28 (2.42 - 4.44)	5.76 (0.81)	21.3
009	Blenheim	25.04.94	0.75 (0.65 - 0.87)	1.70 (1.16 - 2.48)	6.58 (1.52)	12.5
032	Blenheim	27.01.96	0.82 (0.73 - 0.92)	2.02 (1.51 - 2.72)	5.96 (0.65)	13.7
144	Blenheim	03.03.96	1.26 (1.20 - 1.32)	2.38 (2.09 - 2.71)	8.42 (0.79)	13.7
009	Blenheim	05.03.96	2.06 (1.91 - 2.22)	4.69 (3.87 - 5.69)	6.49 (0.42)	34.3
009	Blenheim	10.12.96	0.95 (0.88 - 1.03)	2.26 (1.85 - 2.74)	6.23 (0.44)	15.8
014	Blenheim	12.01.97	1.31 (1.18 - 1.44)	3.54 (2.77 - 4.54)	5.36 (0.61)	21.7
032	Blenheim	20.01.97	0.85 (0.74 - 0.97)	3.23 (2.24 - 4.67)	4.00 (0.50)	14.2
009	Blenheim	14.04.97	1.15 (1.03 - 1.28)	4.25 (3.25 - 5.56)	4.09 (0.37)	19.2
Nelson						
051	Nelson	15.01.96	1.22 (1.14 - 1.31)	3.34 (2.77 - 4.03)	5.32 (0.40)	20.3
051	Nelson	17.04.96	1.10 (1.03 - 1.18)	2.74 (2.30 - 3.26)	5.89 (0.20)	18.3
063	Wakefield	26.02.97	0.76 (0.70 - 0.82)	1.94 (1.63 - 2.31)	5.70 (0.62)	12.7
North Canterbury						
031	Amberley	28.12.91	0.63 (0.60 - 0.67)	1.39 (1.21 - 1.59)	6.85 (0.29)	10.5
005	Waiau	18.01.92	1.06 (0.95 - 1.19)	2.26 (1.67 - 3.06)	7.09 (1.30)	17.7
057	Cheviot	11.12.92	0.94 (0.86 - 1.04)	2.52 (1.90 - 3.32)	5.47 (0.73)	15.7
028	Cheviot	14.12.92	0.87 (0.83 - 0.90)	1.31 (1.19 - 1.44)	13.1 (1.35)	14.5
145	Scargill	01.03.94	0.47 (0.39 - 0.57)	1.26 (0.82 - 1.94)	5.44 (0.68)	7.8
060	Waiau	14.12.96	0.87 (0.79 - 0.97)	2.12 (1.66 - 2.71)	6.04 (0.78)	14.5
021	Amberley	14.12.96	0.83 (0.73 - 0.94)	2.86 (2.12 - 3.84)	4.32 (0.48)	13.8
057	Cheviot	13.01.97	0.80 (0.69 - 0.91)	2.35 (1.63 - 3.40)	4.95 (0.77)	13.3
057	Cheviot	13.04.97	0.64 (0.58 - 0.71)	1.91 (1.49 - 2.45)	4.88 (0.43)	10.7
Mid Canterbury						
146	Darfield	21.11.91	0.57 (0.62 - 0.53)	1.71 (1.40 - 2.08)	4.90 (0.40)	9.5
029	Ashburton	21.12.92	0.76 (0.62 – 0.93)	2.06 (1.17 - 3.64)	5.37 (1.28)	12.7
030	Lincoln	22.03.93	0.69 (0.62 - 0.77)	2.45 (1.86 - 3.24)	4.23 (0.49)	11.5
055	Ashburton	06.01.94	0.37 (0.31 - 0.44)	1.34 (0.89 - 2.03)	4.20 (0.68)	6.2
039	Rakaia	10.01.94	0.40 (0.35 - 0.47)	1.25 (0.86 - 1.82)	4.72 (0.51)	6.7
039	Rakaia	14.02.94	0.38 (0.35 - 0.42)	0.76 (0.61 - 0.94)	7.84 (0.84)	6.3
041	Ashburton	16.02.94	0.60 (0.50 - 0.72)	1.85 (1.12 - 3.04)	4.75 (0.91)	10.0
034	Leeston	18.04.94	0.37 (0.32 - 0.42)	0.96 (0.69 - 1.33)	5.57 (0.61)	6.2
066	Dorie	20.04.94	0.37 (0.33 - 0.43)	0.74 (0.54 - 1.03)	7.81 (1.20)	6.2
055	Ashburton	16.01.95	0.51 (0.46 - 0.57)	1.12 (0.90 - 1.40)	6.87 (0.62)	8.5
065	Ashburton	07.11.95	0.57 (0.51 - 0.64)	1.22 (0.95 - 1.57)	7.01 (0.98)	9.5

055	Ashburton	07.12.95	0.33 (0.29 - 0.38)	0.82 (0.63 - 1.07)	5.95 (0.93)	5.5
114	Ashburton	09.01.96	0.18 (0.13 - 0.24)	1.32 (0.80 - 2.17)	2.67 (0.25)	2.9
047	Ashburton	09.01.96	0.40 (0.35 - 0.45)	1.60 (1.18 - 2.16)	3.84 (0.25)	6.6
055	Ashburton	22.03.96	0.54 (0.50 - 0.59)	1.73 (1.40 - 2.14)	4.63 (0.44)	9.0
092	Ashburton	06.04.96	0.73 (0.65 - 0.83)	1.65 (1.28 - 2.12)	6.60 (0.91)	12.2
055	Ashburton	21.05.96	0.47 (0.43 - 0.51)	1.18 (0.99 - 1.41)	5.79 (0.48)	7.8
092	Ashburton	16.01.97	0.57 (0.53 - 0.62)	1.51 (1.27 - 1.81)	5.52 (0.69)	9.5
065	Ashburton	04.04.97	0.56 (0.50 - 0.64)	1.69 (1.27 - 2.25)	4.89 (0.74)	9.3
055	Ashburton	29.04.97	0.30 (0.24 - 0.37)	1.85 (1.27 - 2.71)	2.94 (0.26)	5.0
South Canterbury						
052	Hakataramea	09.01.96	0.42 (0.35 - 0.50)	0.69 (0.49 - 0.99)	10.7 (0.88)	7.0
148	Temuka	01.05.96	0.52 (0.47 - 0.58)	1.97 (1.59 - 2.44)	4.04 (0.30)	8.7
062	Timaru	27.02.97	0.65 (0.61 - 0.70)	1.75 (1.48 - 2.06)	5.43 (0.38)	10.8
Otago Lakes						
149	Wanaka	13.11.92	0.47 (0.43 - 0.51)	1.18 (0.96 - 1.45)	5.82 (0.64)	7.8
Central Otago						
030	Kurow	10.01.96	0.60 (0.55 – 0.66)	1.41 (1.15 – 1.74)	6.23 (0.62)	10.0
048	Omarama	12.01.96	0.31 (0.27 – 0.35)	1.10 (0.82 – 1.48)	4.21 (0.50)	5.2
050	Cromwell	15.01.96	0.56 (0.52 – 0.61)	2.01 (1.65 – 2.44)	4.19 (0.40)	9.3
150	Cromwell	16.01.96	0.27 (0.24 – 0.30)	1.18 (0.95 – 1.47)	3.62 (0.34)	4.5
054	Alexandra	17.01.96	0.55 (0.50 – 0.60)	1.12 (0.93 – 1.36)	7.45 (0.80)	9.1
147	Omarama	14.02.96	0.38 (0.34 – 0.43)	1.89 (1.47 – 2.44)	3.35 (0.29)	6.3

Appendix 1.1.13

Frequency distribution histograms

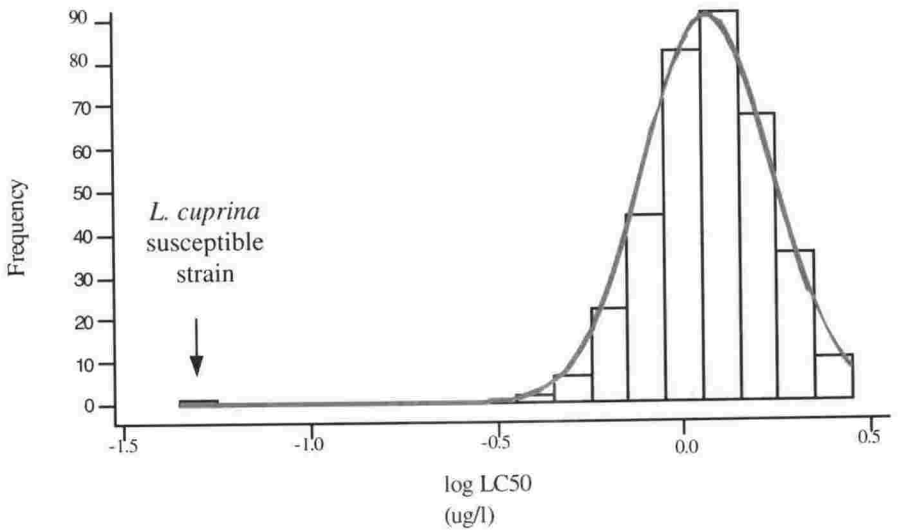


Figure 1 Distribution of LC_{50}s, as a measure of toxicological response to diazinon, in field populations of *Lucilia cuprina* larvae. The laboratory susceptible strain lies to the far left of the normal distribution.

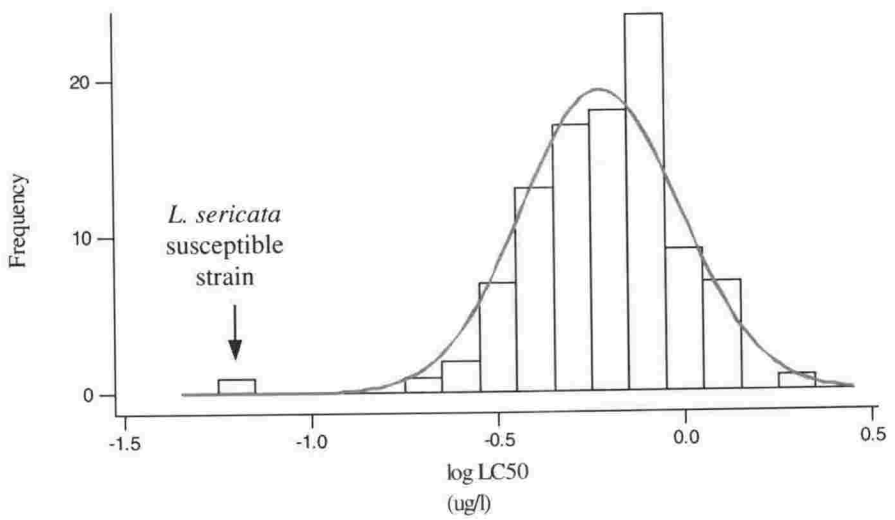


Figure 2 Distribution of LC_{50}s, as a measure of toxicological response to diazinon, in field populations of *Lucilia sericata* larvae. The laboratory susceptible strain lies to the far left of the normal distribution.

Appendix 1.1.14

Analysis of variance tables showing the relationship between slope of ldp-lines and insecticide LC_{50}s and the influence of region and season.

(a) Lucilia cuprina - slope of diazinon ldp-line

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Diazinon LC_{50}	1	1.8470	1.8470	1.2352	0.2671
Region	5	14.1082	2.8217	1.8870	0.0959
Season	3	36.5324	12.1775	8.1438	0.0000
Residuals	349	521.8609	1.4953		

(b) Lucilia cuprina - slope of chlorfenvinphos ldp-line

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Chlorfenvinphos LC_{50}	1	0.8587	0.8587	0.2976	0.5909
Region	5	8.4992	1.6998	0.5890	0.7083
Season	1	9.4202	9.4202	3.2646	0.0845
Residuals	22	63.4825	2.8856		

(c) Lucilia cuprina - slope of chlorpyrifos ldp-line

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Chlorpyrifos LC_{50}	1	12.6016	12.6017	2.9358	0.0937
Region	5	9.2217	1.8443	0.4297	0.8255
Season	2	9.2318	4.6159	1.0754	0.3500
Residuals	44	188.8646	4.2924		

(d) Lucilia cuprina - slope of dichlofenthion ldp-line

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Dichlofenthion LC_{50}	1	1.5484	1.5484	0.7573	0.3885
Region	5	4.6906	0.9381	0.4589	0.8048
Season	2	9.6217	4.8109	2.3531	0.1060
Residuals	48	98.1352	2.0445		

(e) Lucilia cuprina - slope of propetamphos ldp-line

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Propetamphos LC_{50}	1	4.9633	4.9633	0.5703	0.4535
Region	5	33.1897	6.6379	0.7627	0.5808
Season	2	12.3634	6.1817	0.7103	0.4962
Residuals	52	452.5453	8.7028		

(f) Lucilia cuprina - slope of diflubenzuron ldp-line

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Diflubenzuron LC_{50}	1	0.7937	0.7937	0.9904	0.3456
Region	5	3.7780	1.2593	1.5715	0.2631
Season	1	5.9793	5.9793	7.4615	0.0232
Residuals	9	7.2121	0.8013		

(g) Lucilia cuprina - slope of deltamethrin ldp-line

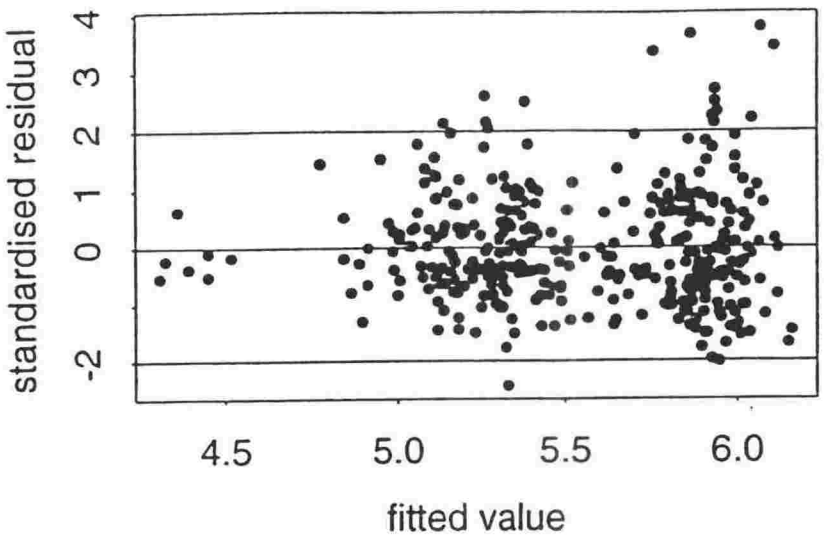
	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Deltamethrin LC_{50}	1	3.0308	3.0308	5.5336	0.0272
Residuals	24	13.1450	0.5477		

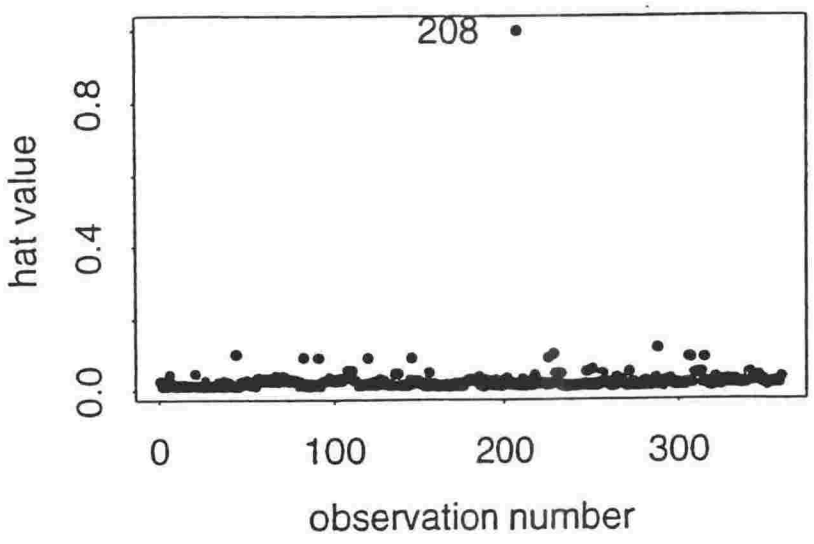
(h) Lucilia sericata - slope of diazinon ldp-line

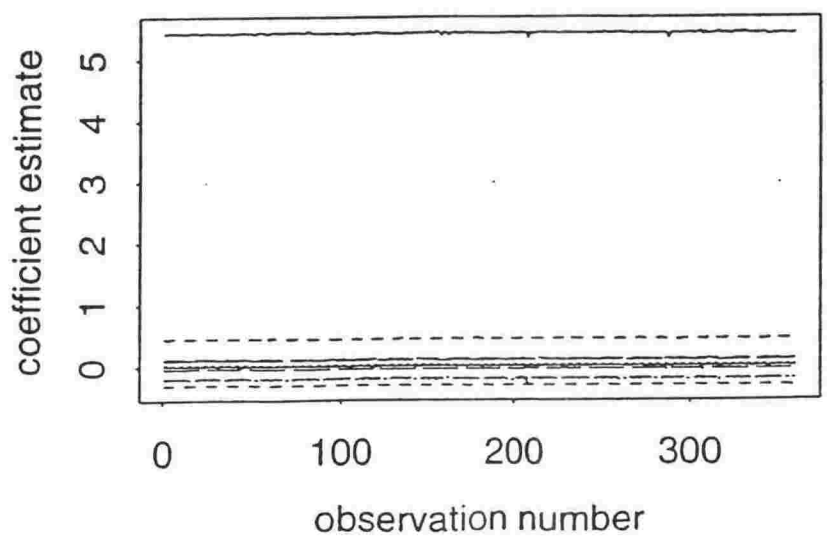
	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Diazinon LC_{50}	1	24.9959	24.9959	10.7989	0.0014
Region	3	5.6898	1.8966	0.8194	0.4865
Season	2	6.5041	3.2521	1.4050	0.2507
Residuals	90	208.3212	2.3147		

Appendix 1.1.15

Examples of ANOVA regression diagnostics for the relationship between slope of ldp-line and diazinon LC_{50} with the influence of region and season.







Appendix 1.1.16

Analysis of variance tables showing the relationship between resistance by strains of *Lucilia cuprina* to pairs of insecticides and the influence of region.

(a) Diazinon and Chlorfenvinphos

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Chlorfenvinphos	1	0.0311	0.0311	0.1559	0.6970
Region	5	0.9983	0.1997	1.0001	0.4180
Residuals	21	4.1922	0.1997		

(b) Diazinon and Chlorpyrifos

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Chlorpyrifos	1	5.7670	5.7670	85.6324	0.0000
Region	5	0.8526	0.1705	2.5319	0.0479
Residuals	35	2.2224	0.0673		

(c) Diazinon and Dichlofenthion

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Dichlofenthion	1	5.5706	5.5706	47.5960	0.0000
Region	5	1.3111	0.2622	2.2405	0.0674
Residuals	43	5.0327	0.1170		

(d) Diazinon and Propetamphos

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Propetamphos	1	2.5714	2.5714	17.8228	0.0001
Region	5	3.3082	0.6616	4.5859	0.0021
Residuals	40	5.7711	0.1443		

(e) Diazinon and Deltamethrin

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Deltamethrin	1	0.0276	0.0276	0.1463	0.7075
Region	5	1.7318	0.3464	1.8387	0.1657
Residuals	15	2.8256	0.1884		

(f) Chlorpyrifos and Chlorfenvinphos

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Chlorfenvinphos	1	0.1194	0.1194	1.9202	0.1910
Region	5	0.3878	0.0776	1.2476	0.3469
Residuals	12	0.7461	0.0622		

(g) Chlorpyrifos and Dichlofenthion

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Dichlofenthion	1	2.2083	2.2083	56.0193	0.0000
Region	5	0.1682	0.0336	0.8533	0.5213
Residuals	37	1.4586	0.0394		

(h) Chlorpyrifos and Propetamphos

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Propetamphos	1	0.2883	0.2883	7.8501	0.0097
Region	5	0.3742	0.0748	2.0374	0.1078
Residuals	25	0.9182	0.0327		

(i) Propetamphos and Chlorfenvinphos

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Chlorfenvinphos	1	0.1225	0.1225	14.8893	0.0019
Region	5	0.0226	0.0045	0.5504	0.7357
Residuals	13	0.1070	0.0082		

(j) Propetamphos and Dichlofenthion

Appendix 1.1.17

Insecticides used for the prevention of flystrike on sheep in New Zealand

Company	Product	Application	Concentration	Active Ingredient
Ancare	Cypercare	Pour-on	25 g/l	Cypermethrin
	Fleececare	Saturation	250 g/l	Diflubenzuron
	Flypel	Spray-on	10 g/l 100 g/l	Chlorpyrifos Cypermethrin
	Xterminate 10	Saturation	100 g/l	Chlorpyrifos
Bayer	Asuntol (P)	Dressing	10 g/l	Coumaphos
	Asuntol (L)	Saturation	160 g/l	Coumaphos
	Asuntol (P)	Saturation	500 g/l	Coumaphos
	Zapp	Pour-on	25 g/l	Triflumuron
FIL	Dokko	Dressing		Natural resins
	Flystrike Dressing	Dressing	10 g/l	Chlorfenvinphos
	Flystrike Powder	Dressing	20 g/l	Diazinon
	Jetting Fluid	Saturation	400 g/l	Diazinon
	SS Docking Fluid	Dressing	10 g/l	Chlorfenvinphos
	Sheardock	Dressing	4.9 g/l	Chlorfenvinphos
Novartis	Vetrazin	Spray-on	60 g/l	Cyromazine
	Ectomin 100 EC	Saturation	100 g/l	Cypermethrin
	Topclip	Saturation	400 g/l	Diazinon
	Vetrazin	Saturation	500 g/l	Cyromazine
	Vetrazin/ Ectomin	Saturation		Cyromazine Cypermethrin
	Banish	Spray-on	28 g/kg	Cypermethrin
	Cypafly	Spray-on/ Pour on	25 g/l	Cypermethrin
	Defiance	Dressing	2.5 g/l 50 g/l	Chlorfenvinphos Orthodichlorobenzene
	Diazinon 40	Saturation	400 g/l	Diazinon
	Maggo	Dressing	16 g/l 400 g/l	Propetamphos Paradichlorobenzene
	Percist	Saturation	40 g/l	Cypermethrin
	Seraphos 1250	Saturation	400 g/l	Propetamphos
	Seraphos 500	Saturation	160 g/l	Propetamphos
	Zenith	Saturation	250 g/l	Diflubenzuron
	Clik	Spray-on	50 g/l	Dicyclanil
	Eureka Gold	Spray-on	93.3 g/l	Diazinon
Schering-Plough	Supreme	Saturation	1000 g/l	Chlorfenvinphos
	Trigon	Saturation	1000 g/l	Dichlofenthion
	Blitz	Saturation	250 g/l	Diflubenzuron
Nufarm	Diazinon 40 sheep dip	Saturation	400 g/l	Diazinon
Pfizer	Duracide	Spray-on	20 g/l	Alphamethrin

Appendix 2.1.1

Table 1 Percentage internal radioactivity two hours after dosing *Lucilia cuprina* larvae with [^{14}C] methyl parathion and percentage organic metabolites excreted, using *method 2* and the 000, susceptible strain (RF = 1.0).

Larva	% internal radioactivity	% organic metabolites	% internal + metabolites
1	12.86	52.60	65.45
2	21.47	32.47	53.94
3	8.63	21.32	29.95
4	38.07	8.23	46.30
5	10.64	15.92	26.56
6	4.56	39.51	44.07
7	20.01	17.29	37.30
8	22.48	29.16	51.64
9	21.97	12.74	34.71
10	19.04	12.61	31.65
11	12.86	17.20	30.06
12	26.22	26.27	52.48
13	4.97	15.52	20.49
14	15.99	14.08	30.07
15	19.04	13.69	32.73
16	11.99	81.03	93.02
17	62.99	16.10	79.08
18	13.39	42.88	56.27
19	17.90	10.32	28.22
20	20.41	12.37	32.78
Mean	**19.27**	**24.57**	**43.84**
SE	**2.87**	**4.00**	**4.20**

Appendix 2.1.1

Table 2. Percentage internal radioactivity two hours after dosing *Lucilia cuprina* larvae with [^{14}C] methyl parathion and percentage organic metabolites excreted using *method 2* and the 058, Takapau strain (RF = 41.2).

larva	% penetrated	% organic metabolites	% penetrated + metabolites
1	23.44	6.70	30.15
2	17.04	17.10	34.14
3	8.83	60.16	68.99
4	10.57	12.97	23.54
5	21.59	25.05	46.64
6	16.27	6.57	22.85
7	25.13	22.48	47.61
8	11.40	22.60	34.01
9	7.84	26.94	34.79
10	11.76	21.95	33.71
11	12.42	13.45	25.87
12	21.61	28.82	50.42
13	6.52	32.37	38.90
14	12.60	11.85	24.45
15	10.08	24.11	34.18
16	16.89	18.55	35.44
17	10.08	9.70	19.78
18	10.15	6.71	16.86
19	62.25	4.99	67.24
20	17.66	35.98	53.64
Mean	**16.71**	**20.45**	**37.16**
SE	**2.69**	**2.92**	**3.27**

Appendix 2.2.1

Table 1. Non-specific esterase activity with the substrates α- and β-naphthyl acetate in adults strains of *L. cuprina*.

Farm	Area	Date	α-NA[a] Activity µmol/mg protein	StDev[b]	β-NA[c] Activity µmol/mg protein	StDev	RF[d]
000	Lab	00.00.00	0.1499	0.0472	0.0408	0.0138	1.0
025	Dargaville	04.12.91	0.0433	0.0150	0.0081	0.0044	21.4
014	Blenheim	15.12.91	0.1472	0.0206	0.0626	0.0223	45.0
024	Masterton	29.12.91	0.0906	0.0180	0.0288	0.0071	27.6
023	Masterton	07.01.92	0.0849	0.0155	0.0430	0.0153	42.6
003	Wanganui	10.01.92	0.1692	0.0364	0.0783	0.0330	41.0
017	Tauranga	10.01.92	0.1737	0.0481	0.0499	0.0141	27.2
004	Bulls	13.01.92	0.0671	0.0103	0.0162	0.0034	42.8
012	Hastings	14.01.92	0.1331	0.0254	0.0717	0.0269	35.4
019	Wakefield	20.01.92	0.1479	0.0496	0.0433	0.0161	31.4
020	Hastings	21.01.92	0.1035	0.0283	0.0566	0.0173	28.6
022	Te Kuiti	23.01.92	0.0791	0.0148	0.0267	0.0058	24.8
014	Blenheim	26.01.92	0.0904	0.0275	0.0390	0.0089	46.6
026	Foxton	27.01.92	0.1494	0.0196	0.0640	0.0168	34.6
010	Te Kuiti	29.01.92	0.1108	0.0188	0.0444	0.0138	25.4
010	Te Kuiti	16.02.92	0.0512	0.0223	0.0338	0.0216	30.0
014	Blenheim	24.02.92	0.1757	0.1155	0.1009	0.0335	20.2
002	Blenheim	25.02.92	0.1951	0.0195	0.0824	0.0181	42.0
016	Kaikohe	28.02.92	0.0680	0.0113	0.0234	0.0065	38.0
015	Nelson	05.03.92	0.1030	0.0298	0.0538	0.0114	23.8
014	Blenheim	14.03.92	0.0844	0.0232	0.0677	0.0112	18.8
012	Hastings	16.03.92	0.0651	0.0120	0.0331	0.0085	27.4
011	Gisborne	19.03.92	0.1380	0.0307	0.0602	0.0204	34.0
009	Blenheim	21.04.92	0.1121	0.0428	0.0854	0.0229	49.6
008	Kihikihi	21.04.92	0.3361	0.1051	0.1409	0.0377	19.0
007	Silverstream	23.04.92	0.1140	0.0420	0.0506	0.0174	27.8

Table 2. Non-specific esterase activity with the substrates α- and β-naphthyl acetate in larval strains of *L. cuprina*.

Farm	Area	Date	α-NA[a] Activity µmol/mg protein	StDev[b]	β-NA[c] Activity µmol/mg protein	StDev	RF[d]
000	Lab	00.00.00	0.4707	0.1152	0.1317	0.0299	1.0
025	Dargaville	04.12.91	0.4088	0.1465	0.1295	0.0558	21.4
014	Blenheim	15.12.91	1.3936	0.4065	0.6428	0.2077	45.0
024	Masterton	29.12.91	0.3910	0.0736	0.1300	0.0302	27.6
023	Masterton	07.01.92	1.4530	0.2136	0.7093	0.1489	42.6
003	Wanganui	10.01.92	0.9745	0.2292	0.4175	0.1017	41.0
017	Tauranga	10.01.92	0.7521	0.1048	0.2242	0.0463	27.2
004	Bulls	13.01.92	0.6973	0.1741	0.2282	0.0389	42.8
012	Hastings	14.01.92	0.6170	0.0761	0.2045	0.0293	35.4
019	Wakefield	20.01.92	2.1287	0.2877	0.9642	0.1671	31.4
022	Te Kuiti	23.01.92	1.3293	0.2047	0.4261	0.0577	24.8
014	Blenheim	26.01.92	0.8147	0.1393	0.3128	0.0412	46.6
026	Foxton	27.01.92	0.5647	0.1670	0.2023	0.0546	34.6
004	Bulls	14.02.92	0.7311	0.2287	0.2698	0.0920	33.4
006	Wairoa	18.02.92	2.1575	0.3526	1.2245	0.2005	41.4
002	Blenheim	25.02.92	1.8553	0.3188	0.5884	0.1132	42.0
016	Kaikohe	28.02.92	0.2430	0.0605	0.1122	0.0344	38.0
015	Nelson	05.03.92	1.3308	0.2597	0.4757	0.0981	23.8
014	Blenheim	14.03.92	0.7911	0.0733	0.3153	0.0373	18.8
012	Hastings	16.03.92	1.0739	0.3196	0.4242	0.1180	27.4
011	Gisborne	19.03.92	0.4420	0.1717	0.3117	0.1372	34.0
009	Blenheim	21.04.92	0.3731	0.0653	0.2257	0.0538	49.6
008	Kihikihi	21.04.92	1.2045	1.8446	0.2958	0.0881	19.0
007	Silverstream	23.04.92	1.0347	0.2230	0.3586	0.0748	27.8

Table 3. Non-specific esterase activity with the substrates α- and β-naphthyl acetate in adult strains of *L.sericata.*

Farm	Area	Date	α-NA[a] Activity µmol/mg protein	StDev[b]	β-NA[c] Activity µmol/mg protein	StDev	RF[d]
001	Lab	00.00.00	0.2353	0.0288	0.0795	0.0163	1.0
003	Wanganui	14.12.91	0.2885	0.0708	0.1254	0.0270	9.2
033	Waipukurau	02.01.92	0.2199	0.0577	0.1109	0.0524	12.2
018	Waipawa	06.01.92	0.4855	0.1204	0.1461	0.0517	11.0
005	Waiau	18.01.92	0.2059	0.0691	0.1043	0.0456	17.7
032	Blenheim	20.01.92	0.5482	0.1227	0.1877	0.0406	12.5
031	Amberley	28.01.92	0.5151	0.0289	0.1844	0.0171	10.5
021	Amberley	04.02.92	0.0524	0.0134	0.0436	0.0105	10.5
030	Lincoln	22.03.93	0.1905	0.0410	0.0733	0.0248	11.5
055	Ashburton	16.01.95	0.2249	0.0867	0.0710	0.0377	8.5
012	Hastings	04.11.95	0.1467	0.0173	0.0440	0.0059	10.7
056	Carterton	19.11.95	0.1277	0.0577	0.0579	0.0167	14.0
018	Waipawa	28.12.95	0.2557	0.0210	0.1001	0.0100	9.8
047	Ashburton	09.01.96	0.1411	0.0253	0.0261	0.0085	6.6
052	Hakataramea	09.01.96	0.0829	0.0093	0.0195	0.0036	7.0
048	Kurow	10.01.96	0.1311	0.0046	0.0279	0.0043	10.0
049	Wairoa	10.01.96	0.0980	0.0156	0.0236	0.0043	12.7
050	Cromwell	15.01.96	0.0548	0.0255	0.0122	0.0061	9.3
051	Nelson	15.01.96	0.1139	0.0551	0.0367	0.0125	20.3
053	Martinborough	17.01.96	0.1156	0.0282	0.0252	0.0061	18.2
054	Alexandra	17.01.96	0.2518	0.0829	0.0711	0.0235	9.1
003	Wanganui	22.01.96	0.1357	0.0306	0.0357	0.0067	20.8
032	Blenheim	27.01.96	0.1533	0.0564	0.0475	0.0147	13.7
040	Wanganui	25.03.96	0.1698	0.0321	0.0479	0.0098	11.2

Table 4. Non-specific esterase activity with the substrates α- and β-naphthyl acetate in larval strains of *L.sericata.*

Farm	Area	Date	α-NA[a] Activity µmol/mg protein	StDev[b]	β-NA[c] Activity µmol/mg protein	StDev	RF[d]
001	Lab	00.00.00	3.2347	0.3970	1.9541	0.1497	1.0
003	Wanganui	14.12.91	1.1364	0.2936	0.4020	0.1103	9.2
033	Waipukurau	02.01.92	0.0532	0.0096	0.0385	0.0088	12.2
018	Waipawa	06.01.92	1.5658	0.1338	0.7105	0.1241	11.0
032	Blenheim	20.01.92	1.1782	0.1713	0.7257	0.1305	12.5
021	Amberley	04.02.92	0.3137	0.0595	0.1159	0.0277	10.5
028	Cheviot	14.12.92	0.2625	0.0224	0.0958	0.0057	14.5
030	Lincoln	22.03.93	1.9184	0.0698	0.6008	0.0312	11.5
055	Ashburton	16.01.95	1.6187	0.3352	0.6633	0.1607	8.5
012	Hastings	04.11.95	1.4984	0.6084	0.3617	0.1288	10.7
056	Carterton	19.11.95	0.8994	0.1277	0.2745	0.0598	14.0
018	Waipawa	28.12.95	1.2639	0.4162	0.4594	0.2022	9.8
047	Ashburton	09.01.96	0.4816	0.1244	0.1608	0.0292	6.6
052	Hakataramea	09.01.96	1.0158	0.1842	0.3279	0.0973	7.0
048	Kurow	10.01.96	1.4742	0.7065	0.3971	0.2105	10.0
049	Wairoa	10.01.96	0.6435	0.2563	0.1557	0.0711	12.7
050	Cromwell	15.01.96	0.4918	0.1492	0.1429	0.0667	9.3
051	Nelson	15.01.96	0.8795	0.1506	0.4074	0.1299	20.3
053	Martinborough	17.01.96	0.4765	0.0620	0.1295	0.0624	18.2
054	Alexandra	17.01.96	1.5905	0.4010	0.6020	0.1957	9.1
003	Wanganui	22.01.96	0.6357	0.1309	0.2141	0.0400	20.8
032	Blenheim	27.01.96	0.6199	0.2254	0.3102	0.1713	13.7
040	Wanganui	25.03.96	0.3307	0.0737	0.0763	0.0188	11.2

[a] α-naphthyl acetate
[b] Standard deviation
[c] β-naphthyl acetate
[d] Resistance factor

Appendix 2.2.2

Analysis of variance tables showing the relationship between resistance to diazinon, total esterase activity and environmental factors

(a) Lucilia cuprina adults

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
α-NA esterase activity	1	0.0682	0.0682	0.0705	0.7935
Region	4	4.5171	1.1293	1.1667	0.3566
Season	1	2.0240	2.0240	2.0911	0.1645
Residuals	19	18.3907	0.9679		

(b) Lucilia cuprina adults

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
β-NA esterase activity	1	0.0018	0.0018	0.0019	0.9659
Region	4	4.7091	1.1772	1.2187	0.3358
Season	1	1.9352	1.9353	2.0033	0.1731
Residuals	19	18.3539	0.9660		

(c) Lucilia cuprina larvae

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
α-NA esterase activity	1	2.5670	2.5170	2.7229	0.1197
Region	3	4.0605	1.5341	1.6597	0.2181
Season	2	0.0150	0.0075	0.0081	0.9919
Residuals	15	13.8655	0.9244		

(d) Lucilia cuprina larvae

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
β-NA esterase activity	1	1.7502	1.7502	2.0870	0.1679
Region	5	4.4596	0.8919	1.0635	0.4162
Season	1	3.3721	3.3721	4.0210	0.0622
Residuals	16	13.4181	0.8386		

(e) Lucilia sericata adults

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
α-NA esterase activity	1	0.4418	0.4418	0.4538	0.5108
Region	3	5.7736	1.9245	1.9768	0.1608
Season	2	0.1813	0.0907	0.0931	0.9116
Residuals	15	14.6033	0.9736		

(f) Lucilia sericata adults

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
β-NA esterase activity	1	0.1353	0.1353	0.1339	0.7195
Region	3	5.5817	1.8606	1.8406	0.1831
Season	2	0.1202	0.0601	0.0595	0.9425
Residuals	15	15.1627	1.0108		

(g) Lucilia sericata larvae

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
α-NA esterase activity	1	2.5670	2.5170	2.7229	0.1197
Region	3	4.0605	1.5341	1.6597	0.2181
Season	2	0.0150	0.0075	0.0081	0.9919
Residuals	15	13.8655	0.9244		

(h) Lucilia sericata larvae

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
β-NA esterase activity	1	1.3162	1.3162	1.4581	0.2459
Region	3	6.1171	2.0390	2.2589	0.1235
Season	2	0.0265	0.0133	0.0147	0.9854
Residuals	15	13.5401	0.9027		

Appendix 2.2.3

Table 1. Percentage occurrence of the staining E_3 esterase band in OP-susceptible and field strains of *Lucilia cuprina.*

Farm	Area	Sample Date	Diazinon RF	Adult % E_3	Larvae % E_3
046	Lab	00.00.00	1.0	100	100
000	Lab	00.00.00	1.0	100	100
Northland					
025	Dargaville	04.12.91	21.4	0	0
016	Kaikohe	28.02.92	38.0	14.3	-
036	Kaiwaka	14.03.94	-	0	0
Bay of Plenty					
017	Tauranga	10.01.92	27.2	10.7	0
Gisborne					
006	Wairoa	18.02.92	41.4	3.6	0
011	Gisborne	19.03.92	34.0	0	0
Waikato					
022	Te Kuiti	23.01.92	24.8	17.9	-
010	Te Kuiti	29.01.92	25.4	14.3	-
010	Te Kuiti	16.02.92	30.0	15.0	-
008	Kihikihi	21.04.92	19.0	0	0
043	Waingaro	31.01.94	24.0	0	-
008	Kihikihi	07.03.94	11.8	0	-
Hawkes Bay					
018	Waipawa	06.01.92		0	-
012	Hastings	14.01.92	35.4	0	0
020	Hastings	21.01.92	28.6	0	-
012	Hastings	16.03.92	27.4	7.1	-
012	Hastings	08.04.94	-	0	0
Wanganui					
004	Bulls	13.01.92	42.8	0	-
003	Wanganui	10.01.92	41.0	10.7	0
004	Bulls	14.02.92	33.4	0	-
045	Feilding	13.04.94	-	0	0
003	Wanganui	16.05.94	-	0	0
Wellington					
026	Foxton	27.01.92	34.6	3.6	-
007	Silverstream	23.04.92	27.8	0	-
037	Levin	15.03.94	16.0	0	0
035	Levin	11.04.94	14.2	0	0
038	Marton	09.05.94	11.8	0	0

Wairarapa					
024	Masterton	29.12.91	27.6	0	7.1
023	Masterton	07.01.92	42.6	0	0
013	Featherston	05.03.92	-	0	-
024	Masterton	28.02.94	-	0	0
Marlborough					
014	Blenheim	15.12.91	45.0	8.7	0
014	Blenheim	26.01.92	46.6	0	-
014	Blenheim	24.02.92	20.2	0	-
002	Blenheim	25.02.92	42.0	17.9	-
014	Blenheim	14.03.92	18.8	4.3	-
009	Blenheim	21.04.92	49.6	0	-
Nelson					
019	Wakefield	20.01.92	31.4	0	0
015	Nelson	05.03.92	23.8	0	10.7
North Canterbury					
042	Waikari	16.02.94	19.8	0	0
Mid Canterbury					
039	Rakaia	14.02.94	18.4	0	0
034	Leeston	18.04.94	8.2	0	0

Appendix 2.2.4

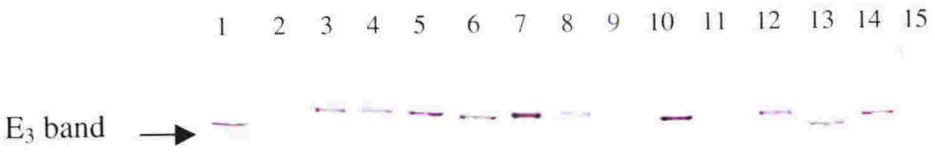


Figure 1. Native PAGE of *Lucilia cuprina* strain 004 from Bulls (13 January 1992). The OP-susceptible laboratory strain is represented lane 1 of the gel and the box acts as a guide to the position of the E_3 in the field strain.

Figure 2. Native PAGE of *Lucilia cuprina* strain 025 from Dargaville (4 December 1991). The OP-susceptible laboratory strain is represented lane 1 of the gel as a comparison with the field strain.

Figure 3. Native PAGE of *Lucilia cuprina* strain 019 from Wakefield (20 January 1992). The OP-susceptible laboratory strain is represented lane 1 of the gel as a comparison with the field strain.

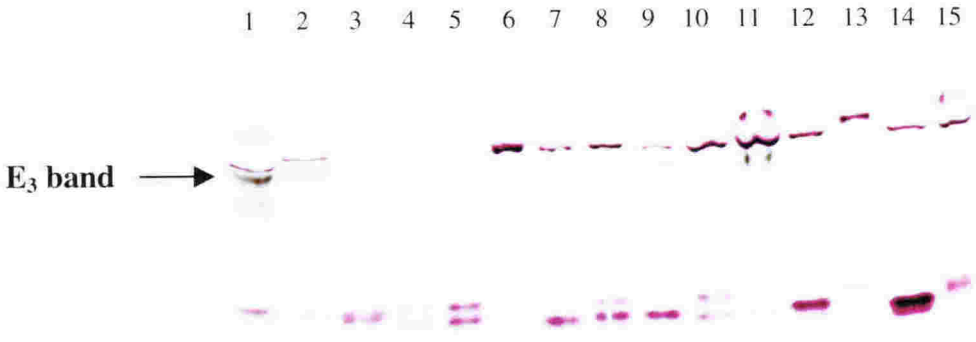


Figure 4. Native PAGE of *Lucilia cuprina* strain 010 from Te Kuiti (16 February 1992). The OP-susceptible laboratory strain is represented in lane 1of the gel as a comparison with the field strain.

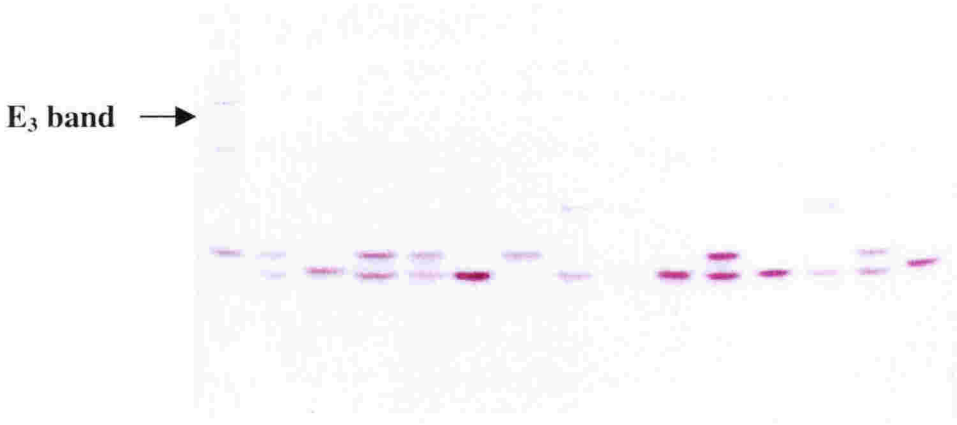


Figure 5. Native PAGE of *Lucilia cuprina* strain 014 from Blenheim (24 February 1992). The OP-susceptible laboratory strain is represented in lane 1of the gel as a comparison with the field strain.

Figure 6. Native PAGE of *Lucilia cuprina* strain 015 from Nelson (5 March 1992). The OP-susceptible laboratory strain is represented in lane 1of the gel as a comparison with the field strain.

Appendix 2.2.5

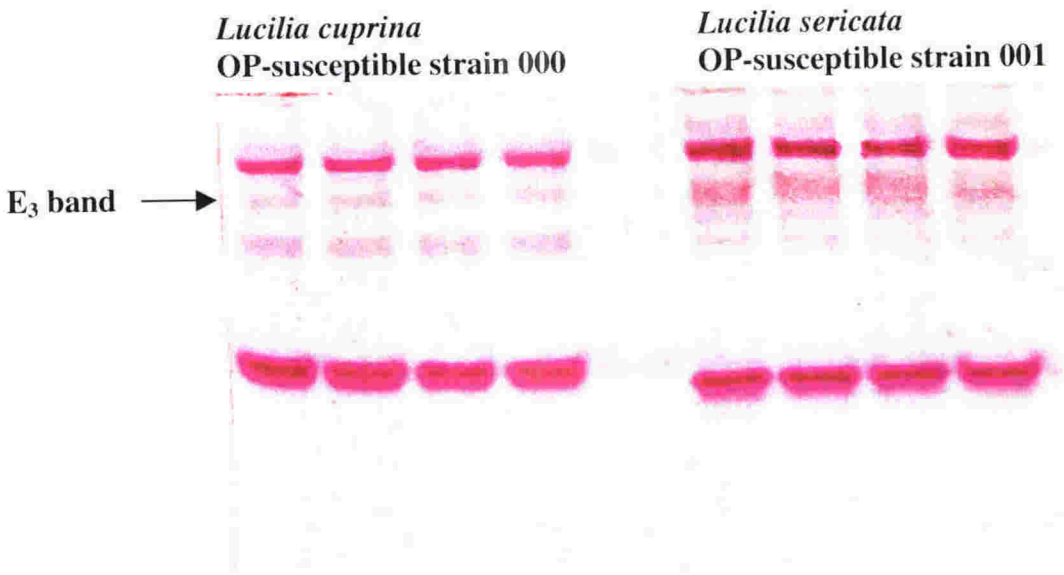


Figure 1. Comparison of esterase patterns from OP-susceptible strains of *Lucilia cuprina* and *Lucilia sericata* visualized by native PAGE. The staining E_3 band found in *L. cuprina* is shown at the left.

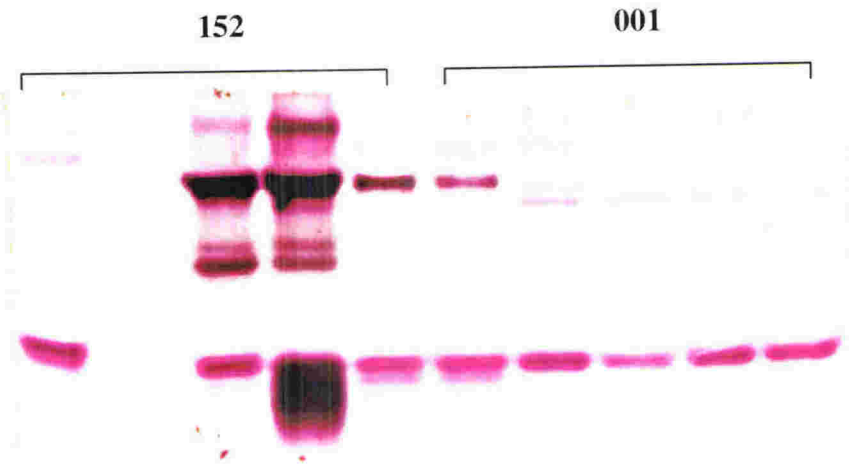


Figure 2. Comparison of esterase patterns of two OP-susceptible strains of *Lucilia sericata* by native PAGE. The 001 (ICI) strain was used as the standard for comparison in toxicological studies and is described in chapter 1.1. The 152 (WE) strain is a spontaneous mutation of the ICI strain and has been reared separately from this strain for several years.

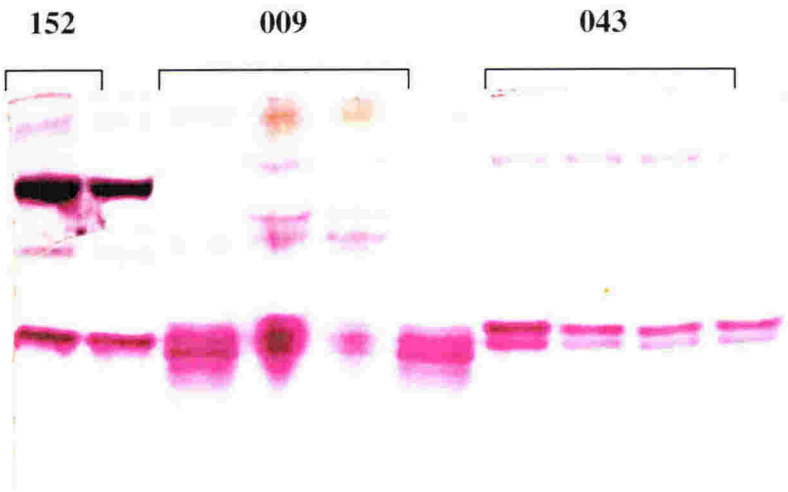


Figure 3. Comparison of esterase patterns from susceptible (152) and resistant field strains of *Lucilia sericata* visualized by native PAGE. Field strains were from Blenheim (009) and Waingaro (043).

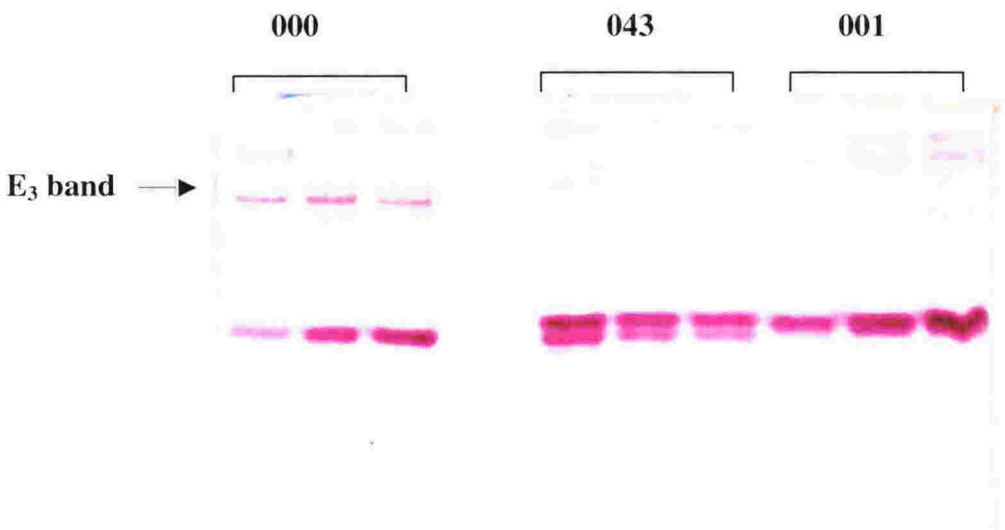


Figure 4. Comparison of esterase patterns from the *Lucilia cuprina* OP-susceptible (000), a *Lucilia sericata* OP-susceptible strain (001) and resistant a field strain of *L. sericata* (043, Waingaro) visualized by native PAGE.

Appendix 2.2.6

Analysis of variance tables showing the relationship between resistance to diazinon and aliesterase activity using methylthiobutyrate as substrate.

(a) Lucilia sericata adults (including susceptibles)

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Regression	1	0.000049	0.000049	0.59	0.456
Residual Error	14	0.001174	0.000084		
Residuals	15	0.001223			

(b) Lucilia sericata adults (excluding susceptibles)

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Regression	1	0.000042	0.000042	0.55	0.474
Residual Error	12	0.000924	0.000077		
Residuals	13	0.000966			

(c) Lucilia sericata larvae (including susceptibles)

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Regression	1	0.007759	0.007759	7.17	0.019*
Residual Error	13	0.014068	0.001082		
Residuals	14	0.021826			

(d) Lucilia sericata larvae (excluding susceptibles)

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Regression	1	0.001005	0.001005	1.20	0.294
Residual Error	12	0.010001	0.000834		
Residuals	13	0.011012			

Appendix 2.2.7

Analysis of variance tables showing the relationship between adult and larval non-specific esterase activities.

(a) Lucilia cuprina adults vs larvae (α-naphthyl acetate activity)

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Regression	1	0.0073	0.0073	1.95	0.177
Residual Error	20	0.0744	0.0037		
Residuals	21	0.0816			

(b) Lucilia cuprina adults vs larvae (β-naphthyl acetate activity)

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Regression	1	0.0002	0.0002	0.26	0.613
Residual Error	20	0.0174	0.0009		
Residuals	21	0.0176			

(c) Lucilia sericata adults vs larvae (α-naphthyl acetate activity)

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Regression	1	0.0096	0.0096	0.50	0.489
Residual Error	20	0.3879	0.0194		
Residuals	21	0.3975			

(d) Lucilia sericata adults vs larvae (β-naphthyl acetate activity)

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Regression	1	0.0031	0.0031	1.16	0.294
Residual Error	20	0.0532	0.0027		
Residuals	21	0.0563			

Appendix 2.2.8 Residual output of the regression of methylthiobutyrate activity and resistance to diazinon in larval strains of *Lucilia sericata*.

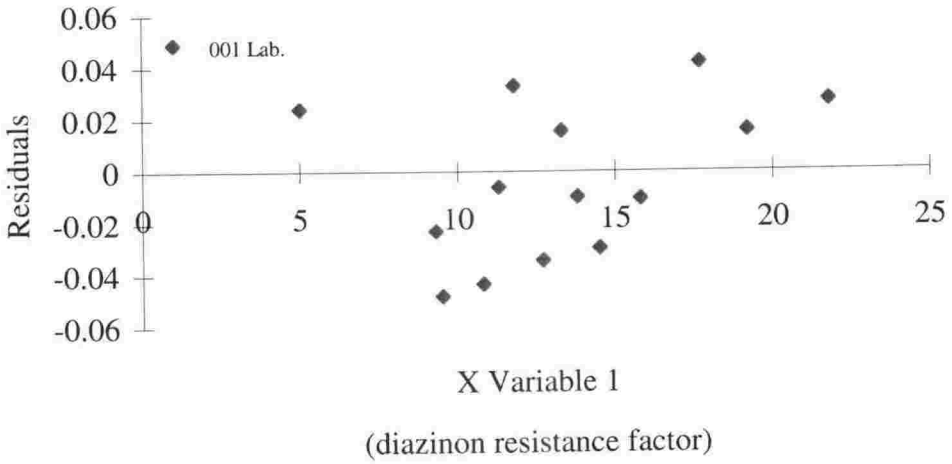


Figure 1. Plot of residuals for the regression of methylthiobutyrate activity and resistance to diazinon in larval strains of *Lucilia sericata*.

Table 1. Residuals output for the regression of methylthiobutyrate activity and resistance to diazinon in larval strains of *Lucilia sericata*.

Farm	Area	Date	Predicted Y	Residuals	Standard Residuals
056	Carterton	15.01.97	0.0523	-0.0061	-0.1913
061	Masterton	15.01.97	0.0501	0.0328	1.0359
063	Wakefield	26.02.97	0.0461	-0.0345	-1.0870
009	Blenheim	10.12.96	0.0323	-0.0106	-0.3348
009	Blenheim	14.04.97	0.0171	0.0158	0.4917
060	Wairoa	14.12.96	0.0381	-0.0298	-0.9388
021	Amberley	14.12.97	0.0412	-0.0099	-0.3119
055	Ashburton	29.04.97	0.0806	0.0241	0.7604
012	Hastings	18.04.97	0.0238	0.0415	1.3106
064	Ashburton	16.02.97	0.0604	-0.0481	-1.5177
057	Cheviot	13.01.97	0.0434	0.0155	0.4903
062	Timaru	27.02.97	0.0546	-0.0436	-1.3765
065	Ashburton	14.04.97	0.0613	-0.0230	-0.7257
014	Blenheim	12.01.97	0.0055	0.0269	0.8478
001	Laboratory	00.00.00	0.0984	0.0491	1.5476

Appendix 2.3.1

Table 1. Glutathione *S*-transferase activities of various strains of adult *L. cuprina*, using DCNB as substrate.

Farm	Area	Date	Activity (μmol/min/mg protein)	StDev	RF
000	Lab	00.00.00	0.001489	0.000342	1.0
025	Dargaville	04.12.91	0.001660	0.000441	21.4
014	Blenheim	15.12.91	0.003993	0.001384	45.0
024	Masterton	29.12.91	0.001691	0.000226	27.6
023	Masterton	07.01.92	0.002333	0.000351	42.6
003	Wanganui	10.01.92	0.002080	0.000751	41.0
017	Tauranga	10.01.92	0.001422	0.000315	27.2
004	Bulls	13.01.92	0.001676	0.000507	42.8
012	Hastings	14.01.92	0.002925	0.000917	35.4
019	Wakefield	20.01.92	0.001728	0.000687	31.4
020	Hastings	21.01.92	0.002113	0.000396	28.6
022	Te Kuiti	23.01.92	0.001973	0.000627	24.8
014	Blenheim	26.01.92	0.003550	0.001172	46.6
026	Foxton	27.01.92	0.001871	0.000476	34.6
010	Te Kuiti	29.01.92	0.002432	0.000461	25.4
004	Bulls	14.02.92	0.001212	0.000233	33.4
010	Te Kuiti	16.02.92	0.001084	0.000343	30.0
006	Wairoa	18.02.92	0.006712	0.003677	41.4
014	Blenheim	24.02.92	0.001575	0.000460	20.2
002	Blenheim	25.02.92	0.001334	0.000394	42.0
016	Kaikohe	28.02.92	0.002512	0.000865	38.0
015	Nelson	05.03.92	0.002593	0.000809	23.8
014	Blenheim	14.03.92	0.001925	0.000370	18.8
012	Hastings	16.03.92	0.001975	0.000517	27.4
011	Gisborne	19.03.92	0.001715	0.000390	34.0
008	Kihikihi	21.04.92	0.002161	0.000905	19.0
009	Blenheim	21.04.92	0.004121	0.001084	49.6
007	Silverstream	23.04.92	0.003456	0.000670	27.8
043	Waingaro	31.01.94	0.002107	0.000565	24.0
039	Rakaia	14.02.94	0.004853	0.001512	18.4
042	Waikari	16.02.94	0.002360	0.000493	19.8
024	Masterton	31.02.94	0.004916	0.001812	15.2
008	Kihikihi	07.03.94	0.003111	0.000464	11.8
036	Kaiwaka	14.03.94	0.002607	0.000248	18.8
037	Levin	15.03.94	0.001850	0.000234	16.0
012	Hastings	08.04.94	0.003269	0.000347	24.0
035	Levin	11.04.94	0.001541	0.000242	14.2
045	Feilding	13.04.94	0.004658	0.001223	44.8
034	Leeston	18.04.94	0.001385	0.000264	8.0
038	Marton	09.05.94	0.001892	0.000308	11.8

Table 2. Glutathione *S*-transferase activities of various strains of larval *L. cuprina*, using DCNB as substrate.

Farm	Area	Date	Activity (µmol/min/mg protein)	StDev	RF
000	Lab	00.00.00	0.005933	0.001683	1.0
025	Dargaville	04.12.91	0.002421	0.000595	21.4
014	Blenheim	15.12.91	0.011201	0.000865	45.0
024	Masterton	29.12.91	0.008714	0.000934	27.6
023	Masterton	07.01.92	0.008003	0.001800	42.6
003	Wanganui	10.01.92	0.006583	0.000975	41.0
017	Tauranga	10.01.92	0.004887	0.001268	27.2
004	Bulls	13.01.92	0.005205	0.001081	42.8
012	Hastings	14.01.92	0.007915	0.002008	35.4
019	Wakefield	20.01.92	0.009454	0.001579	31.4
022	Te Kuiti	23.01.92	0.005949	0.001496	24.8
014	Blenheim	26.01.92	0.008225	0.001114	46.6
026	Foxton	27.01.92	0.006637	0.001868	34.6
010	Te Kuiti	29.01.92	0.007441	0.002461	25.4
004	Bulls	14.02.92	0.006758	0.000826	33.4
006	Wairoa	18.02.92	0.015623	0.002049	41.4
002	Blenheim	25.02.92	0.004840	0.000217	42.0
015	Nelson	05.03.92	0.006130	0.000937	23.8
014	Blenheim	14.03.92	0.008718	0.000629	18.8
012	Hastings	16.03.92	0.011123	0.001487	27.4
011	Gisborne	19.03.92	0.004480	0.000975	34.0
009	Blenheim	21.04.92	0.006695	0.001060	49.6
008	Kihikihi	21.04.92	0.005504	0.000688	19.0
007	Silverstream	23.04.92	0.004335	0.001379	27.8
043	Waingaro	31.01.94	0.003394	0.000332	24.0
039	Rakaia	14.02.94	0.006132	0.002477	18.4
042	Waikari	16.02.94	0.001792	0.000184	19.8
024	Masterton	31.02.94	0.005179	0.001487	15.2
008	Kihikihi	07.03.94	0.003586	0.000770	11.8
036	Kaiwaka	14.03.94	0.004462	0.000866	18.8
037	Levin	15.03.94	0.001870	0.000240	16.0
012	Hastings	08.04.94	0.004384	0.000961	24.0
035	Levin	11.04.94	0.002111	0.000244	14.2
045	Feilding	13.04.94	0.003973	0.000765	44.8
034	Leeston	18.04.94	0.002227	0.000596	8.0
038	Marton	09.05.94	0.003153	0.000070	11.8

Table 3. Glutathione *S*-transferase activities of various strains of adult *L. sericata*, using DCNB as substrate.

Farm	Area	Date	Activity (µmol/min/mg protein)	StDev	RF
001	Lab	00.00.00	0.001519	0.000352	1.0
018	Waipawa	06.01.92	0.001127	0.000184	11.0
005	Waiau	18.01.92	0.001201	0.000483	17.7
032	Blenheim	20.01.92	0.001419	0.000527	12.5
031	Amberley	28.01.92	0.001871	0.000193	10.5
021	Amberley	04.02.92	0.001368	0.000422	10.5
009	Blenheim	07.12.92	0.002134	0.000462	21.3
030	Lincoln	22.03.93	0.001714	0.000423	11.5
040	Wanganui	03.01.94	0.001502	0.000249	8.2
039	Rakaia	10.01.94	0.002112	0.000433	6.7
043	Waingaro	31.01.94	0.003460	0.000626	5.0
041	Ashburton	16.02.94	0.002118	0.000282	10.0
044	Wairoa	23.02.94	0.001765	0.000299	5.5
012	Hastings	08.04.94	0.002658	0.000595	7.5
034	Leeston	18.04.94	0.003427	0.000474	6.2
066	Dorie	20.04.94	0.001876	0.000481	6.2
009	Blenheim	25.04.94	0.002690	0.000154	12.5
067	Gisborne	31.11.94	0.002188	0.000710	7.2
055	Ashburton	16.01.95	0.005818	0.001551	8.5
008	Kihikihi	13.02.95	0.001968	0.000925	9.5
012	Hastings	04.11.95	0.007683	0.002167	10.7
056	Carterton	19.11.95	0.005498	0.001077	14.0
018	Waipawa	28.12.95	0.006981	0.002127	9.8
047	Ashburton	09.01.96	0.015223	0.007843	6.6
052	Hakataramea	09.01.96	0.008580	0.004027	7.0
048	Kurow	10.01.96	0.009790	0.004349	10.0
049	Wairoa	10.01.96	0.006462	0.005420	12.7
050	Cromwell	15.01.96	0.009291	0.004799	9.3
051	Nelson	15.01.96	0.003625	0.000795	20.3
053	Martinborough	17.01.96	0.006398	0.001797	18.2
054	Alexandra	17.01.96	0.008594	0.002852	9.1
003	Wanganui	22.01.96	0.006331	0.003775	20.8
032	Blenheim	27.01.96	0.004697	0.002005	13.7
040	Wanganui	25.03.96	0.007875	0.001963	11.2
060	Wairoa	14.12.96	0.001615	0.000359	14.5
021	Amberley	14.12.96	0.000827	0.000623	13.8
059	Wairoa	08.01.97	0.004026	0.003060	12.2
014	Blenheim	12.01.97	0.001343	0.000237	21.8
057	Cheviot	13.01.97	0.001136	0.000275	13.3
056	Carterton	15.01.97	0.003628	0.000802	11.3

061	Masterton	15.01.97	0.001252	0.000763	11.8
064	Ashburton	16.02.97	0.003007	0.000934	9.5
062	Timaru	27.02.97	0.000763	0.000210	10.8
012	Hastings	18.04.97	0.002975	0.001623	17.7

Table 4. Glutathione *S*-transferase activities of various strains of larval *L. sericata*, using DCNB as substrate.

Farm	Area	Date	Activity (µmol/min/mg protein)	StDev	RF
001	Lab	00.00.00	0.005311	0.000912	1.0
009	Blenheim	07.12.91	0.011329	0.001138	21.3
003	Wanganui	14.12.91	0.007496	0.000655	9.2
033	Waipukurau	02.01.92	0.010386	0.001077	12.2
018	Waipawa	06.01.92	0.007478	0.001327	11.0
005	Waiau	18.01.92	0.006626	0.000520	17.7
032	Blenheim	20.01.92	0.007966	0.000400	12.5
031	Amberley	28.01.92	0.009355	0.000533	10.5
021	Amberley	04.02.92	0.008617	0.002016	10.5
028	Cheviot	14.12.92	0.005239	0.000474	14.5
029	Ashburton	21.12.92	0.006983	0.000672	12.7
030	Lincoln	22.03.93	0.009206	0.001396	11.5
040	Wanganui	03.01.94	0.003727	0.000429	8.2
039	Rakaia	10.01.94	0.003224	0.000547	6.7
043	Waingaro	31.01.94	0.003223	0.000982	5.0
050	Levin	09.02.94	0.005360	0.000386	6.3
041	Ashburton	16.02.94	0.001518	0.000384	10.0
044	Wairoa	23.02.94	0.004177	0.000480	5.5
040	Wanganui	25.03.94	0.005114	0.001509	6.0
012	Hastings	08.04.94	0.004269	0.000982	7.5
034	Leeston	18.04.94	0.005399	0.001181	6.2
066	Dorie	20.04.94	0.007709	0.000898	6.2
009	Blenheim	25.04.94	0.005834	0.000914	12.5
067	Gisborne	31.11.94	0.040732	0.004660	7.2
055	Ashburton	16.01.95	0.008644	0.002096	8.5
008	Kihikihi	13.02.95	0.007055		9.5
012	Hastings	04.11.95	0.011296	0.004530	10.7
056	Carterton	19.11.95	0.015549	0.008168	14.0
018	Waipawa	28.12.95	0.021129	0.013850	9.8
047	Ashburton	09.01.96	0.01979	0.008216	6.6
052	Hakataramea	09.01.96	0.028997	0.015273	7.0
048	Kurow	10.01.96	0.014205	0.003879	10.0
049	Wairoa	10.01.96	0.006952	0.001512	12.7
050	Cromwell	15.01.96	0.010097	0.002943	9.3
051	Nelson	15.01.96	0.024107	0.012270	20.3
053	Martinborough	17.01.96	0.014866	0.005555	18.2
054	Alexandra	17.01.96	0.025950	0.005503	9.1
003	Wanganui	22.01.96	0.017788	0.006347	20.8
032	Blenheim	27.01.96	0.024723	0.010130	13.7
040	Wanganui	25.03.96	0.020857	0.012305	11.2

060	Wairoa	14.12.96	0.003139	0.000650	14.5
014	Blenheim	12.01.97	0.005182	0.000491	21.8
057	Cheviot	13.01.97	0.013339	0.021980	13.3
056	Carterton	15.01.97	0.005405	0.001857	11.3
061	Masterton	15.01.97	0.006060	0.001326	11.8
032	Blenheim	20.01.97	0.004622	0.000859	14.0
064	Ashburton	16.02.97	0.001562	0.000456	9.5
062	Timaru	27.02.97	0.009868	0.003179	10.8
009	Blenheim	14.04.97	0.001648	0.000686	19.2
012	Hastings	18.04.97	0.015825	0.005169	17.7

Table 5. Glutathione *S*-transferase activities of various strains of adult *L. cuprina*, using CDNB as substrate.

Farm	Area	Date	Activity (µmol/min/mg protein)	StDev	RF
000	Lab	00.00.00	0.040338	0.006002	1.0
025	Dargaville	04.12.91	0.048263	0.008557	21.4
024	Masterton	29.12.91	0.037594	0.008826	27.6
023	Masterton	07.01.92	0.049719	0.011160	42.6
003	Wanganui	10.01.92	0.241287	0.032669	41.0
017	Tauranga	10.01.92	0.049996	0.006855	27.2
004	Bulls	13.01.92	0.043842	0.007014	42.8
012	Hastings	14.01.92	0.049675	0.007922	35.4
019	Wakefield	20.01.92	0.036443	0.007480	31.4
020	Hastings	21.01.92	0.063046	0.014608	28.6
022	Te Kuiti	23.01.92	0.047852	0.010257	24.8
014	Blenheim	26.01.92	0.033577	0.008180	46.6
026	Foxton	27.01.92	0.032247	0.004919	34.6
010	Te Kuiti	29.01.92	0.075864	0.006623	25.4
004	Bulls	14.02.92	0.192323	0.023613	33.4
010	Te Kuiti	16.02.92	0.023118	0.006545	30.0
006	Wairoa	18.02.92	0.106692	0.037691	41.4
014	Blenheim	24.02.92	0.029665	0.004062	20.2
002	Blenheim	25.02.92	0.216487	0.096604	42.0
016	Kaikohe	28.02.92	0.051638	0.007649	38.0
015	Nelson	05.03.92	0.047833	0.005187	23.8
014	Blenheim	14.03.92	0.058257	0.011429	18.8
012	Hastings	16.03.92	0.051767	0.007688	27.4
011	Gisborne	19.03.92	0.081588	0.014793	34.0
008	Kihikihi	21.04.92	0.078267	0.009920	19.0
009	Blenheim	21.04.92	0.114735	0.013745	49.6
007	Silverstream	23.04.92	0.110289	0.016415	27.8
014	Blenheim	15.12.92	0.082869	0.028625	45.0

Table 6. Glutathione *S*-transferase activities of various strains of larval *L. cuprina*, using CDNB as substrate.

Farm	Area	Date	Activity (μmol/min/mg protein)	StDev	RF
000	Lab	00.00.00	0.071842	0.017674	1.0
025	Dargaville	04.12.91	0.063343	0.012995	21.4
014	Blenheim	15.12.91	0.070369	0.006482	45.0
024	Masterton	29.12.91	0.036978	0.005414	27.6
023	Masterton	07.01.92	0.097542	0.008754	42.6
003	Wanganui	10.01.92	0.084759	0.032994	41.0
017	Tauranga	10.01.92	0.092689	0.021138	27.2
004	Bulls	13.01.92	0.060248	0.006873	42.8
012	Hastings	14.01.92	0.084268	0.005966	35.4
019	Wakefield	20.01.92	0.077823	0.008529	31.4
022	Te Kuiti	23.01.92	0.063266	0.009530	24.8
014	Blenheim	26.01.92	0.128822	0.013506	46.6
026	Foxton	27.01.92	0.055176	0.011525	34.6
010	Te Kuiti	29.01.92	0.033408	0.005878	25.4
004	Bulls	14.02.92	0.054484	0.007891	33.4
006	Wairoa	18.02.92	0.070981	0.004474	41.4
002	Blenheim	25.02.92	0.057584	0.011362	42.0
015	Nelson	05.03.92	0.070344	0.009449	23.8
014	Blenheim	14.03.92	0.105307	0.011555	18.8
012	Hastings	16.03.92	0.072672	0.005202	27.4
011	Gisborne	19.03.92	0.045756	0.005674	34.0
008	Kihikihi	21.04.92	0.061957	0.010767	19.0
009	Blenheim	21.04.92	0.080352	0.007671	49.6
007	Silverstream	23.04.92	0.043742	0.004962	27.8

Table 7. Glutathione *S*-transferase activities of various strains of adult *L. sericata*, using CDNB as substrate.

Farm	Area	Date	Activity (μmol/min/mg protein)	StDev	RF
001	Lab	00.00.00	0.307392	0.043052	1.0
018	Waipawa	06.01.92	0.062846	0.020702	11.0
005	Waiau	18.01.92	0.338187	0.042524	17.7
032	Blenheim	20.01.92	0.072568	0.008641	12.5
031	Amberley	28.01.92	0.068393	0.009595	10.5
021	Amberley	04.02.92	0.041968	0.007828	10.5
009	Blenheim	07.12.92	0.063674	0.005439	21.3
030	Lincoln	22.03.93	0.068075	0.006589	11.5
043	Waingaro	31.01.94	0.022824	0.001321	5.0
012	Hastings	08.04.94	0.041645	0.004445	7.5
034	Leeston	18.04.94	0.035243	0.007318	6.2
066	Dorie	20.04.94	0.027361	0.006186	6.2
009	Blenheim	25.04.94	0.07494	0.004875	12.5
055	Ashburton	16.01.95	0.053711	0.015155	8.5
008	Kihikihi	13.02.95	0.061904	0.002138	9.5
012	Hastings	04.11.95	0.078876	0.019513	10.7
056	Carterton	19.11.95	0.041997	0.016894	14.0
047	Ashburton	09.01.96	0.082348	0.010197	6.6
052	Hakataramea	09.01.96	0.069221	0.016576	7.0
048	Kurow	10.01.96	0.101364	0.020840	10.0
049	Wairoa	10.01.96	0.149803	0.050813	12.7
050	Cromwell	15.01.96	0.177328	0.088725	9.3
051	Nelson	15.01.96	0.081691	0.015445	20.3
053	Martinborough	17.01.96	0.097995	0.025008	18.2
054	Alexandra	17.01.96	0.055078	0.011531	9.1
003	Wanganui	22.01.96	0.100941	0.025703	20.8
032	Blenheim	27.01.96	0.07269	0.031528	13.7
040	Wanganui	25.03.96	0.077639	0.019991	11.2
021	Amberley	14.12.96	0.011214	0.004871	13.8
060	Wairoa	14.12.96	0.088566	0.119487	14.5
059	Wairoa	08.01.97	0.062103	0.008632	12.2
014	Blenheim	12.01.97	0.020396	0.001676	21.8
057	Cheviot	13.01.97	0.042441	0.008593	13.3
056	Carterton	15.01.97	0.077887	0.021425	11.3
061	Masterton	15.01.97	0.016744	0.003373	11.8
064	Ashburton	16.02.97	0.039226	0.007882	9.5
062	Timaru	27.02.97	0.007605	0.001843	10.8
012	Hastings	18.04.97	0.04194	0.012624	17.7

Table 8. Glutathione *S*-transferase activities of various strains of larval *L. sericata*, using CDNB as substrate.

Farm	Area	Date	Activity (µmol/min/mg protein)	StDev	RF
001	Lab	00.00.00	0.097325	0.007897	1.0
009	Blenheim	07.12.91	0.078064	0.005703	21.3
003	Wanganui	14.12.91	0.061727	0.020573	9.2
033	Waipukurau	02.01.92	0.068292	0.011373	12.2
018	Waipawa	06.01.92	0.076319	0.015883	11.0
005	Waiau	18.01.92	0.081091	0.017869	17.7
032	Blenheim	20.01.92	0.090901	0.003586	12.5
031	Amberley	28.01.92	0.063721	0.006581	10.5
021	Amberley	04.02.92	0.044527	0.010006	10.5
028	Cheviot	14.12.92	0.062270	0.026158	14.5
029	Ashburton	21.12.92	0.069910	0.014253	12.7
030	Lincoln	22.03.93	0.032191	0.007085	11.5
043	Waingaro	31.01.94	0.040874	0.007180	5.0
050	Levin	09.02.94	0.054718	0.005391	6.3
040	Wanganui	25.03.94	0.082607	0.011142	6.0
012	Hastings	08.04.94	0.058078	0.004265	7.5
034	Leeston	18.04.94	0.034828	0.005254	6.2
066	Dorie	20.04.94	0.087981	0.010779	6.2
009	Blenheim	25.04.94	0.095234	0.006395	12.5
055	Ashburton	16.01.95	0.015883	0.005005	8.5
012	Hastings	04.11.95	0.016042	0.002273	10.7
056	Carterton	19.11.95	0.023757	0.012248	14.0
018	Waipawa	28.12.95	0.031991	0.010728	9.8
047	Ashburton	09.01.96	0.023543	0.007049	6.6
052	Hakataramea	09.01.96	0.047601	0.011188	7.0
048	Kurow	10.01.96	0.048810	0.011214	10.0
049	Wairoa	10.01.96	0.009204	0.001392	12.7
050	Cromwell	15.01.96	0.029552	0.004352	9.3
051	Nelson	15.01.96	0.026250	0.004772	20.3
053	Martinborough	17.01.96	0.030500	0.006928	18.2
054	Alexandra	17.01.96	0.023448	0.002864	9.1
003	Wanganui	22.01.96	0.015500	0.002660	20.8
032	Blenheim	27.01.96	0.013494	0.002691	13.7
040	Wanganui	25.03.96	0.018700	0.003593	11.2
060	Wairoa	14.12.96	0.077421	0.016281	14.5
014	Blenheim	12.01.97	0.078706	0.009445	21.8
057	Cheviot	13.01.97	0.147250	0.028442	13.3
056	Carterton	15.01.97	0.138514	0.035051	11.3
061	Masterton	15.01.97	0.133080	0.029570	11.8

032	Blenheim	20.01.97	0.088369	0.011899	14.0
064	Ashburton	16.02.97	0.025856	0.002790	9.5
062	Timaru	27.02.97	0.024161	0.002525	10.8
009	Blenheim	14.04.97	0.024115	0.002245	19.2
012	Hastings	18.04.97	0.029737	0.006070	17.7

Appendix 2.3.2

Analysis of variance tables showing the relationship between resistance to diazinon, glutathione *S*-transferase activity and environmental factors

(a) Lucilia cuprina adults

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
DCNB activity	1	4.29	4.29	8.12	0.008
Region	5	9.03	1.81	3.42	0.015
Season	1	7.85	7.85	14.87	0.001
Residuals	30	15.84	0.53		

(b) Lucilia cuprina adults

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
CDNB activity	1	2.8555	2.8555	3.4692	0.0781
Region	3	3.0083	1.0028	1.2183	0.3303
Season	1	2.4974	2.4974	3.0342	0.0977
Residuals	19	15.6388	3.0342		

(c) Lucilia cuprina larvae

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
DCNB activity	1	9.3105	9.3105	15.416	0.0005
Region	5	5.1446	1.0289	1.7037	0.1677
Season	1	3.2386	3.2386	5.3625	0.0284
Residuals	27	13.3062	0.6039		

(d) Lucilia cuprina larvae

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
CDNB activity	1	1.6483	1.6483	2.3075	0.1483
Region	3	4.5259	1.5086	2.1120	0.1389
Season	1	3.3967	3.3967	4.7551	0.0445
Residuals	16	11.4292	0.7143		

(e) Lucilia sericata adults

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
DCNB activity	1	1.3121	1.3121	1.7298	0.1965
Region	4	13.6157	3.4039	4.4878	0.0047
Season	2	1.0085	0.5043	0.6648	0.5404
Residuals	37	28.0637	0.7585		

(f) Lucilia sericata adults

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
CDNB Activity	1	2.2010	2.2010	3.2199	0.0825
Region	4	13.6332	3.4083	4.9861	0.0032
Season	2	0.9755	0.4878	0.7136	0.4978
Residuals	31	21.1903	0.6836		

(g) Lucilia sericata larvae

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
DCNB activity	1	0.0517	0.0517	0.0694	0.7934
Region	4	17.1734	4.2933	5.7623	0.0008
Season	2	0.9918	0.4959	0.6656	0.5191
Residuals	44	32.7831	0.7451		

(h) Lucilia sericata larvae

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
CDNB Activity	1	0.0703	0.0703	0.0938	0.7611
Region	3	13.5011	4.5004	6.0075	0.0019
Season	2	1.7111	0.8556	1.1421	0.3301
Residuals	37	27.7176	0.7491		

Appendix 2.3.3

Analysis of variance tables showing the relationship between GST activities in adult and larval strains of *L. cuprina* and *L.sericata.* Laboratory OP-susceptible strains are excluded from this analysis.

(a) Lucilia cuprina adults vs larvae (DCNB activity)

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Regression	1	0.00001	0.00001	6.74	0.14
Residual Error	34	0.00005	0.00000		
Residuals	35	0.00005			

(b) Lucilia cuprina adults vs larvae (CDNB activity)

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Regression	1	0.001735	0.00174	0.48	0.498
Residual Error	21	0.076476	0.00364		
Residuals	22	0.078410			

(c) Lucilia sericata adults vs larvae (DCNB activity)

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Regression	1	0.000539	0.00054	17.14	0.000
Residual Error	39	0.001226	0.00003		
Residuals	40	0.001764			

(d) Lucilia sericata adults vs larvae (CDNB activity)

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Regression	1	0.000006	0.00006	0.04	0.847
Residual Error	27	0.042476	0.00157		
Residuals	28	0.042535			

Appendix 2.3.4 Preliminary method for the partial purification of glutathione *S*-transferases from *Lucilia* species.

Method

Sample Preparation

Up to 50g of whole third instar clear-gut stage larvae or 50g of adult abdomens were homogenised in 5 volumes of 10 m*M* Tris-HCl, pH 9.0 buffer containing 1m*M* phenyl thiourea and 1 m*M* PMSF (dissolved in 2-methoxy-ethanol), 1 m*M* EDTA, 5 m*M* cysteine and 0.2 *M* sodium chloride. Adults or larvae were homogenised using a Polytron Kinematica® motorized homogeniser. The homogenate was filtered twice through cotton fibre and centrifuged at 10,000 g for 20 minutes at 4°C. The supernatant was filtered through glass wool and centrifuged at 45,000 g for 1 hour in a Beckman XL-80 ultracentrifuge. A small amount of the supernatant was retained for protein and GSH-transferase activity determinations.

Enzyme Purification

The enzyme was applied to a QAE Sephadex column (50 x 200 mm) that had been equilibrated with 10 m*M* Tris-HCl, pH 9.0 buffer containing 1 m*M* EDTA and 5 m*M* cysteine. The column was washed with the above buffer followed by 0.2 *M* NaCl after the enzyme had passed through. Fractions were collected (5 ml/12 minutes) and assayed for CDNB and DCNB activity. Active fractions were pooled and pH adjusted to 7.4 with 0.1 *M* sodium phosphate.

The sample was then applied to a GSH-Sepharose (3 µmol/ml) affinity column (20 x 15 mm) followed by a BSP-GSH-Sepharose (2 µmol/ml) affinity column (50 x 25 mm) which had been equilibrated with 0.01 *M* sodium phosphate buffer pH 7.4.

After the sample had passed through the columns were washed with 0.01 *M* sodium phosphate buffer, pH 7.4 until no protein remained in the eluent (between 3-5 column volumes, absorbance <0.05). Affinity columns were then uncoupled for the elution procedure. The GSH-Sepharose column was washed with 0.2 *M* NaCl for 30 volumes until protein absorbance (at 280 nm) had stabilised then eluted with 10 mM GSH in 0.1 *M* sodium phosphate buffer pH 7.4. GSH-transferase activities were measured and active fractions were pooled.

A small Sephadex QAE ion exchange column (20 x 15 mm) was connected after the BSP-GSH affinity column to remove dye from eluted protein. The BSP-GSH column was washed with 0.7 *M* NaCl until protein absorbance (at 280 nm) had stabilised. The BSP-GSH column was then eluted with 2 mM BSP in 10 m*M* sodium phosphate buffer, pH 7.4 containing 0.2 *M* NaCl. GSH-transferase activities were measured and active fractions were pooled.

Appendix 2.4.1

Table 1. Total AChE activities and pseudo first-order rate constants governing the inactivation of AChE activity by tetrachlorvinphos in strains of *L. cuprina* adults.

Farm	Area	Date	E_0 avg (μ/mg)[a]	E_0 std[b]	K_i avg (min^{-1})[c]	K_i std[d]	RF[e]
000	Laboratory	00.00.00	0.1525	0.0287	0.4847	0.0459	1.0
022	Te Kuiti	23.01.92	0.1108	0.0348	0.3554	0.0678	24.8
015	Nelson	05.03.92	0.1413	0.0421	0.5246	0.1810	23.8
009	Blenheim	21.04.92	0.1637	0.0248	0.3271	0.0148	49.6
039	Rakaia	14.02.94	0.1627	0.0163	0.2410	0.0092	18.4
004	Bulls	13.01.92	0.1318	0.0158	0.4637	0.0428	42.8
004	Bulls	14.02.92	0.1888	0.0513	0.4211	0.0382	33.4
008	Kihikihi	07.03.94	0.1507	0.0168	0.2820	0.0161	11.8
008	Kihikihi	21.04.92	0.1453	0.0225	0.5316	0.0272	19.0
037	Levin	15.03.94	0.1820	0.0154	0.2903	0.0266	16.0
002	Blenheim	25.02.92	0.1929	0.0198	0.4851	0.0414	42.0
003	Wanganui	10.01.92	0.1554	0.0116	0.2953	0.0344	41.0
012	Hastings	08.04.94	0.2320	0.0130	0.3173	0.0146	24.0
012	Hastings	14.01.92	0.1386	0.0206	0.5985	0.0778	35.4
012	Hastings	16.03.92	0.1493	0.0291	0.4083	0.0409	27.4
035	Levin	11.04.94	0.1517	0.0146	0.2623	0.0202	14.2
036	Kaiwaka	14.03.94	0.2363	0.0338	0.3353	0.0170	18.8
016	Kaikohe	28.02.92	0.1466	0.0136	0.4676	0.0685	38.0
025	Dargaville	04.12.91	0.2053	0.0950	0.4568	0.1547	21.4
010	Te Kuiti	16.02.92	0.1426	0.0231	0.4099	0.0632	30.0
010	Te Kuiti	29.01.92	0.1713	0.0239	0.4996	0.0545	25.4
042	Waikari	16.02.94	0.2190	0.0113	0.3067	0.0163	19.8
043	Waingaro	31.01.94	0.2543	0.0551	0.2933	0.0151	24.0
019	Wakefield	20.01.92	0.1418	0.0308	0.4268	0.0423	31.4
026	Foxton	27.01.92	0.1705	0.0371	0.3525	0.0358	34.6
034	Leeston	16.04.94	0.1657	0.0163	0.2607	0.0074	8.0
006	Wairoa	18.02.92	0.1507	0.0232	0.3701	0.0585	41.4
038	Marton	09.05.94	0.2253	0.0146	0.2977	0.0267	11.8
024	Masterton	29.12.92	0.1446	0.0338	0.5113	0.1053	27.6
024	Masterton	31.02.94	0.1913	0.0051	0.2937	0.0194	15.2
045	Feilding	13.04.94	0.2387	0.0110	0.2853	0.0093	44.8
023	Masterton	07.01.92	0.1367	0.0460	0.4298	0.0889	42.6
011	Gisborne	19.03.92	0.1772	0.0171	0.4242	0.0484	34.0
007	Silverstream	23.04.92	0.1561	0.0257	0.4085	0.0432	27.8
020	Hastings	21.01.92	0.2109	0.0911	0.4542	0.0993	28.6
014	Blenheim	14.03.92	0.1578	0.0248	0.4395	0.0244	18.8
014	Blenheim	15.12.92	0.1885	0.0233	0.4095	0.0465	45.0
014	Blenheim	24.02.92	0.1737	0.0356	0.4124	0.0464	20.2
014	Blenheim	26.01.92	0.1628	0.0229	0.4012	0.0412	46.6
014	Tauranga	10.01.92	0.1447	0.0298	0.3678	0.0554	27.2

Table 2. Total AChE activities and pseudo first-order rate constants governing the inactivation of AChE activity by tetrachlorvinphos in strains of *L. cuprina* larvae.

Farm	Area	Date	E_0 avg (μ/mg)[a]	E_0 std[b]	K_i avg (min^{-1})[c]	K_i std[d]	RF[e]
000	Laboratory	00.00.00	0.0222	0.0080	0.6077	0.1740	1.0
022	Te Kuiti	23.01.92	0.0792	0.0181	0.2283	0.0552	24.8
015	Nelson	05.03.92	0.0416	0.0032	0.1777	0.0277	23.8
009	Blenheim	21.04.92	0.0394	0.0067	0.1827	0.0083	49.6
039	Rakaia	14.02.94	0.0395	0.0063	0.1038	0.0351	18.4
004	Bulls	13.01.92	0.0378	0.0098	0.1860	0.0072	42.8
004	Bulls	14.02.92	0.0505	0.0112	0.2560	0.0351	33.4
008	Kihikihi	07.03.94	0.0232	0.0086	0.1903	0.0285	11.8
037	Levin	15.03.94	0.0466	0.0017	0.1525	0.0049	16.0
002	Blenheim	25.02.92	0.0359	0.0136	0.1940	0.0484	42.0
003	Wanganui	10.01.92	0.0396	0.0122	0.2563	0.0648	41.0
012	Hastings	08.04.94	0.0205		0.2640		24.0
012	Hastings	16.03.92	0.0604	0.0170	0.2307	0.0284	27.4
035	Levin	11.04.94	0.0291		0.1600		14.2
036	Kaiwaka	14.03.94	0.0145	0.0085	0.2170	0.0223	18.8
016	Kaikohe	28.02.92	0.0276	0.0059	0.2630	0.0327	38.0
042	Waikari	16.02.94	0.1255	0.1666	0.1820	0.1075	19.8
043	Waingaro	31.01.94	0.0283		0.0955		24.0
019	Wakefield	20.01.92	0.0318	0.0022	0.1957	0.0515	31.4
026	Foxton	27.01.92	0.0326	0.0095	0.2197	0.0153	34.6
034	Leeston	16.04.94	0.0244	0.0035	0.1175	0.0318	8.0
006	Wairoa	18.02.92	0.0148	0.0074	0.1630	0.0368	41.4
038	Marton	09.05.94	0.0209	0.0008	0.2050	0.0226	11.8
024	Masterton	29.12.91	0.0335	0.0114	0.2877	0.0599	27.6
024	Masterton	31.02.94	0.0179	0.0052	0.1897	0.0775	15.2
045	Feilding	13.04.94	0.0200	0.0011	0.2710	0.0467	44.8
011	Masterton	07.01.92	0.0439	0.0075	0.2137	0.0656	42.6
011	Gisborne	19.03.92	0.0243	0.0020	0.1230	0.0171	34.0
014	Blenheim	14.03.94	0.0638	0.0108	0.3367	0.0333	18.8
014	Blenheim	26.01.92	0.0502	0.0157	0.1950	0.0410	46.6
017	Tauranga	10.01.92	0.0353	0.0068	0.2423	0.0287	27.2

Table 3. Total AChE activities and pseudo first-order rate constants governing the inactivation of AChE activity by tetrachlorvinphos in strains of *L. sericata* adults.

Farm	Area	Date	E_0 avg (μ/mg)[a]	E_0 std[b]	K_i avg (min^{-1})[c]	K_i std[d]	RF[e]
001	Laboratory	00.00.00	0.1777	0.0351	0.5555	0.0636	1.0
030	Lincoln	22.03.92	0.2399	0.0579	0.4318	0.1217	11.5
050	Cromwell	15.01.96	0.1753	0.0159	0.5997	0.0320	9.3
056	Carterton	15.01.97	0.2280	0.0140	0.5400	0.0315	11.3
056	Carterton	19.11.95	0.1680	0.0195	0.5890	0.0221	14.0
032	Blenheim	20.01.92	0.2684	0.0273	0.3886	0.0189	12.5
032	Blenheim	20.01.97	0.2277	0.0176	0.5553	0.0181	14.0
032	Blenheim	27.01.96	0.1063	0.0147	0.4140	0.0209	13.7
018	Waipawa	06.01.92	0.1998	0.0320	0.2527	0.0418	11.0
018	Waipawa	28.12.95	0.1877	0.0404	0.6020	0.0318	11.0
061	Masterton	15.01.97	0.1967	0.0425	0.4480	0.0910	11.8
047	Ashburton	09.01.96	0.1567	0.0287	0.6060	0.0226	6.6
009	Blenheim	10.12.96	0.1707	0.0571	0.4893	0.0250	15.8
009	Blenheim	25.04.94	0.1740	0.0351	0.4863	0.0612	12.5
060	Wairoa	14.12.96	0.2430	0.0122	0.5557	0.0461	14.5
039	Rakaia	10.01.94	0.2617	0.0702	0.3137	0.0336	6.7
021	Amberley	14.12.96	0.1750	0.0078	0.5087	0.0320	13.8
055	Ashburton	16.01.95	0.2107	0.0230	0.6163	0.0606	8.5
055	Ashburton	29.04.97	0.0735	0.0276	0.4753	0.0234	5.0
031	Amberley	28.01.92	0.2049	0.0533	0.2361	0.0213	10.5
008	Kihikihi	13.02.95	0.1610	0.0486	0.4207	0.0431	9.5
003	Wanganui	22.01.96	0.2493	0.0264	0.4893	0.0686	20.8
012	Hastings	04.11.95	0.1803	0.0237	0.5953	0.0061	10.7
012	Hastings	08.04.94	0.0995	0.0477	0.4137	0.0777	7.5
012	Hastings	18.04.97	0.0816	0.0137	0.4410	0.0184	17.7
064	Ashburton	16.02.97	0.2533	0.0251	0.5293	0.0029	9.5
051	Nelson	15.01.96	0.1103	0.0075	0.4027	0.0336	20.3
044	Wairoa	23.02.94	0.1567	0.0332	0.2973	0.0240	5.5
057	Cheviot	13.01.97	0.2072	0.0148	0.5838	0.0285	13.3
066	Dorie	20.04.94	0.1051	0.0319	0.4923	0.0782	6.2
053	Martinborough	17.01.96	0.2343	0.0211	0.5843	0.0165	18.2
033	Waipukurau	02.01.92	0.1860	0.0333	0.2337	0.0304	12.2
054	Alexandra	17.01.96	0.1257	0.0505	0.4707	0.0984	9.1
040	Wanganui	25.03.94	0.1577	0.0075	0.2030	0.0044	6.0
040	Wanganui	25.03.96	0.1490	0.0279	0.5760	0.0229	11.2
043	Waingaro	31.01.94	0.0738	0.0287	0.3237	0.0550	5.0
005	Waiau	18.01.92	0.1200	0.0364	0.3069	0.0298	17.7
052	Hakataramea	09.01.96	0.1050	0.0176	0.3807	0.0100	7.0
041	Ashburton	16.02.94	0.2803	0.0125	0.3220	0.0497	10.0
034	Leeston	18.04.94	0.1910	0.0409	0.5450	0.1091	6.2
062	Timaru	27.02.97	0.1897	0.0189	0.5003	0.0462	10.8
048	Kurow	10.01.96	0.1933	0.0228	0.6460	0.0278	10.0

065	Ashburton	14.04.97	0.1640	0.0416	0.4987	0.0247	9.3
049	Wairoa	10.01.96	0.1983	0.0197	0.6377	0.0140	12.7
014	Blenheim	12.01.97	0.2370	0.0155	0.5197	0.0294	21.8

Table 4. Total AChE activities and pseudo first-order rate constants governing the inactivation of AChE activity by tetrachlorvinphos in strains of *L. sericata* larvae.

Farm	Area	Date	E_0 avg (μ/mg)[a]	E_0 std[b]	K_i avg (min^{-1})[c]	K_i std[d]	RF[e]
001	Laboratory	00.00.00	0.0328	0.0062	0.2863	0.1065	1.0
030	Lincoln	22.03.93	0.0224	0.0040	0.2520	0.0785	11.5
050	Cromwell	15.01.96	0.0253	0.0059	0.0894	0.0256	9.3
050	Levin	09.02.94	0.0301	0.0013	0.0800	0.0054	6.3
056	Carterton	15.01.97	0.0437	0.0044	0.1328	0.0250	11.3
056	Carterton	19.11.95	0.0445	0.0002	0.1180	0.0057	14.0
032	Blenheim	20.01.92	0.0240	0.0122	0.1943	0.0565	12.5
032	Blenheim	20.01.97	0.0327	0.5781	0.1046	0.0737	14.0
032	Blenheim	27.01.96	0.0202	0.0047	0.0600	0.0120	13.5
018	Waipawa	06.01.92	0.0324	0.0038	0.1783	0.0362	11.0
018	Waipawa	28.12.95	0.0369	0.0094	0.1189	0.0444	11.0
061	Masterton	15.01.97	0.0428	0.0014	0.2133	0.0448	11.8
028	Cheviot	14.12.92	0.0129	0.0016	0.2580	0.0704	14.5
047	Ashburton	09.01.96	0.0205	0.0011	0.1073	0.0141	6.6
063	Wakefield	26.02.97	0.0326	0.0037	0.0997	0.0251	12.7
009	Blenheim	07.12.92	0.0197	0.0042	0.1981	0.1309	21.3
009	Blenheim	10.12.96	0.0180	0.0026	0.1165	0.0163	15.8
009	Blenheim	14.04.97	0.0229	0.0017	0.1350		15.8
009	Blenheim	25.04.94	0.0266	0.0040	0.0729	0.0248	12.5
060	Wairoa	14.12.96	0.0799	0.0173	0.2270	0.0799	14.5
039	Rakaia	10.01.94	0.0197	0.0116	0.1396	0.1449	6.7
021	Amberley	14.12.96	0.0482	0.0117	0.1662	0.1074	13.8
055	Ashburton	16.01.95	0.0221	0.0012	0.0729	0.0234	8.5
055	Ashburton	29.04.97	0.0689	0.0075	0.3410	0.0397	5.0
031	Amberley	28.01.92	0.0223	0.0027	0.2603	0.0280	10.5
008	Kihikihi	13.02.95	0.0245	0.0143	0.0898	0.1300	9.5
003	Wanganui	22.01.96	0.0380	0.0056	0.1545	0.0829	20.8
012	Hastings	04.11.95	0.0308	0.0089	0.0853	0.0116	10.7
012	Hastings	08.04.94	0.0182	0.0079	0.1315	0.1663	7.5
012	Hastings	18.04.97	0.0586	0.0118	0.2787	0.0133	17.7
035	Levin	11.04.94	0.0156	0.0001	0.1665	0.0191	14.2
064	Ashburton	16.02.97	0.0200	0.0061	0.2027	0.0961	9.5
051	Nelson	15.01.96	0.0183	0.0037	0.0709	0.0111	20.3
044	Wairoa	23.02.94	0.0298	0.0294	0.1414	0.1564	5.5
057	Cheviot	13.01.97	0.0268	0.0042	0.0797	0.0237	13.3
066	Dorie	20.04.94	0.0344	0.0058	0.0574	0.0044	6.2
053	Martinborough	17.01.96	0.0222	0.0100	0.2274	0.1739	18.2
033	Waipukurau	02.01.92	0.0356	0.0041	0.2717	0.0297	12.2
054	Alexandra	17.01.96	0.0297	0.0082	0.1018	0.0411	9.1
040	Wanganui	03.01.94	0.0390	0.0072	0.0641	0.0168	8.2
040	Wanganui	25.03.94	0.0455	0.0217	0.0600	0.0204	6.0
040	Wanganui	25.03.96	0.0328	0.0002	0.1205	0.0106	11.2
043	Waingaro	31.01.94	0.0214	0.0024	0.0948	0.0078	5.0

005	Waiau	18.01.92	0.0322	0.0147	0.2820	0.0608	17.7
052	Hakataramea	09.01.96	0.0170	0.0033	0.0947	0.0112	7.0
041	Ashburton	16.02.94	0.0163	0.0052	0.1745	0.0856	10.0
034	Leeston	18.04.94	0.0152	0.0018	0.0407	0.0102	6.2
062	Timaru	27.02.97	0.0392	0.0115	0.3005	0.2030	10.8
048	Kurow	10.01.96	0.0298	0.0045	0.1070	0.0234	10.0
065	Ashburton	14.04.97	0.0640	0.3681	0.3643	0.0759	9.3
049	Wairoa	10.01.96	0.0135	0.0066	0.0764	0.0188	12.7
014	Blenheim	12.01.97	0.0368	0.0059	0.0921	0.0267	21.8

[a] E_o avg (μ/mg) is the mean of total acetylcholinesterase activity.
[b] E_o std is the standard deviation of the (E_o avg) mean.
[c] K_i avg is the mean of the pseudo-first-order rate constant for the inactivation of acetylcholinesterase by tetrachlorvinphos. $Ki = K_2/K_d$ where K_2 is the dissociation constant for the Michaelis complex and K_d is the rate of phosphorylation for the reaction.
[d] K_i std is the standard deviation of the (K_i avg) mean.
[e] RF is the resistant factor as determined in chapter 1.1. RF = LC_{50} resistant strain/ LC_{50} susceptible strain.

Appendix 2.4.2

Analysis of variance tables showing the relationship between resistance to diazinon, acetylcholinesterase activity and environmental factors

(a) Lucilia cuprina adults

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
E_o (total activity)	1	0.9914	0.9914	1.3377	0.2163
Region	5	8.4860	1.6972	2.2901	0.0699
Season	1	5.5481	5.5480	7.4861	0.0102
Residuals	31	22.9745	0.7411		

(b) Lucilia cuprina adults

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
K_i (rate of inactivation)	1	4.0222	4.0222	5.4277	0.0265
Region	5	6.0012	1.2002	1.6196	0.1841
Season	1	5.0036	5.0036	6.7519	0.0142
Residuals	31	22.9730	0.7411		

(c) Lucilia cuprina larvae

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
E_o (total activity)	1	0.9914	0.9914	1.3377	0.2563
Region	5	8.4860	1.6972	2.2901	0.0699
Season	1	5.5481	5.5480	7.4861	0.0102
Residuals	31	22.9746	0.7411		

(d) Lucilia cuprina larvae

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
K_i (rate of inactivation)	1	1.0505	1.0505	1.4650	0.2396
Region	4	5.8671	1.4668	2.0455	0.1246
Season	1	5.0238	5.0238	7.0059	0.0151
Residuals	21	15.0586	0.7171		

(e) Lucilia sericata adults

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
E_o (total activity)	1	2.8907	2.8907	3.8157	0.0576
Region	4	12.7627	3.1907	4.2117	0.0060
Season	2	1.2861	0.6431	0.8488	0.4353
Residuals	41	31.0605	0.7576		

(f) Lucilia sericata adults

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
K_i (rate of inactivation)	1	0.7403	0.7403	1.0038	0.3223
Region	4	14.4936	3.6234	4.9130	0.0025
Season	2	2.5279	1.2640	1.7138	0.1928
Residuals	41	30.2382	0.7375		

(g) Lucilia sericata larvae

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
E_o (total activity)	1	0.0517	0.0517	0.0694	0.7934
Region	4	17.1734	4.2933	5.7623	0.0008
Season	2	0.9918	0.4959	0.6656	0.5191
Residuals	44	32.7831	0.7451		

(h) Lucilia sericata larvae

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
K_i (rate of inactivation)	1	2.0819	2.0819	3.3645	0.0732
Region	4	19.6163	4.9041	7.9254	0.0001
Season	2	2.4565	1.2282	1.9849	0.1492
Residuals	45	27.8453	0.6188		

Appendix 2.4.3

Analysis of variance tables showing the relationship between adult and larval acetylcholinesterase activities (Analysis excludes the laboratory OP-susceptible strain).

(a) Lucilia cuprina adults vs larvae (total acetylcholinesterase activity)

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Regression	1	0.0004	0.0004	0.80	0.378
Residual Error	28	0.0139	0.0005		
Residuals	29	0.0143			

(b) Lucilia cuprina adults vs larvae (pseudo-first-order rate constant governing the inactivation of acetylcholinesterase by tetrachlorvinphos)

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Regression	1	0.0123	0.0123	4.44	0.044
Residual Error	28	0.0773	0.0028		
Residuals	29	0.0896			

(c) Lucilia cuprina adults vs larvae (total acetylcholinesterase activity)

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Regression	1	0.0001	0.0001	0.50	0.484
Residual Error	42	0.0093	0.0002		
Residuals	43	0.0094			

(d) Lucilia sericata adults vs larvae (pseudo-first-order rate constant governing the inactivation of acetylcholinesterase by tetrachlorvinphos)

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Regression	1	0.0178	0.0178	2.66	0.110
Residual Error	42	0.2799	0.0067		
Residuals	43	0.2977			

Appendix 2.5.1

Table 1. Effect of different piperonyl butoxide concentrations on control mortality and LC_{50} in two strains of *Lucilia cuprina* larvae.

Strain/ treatment	LC_{50} (95% FL)[1] mg/l	LC_{99} (95% FL) mg/l	Slope(SE)[2]	% PBO[3] Control Mortality
000 Laboratory				
DZ[4]+ 0 mg/ml PBO	0.07 (0.06 – 0.08)	0.13 (0.09 – 0.18)	8.34 (1.92)	0
DZ + 1 mg/ml PBO	0.12 (0.11 – 0.13)	0.32 (0.26 – 0.39)	5.50 (0.56)	0
DZ + 2 mg/ml PBO	0.11 (0.07 – 0.19)	0.94 (0.24 – 3.66)	2.51 (0.72)	5.8
DZ + 5 mg/ml PBO	0.15 (0.12 – 0.20)	0.61 (0.31 – 1.17)	3.91 (0.91)	18.0
DZ + 10 mg/ml PBO	0.12 (0.05 – 0.28)	1.06 (0.15 – 7.66)	2.49 (1.09)	22.1
058 Takapau				
DZ + 0 mg/ml PBO	2.06 (1.91 – 2.22)	7.04 (5.80 – 8.55)	5.62 (0.29)	0
DZ + 1 mg/ml PBO	1.19 (0.81 – 1.75)	5.97 (2.65–13.48)	4.28 (1.35)	3.2
DZ + 2 mg/ml PBO	1.58 (0.98 – 2.57)	7.07 (3.22–15.52)	3.59 (1.16)	10.5
DZ + 5 mg/ml PBO	1.09 (0.66 – 1.81)	16.5 (3.67-74.26)	1.97 (0.56)	11.1
DZ + 10 mg/ml PBO	1.57 (1.08 – 2.28)	8.10 (2.74-23.92)	3.27 (1.07)	12.6

[1] FL, 95% fiducial limits
[2] SE, Standard Error
[3] Piperonyl butoxide
[4] Diazinon

Appendix 2.6.1

Analysis of variance tables showing the influence of various resistance mechanisms on level of diazinon resistance in strains of *L. sericata* adults.

(a) Lucilia cuprina adults

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
GST Activity (CDNB)	1	2.9584	2.9584	3.7689	0.0664
AChE (Ki)	1	1.4593	1.4593	1.8591	0.1879
AChE (Eo)	1	0.0511	0.0510	0.0650	0.8013
Non-specific Esterases (β)	1	0.4108	0.4108	0.5233	0.4778
GST Activity (DCNB)	1	4.9555	4.9555	6.3132	0.0207
Non-specific Esterases (α)	1	0.4686	0.4659	0.5935	0.4501
Residuals	20	15.6990	0.7850		

(b) Lucilia cuprina larvae

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Non-specific Esterases (β)	1	1.2684	1.2684	2.5513	0.1385
GST Activity (CDNB)	1	0.1942	0.1942	0.3907	0.5378
AChE (Ki)	1	9.3220	9.3220	18.7504	0.0012
AChE (Eo)	1	0.7058	0.7058	1.4197	0.2585
Non-specific Esterases (α)	1	0.0286	0.2089	0.0575	0.8149
GST Activity (DCNB)	1	0.0121	0.0121	0.0244	0.8787
Residuals	11	5.4688	0.4972		

(c) Lucilia sericata larvae

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
GST Activity (DCNB)	1	0.2024	0.2024	0.2357	0.6344
GST Activity (CDNB)	1	2.1711	2.1711	2.5285	0.1327
AChE (Eo)	1	0.0629	0.0629	0.0733	0.7903
AChE (Ki)	1	1.5576	1.5576	1.8140	0.1980
Non-specific Esterases (α)	1	4.1250	4.1250	4.8039	0.0446
Non-specific Esterases (β)	1	0.0007	0.0007	0.0009	0.9770
Residuals	15	12.8802	0.8587		

NB: Order of resistance mechanism had little affect on the above multiple regressions.

Appendix 2.6.2

Analysis of variance tables showing the influence of various resistance mechanisms on level of diazinon resistance in strains of *L. sericata* adults. Four ANOVA tables are presented to show the effect that order of independent variable has on the analysis.

(a) Lucilia sericata adults

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
GST Activity (CDNB)	1	0.1653	0.1653	0.1943	0.6660
Non-specific Esterases (β)	1	0.1086	0.1086	0.1277	0.7261
AChE (Ki)	1	2.6365	2.6365	3.0996	0.1001
GST Activity (DCNB)	1	2.2686	2.2686	2.6671	0.1247
AChE (Eo)	1	1.1403	1.1403	1.3406	0.2663
Non-specific Esterases (α)	1	1.1403	1.7725	2.0839	0.1709
Residuals	14	11.9081	0.8506		

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
GST Activity (CDNB)	1	0.1653	0.1653	0.1943	0.6660
AChE (Eo)	1	0.1252	0.1252	0.1472	0.7070
Non-specific Esterases (α)	1	0.7189	0.7189	0.8451	0.3735
GST Activity (DCNB)	1	4.0732	4.0732	4.7888	0.0461
AChE (Ki)	1	2.6816	2.6816	3.1526	0.0975
Non-specific Esterases (β)	1	0.3277	0.3277	0.3852	0.5448
Residuals	14	11.9081	0.8506		

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
Non-specific Esterases (β)	1	0.1009	0.1009	0.1186	0.7356
GST Activity (CDNB)	1	0.1730	0.1730	0.2034	0.6589
GST Activity (DCNB)	1	3.9540	3.9540	4.6486	0.0490
AChE (Eo)	1	0.1121	0.1121	0.1318	0.7220
AChE (Ki)	1	1.9792	1.9792	2.3269	0.1494
Non-specific Esterases (α)	1	1.7725	1.7725	2.0839	0.1709
Residuals	14	11.9081	0.8506		

	DF	Sum of Squares	Mean Sq	F Value	Pr (F)
AChE (Eo)	1	0.1708	0.1708	0.2008	0.6610
Non-specific Esterases (β)	1	0.2362	0.2362	0.2777	0.6065
GST Activity (DCNB)	1	2.7539	2.7539	3.2377	0.0935
AChE (Ki)	1	2.5889	2.5889	3.0436	0.1030
Non-specific Esterases (α)	1	1.1840	1.1840	1.3920	0.2577
GST Activity (CDNB)	1	1.1591	1.1581	1.3615	0.2628
Residuals	14	11.9081	0.8506		

Appendix 3.1

Key to strains used in RAPD-PCR study and their resistance factors

Map Code	Farm	Area	Region	Date	RF[1]	primers
A	009	Blenheim	Marlborough	15.03.94	33.8	3, 4, 7
B	009	Blenheim	Marlborough	12.02.96	-	3, 4, 7
C	014	Blenheim	Marlborough	12.01.94	25.8	3, 4, 7
D	027	Greytown	Wairarapa	07.01.94	-	3, 4, 7
E	027	Greytown	Wairarapa	06.02.92	-	3, 4, 7
F	134	Pokeno	Auckland	26.02.96	15.0	3, 4, 7
G	000	Laboratory	Laboratory	00.00.00	1.0	3, 4, 7
H	066	Dorie	Mid Canterbury	20.04.94	20.2	3, 4
I	038	Marton	Wellington	12.04.94	21.4	3, 4, 7
J	038	Marton	Wellington	26.04.96	23.8	3, 4, 7
K	003	Turakina	Wanganui	10.05.94	21.2	3, 4, 7
L	003	Turakina	Wanganui	22.04.96	31.8	3, 4, 7
M	004	Bulls	Wanganui	21.12.94	40.8	3, 4
N	004	Bulls	Wanganui	23.02.96	41.0	3, 4
O	034	Leeston	Mid Canterbury	18.04.94	8.0	3, 4, 7
P	008	Kihikihi	Waikato	07.03.94	11.8	3, 4
Q	008	Kihikihi	Waikato	10.01.96	14.0	3, 4
R	038	Rangiora	Mid Canterbury	04.01.93	22.2	3, 4

[1]Resistance factor

Appendix 3.2

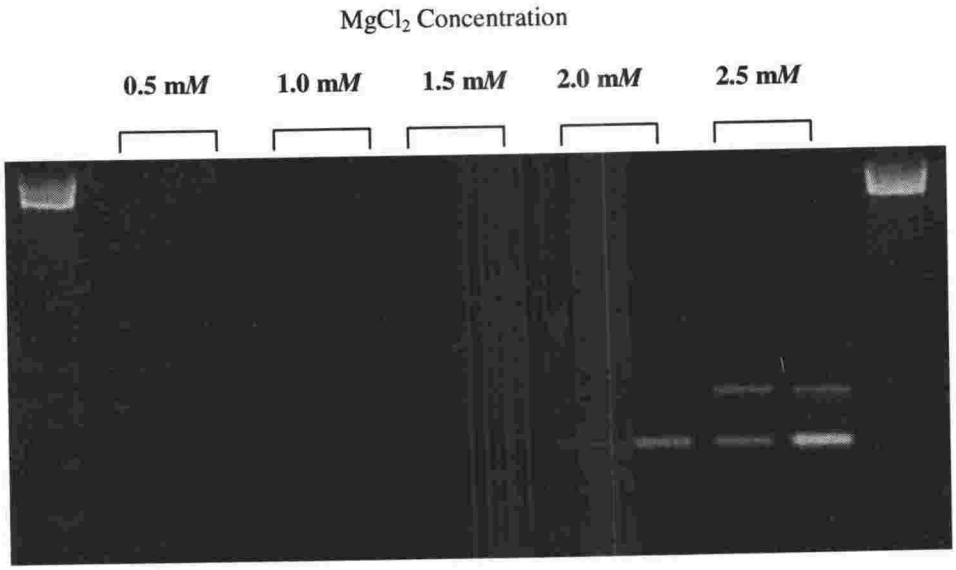


Figure 1. Optimization of $MgCl_2$ concentration (0.5 – 2.5 m*M*) in RAPD-PCR reactions. OPA-3 was used as the primer and DNA from two random individuals was used as template.

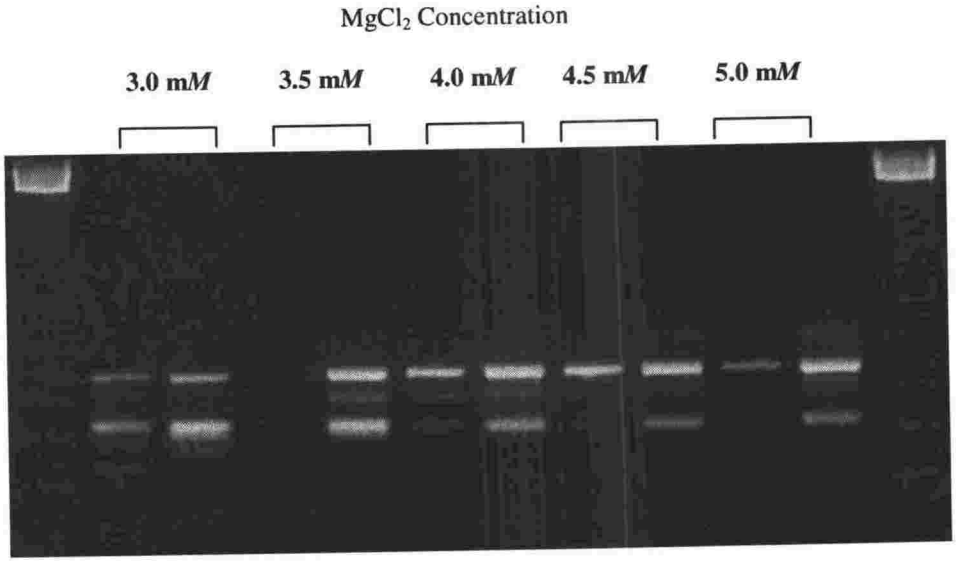


Figure 2. Optimization of $MgCl_2$ concentration (3.0 – 5.0 m*M*) in RAPD-PCR reactions. OPA-3 was used as the primer and DNA from two random individuals was used as template.

Appendix 3.3

Operon oligonucleotides (Kit A) used in RAPD-PCR.

Primer	Base-pair sequence (5' - 3')
OPA-1	CAGGCCCTTC
OPA-2	TGCCGAGCTG
OPA-3	AGTCAGCCAC
OPA-4	AATCGGGCTG
OPA-5	AGGGGTCTTG
OPA-6	GGTCCCTGAC
OPA-7	GAAACGGGTG
OPA-8	GTGACGTAGG
OPA-9	GGGTAACGCC
OPA-10	GTGATCGCAG
OPA-11	CAATCGCCGT
OPA-12	TCGGCGATAG
OPA-13	CAGCACCCAC
OPA-14	TCTGTGCTGG
OPA-15	TTCCGAACCC
OPA-16	AGCCAGCGAA
OPA-17	GACCGCTTGT
OPA-18	AGGTGACCGT
OPA-19	CAAACGTCGG
OPA-20	GTTGCGATCC

Appendix 3.4

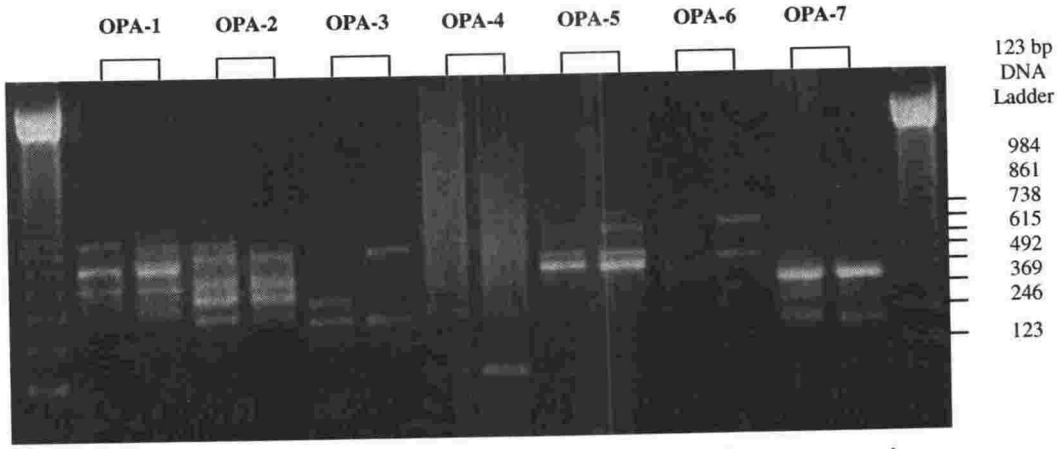


Figure 1. RAPD-PCR profile of the OP-susceptible laboratory strain (000) of *Lucilia cuprina* using Operon primers OPA1 – 7.

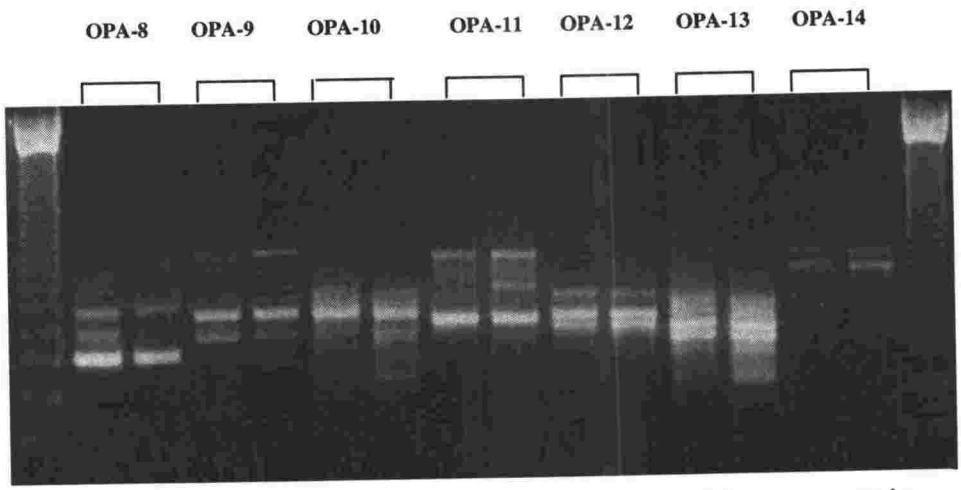


Figure 2. RAPD-PCR profile of the OP-susceptible laboratory strain (000) of *Lucilia cuprina* using Operon primers OPA8 – 14.

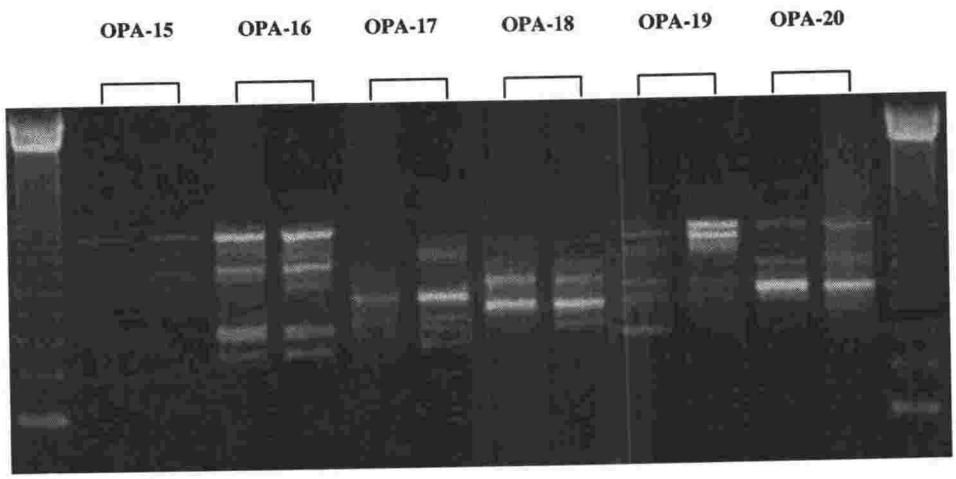


Figure 3. RAPD-PCR profile of the OP-susceptible laboratory strain (000) of *Lucilia cuprina* using Operon primers OPA15 – 20.

Appendix 3.5

Table 1. Summary of bands generated by RAPD-PCR with Operon primer OPA-3 and the percentage observed for each strain.

Fragment size (bp)	000 Lab 00.00.00	009 Blenheim 15.03.94	009 Blenheim 12.02.96	014 Blenheim 12.01.96	004 Bulls 21.12.94	004 Bulls 23.02.96	066 Dorie 20.04.94	027 Greytown 06.02.92	027 Greytown 07.01.94	008 Kihikihi 07.03.94	008 Kihikihi 10.01.96
1082.0	10	-	-	-	-	-	-	-	-	7.1	-
938.1	-	5.6	-	-	-	-	-	-	-	28.6	-
709.1	90	38.9	-	16.7	17.6	-	-	9.1	-	21.4	-
598.6	90	33.3	-	-	11.8	-	57.1	27.3	9.1	21.4	-
528.4	90	-	-	5.6	-	-	100.0	-	-	50.0	16.7
481.8	-	-	-	-	17.6	-	100.0	-	9.1	71.4	83.3
438.2	100.0	55.6	100.0	27.8	-	-	28.6	-	-	-	-
405.5	100.0	-	-	-	47.1	-	100.0	63.6	13.6	21.4	-
360.0	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**54.5**	**100.0**	**100.0**
325.4	-	-	-	11.1	-	-	14.3	-	-	-	-
287.8	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**92.9**	**100.0**
249.3	**100.0**	**66.7**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**78.6**	**79.2**
231.9	100.0	50.0	-	100.0	100.0	100.0	100.0	100.0	100.0	35.7	-
212.6	100.0	-	-	77.8	100.0	100.0	100.0	100.0	100.0	100.0	-
186.7	100.0	-	-	44.4	100.0	100.0	100.0	18.2	-	-	-
169.2	-	-	-	5.6	-	-	-	-	-	-	-
145.2	100.0	100.0	100.0	100.0	-	100.0	100.0	100.0	100.0	-	100.0
Total	**13**	**9**	**5**	**12**	**10**	**7**	**13**	**10**	**9**	**12**	**6**

Table 1. continued

Fragment size (bp)	034 Leeston 18.04.94	038 Marton 12.04.94	038 Marton 26.04.96	134 Pokeno 26.02.96	038 Rangiora 04.01.93	003 Wanganui 10.05.94	003 Wanganui 22.04.96
1082.0	-	-	-	5.6	-	14.3	-
938.1	50.0	-	-	11.1	-	14.3	-
709.1	42.9	-	-	61.1	-	14.3	-
598.6	78.6	-	-	77.8	-	50.0	-
528.4	7.1	-	-	22.2	-	92.9	4.2
481.8	50.0	-	-	16.7	28.6	92.9	75.0
438.2	-	-	-	61.1	-	85.7	-
405.5	100.0	50.0	-	5.6	92.9	92.9	-
360.0	85.7	100.0	73.3	100.0	92.9	100.0	100.0
325.4	-	-	-	-	-	35.7	-
287.8	100.0	100.0	86.7	100.0	100.0	100.0	100.0
249.3	57.1	100.0	93.3	100.0	92.9	78.6	95.8
231.9	92.9	100.0	93.3	72.2	100.0	64.3	66.7
212.6	92.9	100.0	100.0	50.0	100.0	64.3	-
186.7	85.7	100.0	-	-	100.0	-	-
169.2	-	-	-	-	-	-	-
145.2	-	-	100.0	100.0	-	100.0	100.0
Total	**12**	**7**	**6**	**14**	**8**	**15**	**7**

Table 2. Summary of bands generated by RAPD-PCR with Operon primer OPA-4 and the percentage observed for each strain.

Fragment size (bp)	000 Lab 00.00.00	009 Blenheim 12.02.94	009 Blenheim 12.01.96	014 Blenheim 12.01.96	004 Bulls 21.12.94	004 Bulls 23.02.96	066 Dorie 20.04.94	027 Greytown 06.02.92	027 Greytown 07.01.94	008 Kihikihi 07.03.94	008 Kihikihi 10.01.96
901.2	100.0	-	-	-	-	-	-	-	-	-	-
770.3	100.0	-	-	-	35.3	-	-	100.0	13.6	-	-
652.0	-	100.0	-	55.6	100.0	-	-	-	-	-	-
591.3	100.0	100.0	14.3	72.2	100.0	-	-	100.0	31.8	14.3	-
506.0	-	100.0	50.0	83.3	100.0	60.0	-	-	-	14.3	-
451.9	100.0	100.0	64.3	100.0	100.0	80.0	42.9	100.0	68.2	57.1	100.0
432.0	-	-	-	-	-	-	100.0	4.5	18.2	-	-
362.3	-	11.1	-	-	100.0	-	-	-	-	28.6	-
345.0	-	-	-	100.0	-	10.0	-	-	-	7.1	-
308.7	100.0	100.0	78.6	-	100.0	100.0	100.0	100.0	100.0	92.9	100.0
268.7	-	-	-	-	-	-	-	4.5	54.5	-	-
250.8	100.0	-	-	-	100.0	10.0	-	-	-	-	-
219.0	-	-	85.7	-	100.0	10.0	-	-	-	-	8.3
196.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
169.1	-	-	-	16.7	-	-	-	-	-	14.3	-
151.3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
112.5	100.0	-	-	-	-	-	100.0	-	-	-	-
93.8	-	100.0	100.0	100.0	100.0	100.0	-	100.0	100.0	7.1	100.0
Total	**9**	**9**	**8**	**9**	**12**	**9**	**6**	**9**	**9**	**10**	**6**

Table 2. continued

Fragment size (bp)	034 Leeston 18.04.94	038 Marton 12.04.94	038 Marton 26.04.96	038 Rangiora 04.01.93	134 Pokeno 26.02.96	003 Wanganui 10.05.94	003 Wanganui 22.04.96
901.2	7.1	100.0	-	-	-	-	-
770.3	42.9	100.0	-	-	-	-	95.8
652.0	42.9	100.0	-	-	-	-	-
591.3	35.7	100.0	-	-	50.0	7.1	95.8
506.0	35.7	100.0	29.2	14.3	33.3	-	-
451.9	42.9	100.0	8.3	35.7	91.7	21.4	100.0
432.0	-	-	-	-	-	-	-
362.3	-	-	-	-	100.0	100.0	100.0
345.0	-	-	8.3	-	-	-	-
308.7	57.1	100.0	-	35.7	100.0	100.0	100.0
268.7	7.1	-	-	-	-	35.7	-
250.8	-	-	37.5	-	100.0	-	-
219.0	-	-	-	-	41.7	-	-
196.8	92.9	100.0	-	21.4	100.0	100.0	100.0
169.1	14.3	-	-	21.4	-	-	-
151.3	100.0	100.0	50.0	57.1	100.0	100.0	100.0
112.5	100.0	-	-	78.6	100.0	-	-
93.8	100.0	100.0	100.0	100.0	-	100.0	100.0
Total	**13**	**10**	**6**	**8**	**10**	**8**	**8**

Table 3. Summary of bands generated by RAPD-PCR with Operon primer OPA-7 and the percentage observed for each strain.

Fragment size (bp)	000 Lab 00.00.00	009 Blenheim 15.03.94	009 Blenheim 12.02.96	014 Blenheim 12.01.96	066 Dorie 20.04.94	027 Greytown 07.01.94	027 Greytown 06.02.92	038 Marton 12.04.94	038 Marton 26.04.96	134 Pokeno 26.02.96	003 Wanganui 10.05.94	003 Wanganui 22.04.96
749.9	-	-	-	-	14.3	-	-	-		-	-	-
683.2	10.0	-	-	-	14.3	-	-	-		-	7.1	-
590.0	-	-	-	-	7.1	-	22.7	-		-	7.1	-
541.7	100.0	-	-	-	-	-	31.8	-		-	21.4	-
504.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	-		-	100.0	-
444.3	100.0	-	21.4	-	100.0	18.2	-	-		-	-	-
412.0	-	72.2	42.9	100.0	100.0	-	-	16.7		-	-	-
373.7	-	33.3	14.3	5.6	14.3	-	68.2	100.0	62.5	-	35.7	-
359.2	100.0	-	-	-	-	-	-	-	-	-	-	-
352.0	-	-	-	-	-	-	-	79.2	25.0	-	50.0	-
298.5	-	-	-	-	-	-	-	-	-	-	-	-
286.2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	75.0	-	100.0	-
274.2	-	-	-	-	-	-	-	58.3	58.3	-	-	-
257.0	100.0	-	-	-	100.0	-	36.4	25.0	-	-	-	-
240.7	-	-	-	-	14.3	-	-	-	100.0	-	-	-
220.4	-	5.6	100.0	16.7	14.3	100.0	4.5	-	-	-	7.1	-
184.7	-	61.1	100.0	-	14.3	100.0	-	-	-	100.0	7.1	100.0
169.2	-	5.6	100.0	5.6	-	100.0	-	100.0	100.0	100.0	-	100.0
116.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	-		100.0	100.0	100.0
Total	**8**	**8**	**9**	**7**	**13**	**7**	**8**	**7**	**6**	**3**	**10**	**3**

Appendix 3.6

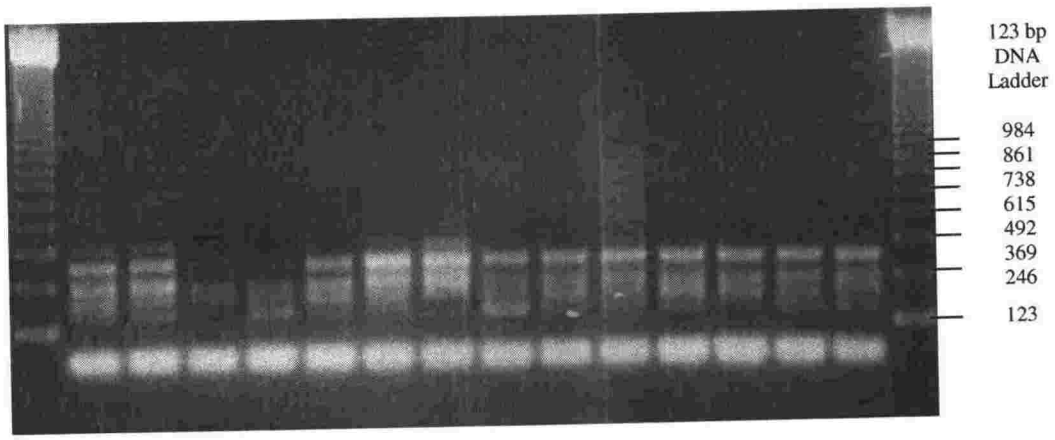


Figure 1. Example of RAPD-PCR profile generated by individuals from an OP-insecticide resistant strain of *Lucilia cuprina* (027, Greytown, 7 January 1994), using primer OPA-3.

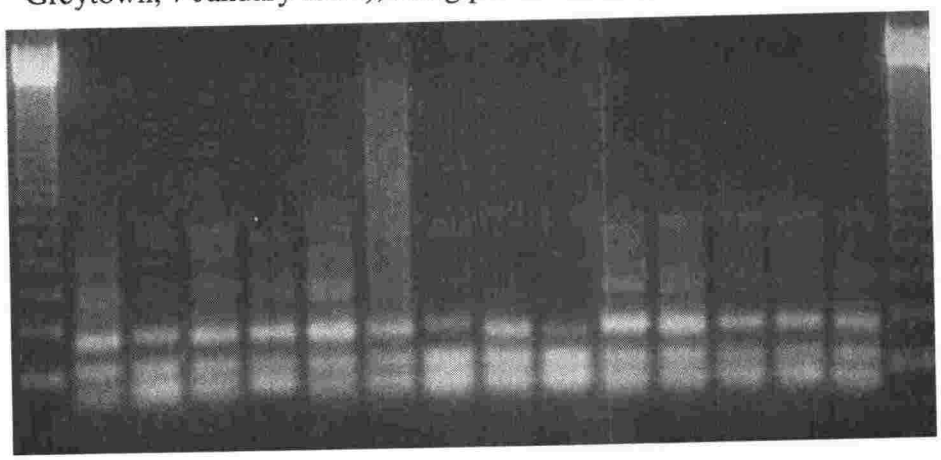


Figure 2. Example of RAPD-PCR profile generated by individuals from an OP-insecticide resistant strain of *Lucilia cuprina* (134, Pokeno, 26 February 1996), using primer OPA-4.

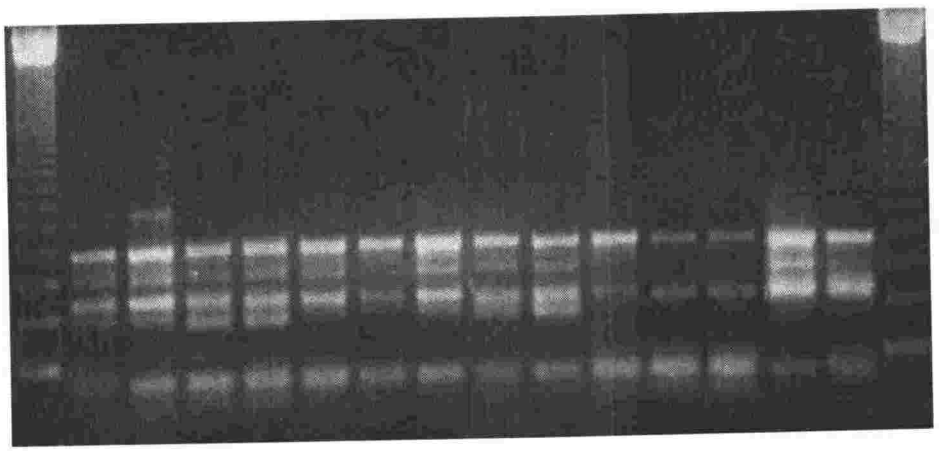


Figure 3. Example of RAPD-PCR profile generated by individuals from an OP-insecticide resistant strain of *Lucilia cuprina* (066, Dorie, 20 April 1994), using primer OPA-7.

Appendix 3.7

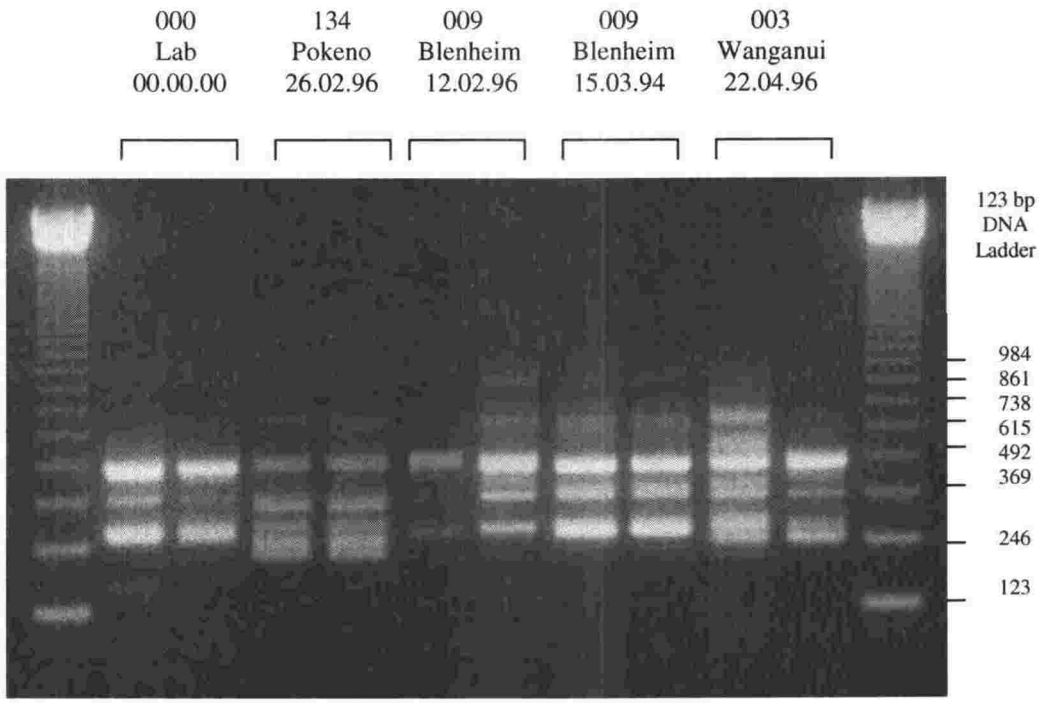


Figure 1. Comparison of RAPD-PCR profiles generated by five strains of *Lucilia cuprina*, using primer OPA-7.

Appendix 3.8

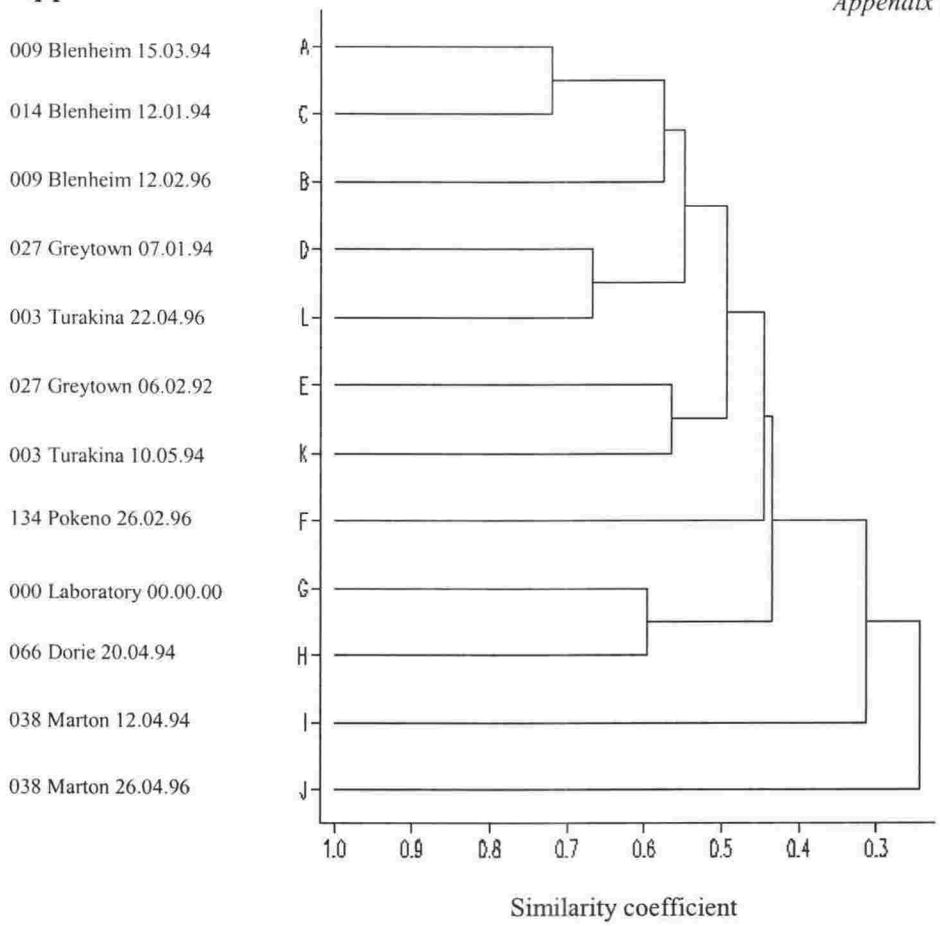


Figure 1. Dendrogram representing genetic similarity of *Lucilia cuprina* strains amplified with three random primers (OPA-3, 4 & 7). Genetic similarities were calculated by the **Jacquard** method of similarity. Dendrograms were constructed using hierarchical cluster analysis with an average link option in the GENSTAT® program.

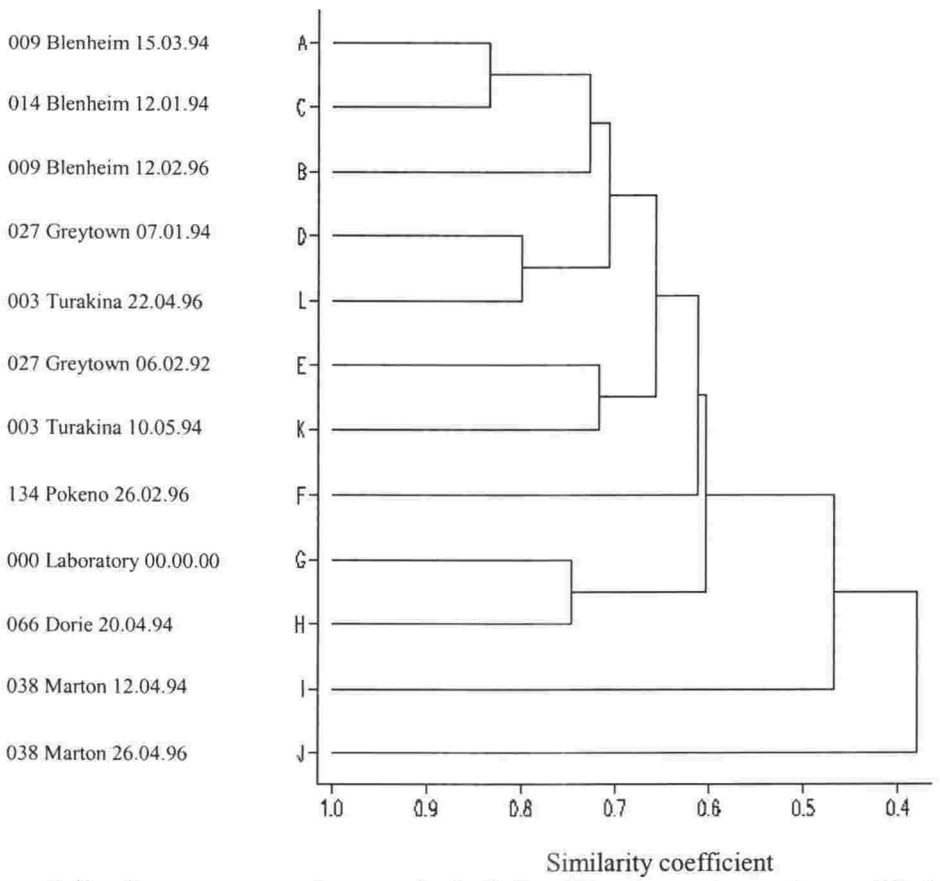


Figure 2. Dendrogram representing genetic similarity of *Lucilia cuprina* strains amplified with three random primers (OPA-3, 4 & 7). Genetic similarities were calculated by the **shared fragments** method of similarity. Dendrograms were constructed using hierarchical cluster analysis with an average link option in the GENSTAT® program.

Appendix 3.9

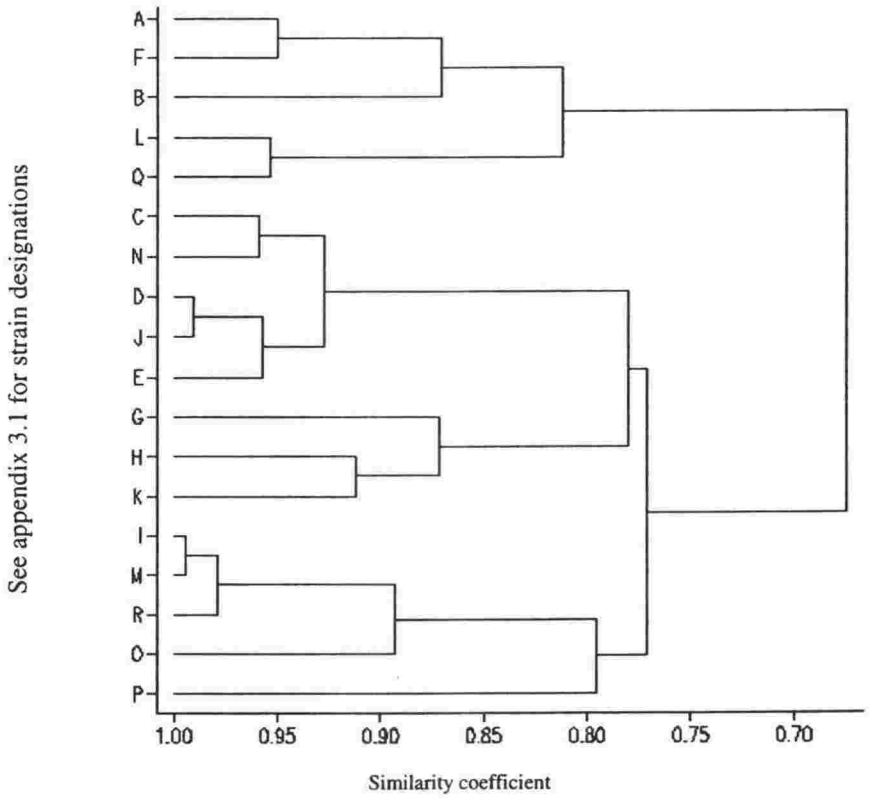


Figure 1. Dendrogram representing genetic similarity of *Lucilia cuprina* strains amplified with random primer OPA-3. Genetic similarities were calculated by the Nei & Li (1979) method of band sharing using positive frequencies. Dendrograms were constructed using hierarchical cluster analysis with an average link option in the GENSTAT® program.

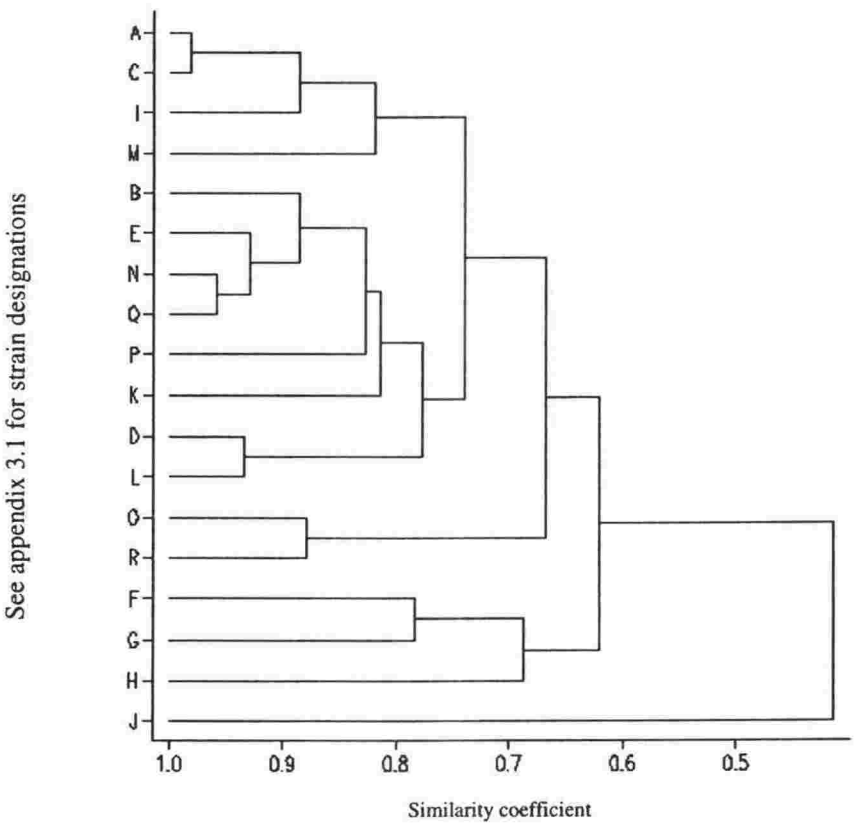


Figure 2. Dendrogram representing genetic similarity of *Lucilia cuprina* strains amplified with random primer OPA-4. Genetic similarities were calculated by the Nei & Li (1979) method of band sharing using positive frequencies. Dendrograms were constructed using hierarchical cluster analysis with an average link option in the GENSTAT® program.

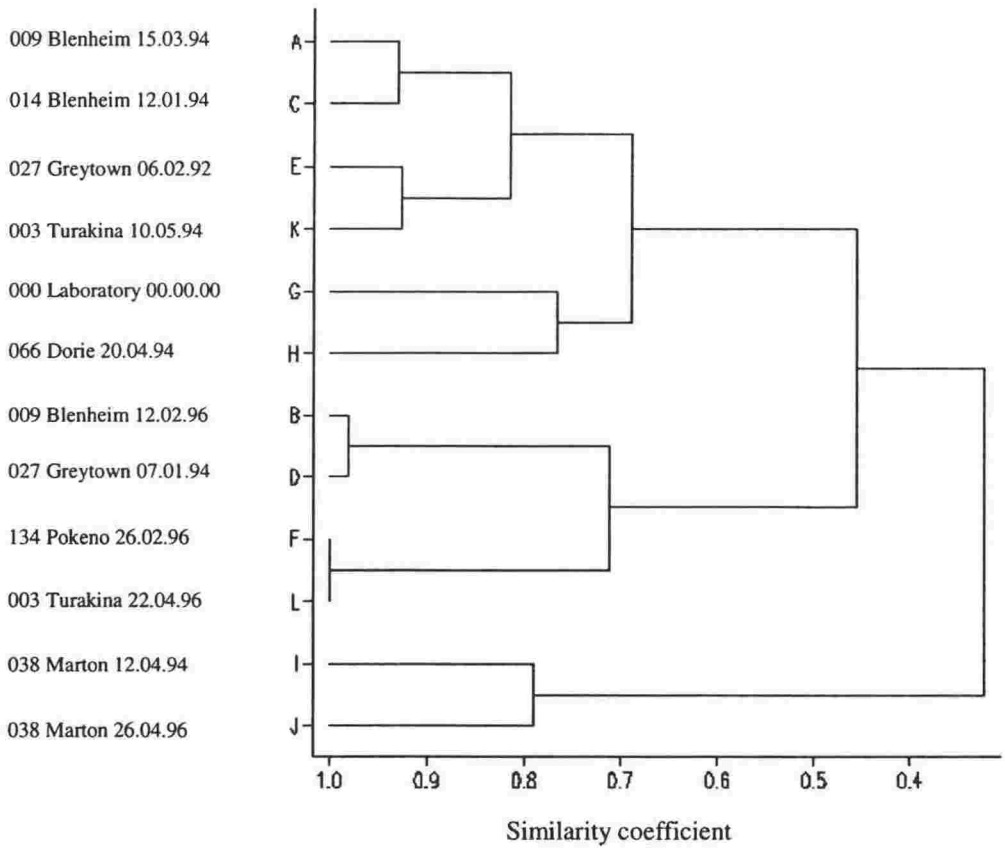


Figure 3. Dendrogram representing genetic similarity of *Lucilia cuprina* strains amplified with random primer OPA-7. Genetic similarities were calculated by the Nei & Li (1979) method of band sharing using positive frequencies. Dendrograms were constructed using hierarchical cluster analysis with an average link option in the GENSTAT® program.

Appendix 3.10

Table 1. Lower triangular similarity matrix generated by Nei & Li's (1979) method of band sharing using positive frequencies, and showing intra-specific variation in a strain of *Lucilia cuprina*, from Blenheim (009 Blenheim 15.03.94).

Fly #	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17
A1	1.000																
A2	0.882	1.000															
A3	0.833	0.833	1.000														
A4	0.889	0.889	0.842	1.000													
A5	0.914	0.914	0.757	0.919	1.000												
A6	0.875	0.937	0.824	0.882	0.909	1.000											
A7	0.875	0.937	0.824	0.882	0.909	1.000	1.000										
A8	0.941	0.941	0.778	0.889	0.971	0.937	0.937	1.000									
A9	0.941	0.882	0.833	0.944	0.914	0.937	0.937	0.941	1.000								
A10	0.889	0.833	0.842	0.842	0.919	0.824	0.824	0.889	0.833	1.000							
A11	0.800	0.850	0.810	0.857	0.878	0.789	0.789	0.850	0.800	0.905	1.000						
A12	0.895	0.842	0.850	0.900	0.923	0.833	0.833	0.895	0.895	0.950	0.909	1.000					
A13	0.914	0.914	0.865	0.865	0.889	0.909	0.909	0.914	0.914	0.865	0.829	0.923	1.000				
A14	0.865	0.811	0.872	0.923	0.842	0.800	0.800	0.811	0.865	0.872	0.884	0.878	0.842	1.000			
A15	0.944	0.889	0.789	0.947	0.973	0.882	0.882	0.944	0.944	0.895	0.857	0.950	0.919	0.872	1.000		
A16	0.919	0.865	0.821	0.923	0.947	0.857	0.857	0.919	0.919	0.923	0.884	0.976	0.895	0.850	0.974	1.000	
A17	0.850	0.850	0.857	0.905	0.878	0.789	0.789	0.850	0.850	0.905	0.913	0.955	0.878	0.884	0.905	0.930	1.000

Table 2. Lower triangular similarity matrix generated by Nei & Li's (1979) method of band sharing using positive frequencies, and showing intra-specific variation in a strain of *Lucilia cuprina*, from Blenheim (009 Blenheim 12.02.96).

Fly #	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14
B1	1.000													
B2	0.966	1.000												
B3	0.933	0.903	1.000											
B4	0.933	0.903	1.000	1.000										
B5	0.875	0.909	0.941	0.941	1.000									
B6	0.903	0.937	0.970	0.970	0.971	1.000								
B7	0.933	0.903	0.937	0.937	0.941	0.909	1.000							
B8	0.824	0.857	0.889	0.889	0.947	0.919	0.889	1.000						
B9	0.848	0.882	0.914	0.914	0.973	0.944	0.914	0.974	1.000					
B10	0.848	0.882	0.914	0.914	0.973	0.944	0.914	0.974	1.000	1.000				
B11	0.800	0.778	0.865	0.865	0.872	0.842	0.865	0.927	0.900	0.900	1.000			
B12	0.824	0.800	0.889	0.889	0.895	0.865	0.889	0.900	0.923	0.923	0.976	1.000		
B13	0.848	0.824	0.914	0.914	0.919	0.889	0.914	0.923	0.947	0.947	0.950	0.974	1.000	
B14	0.875	0.848	0.941	0.941	0.944	0.914	0.941	0.947	0.973	0.973	0.923	0.947	0.973	1.000

Table 3. Lower triangular similarity matrix generated by Nei & Li's (1979) method of band sharing using positive frequencies, and showing intra-specific variation in a strain of *Lucilia cuprina*, from Blenheim (014 Blenheim 12.01.94).

Fly #	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18
C1	1.000																	
C2	0.971	1.000																
C3	0.971	1.000	1.000															
C4	0.865	0.889	0.889	1.000														
C5	0.919	0.944	0.944	0.895	1.000													
C6	0.895	0.919	0.919	0.821	0.923	1.000												
C7	0.811	0.833	0.833	0.789	0.842	0.872	1.000											
C8	0.857	0.882	0.882	0.833	0.889	0.865	0.944	1.000										
C9	0.895	0.919	0.919	0.821	0.923	0.900	0.872	0.919	1.000									
C10	0.833	0.857	0.857	0.865	0.865	0.895	0.865	0.914	0.842	1.000								
C11	0.889	0.914	0.914	0.865	0.919	0.895	0.919	0.971	0.947	0.889	1.000							
C12	0.889	0.914	0.914	0.865	0.865	0.842	0.811	0.857	0.842	0.833	0.889	1.000						
C13	0.865	0.889	0.889	0.895	0.947	0.872	0.895	0.944	0.923	0.865	0.973	0.865	1.000					
C14	0.865	0.889	0.889	0.842	0.895	0.923	0.895	0.944	0.923	0.919	0.973	0.865	0.947	1.000				
C15	0.895	0.919	0.919	0.821	0.923	0.950	0.872	0.919	0.950	0.895	0.947	0.842	0.923	0.974	1.000			
C16	0.833	0.857	0.857	0.811	0.865	0.895	0.919	0.971	0.895	0.944	0.944	0.833	0.919	0.973	0.947	1.000		
C17	0.865	0.889	0.889	0.842	0.895	0.872	0.947	0.944	0.923	0.865	0.973	0.865	0.947	0.947	0.923	0.919	1.000	
C18	0.872	0.895	0.895	0.800	0.900	0.927	0.900	0.895	0.927	0.872	0.923	0.821	0.900	0.950	0.976	0.923	0.950	1.000

Table 4. Lower triangular similarity matrix generated by Nei & Li's (1979) method of band sharing using positive frequencies, and showing intra-specific variation in a strain of *Lucilia cuprina*, from Marton (038 Marton 12.04.94).

Fly #	I1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I14	I15	I16	I17	I18	I19	I20	I21	I22	I23	I24
I1	1.000																							
I2	0.979	1.000																						
I3	0.909	0.933	1.000																					
I4	0.933	0.957	0.977	1.000																				
I5	0.933	0.957	0.977	1.000	1.000																			
I6	0.955	0.933	0.952	0.977	0.977	1.000																		
I7	0.930	0.909	0.927	0.952	0.952	0.976	1.000																	
I8	0.933	0.957	0.977	1.000	1.000	0.977	0.952	1.000																
I9	0.978	0.957	0.884	0.909	0.909	0.930	0.952	0.909	1.000															
I10	0.978	0.957	0.930	0.955	0.955	0.977	0.952	0.955	0.955	1.000														
I11	0.909	0.933	0.952	0.977	0.977	0.952	0.976	0.977	0.930	0.930	1.000													
I12	0.909	0.933	0.952	0.977	0.977	0.952	0.976	0.977	0.930	0.930	1.000	1.000												
I13	0.978	0.957	0.884	0.909	0.909	0.930	0.952	0.909	1.000	0.955	0.93	0.930	1.000											
I14	0.955	0.933	0.952	0.977	0.977	1.000	0.976	0.977	0.930	0.977	0.952	0.952	0.93	1.000										
I15	0.757	0.737	0.800	0.778	0.778	0.800	0.765	0.778	0.722	0.778	0.743	0.743	0.722	0.800	1.000									
I16	0.789	0.769	0.778	0.811	0.811	0.833	0.800	0.811	0.757	0.811	0.778	0.778	0.757	0.833	0.966	1.000								
I17	0.789	0.769	0.778	0.757	0.757	0.778	0.743	0.757	0.757	0.811	0.722	0.722	0.757	0.778	0.966	0.933	1.000							
I18	0.757	0.737	0.743	0.778	0.778	0.800	0.824	0.778	0.778	0.778	0.800	0.800	0.778	0.800	0.929	0.966	0.897	1.000						
I19	0.757	0.737	0.800	0.778	0.778	0.800	0.765	0.778	0.722	0.778	0.743	0.743	0.722	0.800	1.000	0.966	0.966	0.929	1.000					
I20	0.789	0.769	0.778	0.811	0.811	0.833	0.800	0.811	0.757	0.811	0.778	0.778	0.757	0.833	0.966	1.000	0.933	0.966	0.966	1.000				
I21	0.757	0.737	0.743	0.778	0.778	0.800	0.824	0.778	0.778	0.778	0.800	0.800	0.778	0.800	0.929	0.966	0.897	1.000	0.929	0.966	1.000			
I22	0.757	0.737	0.743	0.778	0.778	0.800	0.824	0.778	0.778	0.778	0.800	0.800	0.778	0.800	0.929	0.966	0.897	1.000	0.929	0.966	1.000	1.000		
I23	0.757	0.737	0.743	0.778	0.778	0.800	0.824	0.778	0.778	0.778	0.800	0.800	0.778	0.800	0.929	0.966	0.897	1.000	0.929	0.966	1.000	1.000	1.000	
I24	0.757	0.737	0.800	0.778	0.778	0.800	0.765	0.778	0.722	0.778	0.743	0.743	0.722	0.800	1.000	0.966	0.966	0.929	1.000	0.966	0.929	0.929	0.929	1.000

Table 5. Lower triangular similarity matrix generated by Nei & Li's (1979) method of band sharing using positive frequencies, and showing intra-specific variation in a strain of *Lucilia cuprina*, from Marton (038 Marton 26.04.96).

Fly #	J1	J2	J3	J4	J5	J6	J7	J8	J9	J10	J11	J12	J13	J14	J15	J16	J17	J18	J19	J20	J21	J22	J23	J24
J1	1.000																							
J2	0.929	1.000																						
J3	0.800	0.870	1.000																					
J4	0.889	0.960	0.909	1.000																				
J5	0.846	0.917	0.952	0.957	1.000																			
J6	0.889	0.960	0.909	1.000	0.957	1.000																		
J7	0.750	0.818	0.947	0.857	0.900	0.857	1.000																	
J8	0.750	0.818	0.947	0.857	0.900	0.857	1.000	1.000																
J9	0.750	0.818	0.947	0.857	0.900	0.857	1.000	1.000	1.000															
J10	0.929	1.000	0.870	0.960	0.917	0.960	0.818	0.818	0.818	1.000														
J11	0.929	1.000	0.870	0.960	0.917	0.960	0.818	0.818	0.818	1.000	1.000													
J12	0.714	0.615	0.696	0.640	0.667	0.640	0.727	0.727	0.727	0.615	0.615	1.000												
J13	0.714	0.615	0.522	0.640	0.583	0.640	0.455	0.455	0.455	0.615	0.615	0.769	1.000											
J14	0.897	0.815	0.667	0.769	0.720	0.769	0.609	0.609	0.609	0.815	0.815	0.741	0.815	1.000										
J15	0.667	0.545	0.421	0.571	0.500	0.571	0.333	0.333	0.333	0.545	0.545	0.545	0.818	0.783	1.000									
J16	0.609	0.476	0.333	0.500	0.421	0.500	0.353	0.353	0.353	0.476	0.476	0.571	0.762	0.727	0.941	1.000								
J17	0.636	0.500	0.353	0.526	0.444	0.526	0.375	0.375	0.375	0.500	0.500	0.500	0.700	0.667	0.875	0.933	1.000							
J18	0.500	0.444	0.400	0.471	0.500	0.471	0.429	0.429	0.429	0.444	0.444	0.444	0.556	0.526	0.714	0.769	0.833	1.000						
J19	0.609	0.476	0.333	0.500	0.421	0.500	0.353	0.353	0.353	0.476	0.476	0.571	0.762	0.727	0.941	1.000	0.933	0.769	1.000					
J20	0.571	0.526	0.375	0.556	0.471	0.556	0.400	0.400	0.400	0.526	0.526	0.421	0.632	0.600	0.800	0.857	0.923	0.909	0.857	1.000				
J21	0.636	0.700	0.471	0.632	0.556	0.632	0.375	0.375	0.375	0.700	0.700	0.300	0.600	0.667	0.750	0.667	0.714	0.667	0.667	0.769	1.000			
J22	0.421	0.471	0.571	0.500	0.533	0.500	0.462	0.462	0.462	0.471	0.471	0.353	0.471	0.444	0.615	0.500	0.545	0.667	0.500	0.600	0.727	1.000		
J23	0.609	0.476	0.333	0.500	0.421	0.500	0.353	0.353	0.353	0.476	0.476	0.571	0.762	0.727	0.941	1.000	0.933	0.769	1.000	0.857	0.667	0.500	1.000	
J24	0.526	0.609	0.476	0.333	0.500	0.421	0.500	0.353	0.353	0.353	0.476	0.476	0.571	0.762	0.727	0.941	1.000	0.933	0.769	1.000	0.857	0.667	0.500	1.000

Appendix 3.11

An example of output from principal co-ordinates analysis of *Lucilia cuprina* populations using Nei & Li's (1979) method of band sharing.

Latent roots

1	2	3	4	5	6
1.038	0.9645	0.6448	0.5714	0.3606	0.3009

Percentage variation

1	2	3	4	5	6
20.51	19.06	12.74	11.29	7.13	5.94

Trace

5.061

Latent vectors (co-ordinates)

Population	1	2	3	4	5	6
A	-0.2831	-0.0109	-0.1781	-0.2735	0.0634	0.1187
B	-0.3833	-0.0116	0.1229	-0.1063	0.2090	0.0509
C	-0.0863	0.0151	-0.0744	-0.3200	0.0723	-0.0037
D	-0.1821	0.0496	-0.0278	0.0241	0.0545	-0.3349
E	0.0559	-0.0784	0.1185	-0.1995	-0.0617	-0.1152
F	-0.1443	-0.1969	-0.0906	0.4235	0.2049	0.1385
G	0.2695	-0.3232	-0.2458	0.0284	0.1240	0.0287
H	0.2941	-0.3924	0.1111	-0.0976	-0.0018	0.0005
I	0.0646	0.3990	-0.3635	-0.0533	-0.2671	0.0275
J	0.1670	0.5646	0.3464	0.1245	0.0653	0.1662
K	-0.0187	-0.2728	0.1773	-0.0500	-0.1975	0.1746
L	-0.3416	0.0044	-0.0536	0.3128	-0.1076	-0.1908
M	0.0453	0.1975	-0.3152	0.0478	0.0707	0.1200
N	0.0335	0.1618	0.1031	-0.0951	0.1037	-0.0668
O	0.3544	-0.0113	-0.1011	0.0617	-0.0364	-0.0677
P	0.0257	-0.1738	0.0644	0.1325	-0.2637	0.0630
Q	-0.3228	-0.0204	0.2439	0.0025	-0.1391	0.0160
R	0.4521	0.0998	0.1625	0.0377	0.107	-0.1255