# DIFFERENCES IN SUSCEPTIBILITY TO DIFLUBENZURON BETWEEN POPULATIONS OF THE AUSTRALIAN SHEEP BLOWFLY, *LUCILIA CUPRINA* (WIEDEMANN) AND THEIR INFLUENCE ON FLYSTRIKE PROTECTION

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## Summary

Field populations of the Australian sheep blowfly were found to vary widely in their susceptibility to diflubenzuron. The distribution of LC50s was bunched around that for the reference susceptible laboratory strain. This suggested that most populations were susceptible to diflubenzuron. Toxicological responses were significantly correlated with those to the organophosphorus insecticide diazinon. A small number of populations were resistant to diflubenzuron and were associated with shorter than expected flystrike protection of diflubenzuron treated sheep. Results of a larval implant trial showed that resistant larvae established artificial strikes on diflubenzuron treated sheep significantly earlier than susceptible larvae.

**Keywords:** diflubenzuron, *Lucilia cuprina*, flystrike

## INTRODUCTION

The Australian sheep blowfly, *Lucilia cuprina* (Wied.) has developed resistance to organochlorinc, organophosphate (OP) and carbamate insecticides (Hughes and McKenzie 1987). Resistance to the OPs is primarily due to detoxification by a microsomal esterase (Hughes and Devonshire 1982) $E3_{null}$ (Hughes and Raftos 1985) which is present in about 98% of field blowflies (Shanahan and Roxburgh 1974). Secondarily, monooxygenases augment the esterase-mediated resistance to varying degrees (Hughes and Devonshire 1982, Terras *et al.* 1983). Monooxygenase activity (measured using an *in-vitro* aldrin epoxidase assay) was found to be correlated with increased tolerance to both the OP diazinon, and to diflubenzuron (Kotze *et al.* 1997), so it was not surprising that toxicological responses to these two insecticides were also correlated (Kotze *et al.* 1993).

During spring 1998 severe flywave conditions existed across much of eastern Australia for the first time in several years. Several woolgrowers reported that diflubenzuron products had protected sheep from flystrike for only 7 - 8 weeks, whereas product labels carry a claim of 12 weeks. To investigate these complaints we conducted laboratory bioassays with larvae reared from blowflies sent to us by the affected woolgrowers. We compared the toxicological responses of these larvae with those of numerous other diflubenzuron naïve populations tested between 1996 and 1999. Furthermore, to determine whether resistance contributed to the poor field performance of diflubenzuron on these properties, we conducted a larval implant trial comparing strike establishment by susceptible and Resistant blowfly strains on diblubenzuron treated sheep.

## MATERIALS AND METHODS

### *In-vitro bioassays*

Between November 1996 and June 1999 field populations of *L. cuprina* were sampled as larvae on struck sheep and maintained for two generations in the laboratory. A few samples were received as suspect diflubenzuron failures, however, relatively few of the 77 populations tested had previous exposure to diflubenzuron.

Groups (N=30 - 60) of first instar $F_2$ larvae were exposed to a serial range of concentrations expected to elicit 0 - 100% mortality using a method modified from that of Roxburgh and Shanahan (1973). Briefly, larvae were counted onto rolled strips (12x3 cm) of insecticide impregnated chromatography paper soaked with sheep serum containing 2% (w/v) yeast extract and 0.5% (w/v) potassium dihydrogen phosphate and contained within glass phials (4x1cm). Mortality was assessed after 48 h (diflubenzuron), or 24 h (diazinon). Each concentration was replicated once. Controls consisted of acetone (solvent) treated papers.

### *Analysis*

Data was analysed by the probit method of Finney (1971) to generate LC50 values. Regression analysis was performed on the 77 sets of data (log LC50 values for diflubenzuron and diazinon) obtained from the bioassay results.

### *Larval Implant Trial*

#### *Insecticide treatment of sheep*

Thirty two merino sheep carrying about 6 cm wool growth were hand-jetted along the back with a proprietary diflubenzuron formulation (Fleececare$^{R}$) diluted with water according to label instructions, to give a final concentration of 500 mg/L. Approximately 3L of solution was applied to each sheep. Five additional sheep remained untreated to act as controls.

#### *Larvae*

A field-collected, diflubenzuron susceptible (LC50 = 0.10 - 0.13 mg/L) population and a resistant field population (LC50 = 3.7 - 4.5 mg/L) were used in the larval implant trial. In order to maintain the resistant strain's elevated response to diflubenzuron, only flies reared from larvae capable of surviving 1 mg diflubenzuron/L in the above bioassay were included in the diflubenzuron resistant blowfly colony that provided the larvae for the implants.

#### *Implant technique*

At 6, 9 and 12 weeks after treatment (WAT) larval implants were initiated into superficial wounds made on 18 of the 32 treated, and 3 of the 5 untreated sheep. Batches of about 200 newly hatched larvae of the two blowfly strains were implanted separately but adjacent to each other, on either side of the dorsal midline of the sheep. To minimise any bias that may have resulted from non-uniform insecticide application, resistant and susceptible larvae were implanted alternately on both the left and right hand side of the sheep and at the shoulder, rump and mid back. Hence, at 6 WAT resistant larvae were placed at the right shoulder and the susceptible larvae on the left shoulder. At 9 WAT resistant larvae were implanted onto the left side mid back of the sheep and susceptible larvae onto the right hand side. At 12 weeks resistant larvae were put on the right rump and the susceptible larvae on the left rump.

To minimise unnecessary stress to the animals and to ensure that results for some sheep were collected on consecutive implant dates, the sheep were 'rostered on or off' for implanting. Consequently, 7 previously unchallenged sheep had implants put on them at each date. By week 12, 11 sheep had been implanted on consecutive dates (6 and 9, or 9 and 12 WAT) and 4 sheep had been challenged on each of the 3 implant dates. Similarly, the control sheep were rotated such that 2 sheep were implanted once, 2 sheep were implanted twice and one sheep was implanted on each of the 3 occasions. Strikes were inspected for live larvae 48 h later. Although implants were considered positive if a single live larva was found, a subjective assessment was also made regarding the health of the larvae present. Strike sites were then saturated with chloroform to kill all maggots.

#### *Analysis*

Data (establishment or non-establishment of strike) were analysed using a generalised linear model (McCullagh and Nelder 1989) with errors assumed to have a binomial distribution. The deviance value attributed to the difference between blowfly strains was compared by Chi-square with 1 degree of freedom.

## RESULTS

#### *In-vitro bioassays*

The diflubenzuron LC50 for the laboratory susceptible strain response was 0.14 mg/L. With the exception of one very susceptible population (LC50 = 0.01 mg/L) the LC50s for field populations ranged from 0.10 mg/L to 3.35 mg/L (median 0.32 mg/L), giving a maximum Resistance Factor (LC50 for field strain/LC50 for the susceptible strain) of 24. Generally, the field data approximated a normal distribution (Fig. 1). Diazinon responses (log LC50s) were significantly ($P<0.01$) correlated ($r=0.54$, 75df) with those for diflubenzuron (Fig. 2).

#### *Larval implant trial*

At each of the challenges, larvae implanted on to each of the control sheep developed well into the third instar and formed extensive strikes. The resistant larvae formed strikes on each of the 18 diflubenzuron treated sheep at each challenge.

Most maggots had apparently grown normally and were thought capable of completing development. The susceptible larvae established significantly ($P<0.01$) fewer strikes (12/18) 6 WAT; but strike establishment was not significantly different thereafter: 17/18 at 9 WAT and 18/18 at 12 WAT (Table 1). For the 6 and 9 WAT assessments less than half of these strikes contained normal looking larvae. Larvae in the remainder displayed symptoms of diflubenzuron intoxication (stunted growth, lethargy, not feeding) or were very few in number. For example, at the 9 WAT assessment, of the 17 positive implants on the treated sheep, 8 contained only normal larvae; 4 contained moderate numbers of stunted maggots only; and, 5 contained less that 6 stunted maggots only.

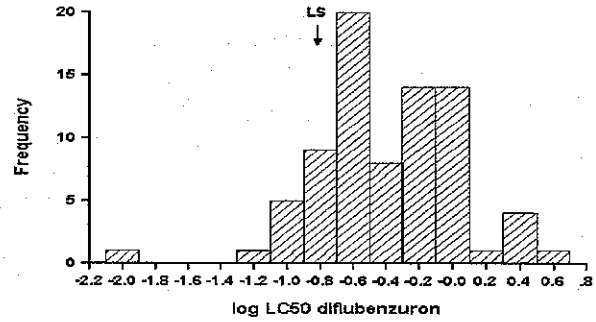


Figure 1. Frequency distribution of log LC50s of diflubenzuron for Australian populations of *Lucilia cuprina*. The arrow indicates where the log LC50 of the laboratory susceptible strain (LS) would lie in the distribution.

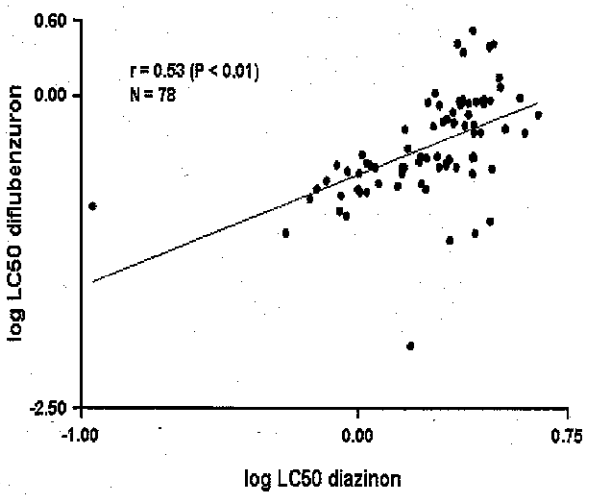


Figure 2. Scatter-plot of log LC50s of diflubenzuron and diazinon for Australian populations of *Lucilia cuprina*, (r = correlation coefficient; N = number of populations in analysis).

## DISCUSSION

Compared to the susceptible strain, the most resistant population was 24 times resistant to diflubenzuron. Although field failures are not necessarily due to resistance, it is significant that the 4 highest diflubenzuron responses were recorded for populations that had survived on diflubenzuron treated sheep. Previous commentary on field responses to diflubenzuron (Hughes and Levot 1987) suggested that the (then) levels of resistance were unlikely to compromise field performance. However, our results indicate that, compared to the susceptible strain, resistant larvae formed significantly more strikes on diflubenzuron treated sheep 6 WAT. Furthermore, this advantage remained for a further 3 weeks. During this period resistant strain larvae on the sheep appeared normal, whereas larvae in about half of the strikes formed by the susceptible strain contained only stunted maggots that were unlikely to develop further.

The implant trial measured the relative abilities of apparently diflubenzuron-resistant and -susceptible sheep blowfly larvae to survive in artificial strikes on diflubenzuron treated sheep. No conclusions should be drawn regarding the absolute length of flystrike protection provided by diflubenzuron against susceptible populations. Larval implant trials are an excellent technique for comparing treatments or blowfly populations but they do not provide an accurate measurement of the flystrike protection that can be expected under field conditions (Hughes and Shanahan 1978). This may be caused by ovicidal, repellent or oviposition deterrent effects being

**Table 1. Flystrike establishment by Resistant and Susceptible maggots on diflubenzuron-treated sheep.**

| Weeks after treatment | Number of +ve implants/number of sheep challenged | |
|---|---|---|
| | Susceptible strain | Resistant strain |
| 6 | 12/18a | 18/18b |
| 9 | 17/18b | 18/18b |
| 12 | 18/18b | 18/18b |

NB. Results followed by the same letter were not significantly (P<0.01) different.
All 3 implants on control sheep were positive at each challenge.

by-passed when first instar larvae rather than eggs, are implanted onto the sheep's skin.

Based on results for a relatively small number of blowfly populations Kotze *et al.* (1997) reported that diflubenzuron responses were correlated with those to diazinon. Our results confirm this correlation ($P < 0.01$). Kotze *et al.* (1997) speculated that 40+ years of OP use had selected enhanced monooxygenase activity in some sheep blowfly populations. This contention is supported by the strong correlation between responses to diazinon and diflubenzuron in blowfly populations with no previous exposure to diflubenzuron. Differences in monooxygenase activity could also explain, at least in part, the variation in diazinon resistance levels between populations (Terras *et al.*, 1983). Although all field populations contain almost only OP resistant individuals, resistance factors to diazinon vary from around 5 to 60 times in the field (Levot unpublished data). It is this variation in diazinon responses that is significantly correlated with responses to diflubenzuron.

Most sheep blowfly populations remain susceptible to diflubenzuron however we have demonstrated that some populations are sufficiently resistant to reduce the protection period provided by this insecticide. There are efficient alternative insecticides available to affected woolgrowers. Unfortunately, if re-treatment is necessary, it will most probably be needed late in the wool-growing cycle. In order to minimise insecticide residues in Australian wool, current recommendations discourage insecticide applications, particularly during the last 3 months before shearing. During this time woolgrowers are encouraged to treat struck sheep individually rather than apply insecticide to all sheep in the mob in an effort to prevent flystrike completely.

## ACKNOWLEDGMENTS

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