NEW HIGH LEVEL RESISTANCE TO DIFLUBENZURON DETECTED IN THE AUSTRALIAN SHEEP BLOWFLY, *LUCILIA CUPRINA* (WIEDEMANN) (DIPTERA: CALLIPHORIDAE)

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Summary

Diflubenzuron normally protects sheep from flystrike by susceptible blowflies (*Lucilia cuprina* Diptera: Calliphoridae) for 12 weeks. Recently however, we tested blowfly larvae collected at Tara (Queensland) from lambs found flystruck less than one week after thorough treatment with a diflubenzuron based jetting product. Resistance was 790 times greater than the reference susceptible strain. Previously the highest reported resistance in *L. cuprina* was 24 fold. This represents a new extreme level of resistance that could have serious pest control implications to the Australian wool industry.

Keywords: diflubenzuron, *Lucilia cuprina*, resistance

INTRODUCTION

Sales *et al.* (2001) reported a small number of diflubenzuron resistant sheep blowfly, *Lucilia cuprina* (Wiedemann) populations during an insecticide resistance survey. Resistance levels were only moderate (maximum 24x) but this was apparently sufficient to shorten the period of flystrike protection on diflubenzuron treated sheep. Reports of field failures with diflubenzuron still occur, with resistance increasingly found to be a contributing factor. Here we report a new and worrying occurrence in the development of diflubenzuron resistance in sheep blowfly.

MATERIALS AND METHODS

Samples of blowfly larvae from Tara (Queensland) (N=39 third instar larvae) collected from flystruck cross-bred lambs and from Emmaville (New South Wales) (N=148 third instar larvae) were submitted for resistance testing as part of the investigations into failure of diflubenzuron to prevent flystrike. Laboratory cultures of both populations were established to rear sufficient larvae for testing. Groups (N=30-60) newly hatched first instar F_2 larvae were exposed to a serial range of concentrations expected to elicit 0 - 100% mortality. The bioassay used was that first developed by Roxburgh and Shanahan (1973) and later modified by Hughes and Levot (1987) especially for testing insect growth regulator insecticides. Briefly, larvae were counted onto rolled strips (12 x 3 cm) of insecticide impregnated chromatography paper soaked with sheep serum containing 2 g/L yeast extract and 0.5 g/L potassium dihydrogen phosphate and contained within glass phials (4 x 1 cm). The phials were plugged with cotton wool and held under lights at 28^{o}C until mortality of larvae was assessed at 48 h (diflubenzuron), or 24 h (diazinon). Each concentration was replicated once. Controls consisted of acetone (solvent) treated papers. Data was corrected for control mortality then analysed by the probit method of Finney (1971).

RESULTS

Control mortality never exceeded 10% and was usually much lower. Results for the Tara blowfly strain indicated an LC50 of diflubenzuron of 110.7 mg/L and failure to kill more than about 45% of larvae at concentrations greater than 32 mg/L (Figure 1). Compared to the susceptible strain, LS (LC50 = 0.14 mg/L and 100% mortality at 0.5 mg/L) the Tara strain was 790 x more resistant to diflubenzuron. The Emmaville strain was 41 x resistant to diflubenzuron. Approximately 5% of these larvae survived exposure to 100 mg/L in the bioassay (Figure 1).

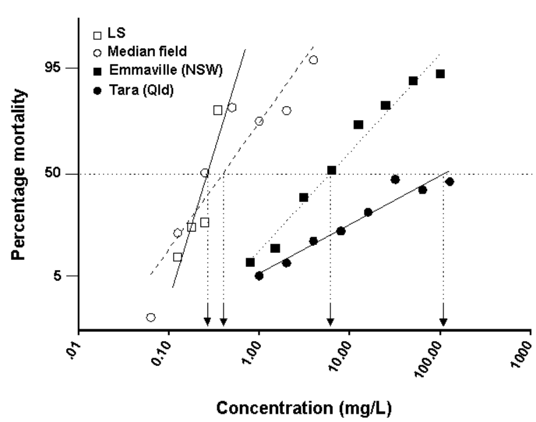

Figure 1. Diflubenzuron dose response lines for sheep blowfly larvae.

Compared to LS and other field populations, the Tara strain's response to diazinon (LC50 = 4.41 mg/L) was high (Resistance Factor = 44 times higher than the susceptible strain response (LC50 = 0.1 mg/L)). The Emmaville strain's response was similar to the median field response reported previously (Sales *et al.* 2001) (Figure 2).

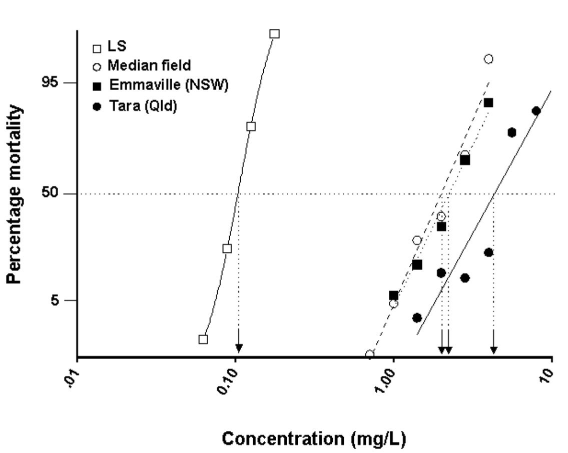

Figure 2. Diazinon dose response lines for sheep blowfly larvae.

DISCUSSION

In terms of practical flystrike control this new high level resistance to diflubenzuron is a very significant development. Previously the highest resistance measured was 24x (Sales *et al.* 2001) for a population from Loomberah near Tamworth in New South Wales. This population was one of a cluster of five whose responses to diflubenzuron formed a 'top end tail' to the normal distribution of field population responses (log LC50s) (Sales *et al.* 2001). Like four of these populations, the Emmaville sample was also collected from diflubenzuron-treated sheep that had become struck during the normal flystrike protection period.

The cluster of 'low' resistant populations, including that from Emmaville, may represent an intermediate stage between a susceptible response and the high diflubenzuron resistance seen in the Tara population. Sheep blowfly larval responses to diflubenzuron and diazinon have been reported to be significantly correlated (Kotze *et al.* 1997; Sales *et al.* 2001). Kotze *et al.* (1997) suggested that 40+ years of organophosphate (OP) use on sheep to control flystrike and lice may have selected for enhanced monooxygenase activity that improved the likelihood of OP resistant larvae surviving on diflubenzuron-treated sheep. The diazinon resistance in the Tara strain (44x) was the highest measured in our laboratory for a field-collected population but was far below the extreme level of the diflubenzuron resistance in this strain. It is likely, though as yet unproven, that a second resistance mechanism, perhaps augmented by the monooxygenases, is responsible for the enormous increase in diflubenzuron resistance.

At Tara, diflubenzuron treated lambs became flystruck within one week of jetting whereas susceptible larvae are unable to establish strikes for at least 12 weeks (Sales *et al*. 2001). Flock management at Tara involved split shearings in May and December and jetting in September and late December targeting flystrike and lice control. This had been the pattern for the past three years with the owner reporting no problem until early 2002 when the major breakdown occurred. Although split shearings sometimes offer more frequent opportunities to access markets, one disadvantage is that ectoparasite control, particularly lice control, becomes extremely difficult. Control programs that minimise selection for resistance in blowflies and lice should be an integral part of flock management but are sometimes compromised to 'fit in' with other farm priorities. At Tara, sole reliance on diflubenzuron to prevent flystrike and to control lice has proved unsustainable. Strategies likely to suit the current flock management program at Tara are now severely curtailed. Flystrike control should be readily achievable with cyromazine-based products, as there were no survivors of a susceptible discriminating concentration bioassay with this insecticide (Levot unpublished data). However, cyromazine has no lousicidal activity. Consequently, even if the producer at Tara continues to use diflubenzuron to control lice he will be forced to apply another treatment to control flystrike. This would increase pesticide residues in his woolclip and increase costs. Other alternatives would be to use ivermectin or spinosad to control both pests but these too, may not be cost-effective.

The incremental rise in diflubenzuron resistance in several blowfly populations and proof that extreme resistance can render diflubenzuron totally ineffective for flystrike prevention provides a timely warning. Producers relying solely on diflubenzuron (or other benzoylphenyl urea insecticide) for blowfly and/or lice control should be mindful that prolonged use, with no rotation to an unrelated insecticide group, is unlikely to be sustainable.

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REFERENCES

Finney, D.J. (1971). *Probit analysis*. 3rd Edition. Cambridge University Press, London.

Hughes, P.B. and Levot, G. (1987). Simulation of fly-waves to assess the ability of diflubenzuron to protect sheep against flystrike by *Lucilia cuprina*. *Veterinary Parasitology* **24**: 275-284.

Kotze, A.C., Sales, N. and Barchia, I.M. (1997). Diflubenzuron tolerance associated with monooxygenase activity in field strain larvae of the Australian sheep blowfly (Diptera: Calliphoridae). *Journal of Economic Entomology* **90**: 15-20.

Roxburgh, N. A. and Shanahan, G.J. (1973). A method for the detection and measurement of insecticide resistance in larvae of *Lucilia cuprina* (Wied.)(Dipt., Calliphoridae). *Bulletin of Entomological Research* **63**: 99-102.

Sales, N., Levot, G.W. and Barchia, I.M. (2001). Differences in susceptibility to diflubenzuron between populations of the Australian sheep blowfly, *Lucilia cuprina* (Wiedemann) and their influence on flystrike protection. *General and Applied Entomology* **30**: 27-30.

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