BACTERIAL CONTROL OF THE SHEEP BLOW FLY

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SUMMARY

The blow flies *Lucilia sericata*, *Calliphora vicina*, *C. stygia*, *Lucilia cuprina* and *Chrysoma rufifacies* were tested for susceptibility to seven strains of the bacterium *Serratia marcescens* and one strain of *Bacillus thuringiensis* in a laboratory experiment. Flies were caged individually and fed bacteria in a sucrose suspension. At 4 days the mean mortality for *L. sericata*, *C. vicina*, *C. stygia* and *L. cuprina* when treated with *S. marcescens* was 74.7%. Results for *C. rufifacies* were more variable, and no significant mortality occurred. The LT_{50} was significantly reduced for *L. sericata*, *C. vicina* and *C. stygia* compared with untreated controls. Treatment with *Bt* had little effect on the flies.

Keywords: *Serratia marcescens*, insect pathogen, *Lucilia*, blowflies

INTRODUCTION

The nationwide cost of ovine myiasis or flystrike is in excess of \$56 million annually (Grace 1989). Deaths of sheep, loss of liveweight and wool and pelt damage make up 30 percent of the cost while insecticides and the cost of their application accounts for \$39 million. The prevalence of flystrike is increasing with between 3-5% of the national flock affected annually compared with 1-2% pre 1980. In addition the duration of the flystrike "season" is now 12 months compared to about 7 or 8 months previously (Heath *et al* 1989).

Fly strike can be caused by a number of blow fly species (Diptera: Calliphoridae) including the green blow fly *Lucilia sericata* (Meigen), brown blow fly *Calliphora stygia* F., the European blow fly *C. vicina* (Robineau-Desvoidy) and hairy maggot blow fly *Chrysoma rufifacies* (Macquart). Recently the Australian green blow fly *Lucilia cuprina* (Wiedemann) has joined this complex.

Flystrike is currently controlled by regular dipping of sheep with residual chemical insecticides, predominantly organophosphates. This had led to the development of resistant fly populations (Hughes 1981). Biological control is an attractive alternative for fly control but there are few obvious candidates. The bacterium *Serratia marcescens* (Bizio) may provide a possibility. Steinhaus (1959) reported isolation of *S. marcescens* from three dipteran hosts. More recently Poinar *et al* (1979), Onoviran *et al* (1985), Kaaya and Darji (1989) and Watson and Petersen (1991) have reported on the pathogenicity of *S. marcescens* to tsetse and stable flies.

In this experiment one *Bacillus thuringiensis* strain and a number of *S. marcescens* isolates, taken from diseased and dead insects, were screened to determine their activity against adults of *L. sericata*, *C. vicina*, *C. stygia*, *C. rufifacies* and *L. cuprina*.

METHODS

All the flies used in these experiments were obtained from cultures held at MAF Animal Research Centre at Wallaceville. Three species, *L. sericata*, *C. vicina* and *C. stygia* were chilled and individually placed in a sterile 30 ml transparent plastic tube with a screw cap, plus an absorbent cotton roll. Two to ten flies were used per treatment depending on availability, and up to 20 flies were used as untreated controls.

The bacteria came from four sources (Table 1). All isolates were aseptically inoculated into 10 ml of sterile Luria Bertani broth and incubated at 30 °C in a shaking water bath at 180 rpm for 24 h. One ml of each bacterial broth ($1-3 \times 10^9$ bacteria) was added to the cotton roll after application of 1 ml of 10% sucrose solution. The

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TABLE 1: Origin and host insect of bacterial strains of *Serratia marcescens* and *Bacillus thuringiensis* used against blow flies.

Treatment	Host	Origin - * Collection
1 <i>S. marcescens</i>	<i>Periplaneta americana</i>	G1405 * Grimont
2 <i>S. marcescens</i>	<i>Nephantis serinopa</i>	89-1-1 * Steinhaus
3 <i>S. marcescens</i>	<i>Hyponomeuta malineus</i>	G1440 * Grimont
4 <i>S. marcescens</i>	<i>Musca domestica</i>	0-34-0 * Steinhaus
5 <i>S. marcescens</i>	<i>Apis mellifera</i>	G1437 * Grimont
6 <i>S. marcescens</i>	<i>Drosophila</i>	* Steinhaus
7 <i>S. marcescens</i>	<i>Paropsis charybdis</i>	MAFTech
8 <i>Bacillus thuringiensis</i>		ABG-6193 * Abbott

untreated controls received 2 ml of a 5% sucrose solution. The flies were incubated at 25 °C on a 12 hour day/night cycle. Mortality was noted daily and dead flies removed and refrigerated for subsequent bacterial isolation. The experiment was continued until all the flies were dead.

L. cuprina and *C. rufifacies* were treated as above and monitored at Wallaceville under quarantine restrictions. These flies were treated with CO₂ to facilitate their transfer to tubes. Upon death, the flies were refrigerated and at the completion of the trial were sent to Lincoln for bacterial isolation.

Bacteria were isolated from the cadavers by surface sterilising the dead flies (Poinar and Thomas 1984) and streaking out haemolymph from the thoracic region onto *Serratia* selective Caprylate-thallos agar and then transferring colonies onto Dnase-toluidine blue agar plates for confirmation of identity (O'Callaghan and Pearson 1989).

Mortality data were analysed using a parametric accelerated failure time model based on the Weibull distribution (Cox and Oakes 1984).

RESULTS AND DISCUSSION

By day four significant mortality was recorded in four of the five fly species treated with *S. marcescens* (Table 2). There was no significant mortality due to *Bt*.

TABLE 2: Percent mortality at day 4 and LT₅₀ (days to 50% mortality) of different blow fly species after treatment with *Serratia marcescens* or *Bacillus thuringiensis*.

Total no. flies treated/species	<i>L. sericata</i> (98)	<i>C. stygia</i> (43)	<i>C. vicina</i> (31)	<i>L. cuprina</i> (91)	<i>C. rufifacies</i> (91)
% Mortality @ 4 days					
<i>S. marcescens</i>	50	100	100	49	25
<i>Bt</i>	0	—	50	10	20
Control	5	31	38	0	33
Analysis					
Treatment v Control	***	***	***	***	ns
<i>Bt</i> v <i>S. marcescens</i>	***	—	*	*	ns
Between <i>S. marcescens</i>	**	ns	ns	**	ns
LT ₅₀					
<i>S. marcescens</i>	4.33	2.63	2.95	4.51	5.29
<i>Bt</i>	7.6	—	3.8	6.6	6.5
Control	8.6	5.7	6.1	7.1	5.0
Analysis					
Treatment v Control	***	***	***	ns	ns
<i>Bt</i> v <i>S. marcescens</i>	***	—	ns	*	***
Between <i>S. marcescens</i>	ns	ns	ns	***	*

*** = P < .001, ** = P < .01, * = P < .05, ns = no significant difference @ 5% level, — = not treated.

Based on the model, an LT_{50} (days until 50% mortality) was calculated for each treatment. *L. sericata*, *C. vicina* and *C. stygia* treated with bacteria had a significantly shorter life than untreated controls (Table 2). The mean LT_{50} for treated flies of these species was 4.7, 3.1 and 2.6 days respectively compared to 8.6, 6.1 and 5.7 days for untreated controls. Results for *L. cuprina* and *C. rufifacies* were more variable but there were significant differences in mortality between different *S. marcescens* isolates based on LT_{50} values (Table 2).

S. marcescens was re-isolated from the haemolymph of more than 50% of the treated flies. In the untreated flies the bacterium was recovered from three individuals of *C. stygia*.

The results of this experiment are consistent with the findings of other studies. Ingestion of the bacterium caused septicaemia and death within a few days. After treatment of tsetse fly, *Glossina* spp., with *S. marcescens*, Onoviran *et al* (1985) reported 65-90% mortality in 4 days and Poinar *et al* (1979) reported 100% mortality in 10 days. Watson and Petersen (1991) have reported 45% mortality of stable flies, *Stomoxys calcitrans* (L.), 4 days after treatment with *S. marcescens*.

The current study indicates that *S. marcescens* could have a role in the biological control of sheep blowflies. Further work is necessary to determine dosage of bacteria required and the process of pathogenicity which could provide a sound basis for strain selection. *S. marcescens* is known to produce several hydrolytic enzymes such as proteases (Lysenko 1976) and a chitinase, some of which have been shown to be toxins (Kaska 1976). If highly toxic strains are isolated it will then be necessary to consider how these can best be used to provide protection for the sheep flock.

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