



## *In vitro* infection of sheep lice (*Bovicola ovis* Schrank) by Steinernematid and Heterorhabditid nematodes

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### ABSTRACT

Control of sheep lice with conventional pesticides can be compromised by difficulty in contacting lice in the dense water repellent fleeces of sheep, particularly when sheep have not been recently shorn. Entomopathogenic nematodes (ENs) are motile and are able to actively seek out insect hosts. They have particular advantages for the control of pests in cryptic habitats, such as the fleeces of sheep and avoid many of the problems frequently associated with chemical controls. This study investigated whether ENs were able to infect and kill *Bovicola ovis* and compared the effectiveness of different species at different temperatures and when applied to wool.

Four species of nematodes, *Steinernema carpocapsae*, *Steinernema riobrave*, *Steinernema feltiae* and *Heterorhabditis bacteriophora* were tested. All were shown to infect and kill lice in Petri dish assays at 30 °C. At 35 °C, the percent infection for *S. carpocapsae* and *S. riobrave* was significantly higher than for the other two species and percent infection by *S. feltiae* was significantly greater than for *H. bacteriophora* ( $P < 0.05$ ). At 37 °C the percent mortality induced by *S. riobrave* was significantly greater than for *S. carpocapsae* ( $P < 0.05$ ). All species were able to locate and infect lice in wool when formulated in water with 8% Tween 80. In wool assays the percent lice infected with nematodes was significantly greater for *S. riobrave* than *H. bacteriophora* at 25 °C, but there were no other differences between species ( $P = 0.05$ ). *S. carpocapsae*, *S. riobrave* and *S. feltiae* caused significantly higher lice mortality than *H. bacteriophora* at both 25 and 35 °C in wool assays, but mortality induced by the three steinernematid species did not differ significantly ( $P > 0.05$ ). It is concluded that of the ENs studied *S. riobrave* is likely to be most effective against *B. ovis* when applied to live sheep because of its greater tolerance to high temperatures and 'cruiser' foraging strategy.

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### 1. Introduction

Sheep lice (*Bovicola ovis*) cause significant economic loss by irritating sheep and reducing wool quantity and quality (James, 2008). Control of sheep lice depends almost exclusively on the application of chemical pediculicides and conventional treatments aim to deliver active concentrations of insecticide to all sites on the sheep where lice

survive. As *B. ovis* is a chewing louse and not blood feeding, systemic chemicals are not effective and treatment is usually by immersion dipping, high volume spraying or through the use of high concentration formulations that diffuse through the wool grease in the fleece and on the skin. Difficulty in completely covering the sheep and contacting all lice, particularly when sheep have not been recently shorn, often compromises treatment effectiveness (Lund et al., 2000). In addition, heavy use of chemicals, particularly with long wool treatments, can leave undesirable chemical residues in the wool (Savage, 1998; Morcombe et al., 1999), contribute to the development of resistance in lice populations (James et al., 2008; Levot and Sales, 2008)

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and pose occupational health and safety and environmental risks during application (Littlejohn and Melvin, 1991; Murray et al., 1992).

Entomopathogenic nematodes (ENs) are microscopic worms that invade and kill a wide range of insect species, including human lice *Pediculus humanus* (Weiss et al., 1993; Doucet et al., 1998). Infective juvenile nematodes (IJs) gain entry to an insect's haemocoel through body openings including the mouth, anus, spiracles or in some instances, directly through the insect's cuticle (Bedding and Molyneux, 1982). Once penetration has been achieved they release a toxin and mutualistic bacteria in the genera *Xenorhabdus* or *Photorhabdus*. These bacteria inhibit the insect's immune defences and the growth of other microorganisms and provide food for the developing nematodes. Death of the insect usually ensues within 24–48 h. ENs can be formulated to provide extended shelf life, can be applied through most conventional spray equipment, do not leave chemical residues and are not a health risk for humans or other vertebrates (Akhurst and Smith, 2002; Bedding, 2006). The motility of ENs and their ability to actively seek out insect targets, makes them particularly attractive for use against pests in 'cryptic habitats' which may be hard to reach with conventional pesticides, as is the case with lice in a sheep's fleece. This paper reports the results of a series of laboratory studies conducted to determine if ENs could infect and kill sheep lice and to provide a preliminary assessment of the relative potential of four species of ENs for use in sheep louse control.

## 2. Materials and methods

### 2.1. Nematodes and lice

Four species of nematodes were tested against *B. ovis*. These were *Steinernema carpocapsae* (ALL strain), *Steinernema feltiae* (NJ), *Steinernema riobrave* (RGV) and *Heterorhabditis bacteriophora* (Otio). Initial stocks of nematodes were sourced from EcoGrow Environment Pty Ltd (Westgate, NSW, Australia), a commercial supplier of ENs, and nematodes used in the assays were maintained in our laboratory by rearing through *Galleria mellonella* according to standard methods (Poinar, 1979). Nematodes were stored at 20 °C in spring water (Mt Franklin®, Sydney, Australia) aerated with aquarium bubblers after emergence from *G. mellonella* larvae and all assays conducted with nematodes within 2 weeks of collection.

All lice were collected directly by vacuum suction from source sheep that had been held at the Animal Research Institute, Yeerongpilly, Queensland without chemical treatment for 6 years (Animal Ethics Approval ARI 055/2004) and all assays commenced within 24 h of lice collection.

### 2.2. Treated surface (TS) assays

#### 2.2.1. TS assay 1

Assays were conducted with the four nematode species and with controls (no nematodes) in 50 mm glass Petri dishes containing a single layer of Whatman No1 filter paper. Nematode suspensions were mixed to contain

1000 nematodes per ml in commercial spring water (Mt Franklin®, Coca-Cola Amatil, Sydney Australia), agitated to disperse the nematodes and 0.5 ml of the suspension applied to the centre of the filter paper from a pipette with widened aperture. Ten mixed sex adult lice were then added to each Petri dish and held in darkness at either 30 or 35 °C for 24 h at which time mortality of the lice was assessed. There were three replicates for all treatment and control groups.

For inspection, lice from each group were rinsed twice in deionised water to remove any adhering nematodes and individually inspected under a microscope to ensure no nematodes were still present on the integument. They were then mounted on a microscope slide in a drop of normal saline under a coverslip and examined for movement of nematodes within. If no nematodes were obvious, pressure was applied to the coverslip to rupture the louse exoskeleton and the released body contents and haemolymph carefully inspected.

#### 2.2.2. TS assay 2

A second Petri dish assay was conducted at 32 and 37 °C with *S. carpocapsae* and *S. riobrave*, which were the most effective species in infecting lice in the first experiment. A slightly different system was used because of the high mortality experienced in control lice in Experiment 1 and the experiment was conducted at higher temperatures to more adequately span the range of temperatures likely at the skin surface of sheep (Lee et al., 1941). Aliquots of 0.3 ml of nematode suspension mixed at 1000 nematodes per ml in mineral water were pipetted onto 50 mm × 50 mm pieces of unbleached calico held tight in 50 mm Petri dishes by 47 mm internal diameter metal rings. The nematodes were distributed approximately equally among five sites, one at the centre of the test arena and four at evenly spaced positions approximately 1 cm inside the ring circumference. Ten mixed sex adult lice were added to each dish and mortality assessed 24 h later. In this assay the Petri dishes were used without lids and held in darkness at 65% RH in a humidity controlled incubator. There were three replicates for the controls and each species at both temperatures. Dead lice were squashed under a coverslip to confirm nematode infection.

### 2.3. Infection of lice in wool

In preliminary studies it was determined that without a wetting agent, droplets of water that formed on or between wool fibres trapped the nematodes, preventing them from moving along the fibres to infect lice. Sometimes lice also got caught in the droplets, which caused significant non-nematode induced mortalities. A series of preliminary tests determined that 8% Tween 80 (Tw80) allowed good wetting of wool fibres without droplet formation and had no effect on nematode viability.

#### 2.3.1. Wool assay 1

To assess the relative effectiveness of *S. carpocapsae*, *S. feltiae*, *S. riobrave* and *H. bacteriophora* in infecting and killing lice in wool, groups of naturally aligned wool fibres (staples) plucked from shorn fleece were cut to

4 cm lengths (approximately 0.22 g) and inserted into 50 mm × 15 mm flat bottomed glass tubes. Ten lice were added to each tube. Dilutions were mixed to deliver 500 nematodes in each 0.5 ml application. Nematodes were applied to the top of the wool and the tubes held at either 25 or 35 °C. There were four replicates for each species or control (8% Tw80, no nematodes) by temperature combination and mortality was assessed at 48 h.

### 2.3.2. Wool assay 2

A second study was carried out with *S. carpocapsae* and *S. riobrave* using a system designed to more closely mimic the situation on the sheep. In this study nymphs and adult lice were tested separately and nematode suspension (0.1 ml in 8% Tw80) mixed to deliver 350 nematodes per tube was applied to the surface of the wool. Ten lice were used in each assay and placed, together with 10 mg lice diet (James et al., 1998), at the base of 50 mm × 15 mm flat bottomed glass tubes. Black electrical tape was wrapped around the basal half of each tube to provide a light gradient. Wool staples were trimmed to 4 cm length, weighed to ensure equal weight (approximately 0.22 g) and inserted into tubes with fibres aligned to approximate the fleece structure on a sheep. The tubes were then placed on a metal tray heated from below to 37 °C, with a fluorescent lamp above. Nematode suspensions were applied slowly to the top of the staple taking care to avoid runoff. After 48 h living and dead lice were harvested and examined for the presence of nematodes. There were four replicates for each nematode by louse age treatment.

### 2.4. Nematode movement in wool

To further investigate the ability of nematodes to move through wool, five lice were added to 50 mm × 15 mm flat based glass tubes. Wool staples were cut to lengths of 2, 3 and 4 cm, immersed and gently agitated in 10% Tw80 for approximately 30 s, removed from the Tw80 and then rolled in paper towelling to remove excess moisture. The staples were removed from the paper and inserted to touch the bottom of each tube, taking care to preserve fibre alignment. The tubes were allowed to stand for at least 2 min to enable the lice to move into the wool. Quantities of 0.5 ml of *S. carpocapsae* solution mixed at concentration of 500 nematodes ml<sup>-1</sup> were applied to either the top (NT) of the staple or to the bottom of the vial (NB) from a 1 ml pipette. For application to the bottom of the vials, the wool with lice was partially removed from the tube to allow insertion of the pipette and then reinserted after dispensation of the nematode suspension. For the 2 and 4 cm wool lengths additional treatments with no lice, but with nematodes applied to the bottom of the tubes were included to assess the degree of nematode migration in the absence of lice. All tubes were held at 25 °C and there were three replicates for each wool length × position of application combination.

Wool staples were examined microscopically and nematodes scored as present in the top, middle and bottom thirds of the staples at 24 and 48 h. The number of lice alive and dead in each tube was also recorded at each inspection. Lice were removed from tubes in the third replicate

after 24 h and examined for the presence of nematodes as previously described.

### 2.5. Statistical methods

For most experiments the data was analysed by fitting a generalised linear model, assuming the distribution of dead and live or infested and non-infested lice to be binomial and using a complementary logarithm–logarithm link function. Interactions were included when significant. When effects were significant ( $P > 0.05$ ), differences between complementary logarithm–logarithm means were determined by pairwise Student *t*-tests. As parameter estimates could not be obtained for groups where either 100% or 0% of lice were dead or infected, these groups were excluded from the generalised linear model analysis. In these instances Fisher's exact test for 2 × 2 contingency tables was used to determine if they were significantly different from other groups. For analysis of the lice mortality data in the nematode movement study an arcsine transformation was applied, wool length was treated as a linear variate and a generalised linear model fitted. All analyses were undertaken using Genstat v11 (Payne et al., 2007).

## 3. Results

### 3.1. Treated surface assays

#### 3.1.1. TS assay 1

This experiment confirmed the ability of all four species of EN to infect *B. ovis*. Some lice were found to be infected with nematodes in all of the treatment groups except for *H. bacteriophora* at 35 °C (Fig. 1). The numbers of lice infected by *S. feltiae* at 30 °C and by both *S. feltiae* and *H. bacteriophora* at 35 °C were significantly lower than for the other two species ( $P < 0.05$ ). As expected, no nematodes were found in any control lice.

Although lice mortality was high in the control groups because of high humidity and condensation within the covered dishes, there was also a clear effect of ENs on lice mortality. At 30 °C all of the lice were dead in all nematode-treated groups compared to 62 ± 8.8% mortality in the controls ( $P < 0.05$ ). At 35 °C, mortality was 100% for *S. riobrave*, 87 ± 6.1% for *S. carpocapsae*, 87 ± 6.0% for *S.*

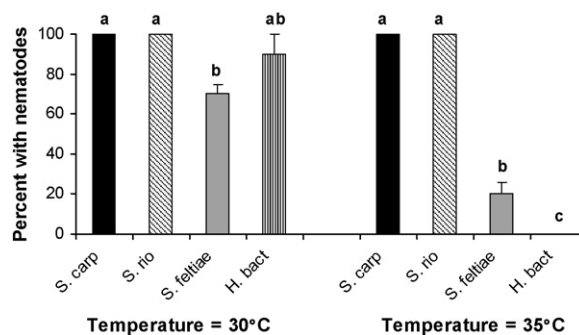
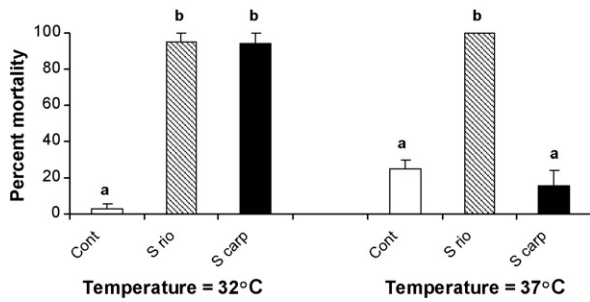


Fig. 1. Mean percent of lice (± s.e.) infected by *Steinernema carpocapsae*, *S. feltiae*, *S. riobrave* and *H. bacteriophora* at 30 and 35 °C in Petri dish assays. Values marked by different letters are significantly different ( $P < 0.05$ ).



**Fig. 2.** Mortality of lice induced by *S. carpocapsae* and *S. riobrave* in Petri dish assays at 32 and 37 °C (mean ± s.e.). Values marked by different letters are significantly different ( $P < 0.05$ ).

*feltiae*,  $90 \pm 5.3\%$  for *H. bacteriophora* and  $60 \pm 8.9\%$  in the controls, which was significantly lower than for the groups with ENs ( $P < 0.05$ ).

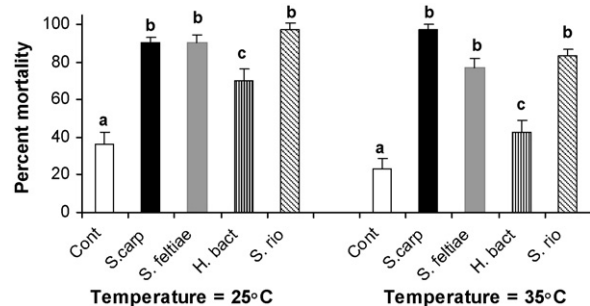
### 3.1.2. TS assay 2

There was a significant interaction between species and temperature in effect on lice mortality in this experiment. At 32 °C there was no difference between *S. carpocapsae* and *S. riobrave* with both species causing mortality close to 100% (Fig. 2). However, at 37 °C mortality induced by *S. riobrave* was significantly higher than for *S. carpocapsae*. It was notable that many *S. riobrave* nematodes were seen nictating on the cotton fibres in the 32 °C treatment, although not at 37 °C, when the dishes were examined at 24 h whereas no live *S. carpocapsae* were observed on the cotton at either 32 or 37 °C. Nematodes were found in all dead lice examined from the nematode treatments, but not in dead lice from the control groups.

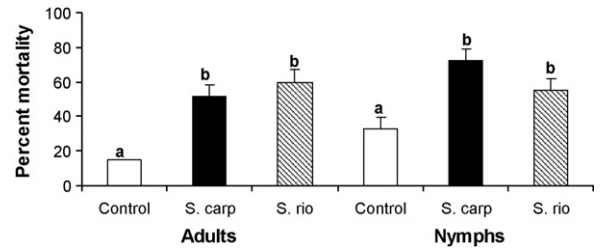
## 3.2. Wool assays

### 3.2.1. Wool assay 1

This study confirmed the ability of all species of nematodes studied to invade and kill lice when applied to wool fibres in formulation with a wetting agent (Fig. 3). Overall mortality at 35 °C was significantly lower than at 25 °C, but there was no interaction between temperature and species. Mortality in the *H. bacteriophora* treatments was significantly lower than with the other three species, reflecting a



**Fig. 3.** Percent mortality of lice exposed to *S. carpocapsae*, *S. feltiae*, *S. riobrave* and *H. bacteriophora* in wool assays at 25 and 35 °C (mean ± s.e.). Values marked by different letters are significantly different ( $P < 0.05$ ).



**Fig. 4.** Percent mortality in adult and nymphal lice exposed to *S. carpocapsae* and *S. riobrave* in wool migration assays (mean ± s.e.). Values marked by different letters are significantly different ( $P < 0.05$ ).

lower infection rate with *H. bacteriophora*, but significantly greater than in controls at both temperatures ( $P < 0.05$ ).

At 35 °C dead lice were often too desiccated for nematodes to be found. At 25 °C most lice were sufficiently hydrated for inspection although difficulties in detecting nematodes in lice which died early could have led to underestimation of the actual numbers infected. The mean percent infection rates of lice at 25 °C (±s.e.) were  $85.2 \pm 11.0$  for *S. riobrave*,  $74.9 \pm 13.5$  for *S. carpocapsae*,  $59.6 \pm 15.3$  for *S. feltiae* and  $13.4 \pm 10.7$  for *H. bacteriophora*. The difference between *S. riobrave* and *H. bacteriophora* was significant ( $P < 0.05$ ) but there were no other differences between species.

### 3.2.2. Wool assay 2

The results of this experiment confirmed the ability of *S. carpocapsae* and *S. riobrave* to infect and kill both adult lice and nymphs in wool, even when lice were added with lice diet to the bottom of 4 cm wool staples and nematodes were added to the top. There was no significant effect of stage and no interaction between species and stage ( $P > 0.05$ ). Louse mortality was significantly higher with both species of nematodes than in the controls (Fig. 4), but again there was no difference between species. The 48-h time frame together with high temperature dehydrated many of the dead lice before they were inspected and made it impossible to determine the proportions infected by nematodes. However, nematodes were found in both nymphs and adult lice confirming nematode activity. Overall mortality was lower in this study than the experiment described in Section 3.2.1 and this may reflect either the different experimental system or the lower dose rate used.

## 3.3. Movement of nematodes in wool

This experiment confirmed the ability of nematodes to migrate through wool and infect lice. The distribution of nematodes through the wool staple in the different staple length and temperature treatments at 24 and 48 h is shown in Fig. 5. For simplicity of presentation and because there was no 3 cm treatment without lice, only the results for the 2 and 4 cm wool lengths are shown.

The presence of lice clearly affected nematode movement. In the NB treatments without lice, no nematodes were found to have moved out of the bottom third of the staple with either wool length at either 24 or 48 h (Fig. 5). However, nematodes applied to the bottom of the staples in

Wool length	Position	Lice present									No lice									
		Nematodes top						Nematodes bottom						Nematodes bottom						
		24 h			48h			24 h			48h			24 h			48h			
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
2 cm	Top	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched
	Middle	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched
	Bottom	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched
4 cm	Top	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched
	Middle	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched
	Bottom	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched	hatched

**Fig. 5.** Presence of *S. carpocapsae* in the top, middle and bottom thirds of 2 and 4 cm length wool staples at 24 and 48 h after application to either the top or the bottom of the staple, with lice present and with no lice. Hatched cells indicate the presence of nematodes, blank cells indicate no nematodes and solid fill cells indicate replicates sacrificed at 24 h.

the treatments with lice were found throughout the staple in two of the three 4 cm replicates at 24 h and in the middle third of the staple in the other 4 cm replicate (Fig. 5). Although no nematodes were found in the top two thirds of the staple in the 2 cm NB treatment with lice at 24 h, by 48 h they were found throughout the staple in one replicate and had moved into the middle third in the other.

There was no significant effect of position of nematode application on louse mortality ( $P > 0.05$ ) but there was a significant negative association between wool length and mortality at 24 h ( $P = 0.046$ ). At 24 h the mean percentage lice mortalities ( $\pm$ s.e.) were 100 for the 2 cm wool length, 90 ( $\pm 4.5$ ) for the 3 cm wool length and 83.3 ( $\pm 8.0$ ) for the 4 cm wool lengths (application positions combined). At 48 h mortality had increased to 100% in all replicates with nematodes applied to the top, but not in the 3 and 4 cm wool lengths with nematodes applied to the bottom where in each instance two of the three replicates had one surviving louse. Nematodes were found in all lice in the replicates sacrificed at 24 h.

#### 4. Discussion

There have been relatively few studies of the suitability of ENs for use in the control of veterinary parasites. Samish et al. (2004) reviewed studies against ticks, Molyneux et al. (1983) reported tests against larvae of the myiasis fly *Lucilia cuprina* and Manweiler (1994) reported use of ENs against the larval stage of fleas. Most of these studies were focused on the off-host stages of these parasites. However, Kocan et al. (1998) showed *Amblyomma americanum* in the later stages of feeding was susceptible to ENs applied to cattle and Hanan and Abdel-Shafy (2006) reported that application of *Heterorhabditis* sp. and *S. carpocapsae* ALL strain ENs to cattle infested with *Boophilus annulatus* caused semi-fed ticks to detach within 12 h of treatment. We believe this to be the first report of ENs infecting chewing ('mallophagan') lice, which include a number of economically important species parasitising livestock and birds.

Our studies confirmed the ability of all four species of nematodes investigated to infect and kill sheep lice both in filter paper assays and when applied to wool, but there were clear differences in efficacy between species, particularly at higher temperature. The significant level of mortality in the control groups in a number of our experiments was not unexpected and should not be seen to compromise the findings reported here. High levels of moisture in the fleece, either following heavy rainfall or from dipping or spraying are well known to induce significant mortality in *B. ovis* populations (Murray, 1963; Heath et al., 1995). Conventional dipping and spraying for sheep louse control requires use of aqueous solutions and wetting agents and wetting per se contributes to the effectiveness of these methods (Heath et al., 1995). Use of an aqueous carrier with a wetting agent was necessary in our assays to allow movement and survival of nematodes and is also likely to be necessary for any future contemplated animal applications of ENs.

Weiss et al. (1993) and Doucet et al. (1998) previously demonstrated the ability of nematodes to infect and kill human lice. Similar to our results, Weiss et al. (1993) found that *H. bacteriophora* was less effective at infecting and killing *P. h. humanus* than *S. carpocapsae*, although they found clear differences between strains of *S. carpocapsae*. Doucet et al. (1998) reported good results against *P. h. capitatus* with the RN strain of *H. bacteriophora*, but relatively poor effectiveness with the ON strain.

In contrast to our findings, these authors found that *S. feltiae* was not able to infect human head lice. They suggested that this was because the architecture of the blood feeding mouthparts and the intensive excretory activity of the haematophagous lice prevented EN entry by oral and anal routes. The main point of entry by other nematode species was thought to be through the spiracles and they considered *S. feltiae* too large to gain entry by this means. *B. ovis* are chewing lice that feed on scurf and superficial cells of the stratum corneum, bacteria and lipid at the surface of sheep skin (Sinclair et al., 1989; Murray and Edwards, 1987). In *B. ovis* it is

likely that nematodes can enter by both oral and anal routes.

The temperature in the fleece of sheep, the louse habitat, can be quite variable and there is often a temperature gradient from skin level to the surface of the fleece. The size and direction of this gradient depends on prevailing environmental conditions. In addition, evaporative cooling following wetting during dipping or spraying will significantly decrease fleece temperatures and provide a window of lower temperatures for nematode activity until drying is complete. However, at skin level where most lice feed the temperature is commonly between 34 and 38 °C, just below sheep body temperature (Lee et al., 1941). Thus for good effectiveness in killing lice nematodes will probably need to be active at relatively high temperatures.

Our study showed a clear advantage for *S. carpocapsae* and *S. riobrave* over the other two species tested at 35 °C and above in most assays and *S. riobrave* caused a significantly higher mortality of lice than *S. carpocapsae* at 37 °C in Petri dish assays. Our observation that numerous *S. riobrave* were still nictating at 32 °C after 24 h, in contrast to *S. carpocapsae* where none were seen nictating, also suggests greater temperature tolerance for *S. riobrave*. Previous reports have also suggested that *S. riobrave* is more efficient than *S. carpocapsae* against insects at temperatures above 30 °C, although there are significant strain effects in this (Grewal et al., 1994; Gouge et al., 1999). *S. riobrave* (RGV strain) was shown to infect and kill *G. mellonella* larvae at temperatures as high as 39 °C (Grewal et al., 1994).

ENs have been categorized on the basis of their host finding strategies as broadly 'ambushers' which sit, nictate and wait for a host to pass by and 'cruisers', which actively move through their environment in search of host cues. Ambushers are most effective against relatively motile hosts whereas cruisers are more effective against less active arthropods. In practice there is a probably a continuum between the two extremes and most nematode species use a mix of strategies. Campbell et al. (2003) categorized *S. carpocapsae* as primarily ambushers, whereas *S. riobrave* was considered to utilize a mix of both strategies. The wool movement study demonstrated that *S. carpocapsae* would move through the wool, and in particular down wool fibres, to infect lice. The significant negative relationship that we observed between wool length and louse mortality may reflect dilution of the density of both ENs and lice in the greater volume of wool present. A species with a better developed search strategy than *S. carpocapsae* may have been more effective.

On numerous occasions we have attempted to rear *S. carpocapsae* and *S. riobrave* through a generation in *B. ovis* and although we commonly found first generation male and female adults, on no occasion have we found a second generation of juvenile nematodes. Therefore it seems highly unlikely in a practical context that there would be a second generation of nematodes produced to aid in achieving louse control. Although lice move up and down wool fibres in response to temperature gradients (Murray, 1968) and also disperse laterally over the sheep's body (James and Moon, 1999) lateral movement does not occur rapidly and a species of nematode with well-developed search strategies would have obvious advantages.

Our studies indicated that all four species tested were able to locate and infect lice in wool, although *S. carpocapsae* and *S. riobrave* were more effective at higher temperatures. Although there were only minor differences between *S. carpocapsae* and *S. riobrave* in our study a consideration of previously reported temperature ranges and search strategies suggests *S. riobrave* (Grewal et al., 1994; Gouge et al., 1999; Campbell et al., 2003) as the species of choice for further testing in live animal applications.

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