

Strategies for nematode transmission: selective migration of *Trichostrongylus tenuis* infective larvae

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Abstract

Successful transmission of macroparasites is dependent on exposure of susceptible hosts to free-living infective stages. When these hosts are herbivores that feed mostly on a single food plant then natural selection should favour those infective larvae that selectively ascend this main food plant. Red grouse feed predominantly on heather, *Calluna vulgaris*, so we predict that the infective larvae (L3) of the caecal nematode *Trichostrongylus tenuis* selectively locate and ascend heather plants. To determine whether the presence of heather influences the horizontal dispersal of *T. tenuis* L3 across soil, the movement of L3 across trays of soil with and without heather was investigated in the laboratory. More *T. tenuis* L3 were recovered from soil when heather was present, implying that larval migration may be influenced by chemical cues produced by heather plants. This was investigated in a second experiment, in which the horizontal dispersal of *T. tenuis* larvae was examined in the presence of heather and grass vegetation. This trial was repeated with larvae of a second species, *Haemonchus contortus*, a nematode whose hosts feed on a wide range of grass and shrub species. Significantly more larvae of both nematode species were recovered in the region of the heather than the grass or controls. This implies that *T. tenuis* and *H. contortus* L3 exhibit selective migration towards heather, perhaps reflecting a general response to plant cues which may be stronger for heather than for grass.

Introduction

To maximize transmission success, natural selection should favour parasites whose behaviour increases exposure of the infective stages to susceptible hosts. To this end, many macroparasites have developed complex life cycles where the definitive host will eat a prey item that carries the infective stage. Many of the herbivorous vertebrates are infected with gastrointestinal nematodes that have simple direct life cycles where infection occurs through the ingestion of the motile infective larvae with the food of the hosts. For those host species whose diet is

comprised principally of a single plant type, we would predict selection to favour those larvae that are able to locate that specific food plant and thus maximize their chances of being ingested.

Trichostrongylus tenuis is a common parasite of the red grouse (*Lagopus lagopus scoticus*), and one in which the relationship between the parasite and its host has been intensively studied (Hudson, 1986; Dobson & Hudson, 1992; Hudson *et al.*, 1992, 1998). Transmission takes place when a host ingests third-stage infective larvae (L3) that migrate to the growing shoots of heather (*Calluna vulgaris*), the preferred food of red grouse (Hudson, 1986; Hudson *et al.*, 1992). Since red grouse feed predominantly on heather, particularly during the periods of peak infection (Hudson, 1992; Hudson & Dobson, 1997), natural selection should favour those *T. tenuis* infective larvae that selectively locate and ascend

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heather plants. In a previous study on vertical migration of *T. tenuis* larvae on three different plant species, larvae were found to have greater efficiency of migration up heather than other plant species (Saunders *et al.*, 1999). This suggested that larvae might respond to a cue or stimulus produced by heather, which may also influence their horizontal migration. Such behavioural responses to host cues have been documented for entomopathogenic (Lewis *et al.*, 1992, 1993; Grewal *et al.*, 1997) and phytoparasitic nematodes (Grundler *et al.*, 1991).

While the infective larvae of *T. tenuis* can regularly migrate up and down plants they are still highly aggregated in the field (Saunders *et al.*, 2000). This suggests that, even though the larvae can disperse, they do not move far from the host's caecal droppings in which they were deposited as eggs. Indeed, previous studies have shown limited horizontal migration of infective larvae of the genera *Trichostrongylus*, *Haemonchus*, *Ostertagia* and *Chabertia*, with the greatest larval recovery from vegetation near the faeces (Rose, 1961; Holasova *et al.*, 1989). Thus, in contrast to the hypothesis that larvae select heather to ascend, we can propose the null hypothesis that larvae simply migrate towards the closest vegetation.

To determine whether the presence of heather influences the horizontal dispersal of *T. tenuis* L3, larval movement across soil was quantified over time in both the presence and absence of heather. To test the hypothesis that *T. tenuis* L3 selectively locate and ascend heather plants, a second experiment investigated the horizontal dispersal of both *T. tenuis* and *Haemonchus contortus* L3 in the presence of both heather and grass vegetation. *Haemonchus contortus* is a closely related parasitic nematode occurring primarily in the abomasum of sheep and goats, who feed on both grass species and shrubs.

Materials and methods

Culture of nematode larvae

Third stage infective *T. tenuis* larvae (L3) were cultured from red grouse caecal faeces collected from Gunnerside moor in North Yorkshire. Cultures were incubated at 22°C for 7 days to allow nematode eggs to hatch and develop (Wilson, 1979) and were then mixed together to give a homogeneous suspension. Third-stage infective *H. contortus* larvae (L3) were obtained in suspension from the Moredun Research Institute, Edinburgh. Larval suspensions were refrigerated until required and the number of larvae per ml estimated using the modified McMaster technique (MAFF, 1978).

Does the presence of heather influence the horizontal dispersal of T. tenuis L3?

To answer this question, three groups of trays (each measuring 22 × 34 cm) were set up. In the first group of eight trays, five heather stems were planted at distances 2, 4, 6, 8 and 10 cm from the centre. The heather stems had been trimmed so that each had a vertical stem that extended 7 cm above the soil surface. Approximately 500 *T. tenuis* infective larvae were introduced in a single

faecal pat (approximately 1 cm diameter) at the centre of each tray. The second group of two control trays was set up in a similar way to the first group but received 1 ml of water instead of *T. tenuis* larvae. The third group of eight trays was set up in the same way, with approximately 500 *T. tenuis* L3 introduced at the centre of each tray, but contained no heather stems. All trays were kept under optimum conditions for larval migration (constant 12°C with a 12L:12D photoperiod) and sprayed twice daily with water to maintain a high relative humidity (75–90%). The temperature of 12°C was within the range for proficient migration of trichostrongylid larvae (Callinan & Westcott, 1986) and the high relative humidity allowed the formation of a moisture film on the vegetation that is essential for trichostrongyle migration (Callinan, 1979; McGladdery, 1984).

On each of two sampling dates, 5 and 12 days after the introduction of infective larvae, four replicate trays with heather present (soil, heather and L3), four with heather absent (soil and L3) and one control tray (soil and heather) were removed and processed. Previous experiments with *T. tenuis* have identified a maximum recovery rate at 10 days after the introduction of larvae, hence days 5 and 12 were chosen to span this period (Saunders *et al.*, 1999). For each tray, larvae were recovered from heather stems (when present), and from rings of soil 0–2 cm, 2–4 cm, 4–6 cm, 6–8 cm, 8–10 cm and >10 cm from the faecal pat using a standard Baermann technique (Saunders *et al.*, 1999).

Do T. tenuis L3 selectively locate and ascend heather plants?

To answer this question, 24 trays containing peat soil were set up with three vertical cane rods, 15 cm tall, placed in each at equal distances around a 10 cm radius from the centre. In 20 of the trays, 0.5 g of fresh heather leaflets, ground up in 0.5 ml of water using a pestle and mortar, was placed at the base of one rod and 0.5 g of grass, again ground up in 0.5 ml water, was placed at the base of another rod. One ml of water was placed at the base of the remaining rod in each tray. After 24 h (to allow chemical gradients to form), approximately 500 *T. tenuis* L3 were introduced to the central point of ten trays, approximately 500 *H. contortus* L3 were introduced to the central point of ten further trays and the four remaining trays were left untreated as controls.

All trays were kept under optimum conditions for larval migration (constant 12°C with a 12L:12D photoperiod) and sprayed twice daily with water to maintain a high relative humidity (75–90%). Twenty-four hours after the addition of the infective larvae, and every 24 h thereafter, rods were removed from each tray and larvae washed into a Petri dish. The rods were replaced and the number of L3 recovered from each was counted under a ×40 binocular microscope. On each of two sampling dates, 5 and 12 days after the introduction of L3, larvae were recovered from soil in five *T. tenuis* trays, five *H. contortus* trays and two control trays. In each tray, soil was ringed into zones of 0–4 cm, 4–8 cm and >8 cm from the central faecal pat and divided into three 120° arcs, with each arc centred on one of the three rods. Infective larvae were recovered from soil samples using the standard Baermann technique.

Statistical analyses

Unless stated otherwise, data were analysed in SPLUS™ using Generalised Linear Models with negative binomial error distributions. Significance levels were calculated, using chi-squared tests, from the deviance explained by each factor following stepwise deletion (Crawley, 1993). During the examination of samples there were unavoidable time delays between actual sampling and the processing of samples for the recovery of larvae (≤5 days). In addition, the length of time for which samples were processed in the Baermann apparatus varied (8–15.5 h). Since these factors may have influenced numbers of larvae recovered, time delay, processing time and sample mass were included as covariates in analyses whenever relevant.

Results

Does the presence of heather influence the horizontal dispersal of *T. tenuis* L3?

No *T. tenuis* larvae were recovered from heather in the control trays. The number of larvae recovered from heather stems in the experimental trays was significantly related to the distance from the caecal pat, with numbers of larvae recovered per gram of heather decreasing with increasing distance from caecal faeces (fig. 1; deviance = 49.91, d.f. = 1, $P < 0.001$). Numbers of larvae recovered on the two sampling dates were not significantly different (fig. 1; deviance = 0.28, d.f. = 1, $P = 0.595$). However, there was a significant interaction between day and distance from caecal faeces – more larvae were recovered from heather at middle distances on day 5 than on day 12 (fig. 1; interaction deviance = 17.32, d.f. = 1, $P < 0.001$).

The number of larvae recovered from soil in trays with heather present also decreased with increasing

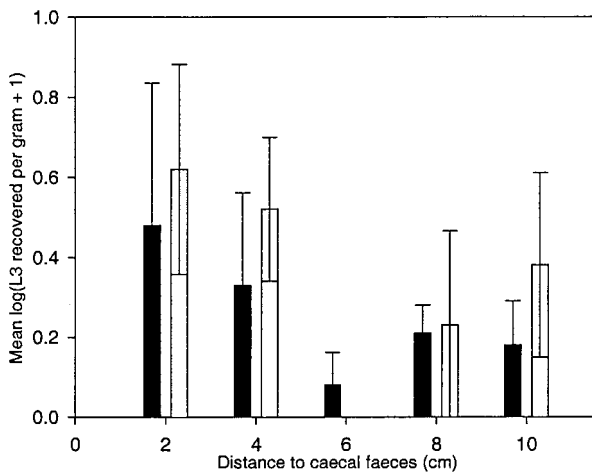


Fig. 1. Recovery of *Trichostrongylus tenuis* infective larvae (L3) from heather stems 5 (■) and 12 (□) days after red grouse caecal faeces were introduced to experimental trays. Numbers recovered (plotted as mean ± S.E.) fitted a negative binomial distribution with arithmetic mean = 7.73 and $k = 1.06$ (Chi-squared test; $\chi^2 = 0.03$, d.f. = 1, $P = 0.862$).

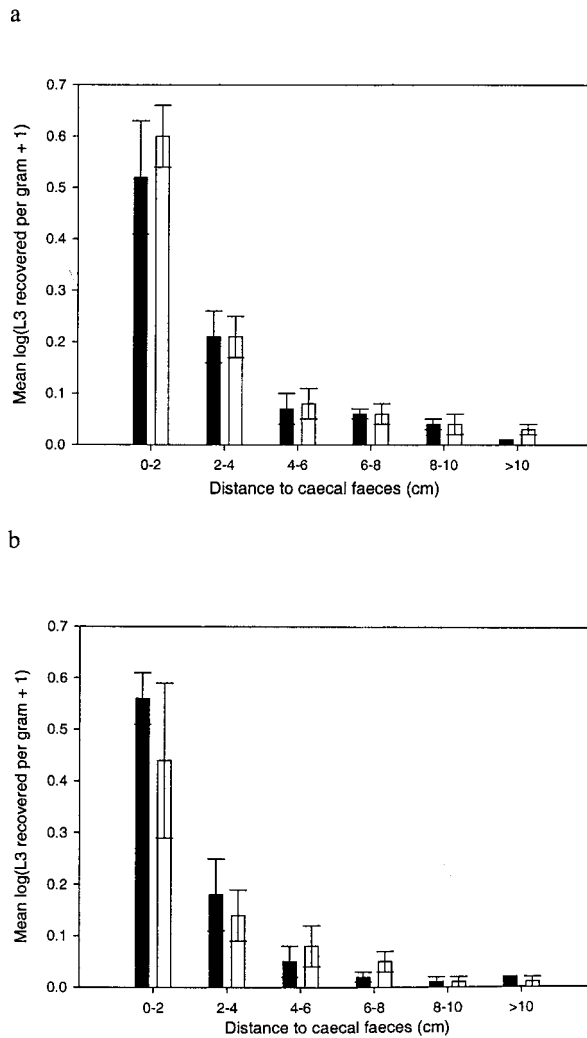


Fig. 2. Recovery of *Trichostrongylus tenuis* infective larvae (L3) from soil 5 (■) and 12 (□) days after red grouse caecal faeces were introduced to experimental trays containing (a) heather stems (numbers recovered (plotted as mean ± S.E.) fitted a negative binomial distribution with arithmetic mean = 16.43 and $k = 0.87$ (Chi-squared test; $\chi^2 = 0.06$, d.f. = 1, $P = 0.802$)) and (b) soil only (numbers recovered (plotted as mean ± S.E.) fitted a negative binomial distribution with arithmetic mean = 15.33 and $k = 0.891$ (Chi-squared test; $\chi^2 = 0.198$, d.f. = 1, $P = 0.657$)).

distance from caecal faeces (fig. 2a; deviance = 98.57, d.f. = 1, $P < 0.001$). Furthermore, significantly more larvae were recovered from soil in trays where *T. tenuis* larvae were added, than from controls (deviance = 77.52, d.f. = 1, $P < 0.001$).

The presence of heather stems had a significant effect on larval recovery, with more larvae being recovered from soil in trays with heather present than from trays with heather absent (fig. 2a,b; deviance = 26.74, d.f. = 1, $P < 0.001$). As before, the number of larvae recovered from soil decreased as the distance from caecal faeces increased (fig. 2a,b; deviance = 46.97, d.f. = 1, $P < 0.001$).

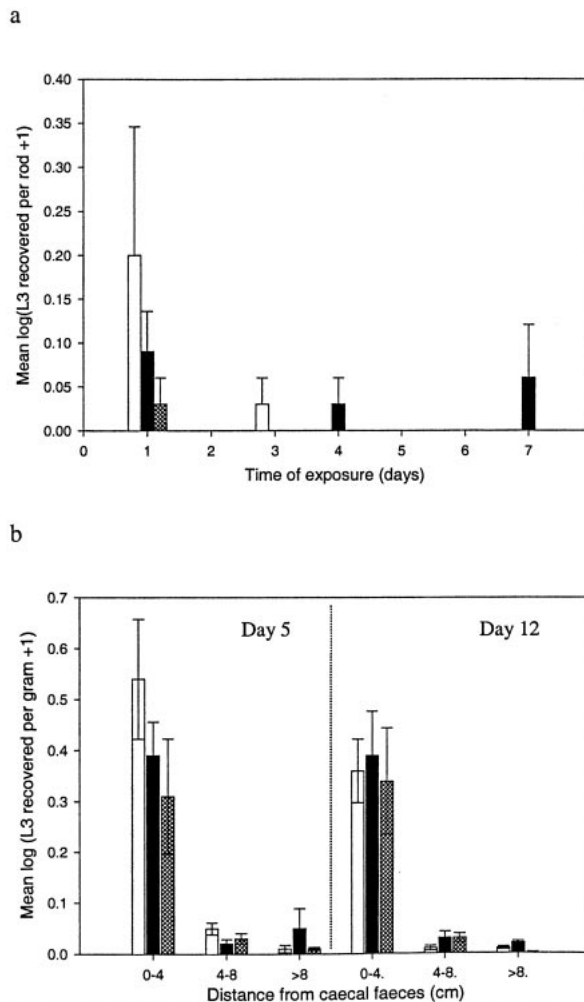


Fig. 3. Recovery of *Trichostrongylus tenuis* infective larvae (L3) from (a) heather (□), grass (■) and control (⊗) rods for 7 days after L3 were introduced to experimental trays (plotted as mean \pm S.E.) and (b) soil closest to heather (□), grass (■) and control (⊗) rods 5 and 12 days after larvae were introduced to experimental trays. Numbers recovered from soil (plotted as mean \pm S.E.) fitted a negative binomial distribution with arithmetic mean = 18.57 and $k = 1.24$ (Chi-squared test; $\chi^2 = 2.16$, d.f. = 3, $P = 0.539$).

Do *T. tenuis* L3 selectively locate and ascend heather plants?

No infective larvae were recovered from either rods or soil in the untreated control trays. Small numbers of *T. tenuis* larvae and only one *H. contortus* larva were recovered from rods over the 12 days of the experiment (fig. 3a), with significantly more *T. tenuis* L3 recovered from the heather and grass rods than from control rods during the first 7 days of the experiment (fig. 3a; deviance = 37.60, d.f. = 1, $P < 0.001$).

For *T. tenuis*, larval recovery from soil decreased as the distance from the caecal faeces increased (fig. 3b; deviance = 891.25, d.f. = 1, $P < 0.001$), with more larvae being recovered on day 5 than on day 12 (fig. 3b;

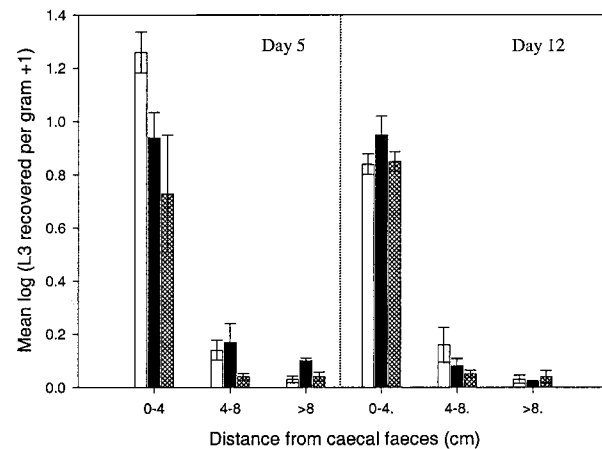


Fig. 4. Recovery of *Haemonchus contortus* infective larvae (L3) from soil closest to heather (□), grass (■) and control (⊗) rods 5 and 12 days after larvae were introduced to experimental trays. Numbers recovered (plotted as mean \pm S.E.) fitted a negative binomial distribution with arithmetic mean = 9.94 and $k = 2.01$ (Chi-squared test; $\chi^2 = 0.389$, d.f. = 2, $P = 0.823$).

deviance = 12.48, d.f. = 1, $P < 0.001$). Greater numbers of L3 were recovered from the soil closest to the heather rods than from the soil closest to the grass or control rods (fig. 3b; deviance = 21.15, d.f. = 1, $P < 0.001$), although the difference was only present at day 5 of the experiment and not at day 12 (fig. 3b; interaction deviance = 31.45, d.f. = 1, $P < 0.001$).

As with *T. tenuis*, *H. contortus* larval recovery from soil decreased as the distance from the caecal faeces increased (fig. 4; deviance = 500.13, d.f. = 1, $P < 0.001$), with more larvae being recovered on day 5 than on day 12 (fig. 4; deviance = 15.59, d.f. = 1, $P < 0.001$). Again, greater numbers of larvae were recovered from the soil closest to the heather rods than from the soil closest to the grass or control rods (fig. 4; deviance = 18.46, d.f. = 1, $P < 0.001$), with the difference only present at day 5 of the experiment and not at day 12 (fig. 4; interaction deviance = 10.36, d.f. = 1, $P = 0.001$).

Discussion

To maximize transmission success, natural selection should favour parasites whose behaviour increases exposure of the parasite to susceptible hosts. Evidence presented here suggests that *T. tenuis* larvae selectively migrate towards heather in response to a specific cue produced by the vegetation, a process which would increase exposure of grouse to infection since red grouse feed predominantly on heather. However, results from a second nematode species, *H. contortus*, whose hosts do not infect animals that feed predominantly on heather, provided similar results indicating that there may be no difference in the selective power of infective larvae.

The first experiment demonstrated that *T. tenuis* larvae can disperse horizontally, over a relatively long period of time. Although *T. tenuis* recovery from heather (fig. 1) and soil (fig. 2) showed no decline with time,

larval migration was limited to just 10 cm from the faecal pat. Infective larvae may be capable of migration over greater distances in the field than observed here, although the high humidity during these experiments provided optimum conditions for dispersal (Callinan, 1979; McGladdery, 1984). Furthermore, Holasova *et al.* (1989) reported that in general, in experiments conducted directly on pastureland, trichostrongyle infective larvae migrate horizontally to a distance of 10–12 cm. Lengthy horizontal migration followed by vertical migration to the heather tips may place heavy demands on larval lipid reserves, and would perhaps leave the larvae with insufficient reserves to successfully infect a new host. The limitation of migration by lipid levels is a likely explanation for the observed limited horizontal dispersal of L3 in both the first and second experiments.

In the field, those *T. tenuis* infective larvae that climb heather in preference to non-food species would be at a selective advantage in terms of transmission to red grouse hosts. In a previous study in which *T. tenuis* L3 were introduced at the base of plant stems, greater numbers of larvae were subsequently recovered from heather than from either a monocotyledon or a dicotyledon with simpler structure (Saunders *et al.*, 1999), suggesting that *T. tenuis* infective larvae selectively ascend heather. Both experiments in this study provide evidence to support the hypothesis that *T. tenuis* L3 exhibit selective migration towards heather.

In the first experiment, the presence of heather stems had a significant effect on larval recovery, with soil yielding greater numbers when heather stems were present than when they were absent (fig. 2). This is surprising, as one would expect fewer and not more larvae in the soil of trays with heather present if larvae were adapted to climb heather. However, the presence of heather could make *T. tenuis* infective larvae proportionately more active in response to chemical cues and in the absence of such cues a proportion of the larvae remained in the caecal pat. If *T. tenuis* L3 move onto heather in response to cues produced by the plants, one would expect them to migrate further in trays with heather stems present than in trays with no heather and thus no cues. However, more larvae were recovered from soil closest to caecal faeces in trays with heather present than in trays without (fig. 2). A possible explanation is that the presence of heather may stimulate the larvae to migrate directly to the nearest stems and L3 were therefore recovered from soil close to the caecal faeces, whereas in the absence of heather cues larvae migrated further from the caecal pat in search of heather.

In the second experiment, both *T. tenuis* and *H. contortus* L3 recovery was greatest from soil in the region of heather rods (figs 3b and 4), implying that larvae selectively migrated in response to a cue produced by the ground-up heather leaflets. Furthermore, of the small numbers of L3 recovered from the rods, significantly more *T. tenuis* L3 were recovered from heather and grass rods with maximum recovery from heather rods (fig. 3a). Since the free-living stages of *H. contortus* achieve transmission to hosts by ascending grass swards on pasture, one may have expected the infective larvae of this species to selectively migrate towards the rods with ground grass at the base. However, *H. contortus* L3 may

be unable to distinguish between heather and grass, perhaps since ruminants will eat both or because grass swards tend to be homogenous and there may not be any need for selection, and, of the two, heather may produce a stronger chemical stimulus thus eliciting a greater migratory response from both species. The fall in *T. tenuis* and *H. contortus* larval recovery from soil with time of exposure (figs 3b and 4) may be accounted for by larval mortality, although the recovery of similar numbers of L3 from soil in the heather, grass and control rod regions on day 12 (figs 3b and 4) suggests that the stimulus produced by the ground heather vegetation in this experiment was relatively short-lived and by day 12 no longer influenced migration patterns.

Since red grouse faeces are highly aggregated on moors (Hudson, 1986), and the distribution of larvae in soil and on heather is shown here to be still highly aggregated even after horizontal migration, the limited dispersal of *T. tenuis* L3 is insufficient to prevent larvae from having a highly aggregated distribution around caecal faeces in the field. We have acquired evidence to suggest, however, that the larvae compensate for this by selectively migrating towards heather, perhaps in response to a stimulus produced by the vegetation, thereby increasing their exposure to red grouse and subsequently increasing their transmission success.

Acknowledgements

We would like to thank Dave Newborn and Rob Foster (Game Conservancy Trust) for collecting red grouse caecal faeces and David Bartley (Moredun Research Institute, Edinburgh) for providing *H. contortus* larvae. Thanks to Ken Wilson for statistical advice and Darren Shaw for the use of his negative binomial goodness of fit test written for SPLUS™. A NERC studentship and support from English grouse moor owners through the Game Conservancy Trust funded this study.

References

- Callinan, A.P.L. (1979) The ecology of the free-living stages of *Trichostrongylus vitrinus*. *International Journal for Parasitology* **9**, 133–316.
- Callinan, A.P.L. & Westcott, J.M. (1986) Vertical distribution of trichostrongylid larvae on herbage and soil. *International Journal for Parasitology* **16**, 241–244.
- Crawley, M.J. (1993) *GLIM for ecologists*. Oxford, Blackwell Scientific Publications.
- Dobson, A.P. & Hudson, P.J. (1992) Regulation and stability of a free-living host-parasite system: *Trichostrongylus tenuis* in red grouse. II. Population models. *Journal of Animal Ecology* **61**, 487–498.
- Grewal, P.S., Lewis, E.E. & Gaugler, R. (1997) Response of infective stage parasites (Nematoda: Steinernematidae) to volatile cues from infected hosts. *Journal of Chemical Ecology* **23**, 503–515.
- Grundler, F., Schnibbe, L. & Wyss, U. (1991) *In vitro* studies on the behaviour of second-stage juveniles of *Heterodera schachtii* (Nematoda: Heteroderidae) in response to host plant root exudates. *Parasitology* **103**, 149–155.

- Holasova, E., Pavlasek, I. & Kotrla, B.** (1989) Migration of infective larvae of sheep gastrointestinal nematodes. *Acta Veterinaria Brno* **58**, 369–378.
- Hudson, P.J.** (1986) *The red grouse. Biology and management of a wild gamebird*. Fordingbridge, Game Conservancy Trust.
- Hudson, P.J.** (1992) *Grouse in space and time*. Fordingbridge, Game Conservancy Trust.
- Hudson, P.J. & Dobson, A.P.** (1997) Transmission dynamics and host-parasite interactions of *Trichostrongylus tenuis* in red grouse (*Lagopus lagopus scoticus*). *Journal of Parasitology* **83**, 194–202.
- Hudson, P.J., Newborn, D. & Dobson, A.P.** (1992) Regulation and stability of a free-living host-parasite system, *Trichostrongylus tenuis* in red grouse. I: Monitoring and parasite reduction experiments. *Journal of Animal Ecology* **61**, 477–486.
- Hudson, P.J., Dobson, A.P. & Newborn, D.** (1998) Prevention of population cycles by parasite removal. *Science* **282**, 2256–2258.
- Lewis, E.E., Gaugler, R. & Harrison, R.** (1992) Entomopathogenic nematode host finding: response to host cues by cruise and ambush foragers. *Parasitology* **105**, 309–315.
- Lewis, E.E., Gaugler, R. & Harrison, R.** (1993) Response of cruiser and ambusher entomopathogenic nematodes (Steinernematidae) to host volatile cues. *Canadian Journal of Zoology* **71**, 765–769.
- MAFF** (1978) *Manual of veterinary investigation laboratory techniques, Part 7 Parasitology*. Reference Book no. 368. Ministry of Agriculture, Fisheries and Food, London, Her Majesty's Stationery Office.
- McGladdery, S.E.** (1984) Behavioural responses of *Trichostrongylus tenuis* larvae in relation to their transmission to the red grouse, *Lagopus lagopus scoticus*. *Journal of Helminthology* **58**, 295–299.
- Rose, J.H.** (1961) Some observations on the free-living stages of *Ostertagia ostertagi*, a stomach worm of cattle. *Parasitology* **51**, 295–307.
- Saunders, L.M., Tompkins, D.M. & Hudson, P.J.** (1999) The dynamics of nematode transmission in the red grouse (*Lagopus lagopus scoticus*): studies on the recovery of *Trichostrongylus tenuis* larvae from vegetation. *Journal of Helminthology* **73**, 171–175.
- Saunders, L.M., Tompkins, D.M. & Hudson, P.J.** (2000) Spatial aggregation and temporal migration of free-living stages of the parasitic nematode *Trichostrongylus tenuis*. *Functional Ecology* **14**, 468–473.
- Wilson, G.P.** (1979) Effects of the caecal threadworm *Trichostrongylus tenuis* on red grouse. Unpublished PhD thesis, University of Aberdeen.

(Accepted 27 November 2000)

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