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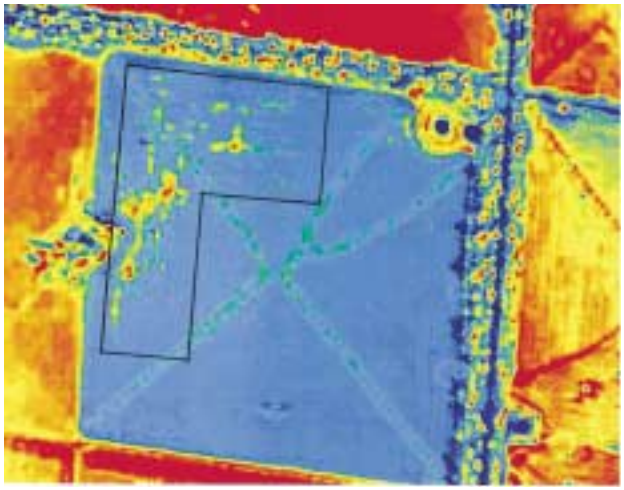

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Nematophagous fungi in pasture: colonisation of sheep faeces and their potential for control of free-living stages of gastro-intestinal nematode parasites of sheep

F. S. Hay[ABC], *J. H. Niezen*[A], *D. Leathwick*[A] *and R. A. Skipp*[A]

[A]AgResearch Grasslands Research Centre, Private Bag 11008, Palmerston North, New Zealand.
[B]Present address: Tasmanian Institute of Agricultural Research, University of Tasmania, North West Centre, PO Box 447, Burnie, Tas. 7320, Australia.
[C]Author for correspondence; e-mail: frank.hay@utas.edu.au

Abstract. Sheep faeces placed on a pasture in New Zealand in February and April were retrieved at intervals up to 28 days and examined for fungi that parasitise nematodes (nematophagous fungi). *Monacrosporium haptotylum*, *Harposporium bysmatosporum*, *H. helicoides* and a *Nematoctonus* sp. were present, respectively, in 37, 22, 13 and 8% of 283 samples retrieved in February and from 29, 14, 11 and 15% of 200 samples retrieved in April. Fungi entered faeces rapidly, being present in 35 and 54% of samples retrieved after 3 days in February and April, respectively. The ability of migrating soil nematodes to carry fungal propagules from soil into faeces was assessed in 2 laboratory experiments. Sheep faeces separated from soil by filters that allowed passage of both nematodes and fungi was colonised by *H. helicoides* more frequently than faeces on filters that excluded nematodes, indicating nematodes were vectors. However, *Arthrobotrys oligospora*, *M. haptotylum* and *A. brochopaga* occurred in faeces independently of filter type, indicating that these species grew into faeces. Two laboratory experiments examined the ability of nematophagous fungi, when inoculated into sheep faeces containing eggs of the gastro-intestinal nematode parasite *Trichostrongylus colubriformis* to reduce the number that developed to third-stage larvae. *Monacrosporium gephyropagum*, *A. cladodes*, *A. conoides* and *A. oligospora* reduced the number of third-stage larvae that emerged by 54–91% (P<0.05) in comparison to a control; however, *M. haptotylum* and *H. bysmatosporum* were ineffective. In a second experiment, several species of nematophagous fungi reduced the number of third-stage larvae that emerged by 41–96% (P<0.05) in comparison to a control, while others had no effect.

Additional keywords: biological control, endoparasitic fungi, nematode-trapping fungi.

Introduction

Gastro-intestinal nematodes of livestock develop from eggs within faeces through 2 bacterial feeding stages (L_1 and L_2) to third-stage larvae (L_3) which migrate out of the faeces into the soil or onto herbage where they can be ingested by livestock and complete the life cycle. Recent studies have shown that emergence of L_3 can be reduced by dosing livestock with fungal parasites of nematodes (nematophagous fungi) that can survive passage through the gut to parasitise nematodes developing in the faeces (Larsen *et al.* 1997). Nematophagous fungi occur naturally in pasture soil and animal faeces (Duddington 1951; Juniper 1957; Fowler 1970; Gray 1983; Mahoney and Strongman 1994). However, the effect of naturally occurring populations of nematophagous fungi on the development of free-living gastro-intestinal nematodes on pasture has not been assessed. Species of gastro-intestinal nematodes can develop within a few weeks to migratory L_3 under optimum conditions. Therefore, the rate at which particular species of nematophagous fungi invade faeces is likely to be an important determinant of their impact on larval emergence. The purpose of these trials was to examine the rate and means by which nematophagous fungi colonise sheep faeces and to assess the ability of nematophagous fungi to reduce emergence of gastro-intestinal nematodes *in vitro*.

Materials and methods

Nematophagous fungi

The following nematophagous fungi were used in this study: endoparasitic nematophagous fungi producing adhesive conidia [*Drechmeria coniospora* (Drechsler) Gams & Jansson, *Hirsutella rhossiliensis* Minter & Brady, *Verticillium balanoides* (Drechsler) Dowsett *et al.*] or ingested conidia [*Harposporium anguillulae* (Lohde) Karling, *H. arthrosporum* Barron, *H. bysmatosporum* Drechsler, *H. helicoides* Drechsler, *H. leptospira* Drechsler, *H. oxycoracum* Drechsler]. Nematode-trapping fungi used were those producing adhesive nets [*Arthrobotrys cladodes* Drechsler, *A. conoides* Drechsler, *A. musiformis* Drechsler, *A. oligospora* Fresenius, *Monacrosporium*

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gephyropagum (Drechsler) Subram.], adhesive knobs [*M. ellipsosporum* (Preuss) Cooke & Dickinson, *Nematoctonus* sp.], adhesive non-constricting rings [*M. leptosporum* (Drechsler) A. Rubner comb. nov.], constricting rings [*A. brochopaga* (Drechsler) Schenck, Kendr. & Pramer], adhesive knobs and non-constricting rings [*M. haptotylum* (Drechsler) Liu & Zhang], adhesive hyphae (*Stylopage* sp.) or adhesive stephanocysts (unidentified resupinate species). All fungi were isolated from sheep faeces on pastures near Palmerston North, New Zealand, except for *H. rhossiliensis*, which was isolated from soil. Nomenclature of nematode-trapping fungi is as described by Rubner (1996).

Field experiment

Eight plots (each 2.5 m^2) were established on an experimental hill-country farm located at Woodville, New Zealand. The predominant pasture species was browntop (*Agrostis capillaris* L.) and plots were mown before the start of experiment. Sheep faeces was collected from bags attached to 6-month-old Romney ram lambs housed indoors and fed a mixture of chaffed meadow hay and lucerne pellets. On 20 February 1995, 36 faecal samples (each about 20 g) were placed 30 cm apart in a 6 by 6 grid pattern within each plot. Six faecal samples from pre-designated points on the grid were re-collected from each plot at 3, 7, 10, 14, 21 and 28 days after placement. On 10 April 1995, the experiment was repeated on separate plots established in close proximity to the original plots. Weather data were collected from a station located about 5 km from the trial site.

Nematophagous fungi were isolated using the soil-sprinkling method (SSM) of Duddington (1955). For each faecal sample, 3 pellets were crushed in a McCartney bottle and half of this mixture sprinkled on the surface of 2% water agar (WA) in a Petri plate. A suspension of about 4000 nematodes (*Cephalobus* sp.) was pipetted onto each plate. *Cephalobus* sp. were maintained on bacteria grown on WA. Plates containing faeces and nematodes were incubated at 21°C for 6 weeks and observed microscopically (×63) at weekly intervals for signs of nematophagous activity. The identity of fungi was confirmed by direct observation of infected nematodes and spores with a compound microscope (×400 and ×1000) or after isolation onto acidified potato dextrose agar (500 mL of 20% lactic acid per 100 mL PDA). Subsamples of faeces were checked by this method before deposition to ensure that faeces was initially free of nematophagous fungi.

Nematode transmission of nematophagous fungi (*experiments 1 and 2*)

Moist Karapoti silt loam from pasture (pH 5.5, 3.0% C, 0.28% N) was pasteurised by microwaving 1 kg quantities for 3 min on full power in a 650-Watt oven to a temperature of 82°C. Faeces was collected in bags attached to 6-month-old Romney ram lambs as described previously. Subsamples of faeces and soil subjected to the SSM before the experiments were shown to be free of nematophagous fungi. Fresh faeces was shown to be free of nematodes by placing subsamples in 9-cm Petri dishes containing 15 mL sterile water at 20°C and examining for nematodes with a microscope (×63) at weekly intervals for up to 3 weeks.

Faeces was placed inside 75-mL screw-top containers (Labserv Ltd) modified by cutting a 35-mm-diameter hole in the lid and affixing filters over the hole with neutral cure silicone rubber (Selleys Ltd). Lids were fitted with either Whatman No. 1 qualitative filters (WF) with a particle retention of 11 µm or cellulose nitrate filters (CNF) with a pore size of 3 µm. Preliminary trials (unpublished results) showed that *Cephalobus* sp. could migrate through WF within 1 day but not through CNF after several days and that all fungi could grow from pieces of PDA colony through WF and CNF filters within 4 days at 20°C.

In experiment 1, nematophagous fungi (*H. helicoides*, *A. oligospora*, *A. brochopaga* and *M. haptotylum*) were subcultured from PDA by aseptically transferring small blocks of agar containing fungal hyphae onto WA in 3 Petri plates. Plates were inoculated with *Cephalobus* sp. (about 3000/plate) and incubated (5 weeks at 20°C). Plates were flooded with sterile water and hyphae, conidia and dead nematodes scraped into suspension. Soil (3.9 kg) was mixed with a total of 79 mL of fungal suspension and 77 mL of a suspension containing a total of 1.1×10^3 *Cephalobus* sp. and placed in a galvanised steel tray. Six faecal pellets were placed in 10 containers of each filter type, inverted and placed onto the soil surface with filters in contact with the soil. Containers were incubated at 20°C in the dark, and soil maintained moist with sterile water. At 47 days, 3 faecal pellets from each container were crushed and half of the faeces examined for nematophagous fungi by the SSM. The presence of *Cephalobus* sp. in faeces was checked by placing 3 faecal pellets from each container in a Petri plate containing 15 mL sterile water at 20°C for 3 weeks and examining the plates microscopically at weekly intervals. To confirm the presence in soil of nematophagous fungi, soil was mixed and 25 g placed in a 250-mL flask with 25 mL sterile water and shaken on an orbital shaker (150 rpm for 15 min). Ten aliquots (100 µL) of a 1/10 dilution of the supernatant were pipetted onto WA and tested for the presence of nematophagous fungi by the SSM.

In experiment 2, suspensions of fungi (*H. helicoides* and *A. oligospora*) were prepared as for experiment 1, following incubation for 2 weeks at 20°C. About 17.4 kg of soil was mixed with 95 mL of fungal suspension and 33 mL of a suspension containing a total of 1.4×10^3 *Cephalobus* sp. Equal quantities of soil were placed in each of 4 galvanised steel trays. Four faecal pellets were placed inside the lid of containers fitted with WF or CNF filters and 10 containers of each filter type were placed on each of the 4 trays. Trays were incubated in the dark at 20°C and soil maintained moist with sterile water. At 47 days, 2 faecal pellets from each container were crushed and half was examined for nematophagous fungi by the SSM. To check for *Cephalobus* sp., 2 faecal pellets from each container were treated as for the first experiment. The presence of nematophagous fungi in soil was checked as for the first experiment, except that 25 g of soil from each tray was extracted and a 1/100 dilution made from the supernatant. Data for experiments 1 and 2 were analysed by Genstat statistical software (Lane *et al.* 1987) by log-linear modelling regression using the model constant + fungal species + filter type + fungal species × filter type. Successive terms were dropped from the model and the model tested for significance by calculating analysis of deviance and a likelihood χ^2.

Pathogenicity of nematophagous fungi to gastro-intestinal nematodes (*experiments 3 and 4*)

In experiment 3, five Romney ram lambs (7 months old) were treated orally with the anthelmintic Arrest (37.5 g/L levamisole and 23.8 g/L albendazole) at the recommended dose of 1 mL/5 kg liveweight. Lambs were housed indoors as described previously. At 5 days after drenching, each lamb was dosed orally with a suspension containing about 5000 third-stage larvae (L_3) of *Trichostrongylus colubriformis*. At 23 days after dosing, faeces was collected in bags attached to lambs at intervals over 12 h and stored at 5°C to slow egg hatch before use the following day. Faeces was mixed and faecal egg count (FEC) determined in 5 subsamples of 10 g using a McMaster counting chamber (Anon. 1986). Faeces was estimated to have a mean of 1393 eggs/g faeces (s.d. = 907). To ensure that faeces was initially free of nematophagous fungi, 2 samples of 3 faecal pellets from each animal were crushed and half of each sample sprinkled onto water agar (2%) and subjected to the SSM.

Fungi were isolated as described previously and maintained on slopes of PDA at 5°C. Fungi were subcultured onto PDA in Petri plates (9 cm diameter) and grown for 4 weeks at 20°C before use. A plug of agar (5 mm diameter) was taken from the edge of each fungal colony and macerated in an eppendorf tube in 700 µL sterile water and added to 9.5 mL sterile water. To check the viability of inoculum, 50 mL of a 1/10 dilution of each fungus was pipetted onto WA in a Petri plate and subjected to the SSM. For each treatment, weighed amounts of faeces

(3.6–4.2 g) were placed in 6 plastic containers and each inoculated with 1 mL of fungal macerate or a macerate of PDA only for the control. Containers were gently rotated to ensure that faecal pellets came into contact with suspension. Faeces was placed in the lid of a 6-cm-diameter Petri plate inside a 9-cm-diameter Petri plate. The cavity between the outer and inner plate was filled with sterile water to about one-third of the height of the Petri plate to trap L_3 migrating from the faeces. The lid of the outer Petri plate was fitted and plates incubated unsealed at 20°C for 28 days. The cavity between the outer and inner plates was topped up with water as required. Larvae that had migrated into the cavity were concentrated to a standard volume by sedimentation and counted in a slide chamber under a microscope (×63). Three faecal pellets from each plate were subjected to the SSM to check for the presence of inoculated fungi within faeces. For each plate, the mean number of L_3 that emerged per gram of faeces was analysed by single-factor ANOVA using the Genstat statistical package (Lane *et al.* 1987) and the means were separated by least significant difference (l.s.d.).

In experiment 4, nematophagous fungi were obtained and cultured as described for experiment 3. For inoculum, 2 plugs (5 mm diameter) from the edge of each fungal colony were macerated in an eppendorf tube in 500 mL sterile water and added to 9.5 mL sterile water. To check the viability of inoculum, 50 mL of a 1/10 dilution of each fungus was pipetted onto WA in a Petri plate and subjected to the SSM. Faeces was collected from a single Romney ram lamb as described for experiment 3, except that the lamb received 10000 L_3 *T. colubriformis* at 4 days after oral administration of anthelmintic. At 24 days after dosing, faeces was collected over a period of 24 h in bags attached to lambs. Faeces was stored at 10°C to slow egg hatch and used for experiments within 2 days of collection. FEC was determined in 7 subsamples (10 g) of faeces and the mean number of eggs calculated as 379 eggs/g (s.d. = 52). To ensure that faeces was initially free of nematophagous fungi, 6 samples of faeces consisting of 3 pellets each were crushed in McCartney bottles and half of each sample sprinkled over the surface of water agar (2%) in Petri plates and subjected to the SSM.

Six faecal pellets, totalling between 1.9 and 2.8 g, were weighed and placed in a 6-cm-diameter Petri plate placed inside a 9-cm-diameter Petri plate. Faeces in each Petri plate was inoculated with 750 mL of a suspension of fungal macerate or of PDA for the control, prepared as above and the outer plate covered with a lid. For each fungus, 5 Petri plates were incubated at each of 15 and 20°C. After 44 days of incubation, water from the moat between the inner and outer Petri plates containing nematodes was poured into McCartney bottles and stored (10°C). Nematodes were also extracted from crushed faecal pellets placed on Whatman No. 1 filter paper suspended over sterile water by a plastic grid in a Petri plate. Suspensions of nematodes were collected after 48 h at room temperature and added to those collected previously. Suspensions were concentrated by sedimentation to 10 mL and the number of nematodes counted in a modified slide chamber. The number of nematodes was expressed per gram of faeces, analysed by the Genstat statistical package (Lane *et al.* 1987) and means separated by least significant difference (l.s.d.).

Results and discussion

Field experiment

Several species of nematophagous fungi with different mechanisms of parasitising nematodes were found to colonise faeces placed on pasture (Tables 1 and 2). *Monacrosporium haptotylum*, *H. bysmatosporum*, *H. helicoides* and a *Nematoctonus* sp. were the most common colonists. In agreement with other studies (Hay *et al.* 1997*a*, 1997*b*, 1998), fungi entered faeces rapidly, with 35 and 54% of faecal samples colonised at 3 days after placement in February and April, respectively. A high proportion of faecal samples became colonised, with 64–76% of samples colonised between 7 and 21 days after placement in February and 50–61% colonised over the same period after placement in April. Some differences in colonisation time were apparent between the times of placement, with the *Nematoctonus* sp., *H. anguillulae* and *V. balanoides* present in faeces at 10 days after placement in February and only 3 days in April. Similarly, more species of fungi were present in April in comparison to February. This suggested that conditions were more favourable for fungal invasion in April. The low level of recovery of these fungi in

Table 1. The percentage of faecal samples colonised by nematophagous fungi at intervals after placement in February and the percentage of the total number of faeces colonised

	Faecal samples colonised (%)						Total no. of samples colonised (%)
	3 days	7 days	10 days	14 days	21 days	28 days	
All nematophagous fungi	35.4	68.8	63.8	71.7	66.7	76.1	63.6
Endoparasitic fungi	20.8	33.3	38.3	52.2	33.3	50.0	37.8
H. bysmatosporum	8.3	18.8	23.4	30.4	16.7	32.6	21.6
H. helicoide	12.5	10.4	17.0	17.4	10.4	8.7	12.7
H. anguillulae	0	2.1	2.1	2.2	6.3	2.2	2.5
H. leptospira	0	0	0	2.2	0	2.2	0.7
Unidentified *Harposporium* spp.	0	0	0	2.2	0	2.2	0.7
V. balanoides	0	2.1	2.2	2.2	0	6.5	2.1
D. coniospora	0	0	0	2.2	0	0	0.4
Nematode-trapping fungi	18.8	47.9	51.1	32.6	47.9	54.3	42.0
M. haptotylum	18.8	41.7	42.6	32.6	45.8	41.3	37.1
Nematoctonus sp.	0	8.3	14.9	2.2	10.4	10.9	7.8
Unidentified net-forming fungus	0	0	0	0	0	2.2	0.4
Number of faecal samples retrieved	48	48	47	46	48	46	237

our experiments makes it difficult to determine whether this seasonal difference represents a real effect. However, similar seasonal effects have been obtained in other studies (Hay *et al.* 1997*a*, 1997*b*, 1998) and Persmark *et al.* (1996) also reported seasonal fluctuations in nematophagous fungi in soil with higher densities and a greater variety of fungi present in autumn.

Soil and air temperatures were similar in February and April before and following the placement of faeces. The average mean daily soil and air temperatures for 15 days before placement of faeces in February were 18.5 and 16.8°C, respectively, and in April 17.3 and 16.6°C, respectively. The average mean daily soil and air temperatures for 15 days following placement of faeces were 18.2 and 16.3°C, respectively, in February and 15.4 and 13.9°C, respectively, in April. However rainfall was higher in April. Cumulative rainfall for the 15 days preceding placement of faeces in February was 8 mm compared with 102 mm in April, while for the 15 days following placement cumulative rainfall was 54 mm in February and 35 mm in April. Increased soil moisture in April would have been conducive to fungal growth and movement of potential vectors of nematophagous fungi such as soil nematodes. In addition, faeces would have been prevented from drying and hardening in April, thereby facilitating entry. Faeces also disappeared more rapidly in April compared with February, with few faecal samples retrieved on day 21 and day 28 (Table 2). Earthworms and their castes were noted frequently amongst faeces in April suggesting that faeces disappearance was related to increased earthworm activity as a result of higher soil moisture. Few species of dung burying beetles are present in New Zealand and faecal degradation arises mainly from weathering and the activity of micro-organisms and invertebrates such as earthworms (Dymock 1993). The rate of faeces burial is also likely to have a major influence on the interaction between nematophagous fungi and survival of gastro-intestinal nematodes.

Fungi reached a high level of infestation in faeces by 7 days, with little or no further increase with longer periods of deposition. The nematophagous fungi that invade faeces soon after deposition may be better able to establish than fungi that enter later, because of less competition from other organisms. Alternatively, the absence of increased faecal infestation with time may result from aged faeces becoming less attractive to potential vectors of nematophagous fungi such as soil nematodes, leading to less carriage of fungi into it.

Nematode transmission of nematophagous fungi

The rapid appearance in faeces of endoparasitic fungi such as *Harposporium* spp. reported in this study and elsewhere (Hay *et al.* 1997*a*, 1998) is surprising, given that such species are essentially obligate in soil. Nansen *et al.* (1988) suggested that conidia and traps of nematophagous fungi might be carried by nematodes that become infected in the soil and migrate into the faeces. Soil nematodes can enter faeces rapidly following placement (Hay *et al.* 1998) and nematophagous fungi produce a number of propagules that can be transported by soil nematodes *in vitro* (Barron 1977). All fungi were retrieved from soil by the SSM in experiments 1 and 2. In both experiments, nematodes were obtained only

Table 2. The percentage of faecal samples colonised by nematophagous fungi at intervals after placement in April and the percentage of the total number of faeces colonised

+, fungi present but insufficient samples to calculate percentage colonisation; —, fungi not present

	Faecal samples colonised (%)						Total no. of samples
	3 days	7 days	10 days	14 days	21 days	28 days	colonised (%)
All nematophagous fungi	54.2	60.9	50.0	60.4	+	—	55.5
Endoparasitic fungi	33.3	32.6	14.6	47.9	+	—	32.5
H. bysmatosporum	18.8	6.5	6.3	20.8	+	—	13.5
H. helicoides	8.3	17.4	4.2	14.6	—	—	10.5
H. anguillulae	4.2	6.5	6.3	8.3	+	—	7.5
Unidentified *Harposporium* spp.	0	0	0	2.1	—	—	0.5
V. balanoides	2.1	2.2	0	0	—	—	1.0
D. coniospora	0	0	0	4.2	—	—	1.0
Total nematode-trapping fungi	37.5	45.7	43.8	37.5	—	—	39.0
M. haptotylum	27.1	39.1	25	31.3	—	—	29.0
Nematoctonus sp.	18.8	13.0	20.8	8.3	—	—	14.5
M. gephyropagum	2.1	2.2	0	0	—	—	1.0
Unidentified net-forming fungus	0	2.2	0	0	—	—	0.5
Stylopage sp.	0	2.2	0	0	—	—	0.5
Unidentified constricting ring-forming fungus	0	0	2.1	0	—	—	0.5
Number of faecal samples retrieved	48	46	48	48	8	2	200

from faeces placed on 11-μm filters and not on 3-μm filters, indicating that 3-μm filters had successfully prevented nematode movement (Table 3). The deviance statistic associated with dropping the interaction term from the log-linear model (constant + fungal species + filter type + fungal species × filter type) had a probability of 0.05 in experiment 1 and <0.001 in experiment 2. Therefore, both experiments indicated a statistically significant interaction between fungal species and filter type. This suggested significantly fewer *H. helicoides* and significantly more other fungal species passed through the 3-μm filter than would be expected under the null hypothesis, indicating that nematodes were important for the movement of *H. helicoides* but not the other fungal species tested.

Harposporium helicoides produces conidia that are ingested by bacterial feeding nematodes such as *Cephalobus* sp. and nematodes could migrate some distance before infection and death. However, the presence of *H. helicoides* in a few faecal samples on 3-μm filters that excluded nematodes indicated that this fungus could also grow from soil into faeces, perhaps from parasitised nematodes that had migrated close to the filter. Under certain conditions, *A. oligospora* can produce conidial traps in the presence of faeces that can be transported by nematodes (Dackman and Nordbring-Hertz 1992). However, the entry of *A. oligospora* into faeces was not enhanced by nematodes in our experiments indicating that it grew into faeces. *Monacrosporium haptotylum* produces adhesive knobs and non-constricting rings which can attach to nematodes and be broken away from the parent hyphae, suggesting that it could be transported by nematodes. In contrast, *A. brochopaga* produces constricting rings either on hyphae or as conidial traps. The nature of the trap is to crush the nematode so nematodes are unlikely to act as vectors of this fungus. The presence of *M. haptotylum* and *A. brochopaga* in a similar number of faecal samples on either filter type in experiment 1 suggested that they also grew into faeces. The presence of nematodes in soil is probably important for faecal colonisation even for those species of nematophagous fungi that are not transported by nematodes, by acting as a nutrient source from which fungi can grow from soil into faeces.

While the transmission experiments demonstrated that some fungi could be transported by nematodes from soil into faeces or could grow from soil into faeces, other means of entry may occur in the field. Splash dispersal of fungal propagules by rainfall and carriage by soil and faecal-dwelling invertebrates such as mites and earthworms might also assist rapid entry. Some nematophagous fungi occur amongst the foliage of plants (Goodey 1938; Hay and Regnault 1995) or are present in litter layers (Gray and Bailey 1985) where they would be well-placed to grow into faeces. In addition, some species of nematophagous fungi can survive ingestion and excretion by livestock, although ingestion and excretion of naturally occurring nematophagous fungi is believed to occur infrequently (Larsen *et al.* 1994).

Pathogenicity of nematophagous fungi to gastro-intestinal nematodes

In experiment 3, 2 isolates each of *A. cladodes*, *A. oligospora* and *M. gephyropagum* and one isolate of *A. musiformis* and *A. conoides* were able to reduce the number of *T. colubriformis* which developed from egg to L_3 in comparison to untreated faeces by 54–91% (Table 4). Three *Harposporium* spp. and *M. haptotylum* had no effect

Table 3. Transmission of nematodes (*Cephalobus* sp.) and nematophagous fungi into faeces separated from infested soil by Whatman filters (WF) or cellulose nitrate filters (CNF) after 47-day exposure

Organism isolated	No. of samples from which organism isolated	
	WF (11 μm)[A]	CNF (3 μm)
Experiment 1, total faecal samples = 10		
Cephalobus sp.	10	0
H. helicoides	8	0
A. oligospora	7	5
A. brochopaga	4	3
M. haptotylum	3	3
Likelihood $\chi^2 = 7.83$, 3 d.f. ($P = 0.05$)		
Experiment 2, total faecal samples = 40		
Cephalobus sp.	40	0
H. helicoides	40	7
A. oligospora	14	16
Likelihood $\chi^2 = 12.9$, 1 d.f. ($P<0.001$)		

[A]Pore size of filter.

Table 4. Number of third-stage larvae (L_3) of *Trichostrongylus colubriformis* emerging from sheep faeces treated with different isolates of species of nematophagous fungi after 28 days at 20°C (experiment 3)

	Mean no. of larvae (L_3/g faeces)	Significance
Control	51.0	
H. leptospira	58.6	n.s.
M. haptotylum	53.0	n.s.
H. leptospira	46.0	n.s.
H. bysmatosporum	36.4	n.s.
M. gephyropagum	23.6	*
A. cladodes	20.9	*
A. musiformis	16.2	*
M. gephyropagum	15.1	*
A. cladodes	14.8	*
A. conoides	13.4	*
A. oligospora	5.7	*
A. oligospora	4.4	*

n.s., means not significantly different from the control; *, means significantly ($P<0.05$) different from the control.

Table 5. Number of third-stage larvae (L_3) of *Trichostrongylus colubriformis* emerging from sheep faeces treated with different isolates of species of nematophagous fungi after 44 days (experiment 4)

	Mean no. of larvae (L_3/g faeces)	Significance
Control	209.6	
H. arthrosporum	271.7	n.s.
Unidentified resupinate fungus	256.4	n.s.
H. oxycoracum	249.6	n.s.
H. anguillulae	218.8	n.s.
M. ellipsosporum	209.1	n.s.
H. rhossiliensis	208.5	n.s.
Nematoctonus sp.	205.8	n.s.
H. anguillulae	180.7	n.s.
M. ellipsosporum	177.4	n.s.
M. leptosporum	171.1	n.s.
H. helicoides	169.1	n.s.
H. helicoides	168.1	n.s.
D. coniospora	168.0	n.s.
H. leptospira	151.0	n.s.
H. leptospira	145.7	n.s.
M. haptotylum	134.1	n.s.
V. balanoides	123.6	*
M. haptotylum	121.1	*
A. cladodes	117.8	*
H. bysmatosporum	116.5	*
H. bysmatosporum	112.7	*
H. arthrosporum	102.0	*
Nematoctonus sp.	98.7	*
A. musiformis	84.2	*
A. conoides	58.0	*
M. gephyropagum	33.4	*
A. oligospora	20.6	*
M. gephyropagum	16.8	*
A. cladodes	8.1	*

n.s., means not significantly different from the control; *means significantly (P<0.05) different from the control.

on larval emergence. In experiment 4, incubation temperature (15 or 20°C) had no significant effect (P = 0.756) on the number of *T. colubriformis* developing from egg to L_3 emerging from faeces treated with different isolates of species of nematophagous fungi. The main effect of isolate (averaged over temperature) was statistically significant (P<0.001). Two isolates each of *A. cladodes*, *M. gephyropagum*, and *H. bysmatosporum* and one isolate each of *A. oligospora*, *A. conoides*, *A. musiformis*, *H. arthrosporum*, *M. haptotylum* and *V. balanoides* reduced the number of by 41–96% (Table 5). Several other fungi were ineffective. The interaction between fungi and temperature was not significant (P = 0.11) (data not shown).

This study and several other laboratory studies (e.g. Waller and Faedo 1993) have demonstrated that a wide variety of species of nematophagous fungi are active against the free-living stages of gastro-intestinal nematodes of livestock. Endoparasitic fungi generally did not perform as well as nematode-trapping types against *T. colubriformis* in our study. Endoparasitic species which parasitise nematodes by means of ingested conidia would only be active against the L_1 and L_2 bacterial-feeding stages, while nematode trapping fungi are active against all stages. Inoculation of nematophagous fungi to the faeces surface would have disadvantaged endoparasitic fungi that have poor saprophytic ability and therefore less ability to grow into the faeces and contact nematodes in comparison to nematode-trapping fungi. In nature there would be opportunity for infected nematodes to carry endoparasitic fungi from the soil to inside the faeces where they may be more effective. When mixed with faeces, endoparasitic species such as *H. anguillulae* have been shown to be particularly effective against gastro-intestinal nematodes in laboratory tests (Charles *et al.* 1996).

Conclusions

Niezen *et al.* (1998) demonstrated that on a hill-country farm in New Zealand, larvae of *T. colubriformis* appeared on herbage at 2 weeks after placement of faeces in summer and autumn. In summer, significant numbers of larvae continued to be recovered from faeces at 2 and 4 weeks after placement, but in autumn faeces had disappeared from the pasture by 4 weeks. Our results demonstrate that a range of species of nematophagous fungi can enter faeces naturally from the soil within a few days of faecal deposition and that many of these fungi are active against free-living stages of *T. colubriformis*. Furthermore, it has been shown that the rapid entry of some fungi could at least in part be explained by microbial-feeding nematodes carrying propagules of nematophagous fungi from the soil as they migrate into the faeces. The rapid entry of naturally occurring nematophagous fungi suggests that they could have an impact on populations of gastro-intestinal nematodes on pasture. However, the level of control is unknown. Trials in which the fungus *Duddingtonia flagrans* (Duddington) Cooke has been incorporated into faeces either artificially or through ingestion by livestock before deposition have shown a significant reduction in the number of gastro-intestinal nematodes, which subsequently develop on pasture plots in comparison to plots receiving faeces not containing the fungus (Grønvold *et al.* 1993). Such trials demonstrate that the level of naturally occurring biological control could be significantly enhanced by introducing a particular species of fungus to faeces at, or soon after, deposition. This might indicate that although naturally occurring nematophagous fungi are entering faeces at an early stage, they may be at too low a population density to exert a major influence on nematode numbers in comparison to fungi that are artificially inoculated into faeces. However, the development of pasture management practices that increase populations of soil-living nematodes and their associated nematophagous fungi and other predators might

enhance the colonisation of parasite-infested faeces by nematophagous fungi. Such practices may provide options for integrated parasite control in organic or low chemical-input systems. Further studies are required to determine the extent to which naturally occurring nematophagous fungi influence populations of gastro-intestinal nematodes on pasture.

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