The ability of the nematode-trapping fungus *Duddingtonia flagrans* to reduce the transmission of infective *Ostertagia ostertagi* larvae from faeces to herbage

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Abstract

The ability of two isolates of the nematode-trapping fungus *Duddingtonia flagrans* to reduce the numbers of gastrointestinal nematode larvae on herbage was tested in three plot studies. Artificially prepared cow pats containing *Ostertagia ostertagi* eggs, with and without fungal spores, were deposited on pasture plots two or three times during the grazing season in 1995, 1996 and 1997. The herbage around each pat was sampled fortnightly over a period of 2 months and the number of infective larvae was recorded. At the end of the sampling period, the remainder of the faecal pats was collected to determine the wet weight, dry weight, and content of organic matter. The infective larvae remaining in the pats were extracted. Faecal cultures showed that both fungal isolates significantly reduced the number of infective larvae. Significantly fewer larvae were recovered from herbage surrounding fungus-treated pats compared with control pats in all three experiments, reflecting the ability of the fungus to destroy free-living larval stages in the faecal pat environment. After 8 weeks on pasture there were no differences between control and fungus-treated pats with respect to wet weight, dry weight, and organic matter content. This indicates that the degradation of faeces was not negatively affected by the presence of the fungus.

Introduction

Duddingtonia flagrans is a nematode-trapping fungus which forms adhesive three-dimensional hyphal nets in which nematodes are trapped (Cooke, 1969). Its role as a biological agent against parasitic nematodes of cattle has

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been studied in Denmark since Larsen et al. (1991) demonstrated that different isolates of this fungus survive in vitro stress selection simulating the passage through the gastrointestinal tract of cattle, and trap third stage larvae of Ostertagia ostertagi in a cow dung pat bioassay. Later, Larsen et al. (1992) confirmed these results in an in vivo experiment, feeding calves with fungal material and re-isolating six out of seven isolates of D. flagrans given to the animals. When tested for their capacity to reduce the number of nematode free-living larvae, the six fungal isolates showed a reduction percentage between 76 and 99%. Grønvold et al. (1993) tested two of these isolates in a plot experiment designed to simulate natural conditions, by depositing faecal pats on pasture. This study showed that herbage surrounding faeces collected from calves that were fed fungi contained 74–85% fewer O. ostertagi L_3 than that surrounding not-fungus-treated faeces. However, the deposition of pats took place only once in the early part of the grazing season.

The objectives of the present experiments were to study the capability of D. flagrans to reduce the transmission of infective larvae of O. ostertagi to the herbage around cow pats when deposited two or three times during three consecutive grazing seasons in Denmark, and to determine whether or not the presence of the fungus affects the degradation of faeces. This study involved testing two of the D. flagrans isolates separately (1995 and 1996) and in a combined experiment (1997).

Materials and methods

Experimental design

In 1995 and 1996, faeces were collected from two groups of ten grazing calves each infected with O. ostertagi. One group was fed fungal material plus a commercial supplement daily at a concentration of 10^6 fungal units/kg of body weight, while the other group received only the supplement. In 1997, faeces from fungus-fed, parasite-free calves were mixed 1:1 (w:w) with faeces containing O. ostertagi eggs from infected donor calves. A final concentration of approximately 150–300 eggs per gram (epg), and an amount of fungal spores equivalent to daily treatment of the animals with the concentration used in 1995/96 was established. Faeces from parasite-free animals not fed with the fungus were mixed with faeces containing O. ostertagi eggs to produce material for control pats.

On two (1996) or three (1995 and 1997) occasions during the grazing season, manually formed faecal pats were randomly deposited on newly mown pasture plots. Each individual square plot was spaced from the neighbouring plots so the herbage in between could be mowed by a lawnmower at regular intervals during the trials. The plot areas were covered with a net to protect them from birds. In the centre of each plot area a 1 kg faecal pat was deposited. Following deposition, herbage was sampled every 14 days for a period of 2 months by collecting $2 \times 1/8$ of a circle area extending 25–30 cm from the periphery of each pat, as previously described by Fernández et al. (1997). The herbage was washed and the total number of O. ostertagi L_3 determined. There were

five replicates/group in 1995 and 1997, and ten replicates/group in 1996.

In 1996 and 1997, the faecal pats were deposited onto a nylon net, mesh size 8 mm. At the time of the last herbage collection, the remaining faeces on the net from each individual plot were collected and weighed. The dry weight was determined (except in 1996) by drying a small portion (< 50 g) of the faecal material overnight at 110°C. Following this, the organic matter content was determined according to the method of Sommer et al. (1992). From the remaining faecal material, larvae were extracted by baermannization (Grønvold, 1984) and subsequently counted.

Faecal cultures of the same isolate mixtures were set up each time faeces were deposited on the herbage plots, and the number of developed infective O. ostertagi larvae determined.

Fungal material

The D. flagrans isolates used were 'CI3', previously selected by Larsen et al. (1991, 1992), and 'Troll A', described by Grønvold et al. (1996). In 1995 and 1996, the D. flagrans isolates were cultivated for 3–4 weeks on barley grains (Grønvold et al., 1993). After washing off in tap water and straining through a nylon mesh (100 μm mesh size), the chlamydospores were collected and counted using a haemocytometer (Fuchs-Rosenthal). Fungal material of both isolates, cultivated on millet seeds, was provided by CHBS A/S, Hørsholm, Denmark.

Experimental animals

Parasite-naive Friesian and Jersey calves, 6–8 months old, were used in 1995 and 1996. The animals were artificially infected by oral administration of 5000 L_3 of O. ostertagi and after nematode eggs started to appear in their faeces, half of them were fed 10^6 fungal units/kg of body weight daily for 2 months. In 1997, 6- to 9-month-old bull Friesian calves were used either as O. ostertagi egg donor calves (each calf receiving 5000 L_3 per os) or as experimental calves, which were fed 2×10^6 units/kg of body weight daily for 4 consecutive days. Faeces from the fungus-fed animals were collected on the third day. Faeces without parasite eggs were collected from non-infected bullocks housed at the same facility.

Experimental plots

Three different pastures at the Royal Veterinary and Agricultural University Research Farm, situated outside Copenhagen, were used for this study. The vegetation at Pasture 1 was poor, and comprised mainly dandelion and some clover and grasses. The pasture had been grazed by cattle the year before, but no trace of nematode infectivity was detected when the pasture was sampled prior to the start of the experiment. Pasture 2 presented a much more dense ground cover, and comprised primarily couch grass. The vegetation in Pasture 3 was again mainly couch grass, but with some clover and grasses. These two pastures had not been previously grazed by animals.

The plots (approximately 85×85 cm each) were mowed

just prior to deposition of faeces to a herbage height of approximately 10 cm. In 1997, Pasture 3 was artificially irrigated. At the beginning of September, 30 mm of water were applied three times over a period of 14 days.

Parasitological procedures

The number of nematode eggs was determined using a modified McMaster method (Roepstorff & Nansen, 1998). Faecal cultures were established using a modification of the technique described by Henriksen & Korsholm (1983). Instead of placing the incubation chambers in moisture boxes, polystyrene cups containing 8 ml water were used. Cultures were kept for 2 weeks in a climatic chamber (Termak A/S, model KBP 6395 FL) at 25°C and 95% humidity before harvest and counting. The number of larvae found in herbage was determined using an agar-gel method (Jørgensen, 1975; Mwegoha & Jørgensen, 1977).

Statistical analysis

The statistical software package GraphPad Prism (GraphPad Software, Inc.), version 2.00, was used for data storage and analysis. The difference in cumulative numbers of infective larvae recovered from herbage around deposited dung from fungus-treated and untreated control animals, respectively, were tested using the Mann-Whitney U-test (Siegel, 1956). One-way analysis of variance and t-tests were performed to detect differences between the groups for faecal cultures, pat weights, dry weight of faeces, organic matter content, and infective larvae per gram of remaining pats.

Meteorological observations

Precipitation and temperature data (fig. 1) were provided by the Laboratory for Agrohydrology and Bioclimatology, Department of Agricultural Sciences, Royal Veterinary and Agricultural University. The climate station was located 500 m from the nearest pasture and 1500 m from the farthest one.

Results

There were significant differences in larval recovery between cultures derived from untreated animals and cultures from fungus-treated groups, except for Troll A-treated calves in Series I, 1996 and Series II, 1997 (table 1). With isolate CI3, larval reduction ranged from 30.3% to 95.7%, while for the Troll A isolate the reduction varied from 18.1% to 98.1%.

Fewer larvae were recovered from herbage around fungus-treated pats compared with control pats in all three studies (figs 2–4). Significant differences in the cumulative number of infective larvae (table 2) were detected between treated and control groups for both fungi and in all three studies, except in Series I, 1995, and Series II, 1996. For these series, statistical analysis was not performed and any reduction in larval numbers was not calculated due to the very low number of larvae recovered from herbage samples. No significant differences with regard to parasite reduction were found
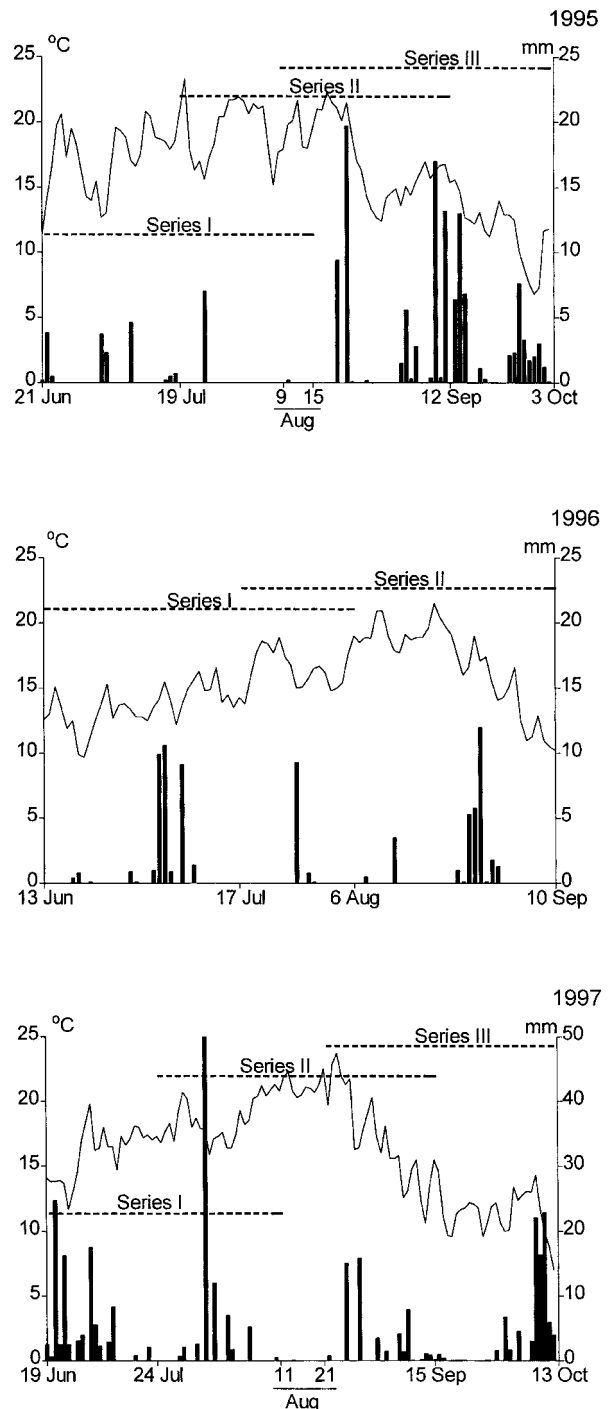

Fig. 1. Meteorological data. Daily precipitation and daily mean temperature recorded during the three plot trials conducted in 1995–1997. Lines represent the temperature, in Celsius grades; bars represent the precipitation, in mm.

Table 1. Reduction effect of *Duddingtonia flagrans*, isolates CI3 and Troll A, upon free-living stages of *Ostertagia ostertagi* in faecal cultures.

	1995					1996					1997				
	EPG	LPG	Yield %	Relative %	Reduct. %	EPG	LPG	Yield %	Relative %	Reduct. %	EPG	LPG	Yield %	Relative %	Reduct. %
Series I															
Control	80	77	96.3	100.0	–	287	245	85.4	100.0	–	167	244	146.1	100.0	–
CI3	100	26	26	26.9	73.1***	–	–	–	–	–	107	109	101.8	69.7	30.3*
Troll A	–	–	–	–	–	60	42	70.0	81.9	18.1	193	107	55.4	37.9	62.1***
Series II															
Control	80	55	68.8	100.0	–	27	19	70.4	100.0	–	284	223	78.5	100.0	–
CI3	80	3	3.8	5.5	94.5***	–	–	–	–	–	276	35	12.7	16.2	83.8***
Troll A	–	–	–	–	–	7	0.1	1.4	1.9	98.1	244	135	55.3	70.4	29.6
Series III															
Control	50	15	30.0	100.0	–						104	99	95.2	100.0	–
CI3	40	0.5	1.3	4.3	95.7***						128	75	58.6	61.5	38.5***
Troll A	–	–	–	–	–						116	41	35.3	37.1	62.9***

Faecal cultures were set up in triplicate (1995–1996) or quintuplicate (1997) at the beginning of each series of the plot trials. The larvae yield was calculated based on the number of eggs per gram of faeces (epg) and the number of infective larvae per gram of faeces (lpg) extracted after 2 weeks of incubation. The relative percentage of larval development in fungus-treated cultures was estimated with the yield of the control cultures as 100% of relative development. *$P < 0.05$; ***$P < 0.001$.

between the two fungal isolates in 1997. The herbage infectivity around faecal pats treated with the CI3 isolate was reduced by 85.2% in both Series II and III in 1995, and by 76.0–84.0% in 1997. For the Troll A isolate, this reduction was 40.8% (Pasture 1) and 30.1% (Pasture 2) in Series I, 1996, and 73.0–81.9% in 1997. Since the initial numbers of eggs in faecal pats were different for both groups in 1996, the reduction percentage for Series I was calculated based on the assumption that the faecal pats of both groups contained equal numbers of eggs at the time of deposition, thus extrapolating the actual numbers of larvae found in the herbage.

No significant differences were detected between the mean weights of fungus-treated pats and their controls in 1996 (table 3). In the first series of this year, the faecal pats recovered from both fungus-treated and control groups in Pasture 2 were bigger and heavier than those in Pasture 1, but no statistical difference was detected between the two pastures where the pats were located. In 1997, the CI3 isolate-treated pats weighed significantly less than the control pats only in Series III.

The mean dry weight values for control, CI3 isolate-treated, and Troll A isolate-treated groups in 1997, respectively, were as follows: 54.0, 54.3, and 51.0% for Series I, 32.9, 29.5, and 24.6% for Series II, and 19.1, 17.1, and 20.9% for Series III. No statistical differences between groups were found, except in Series II for pats treated with the isolate Troll A compared with control pats ($P < 0.05$).

The percentage of organic matter content in pats in 1996 varied from 71.0 to 72.6% (Series I) and 74.4 to 75.6%

Table 2. Cumulative numbers of infective larvae recovered from herbage after four samplings.

		1996		
	1995	Pasture 1	Pasture 2	1997
Series I				
Control	11 (3.4)	220 (58.2)	47 (8.5)	1017 (63.5)
CI3	5 (2.6)	–	–	122 (32.1)**
Troll A	–	27 (10.8)***	7 (8.0)***	212 (67.7)**
Series II				
Control	296 (39.7)	7 (15.7)	11 (11.9)	1548 (597.6)
CI3	44 (13.5)**	–	–	360 (111.8)**
Troll A	–	2 (6.3)	1 (3.1)	361 (142.5)**
Series III				
Control	301 (52.5)		2140 (322.2)	
CI3	35 (17.1)**		427 (58.9)**	
Troll A	–		456 (284.7)**	

Each number represent the mean of five pats (1995 and 1997) or ten pats (1996). Standard deviations are shown in parentheses. **$P < 0.01$; ***$P < 0.001$. No statistical analysis was performed for Series I, 1995, and Series II, 1996, due to the low number of larvae recovered.
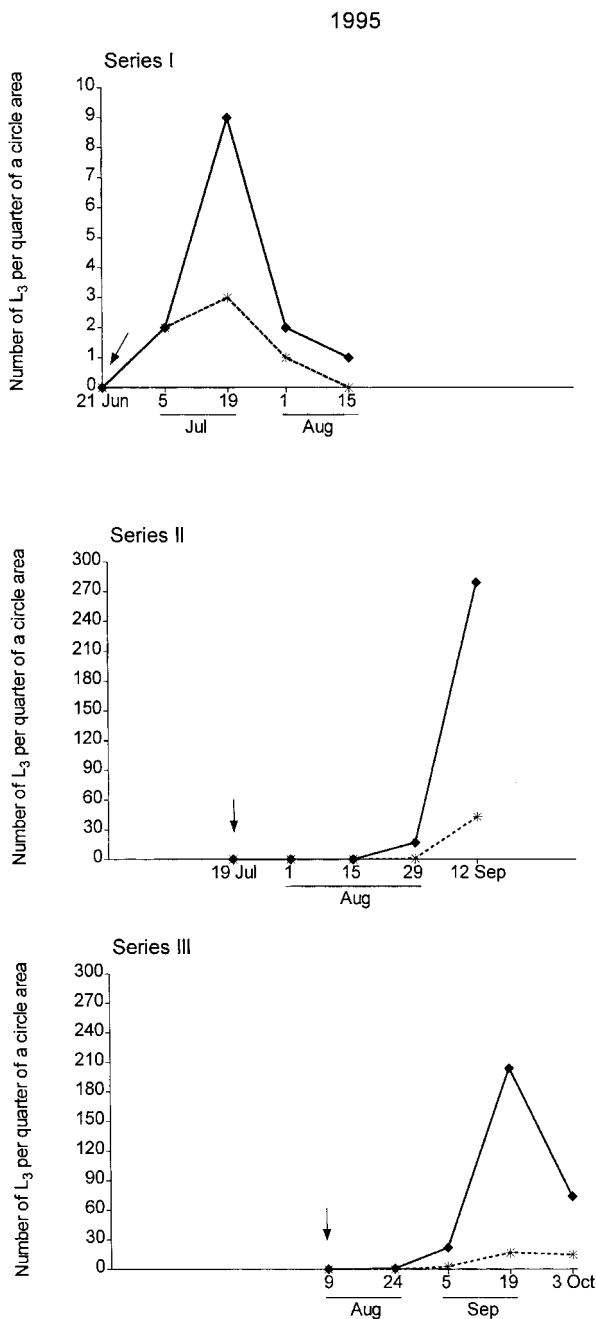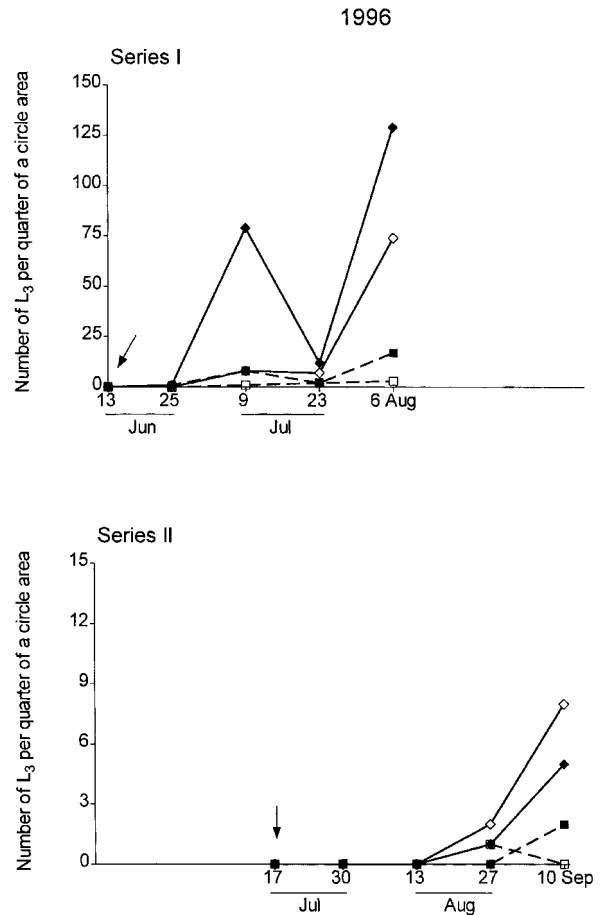
Fig. 2. Numbers of larvae recovered from herbage surrounding faecal pats during the grazing season, 1995, in plot areas located in Pasture 1. The samples were taken every 2 weeks by quarters of a circular area around faecal pats, which extended 25–30 cm from the edge of the pats. Each point represents the mean of five samples. Arrows indicate the date when faecal pats were deposited on plots. ◆, Control group; *, CI3 isolate-treated group. Notice Y-axis scale for Series I is 30 times less than for Series II and III.

Fig. 3. Numbers of larvae recovered from herbage surrounding faecal pats during the grazing season, 1996, in plot areas located in Pasture 1 and Pasture 2. The samples were taken every 2 weeks by quarters of a circular area around faecal pats, which extended 25–30 cm from the edge of the pats. Each point represents the mean of ten samples. Arrows indicated the date when faecal pats were deposited on plots. ◆, Control group located in Pasture 1; ■, Troll A isolate-treated group located in Pasture 1; ◇, control group located in Pasture 2; □, Troll A isolate-treated group located in Pasture 2. Notice Y-axis scale for Series I is 10 times higher than for Series II.

(Series II). In 1997 these percentages varied from 57.6 to 60.3% (Series I), 61.0 to 73.1% (Series II), and 67.0 to 74.9% (Series III), but no significant differences were found.

The number of infective larvae remaining in the faecal pats after the herbage collection period (table 3) was lower in all fungus-treated pats compared with controls. Significant differences were found in Series II, 1996 for pats located in Pasture 2, and in 1997.

Discussion

This plot study demonstrated that there are no apparent differences in nematode-trapping capacity between the CI3 and Troll A isolates of *D. flagrans*. The
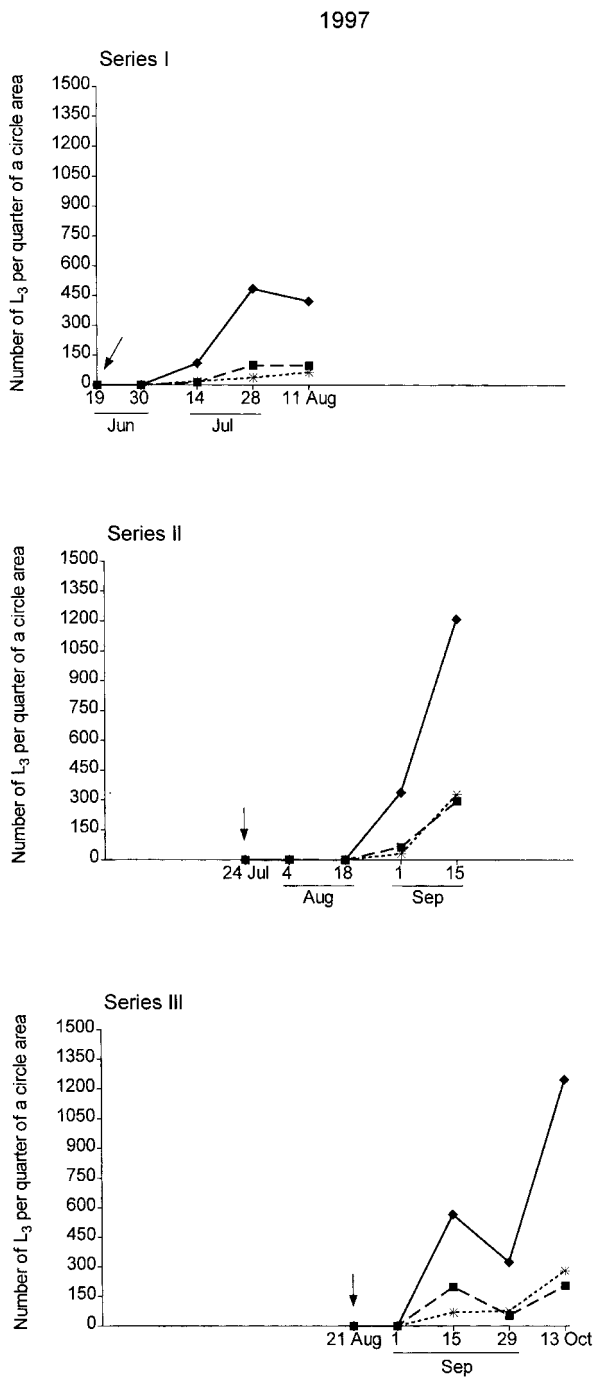
Fig. 4. Numbers of larvae recovered from herbage surrounding faecal pats during the grazing season, 1997, in plot areas located in Pasture 3. The samples were taken every 2 weeks by quarters of a circular area around faecal pats, which extended 25–30 cm from the edge of the pats. Each point represents the mean of five samples. Arrows indicate the date when faecal pats were deposited on plots. ◆, Control group; *, CI3 isolate-treated group; ■, Troll A isolate-treated group.

reducing effect of the two fungal isolates on free-living stages of *O. ostertagi* was confirmed in faecal cultures (except for the low values recorded in 1996 and 1997). Larsen *et al*. (1992) reported a 96% reduction in the number of infective larvae in faecal cultures containing *D. flagrans* CI3, but it is difficult to establish a direct comparison with our results since they did not use a fungal dose adjusted to the body weight of the animals. Nansen *et al*. (1995) showed that larval counts recorded from cultures containing *D. flagrans* CI3 were reduced by more than 95% compared with cultures without fungus. The situation was reflected under field conditions, where the fungus was able to reduce larval populations of *Ostertagia* spp. and *Cooperia* spp. on the herbage and clinical disease in calves was prevented. The lower activity of the fungus in faecal cultures in 1997 compared with the previous two experiments could be explained by the different types of fungal material used. In 1997 the material used was dry, whereas in 1995–1996 it comprised a suspension of fungus washed from barley grains. However, this difference was not reflected in reductions in larval numbers on the herbage. The question remains whether the processing of the fungal material in 1997 influenced the biology and/or trapping-efficacy of the fungus under laboratory conditions. Further studies are needed to determine the correct type of fungal material as a final product given to the animals without compromising the efficacy of the fungus.

It is not surprising that very low numbers of larvae were found in the herbage during the first half of the experiment in 1995 as weather conditions were characterized by very little rain and high temperatures. Larvae could have reached the infective stage because the moisture in fresh faeces is sufficient to support this development (Durie, 1961), but some larvae presumably died due to the rapid desiccation of the pats (Rose, 1962). The lower numbers of larvae found in control pats from Series I compared with those from Series II and III seem to support this. The relatively low activity of the fungus in Series I, 1996, follows the low reduction found in the faecal cultures using the same material. There is no suitable explanation for the low performance of the fungus in this specific series, but it must be remembered that the reduction percentage estimated for the respective herbage larval numbers is an extrapolation of the actual figures, as explained in the results section. The small number of larvae recovered from herbage during Series II in 1996 can be explained by the initial low number of eggs present in the faeces at the time of deposition on the plots. The administration of artificial irrigation in 1997 enhanced larval transmission. The reduced numbers of larvae in herbage due to the fungal activity in faecal pats found in this study (except Series I, 1996) are largely in agreement with the results obtained by Grønvold *et al*. (1993), who reported that *D. flagrans* CI3 reduced herbage larval counts by 74–85% in the very early season. Furthermore, our results show that fungal activity is not restricted to specific parts of the grazing season.

The present study suggests that there is a reduced risk for grazing calves to become clinically infected with trichostrongyle nematodes when *D. flagrans* is used, as was reported in field experiments in Denmark (Wolstrup *et al*., 1994; Larsen *et al*., 1995; Nansen *et al*., 1995).

Table 3. Faecal pats and larvae remaining in faeces after 8 weeks on pasture plots.

	1996				1997		
	Pasture 1		Pasture 2		Pasture 3		
	Control	Troll A	Control	Troll A	Control	CI3	Troll A
Series I							
Faecal pag (g)	147.2 (9.1)	133.0 (10.1)	180.5 (50.8)	178.4 (29.6)	242.2 (47.0)	245.4 (15.6)	226.4 (40.9)
Larvae	410 (554.8)	22 (19.2)	248 (200.7)	8 (10.9)	1869 (897.1)	187 (82.7)**	85 (41.9)**
Series II							
Faecal pat (g)	150.6 (23.8)	161.6 (13.6)	176.5 (18.9)	142.3 (15.6)	298.4 (23.3)	252.2 (39.6)	330.0 (57.1)
Larvae	394 (383.1)	14 (26.1)	1076 (732.3)	0 (0)*	4175 (2821.6)	244 (170.7)*	233 (44.1)**
Series III							
Faecal pat (g)					624.9 (33.4)	495.0 (21.6)***	557.5 (57.3)
Larvae					528 (204.3)	50 (41.6)***	43 (49.5)***

The faeces remaining on a nylon net, mesh size 8 mm, were recovered after the herbage collection period, and the infective larvae were extracted by macro-Baermann technique. The values represent the mean of ten pats (1996) or five pats (1997). Standard deviations are shown in parentheses. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

The differences between Pasture 1 and Pasture 2 (1996) in terms of the wet weight of remaining faecal pats in Series I might be explained by differences in vegetation cover on both pastures. The herbage in Pasture 2 was more dense and taller than in Pasture 1, especially during the first part of the experiment. Thus, faecal pats in Pasture 2 were more protected from the sun, and desiccation was less evident than in Pasture 1, where the pats had little protection. Whether or not the type of soil is a factor influencing the activity of the fungus in faecal pats was not investigated in this experiment, but deserves further attention. The similarities in weight, dry weight, and organic matter content of control and fungus-treated faecal pats indicate that faecal degradation was not affected by the presence of the fungus.

Acknowledgements

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