

## Predacious activity of *Duddingtonia flagrans* within the cattle faecal pat

M. Faedo<sup>1</sup>, M. Larsen<sup>1\*</sup> and J. Grønvold<sup>2</sup>

<sup>1</sup>Danish Centre for Experimental Parasitology, The Royal Veterinary and Agricultural University, 100 Dyrhøvej, DK-1870. Frederiksberg C, Denmark; <sup>2</sup>The Department of Ecology, The Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C, Denmark

### Abstract

Two studies were conducted to investigate the growth and activity of the fungus, *Duddingtonia flagrans*, within cattle faecal pats. Artificial faecal pats were constructed with the centre separated from the outer layer by a nylon mesh. Eight treatments were tested, by varying the presence/absence of *Cooperia oncophora* eggs and fungal spores within each layer. With parasite eggs in the centre layer, a statistically lower recovery of larvae was observed compared to both pats with parasite eggs in the periphery and pats with parasite eggs throughout both layers. Regardless of location within the pat, if co-located with the parasite egg, *D. flagrans* was found to be effective in trapping developing larvae. The reduction in recovery of larvae from pats with parasite eggs and fungal spores in the centre was found to be significantly higher than when parasite eggs were in the centre and fungal spores in the periphery. In the second study, pats were made up in two treatments: pats containing fungal spores and *C. oncophora* eggs (fungus) and pats containing *C. oncophora* eggs (control). The pats were incubated at low or high humidity. Ten pats were used in a cross over where five pats incubated at low humidity for 7 weeks were removed, water added and then incubated at a high humidity for 1 week. Another five pats were incubated at a high humidity for 7 weeks, aerated and incubated at a low humidity for 1 week. There was no apparent growth of fungus in faecal pats incubated at a high humidity and less than 20% of larvae were recovered. The growth of *D. flagrans* was observed in faecal pats incubated at a low humidity, but a corresponding reduction in the percentage recovery of larvae did not occur, except in week 4. No statistical difference between fungal and control pats was seen in the change over pats. Nematophagous activity was assessed throughout the study and observed in the first 4 weeks within the pats containing fungus.

### Introduction

Introduction of the nematode-destroying fungus, *Duddingtonia flagrans*, as a biological control agent against animal parasitic nematodes has become increasingly feasible but questions still arise as to the performance of the fungus under different climatic conditions. It is

known, for example, that parasite eggs can survive for up to 6 weeks or even longer within the cattle faecal pat during long periods of a dry climate, then hatch when ideal conditions for development are available, causing serious problems in their hosts (Hansen & Perry, 1994). However, *D. flagrans* is administered as resting chlamydo-spores, a stage designed to withstand harsh conditions. It is reasonable to assume that, just as the parasite egg remains dormant until abiotic factors such as oxygen, temperature and humidity are conducive to egg hatching, *D. flagrans* chlamydo-spores would germinate when

\*Author for correspondence  
Fax: +45 35 28 27 74  
E-mail: mila@kvl.dk

abiotic factors allow the chlamydo spores to do so, followed by trapping and killing the developing nematode larvae. Incidental evidence of delayed germination of *D. flagrans* has been observed in field trials. Wolstrup *et al.* (1994) fed *D. flagrans* chlamydo spores daily for a two-month period to grazing calves. The spores were administered during a period of unusually hot and dry weather conditions, containing the driest period ever recorded in Denmark. In the following months of wet weather, larval infectivity on the pasture increased but not as much as in the corresponding group of calves not fed fungus. This suggests that the fungus trapped developing larvae before the pats dried out or that the fungus remained in the resting stage until weather conditions were favourable for germination and growth. If the latter, the fungal germination coincided with larval transmission.

Other abiotic factors can also influence the performance of *D. flagrans* spores administered to livestock as a biological control agent. Of particular importance with regard to the treatment of cattle with *D. flagrans* is the effect of CO<sub>2</sub> and CH<sub>4</sub> on the growth and trapping of the fungus. Holter (1991) observed that dung pats became anaerobic almost immediately with low O<sub>2</sub> concentrations and an accumulation of CH<sub>4</sub>. There are no published data on the effect of such anaerobic conditions on the growth and trapping of *D. flagrans* within the faecal pat. However, Grønvold *et al.* (1999) found that *D. flagrans* could not grow or produce trapping networks in anaerobic conditions. However, when returned to aerobic

conditions (21 vol.% O<sub>2</sub> and 0.03 vol.% CO<sub>2</sub>) *D. flagrans* was able to grow and produce trapping nets, although the propensity of the fungus to produce nets was reduced after 3 weeks of prior exposure to anaerobic conditions.

The aims of the present study were: (i) to investigate the activity of *D. flagrans* within the centre of the cattle faecal pat in comparison to periphery of the cattle pat; and (ii) to monitor the growth and activity of *D. flagrans* within the faecal pat over time during exposure to dry conditions.

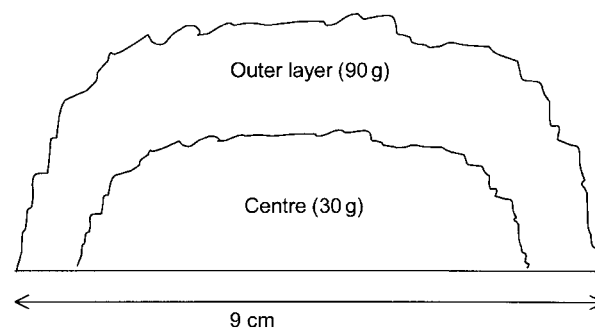
## Materials and methods

### Parasite material

The Danish Veterinary Laboratory provided cattle faeces. Danish Red Dairycow (RDM) calves were housed separately in straw bedded stalls and were fed a standard ration of feed pellets, supplemented with oats plus straw and hay. Those calves acting as parasite donors were excreting a monoculture of *Cooperia oncophora* eggs. During the days of faeces collection, the straw bedding was removed from the stalls.

### Fungal material

A Danish isolate of *D. flagrans* (Larsen *et al.*, 1991) was cultivated on millet seed, dried and packaged into individual doses by Christian Hansen BioSystems A/S. Since the faecal material collected from calves was diluted (1:1) with other faecal material, a dose of  $2 \times 10^6$



Treatment	Centre	Outer layer
1	Faeces with parasite eggs	Faeces with parasite eggs
2	Faeces with parasite eggs + fungal spores	Faeces with parasite eggs + fungal spores
3	Faeces with parasite eggs	Faeces alone
4	Faeces with parasite eggs + fungal spores	Faeces alone
5	Faeces with parasite eggs	Faeces with fungal spores
6	Faeces alone	Faeces with parasite eggs
7	Faeces alone	Faeces with parasite eggs + fungal spores
8	Faeces with fungal spores	Faeces with parasite eggs

Fig. 1. Diagram of faecal pat, outlining the eight treatments tested in the anaerobic layers study.

chlamydo spores per kg body weight was administered to the donor calves to produce an effective dose of  $10^6$  chlamydo spores per kg body weight.

#### *Anaerobic layers*

Artificial cattle faecal pats were constructed such that the centre (30 g) was separated from the outer layer (90 g) by a nylon mesh ( $100\ \mu\text{m}$ ). Five replicates of eight treatments (fig. 1), were incubated at  $25^\circ\text{C}$  and 95% relative humidity. A small pilot study had shown that such conditions allowed for the formation of a crust with a moist centre similar to that seen in pats deposited naturally in the field. After 14 days incubation, the outer layer of each pat was separated from the centre. Egg counts of each treatment were performed according to a modified McMaster technique (Roepstorff & Nansen, 1998). Each pat section was weighed and 4 g removed for egg counts. The remaining portion was placed into a Baermann funnel and left overnight to settle. Counts were performed to estimate the number of larvae present.

#### *Fungal persistence*

Artificial faecal pats (120 g) were constructed for two test treatments (50 pats per treatment): fungal and control. Fungal treatment consisted of a faecal pat containing fungal spores and parasite eggs. Control faecal pats contained only parasite eggs. In each treatment 20 pats were incubated at  $20^\circ\text{C}$  and low humidity (group A), another 20 pats at  $20^\circ\text{C}$  and high humidity (group B), and 10 pats were used in a cross over manipulation. The cross over involved incubating five pats at  $20^\circ\text{C}$  and low humidity for 7 weeks, adding water to each pat (50 ml) and then incubating at  $20^\circ\text{C}$  and high humidity for another week (group C). The remaining five pats were incubated at  $20^\circ\text{C}$  and high humidity for 7 weeks, aerated by piercing small holes through the surface of the pat and then incubated at  $20^\circ\text{C}$  and low humidity for 1 week (group D). Exposure to high humidity was created by placing pats on folded chicken wire within sealed boxes containing water (care was taken to ensure the pats were not in contact with the water in the bottom of the box) and incubating at  $20^\circ\text{C}$ . Low humidity was created by placing pats in boxes which contained saturated NaCl solution and incubating at  $20^\circ\text{C}$ . For the first week of the experiment, boxes containing saturated NaCl solution were left unsealed to allow movement of air and hence, drying of the pat surface. The relative humidity was monitored throughout the experiment using hair hygrometers and was consistently 100% (high) and 68% (low). Throughout the experiment the sealed boxes were opened two to three times per week to ensure adequate oxygen was available. An estimate of a most probable number (MPN) of *D. flagrans* present in both treatments was performed. Faeces (25 g) were diluted in water (50 ml) and five replicate sub-samples (0.1 ml) were plated onto 4 cm diameter Petri dishes containing 20% corn meal agar (CMA). Two further dilutions of this solution (1/10 and 1/100) were made and five replicate sub-samples were likewise plated. Approximately 3000 infective larvae of *C. oncophora* were added to each MPN plate. Each plate was sealed and incubated at  $25^\circ\text{C}$  and 95%

humidity for a total of 3 weeks. The plates were inspected weekly under a stereomicroscope for the trapping of infective larvae and the growth of *D. flagrans*. The abundance of fungus was calculated by determining the number of plates positive for growth of each fungus and comparing those to statistical MPN tables (Oblinger & Koburger, 1975).

Samples were taken at 1, 2, 4 and 8 weeks after set-up for Groups A and B. Groups C and D were only sampled at week 8. On each sampling occasion, five pats from each group and treatment sampled were weighed and half of each pat placed into a Baermann funnel to allow the larval parasites to migrate and settle overnight. Counts were performed to estimate the number of infective larvae present. The remainder of the pat was then weighed, thoroughly mixed and a sample removed (25 g) for MPN estimation. As the pats incubated at a low humidity lost moisture, water (30 ml) was added to group A, C and D pats, and left until the added moisture was adsorbed. To ascertain whether *D. flagrans* within faecal pats were still able to trap larvae, two small cultures were set up containing a mixture of faeces from the remainder of the pat (5 g) and cattle faeces (5 g) known to contain *C. oncophora* eggs. After 2 weeks incubation at room temperature, these cultures were placed into a Baermann funnel and left to settle overnight. Counts were performed to estimate the number of larvae present.

#### *Statistical analysis*

All statistical comparisons were performed in SAS (version 8.01, SAS Institute Inc., Cary, North Carolina, USA). For the anaerobic layers study, four tests were performed. To ascertain if the position of parasite eggs within the faecal pat influenced the number of larvae which subsequently developed, the percentage larval recovery (both second and third stage) from pats in treatments 1, 3 and 6 were compared. To determine whether the nematophagous activity of *D. flagrans* was affected by its position within the faecal pat, the 'reduction' in larval recovery relevant to controls in treatments 2 (treatment 1), 4 (treatment 3) and 7 (treatment 6) was compared. For example, the 'reduction' of treatment 2 with respect to treatment 1 was defined as the percentage development in treatment 1 minus the percentage development in treatment 2. To test if the separation of parasite eggs and fungal spores within the faecal pat affected the nematophagous activity of *D. flagrans* two tests were performed. The 'reduction' in treatments 4 and 5 (relative to treatment 3) were compared. Likewise, the 'reduction' in treatments 7 (treatment 6) and 8 (treatment 6) were compared. All statistical comparisons were performed using proc glm with treatment as a class variable. A plot of the residuals versus predicted values was performed indicating that resulting data were normally distributed.

For the persistence study only the % recovery of larvae from faecal pats incubated at low humidity was tested as recoveries from high humidity were extremely low (fig. 4b). A split-model design was used to analyse data with treatment as the main factor and time (weeks) as a subplot factor. A plot of residuals versus predicted values was performed and the results indicated that data were

best described by a log transformation (% recovery + 1). Since time (weeks) was found to be significant, a Student's *t* test was performed at each sampling week (1, 2, 4 and 8) to determine if there was a significant difference between fungal and control treatments. The changeover results (group C) were also tested using a Student's *t* test. The Shapiro-Wilk statistic was calculated at each sampling week and data found to be normally distributed (change over data were log transformed).

## Results

### Anaerobic layers

Faecal pats in all treatments formed a hard crust. Moisture was lost from both the centre and periphery of each pat; although the loss was greater in the periphery (mean of  $77\% \pm 2\%$  compared to  $61\% \pm 5\%$ ). Mean recoveries of parasite eggs and larvae from all treatments are presented in fig. 2. Parasite eggs were recovered, at a level of 1%, only from treatments 3 and 4, in which the parasite eggs were located in the centre of the pat. Treatments 1, 2 and 5 also had parasite eggs placed in the centre of the pat; however, no eggs were recovered. Second stage larvae were recovered from treatments 1, 3, 4 and 5, all of which had parasite eggs placed within the centre of the pat. Treatment 3 had the greatest recovery of second stage larvae (20%); the remaining treatments yielded less than 10%.

In comparing the total recovery of larvae from pats not containing fungal spores, treatment 3 (eggs in the centre of the pat) had a statistically lower % recovery of larvae than both treatments 1 (eggs through the pat) and 6 (eggs in the periphery) ( $P < 0.01$ ). There was no statistical

difference between the % recovery of larvae from treatments 1 and 6 ( $P = 0.86$ ). Fungal activity was assessed by comparing the 'reduction' of treatments with respect to their various controls. No statistical difference could be found between treatments 2, 4 and 7 ( $P = 0.71$ ), neither could a statistical difference be found when comparing the 'reduction' of treatments 7 and 8 ( $P = 0.12$ ). However, the 'reduction' of treatment 4 was found to be significantly greater than that of treatment 5 ( $P < 0.05$ ).

### Persistence study

Faecal pats incubated at a high humidity (100% RH) did not form a crust and after 8 weeks had only lost minimal moisture (mean of  $7\% \pm 4\%$ ). Faecal pats incubated at low humidity (68% RH) became very dry throughout the experiment losing a mean of  $59\% \pm 6\%$  moisture in the first week when the boxes were unsealed, with a final mean moisture loss of  $92\% \pm 1\%$ . The mean MPN values for *D. flagrans* presence are presented in fig. 3. From a dose of  $1 \times 10^6$  chlamydo spores per kg body weight, the resulting concentration in cattle faeces was just under 1200 chlamydo spores per g (139, 770 per 120 g). Despite the rapid loss of moisture observed in pats incubated at low humidity, the mean MPN value for growth units of *D. flagrans* increased approximately 2.5 times over the first week. This was followed by a drop of similar magnitude to a final value of approximately 40,000 at week 8. For pats incubated at high humidity there was an 80% drop in MPN value in the first week and the level remained at 10,000 for most of the experiment. The increase in growth units seen in week 8 was due to one pat with a high MPN value (495, 406). The change over cattle pats (groups C

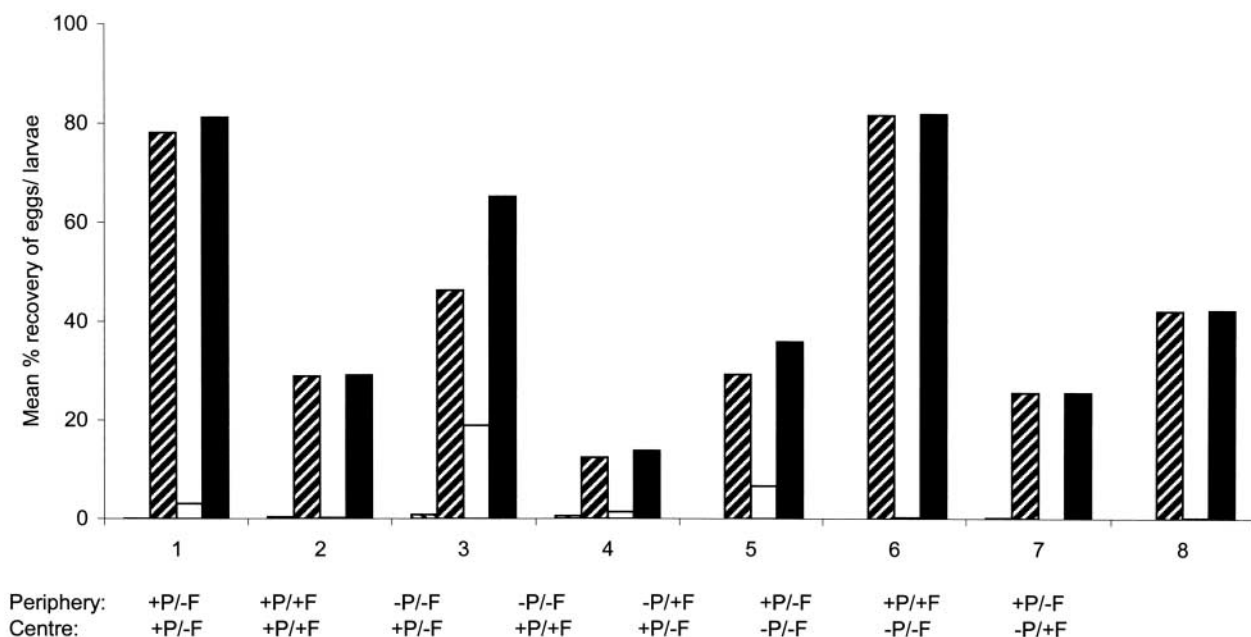


Fig. 2. The mean % recovery of eggs (▨), infective third stage larvae (▧), second stage larvae (□) and total larvae (■) of *Cooperia oncophora* from faecal pats (total of both layers) in all eight treatments tested in the anaerobic layers study. +P/-P, contains/does not contain parasite eggs; +F/-F, contains/does not contain *Duddingtonia flagrans* spores.

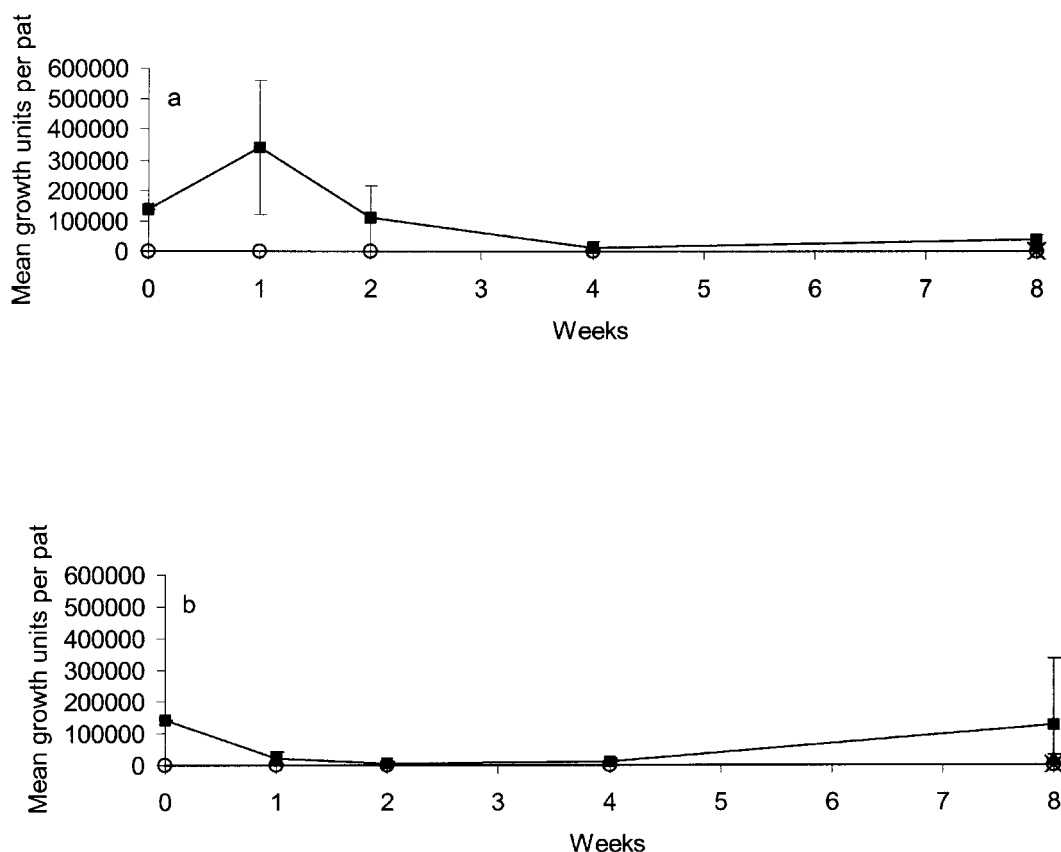


Fig. 3. The mean number of growth units/pat of *Duddingtonia flagrans* on different sampling occasions in faecal pats incubated at (a) 68% relative humidity: fungus - group A (■), control - group A (○), fungus - group C (▲), control - group C (×); and (b) 100% relative humidity: fungus - group B (■), control - group B (○), fungus - group D (▲), control - group D (×). See text for description of groups.

and D) showed similar MPN values as the corresponding treatments at week 8 (groups A and B). The fungus was detected at low levels (60–300 MPN per 120 g) on a few plates from the control treatment incubated at both low and high humidity.

Fungal activity throughout the experiment was assessed by culturing samples from faecal pats with faeces containing eggs (table 1). The percentage reduction observed in faecal pats incubated at low humidity was high throughout the experiment (83–94%). However, the reduction at week 1 and 8 for faecal pats incubated at high humidity was low (59% and 33% respectively), although the intervening samples had high reduction (81% and 88%). It should be noted that the recovery of larvae from control cultures in week 8 were also very low, below 20%.

The mean percentage recoveries of larvae from all faecal pats are presented in fig. 4. The pats incubated at high humidity had low recoveries throughout the experiment, less than 20%. When comparing fungal and control treatments in pats incubated at low humidity, there was no significant difference between the percentage recovery at weeks 1, 2 and 8. However, the reduction at week 4 was significant ( $P < 0.05$ ). An increase in percentage recovery of larvae from control pats occurred in the change over group. Statistical analysis of the low

humidity data showed both treatment ( $P < 0.05$ ) and time ( $P < 0.01$ ) to be significant. No statistical difference between fungal and control pats was seen in the change over pats.

## Discussion

A number of important observations were made in the anaerobic layers study. The majority of larvae recovered from cattle faecal pats originated from the periphery of the pat. This is demonstrated by the statistically lower recovery in treatment 3 compared to treatments 1 and 6 (fig. 2), coupled with the lack of statistical difference between treatment 1 and 6. This is supported by the observation that parasite eggs were only recovered from pats where eggs were located in the centre of the pat. This decrease in the development of larvae from eggs within the centre of the pat may be due to an anaerobic environment (Holter, 1991).

Regardless of location within the cattle pat, if co-located with parasite eggs, the fungus is effective in trapping developing larvae. This is supported by the lack of statistical difference between treatments 2, 4 and 7, coupled with the statistical difference found between 4

Table 1. Percent recovery of *Cooperia oncophora* larvae from cattle faecal cultures containing *Duddingtonia flagrans* incubated at either low (68%) or high (100%) relative humidity.

Weeks	68% RH				68% RH (change over)				100% RH				100% RH (change over)			
	% Recovery <sup>1</sup>		Mean % reduction <sup>2</sup>	Control	% Recovery		Mean % reduction	Control	% Recovery		Mean % reduction	Control	% Recovery		Mean % reduction	
	Fungus	Control			Fungus	Control			Fungus	Control			Fungus	Control		
1	3 ± 3	45 ± 7	93	-	-	-	-	18 ± 9	44 ± 15	59	-	-	-	-	-	
2	2 ± 2	25 ± 14	92	-	-	-	-	9 ± 7	47 ± 8	81	-	-	-	-	-	
4	2 ± 1	36 ± 15	94	-	-	-	-	4 ± 3	32 ± 14	88	-	-	-	-	-	
8	2 ± 1	12 ± 3	83	6 ± 4	18 ± 9	67	4 ± 3	6 ± 3	33	1 ± 1	16 ± 5	94	-	-	-	

<sup>1</sup> Presented as mean ± standard deviation.

<sup>2</sup> [(% recovery control - % recovery fungus) / % recovery control] × 100.

<sup>3</sup> Change over samples were only taken in week 8.

and 5. This agrees with the findings of Faedo *et al.* (2000) that when fungus spores and parasite eggs (in this case within sheep faeces) were deposited simultaneously trapping was efficient; however, if parasite eggs preceded fungal spores, or vice versa, trapping was not seen. On first observation, the lack of statistical difference between treatments 7 and 8 contradicts this finding. In fact, the reverse was expected. As Grønvold *et al.* (1999) showed that anaerobic conditions prevented the growth and trapping of *D. flagrans*, we would expect treatments 4 and 5 to be the same. One explanation could be that growth from the periphery to the centre was deterred by the difference in oxygen content. Hence, the majority of fungal growth occurred in the periphery trapping the few larvae that migrated from the centre of the pat.

When interpreting the results of the two studies presented in this paper it should be remembered that many coinciding events were taking place. For example, the lack of recovery of larvae may not only be due to fungal activity but also to the lack of hatching of parasite eggs. It would appear from the results of the persistence study that anaerobic conditions developed within cattle faecal pats incubated at a high humidity. Not only was there no apparent fungal growth, except for one pat at week 8, but also very few larvae were recovered (less than 20%). This is likely to be attributed to the lack of air movements which normally dry the surface of the cattle pat creating a crust. This porous crust allows penetration of oxygen into the outer layers. In the present study it was necessary to store pats in sealed containers in order to create a high humidity, resulting in the surface of faecal pats remaining moist throughout the 8 weeks of the experiment. Piercing holes in the change over pats in an attempt to aerate the pats was not sufficient to allow development of larvae or growth of the fungus.

Despite the growth *D. flagrans* observed in faecal pats incubated at low humidity, a corresponding reduction in the percentage recovery of larvae did not occur, except in week 4. This is also true of change over pats, although not obvious from fig. 4, and no statistical difference between % recovery from fungal and control pats was found. This may be attributable to the lack of movement of larvae under dry conditions. *Duddingtonia flagrans* is not an obligate parasitic fungus and needs to be triggered to produce trapping networks. If larvae are immobile they will not induce net formation. This is supported by the findings of Nordbring-Hertz (1968) who observed the trapping activity in a closely related fungus *Arthrobotrys oligospora*. In her study, nematodes were killed either by heating in a water bath (65°C), addition of iodine, lyophilizing and re-hydrating, deep freezing and thawing, or air drying at room temperature and re-hydrating. The nematodes were then added to Petri dishes containing *A. oligospora* but the fungus did not produce trapping organs regardless of the method of killing. The lack of statistical difference in the change over faecal pats is of some concern; however, the final weights of the pats suggest an insufficient increase in moisture to allow movement of larvae and stimulation of trapping.

An assessment of fungal activity was hindered somewhat by limited resources. Unfortunately, it was only possible to obtain faeces on one occasion and 'fresh' faeces used in fungal activity cultures were 1 week old at

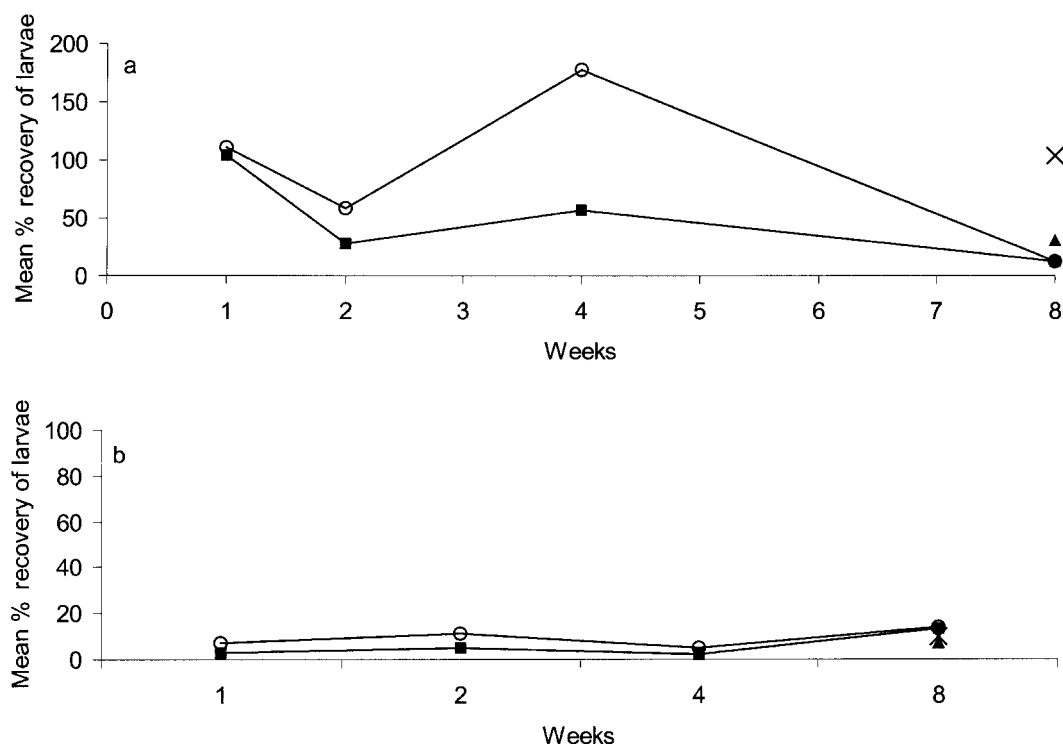


Fig. 4. The mean % recovery of larvae of *Cooperia oncophora* on different sampling occasions in faecal pats incubated at (a) 68% relative humidity: fungus – group A (■), control – group A (○), fungus – group C (▲), control – group C (X); and (b) 100% relative humidity: fungus – group B (■), control – group B (○), fungus – group D (▲), control – group D (X). See text for description of groups. Note different scales used on the y-axis.

the first culturing and 8 weeks at the final. The storage of the faecal material at refrigeration temperatures led to the lower percentage recoveries observed in week 8. A similar result was observed in a pilot study where cattle faeces containing *C. oncophora* eggs were stored at refrigeration temperatures and cultured on different occasions over a 6-week period. A significant drop in larval recovery was detected from day 8 onwards (M. Larsen, personal communication). Regardless, adequate larval recoveries could be observed during the first four sampling occasions, all demonstrating a reduction in the number of larvae recovered from cultures containing fungus compared to those with parasite eggs alone.

It should be emphasized that *D. flagrans* is able to survive the same extremes of conditions in which larvae survive, thus retaining the ability to trap larvae in order to prevent transmission of larvae onto pasture. The fact that *D. flagrans* did not grow or produce traps under anaerobic conditions is of no consequence if larvae are likewise inhibited from developing. By the same token, if larvae are immobile within the faecal pat, larval transmission to the pasture will be prevented and the nematophagous activity of *D. flagrans* will not be needed. Throughout the persistence study *D. flagrans* was present and, for up to 4 weeks within the experiment, was able to trap larvae. The results of these studies conducted in cattle faecal pats largely agree with the findings of Grønvold *et al.* (1999) conducted on CMA. The present studies are somewhat artificial making further observations in the field

desirable before predictions on the performance of the fungus can be made. A preliminary conclusion could be that *D. flagrans* survives long periods of dry conditions, retaining the propensity to prevent transmission of larvae to pasture and again substantiating is the need to ensure co-location of fungal spores with parasite eggs.

### Acknowledgements

We wish to acknowledge the competent technical assistance of Tina Skov, Margrethe Pearman, Marlene Høg and Kamilla Henriksen. The authors would also like to thank Christian Hansen's BioSystems A/S for the supply of *Duddingtonia flagrans* spores. This study was financially supported by the Danish National Research Foundation, the Research Council and Lundbeck.

### References

- Faedo, M., Larsen, M. & Thamsborg, S.M. (2000) Effect of different times of administration of the nematophagous fungus *Duddingtonia flagrans* on the transmission of ovine parasitic nematodes on pasture – a plot study. *Veterinary Parasitology* **94**, 55–65.
- Grønvold, J., Wolstrup, J., Nansen, P., Larsen, M., Henriksen, S.A., Bjørn, H., Kirchheiner, K., Lassen, K., Rawat, H. & Kristiansen, H.L. (1999) Biotic and abiotic factors influencing growth rate and production

- of traps by the nematode-trapping fungus *Duddingtonia flagrans* when induced by *Cooperia oncophora* larvae. *Journal of Helminthology* **73**, 129–136.
- Hansen, J. & Perry, B.** (1994) *The epidemiology, diagnosis and control of helminth parasites of ruminants. A handbook*. Nairobi, International Laboratory for Research on Animal Diseases.
- Holter, P.** (1991) Concentration of oxygen, carbon dioxide and methane in the air within dung pats. *Pedobiologia* **35**, 381–386.
- Larsen, M., Wolstrup, J., Henriksen, S.A., Dackman, C., Grønvold, J. & Nansen, P.** (1991) *In vitro* stress selection of nematophagous fungi for biocontrol of parasitic nematodes in ruminants. *Journal of Helminthology* **65**, 193–200.
- Nordbring-Hertz, B.** (1968) The influence of medium composition and additions of animal origin on the formation of capture organs in *Arthrobotrys oligospora*. *Physiologia Plantarum* **21**, 52–65.
- Oblinger, J.L. & Koburger, J.A.** (1975) Understanding and teaching the Most Probable Number technique. *Journal of Milk Food Technology* **38**, 540–545.
- Roepstorff, A. & Nansen, P.** (1998) *Epidemiology, diagnosis and control of helminth parasites in swine*. Animal Health Manual, No. 3, Rome, Food and Agricultural Organization of the United Nations.
- Wolstrup, J., Grønvold, J., Henriksen, S.A., Nansen, P., Larsen, M., Bøgh, H.O. & Ilsoe, B.** (1994) An attempt to implement the nematode-trapping fungus *Duddingtonia flagrans* in biological control of trichostrongyle infections of first year grazing calves. *Journal of Helminthology* **68**, 175–180.

(Accepted 5 June 2002)  
© CAB International, 2002