Biotic and abiotic factors influencing growth rate and production of traps by the nematode-trapping fungus *Duddingtonia flagrans* when induced by *Cooperia oncophora* larvae

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Abstract

A series of experiments on corn meal agar was carried out to evaluate the efficacy of the nematode-trapping fungus *Duddingtonia flagrans* in different abiotic and biotic conditions which occur in cow pats. Above a concentration of 50 parasitic larvae (L_3) cm^{-2} the fungus produced a maximum of between 500 and 600 nets cm^{-2} at 20°C in 2 days on the surface of corn meal agar. There were no differences in the trap-producing capacity of three strains of *D. flagrans* (CIII4, CI3 and Trol A). On agar at 30° and 20°C, the fungus responded to *Cooperia oncophora* L_3 very quickly producing a maximum of trapping nets 1 day after induction. At 10°C, traps were produced slowly starting on day 4 after induction and continued over the following week. *Duddingtonia flagrans* (CI3) grew at a normal rate at least down to an oxygen concentration of 6 vol.% O_2, but it did not grow anaerobically. On agar, *D. flagrans* (CI3) did not produce trapping nets in an anaerobic atmosphere. Moreover, *C. oncophora* L_3 stopped migration under anaerobic conditions. When the fungal cultures were transferred to a normal aerobic atmosphere, after 1 and 2 weeks under anaerobic conditions, the *C. oncophora* L_3 resumed migrating on the agar and, in response, *D. flagrans* produced traps in the same amount as when it had not been under anaerobic stress. Under microaerophilic conditions (6 vol.% O_2) *D. flagrans* was able to grow, but the *C. oncophora* L_3 were not able to induce trapping nets in *D. flagrans* (Trol A) because of larval immobility. But, as under anaerobic conditions, the fungus could return to a nematode-trapping state when transferred to a normal aerobic atmosphere within 1 or 2 weeks if migrating nematodes were present. Under natural conditions in the cow pat it is expected that the fungus will be ready to attack parasitic larvae, when the oxygen tension increases as a result of,

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for example the activity of the coprophilic fauna. Artificial light giving 3000–3400 Lux on the surface of the agar significantly depressed the growth rate and the production of trapping nets in *D. flagrans* (CI3). On agar, *D. flagrans* (CI3) could grow and produce trapping nets at pH levels of 6.3 to 9.3. Net-production has its optimum between pH 7 and 8. On dry faeces mycelial growth was 7–10 mm during a 15 day period while on moist faeces the fungus expanded 15–20 mm during the same period. Based on the parameters investigated, *D. flagrans* is expected to be especially active in the well aerated surface layer of a cow pat, an area which normally contains a high concentration of infective nematode parasite larvae, but also an area where the temperature can be high and the water content low.

Introduction

This is the second of three planned series of basic experiments on biotic and abiotic factors influencing the predacious efficiency of the nematode-trapping fungus *Duddingtonia flagrans* (Cooke, 1969). The first series of experiments has been published by Grønvold *et al*. (1996).

Duddingtonia flagrans produces adhesive traps (nets) which are induced when there is physical contact with migrating nematodes. *Duddingtonia flagrans* (strain CI3) fed to animals is able to grow, trap and kill different nematode parasite larvae in faeces of cattle, sheep, horses and pigs (Grønvold *et al*., 1993; Wolstrup *et al*., 1994; Larsen *et al*., 1995, 1996; Nansen *et al*., 1995, 1996; Githigia *et al*., 1997). Chlamydospores fed to the animals survive gut passage and grow in the faeces to attack parasitic larvae.

The gastrointestinal nematode parasites *Ostertagia ostertagi* and *Cooperia oncophora* of cattle produce eggs which are released in host faeces. In the cow pat, eggs hatch and develop through three free-living larval stages to the infective stage (L_3), which is transmitted, during rainy weather, to the surrounding herbage to continue the life-cycle (Grønvold, 1987). To successfully control parasitic larvae in faeces, the fungus must be able to germinate and grow in the area of the pats where parasitic larvae are numerous, and it has to be inducible, i.e. able to produce trapping nets in the time period when parasite larvae are present in the faeces.

In the first experimental series (Grønvold *et al*., 1996), the ageing *D. flagrans* mycelium did not retain the ability to produce trapping nets for more than 3 weeks in the temperature interval between 10° and 30°C. After that period the fungus became senile. However, this conclusion is only valid when *D. flagrans* has been kept sterile until induction with non-sterile *O. ostertagi* L_3.

This second series of experiments has been planned primarily to evaluate how the efficacy of *D. flagrans* is affected by abiotic and also some biotic factors prevailing in the cow pat. The present experiments were performed on diluted CMA except for one which took place in cattle faeces. With certain limitations, the results from agar are expected to reflect how different factors influence the fungus in cow pats. One important abiotic factor is the water content of the dung. Under Danish conditions, the cow pat normally loses water fairly slowly (Holter, 1991). However, the surface layer loses water faster than the centre/bottom layer (fig. 1), which normally results in a solid dry crust, covering the moist central dung (table 1). Other important abiotic factors to be taken into consideration are temperature, oxygen, light and pH (table 1). Biotic factors such as the influence of L_3 concentrations and the reaction of different *D. flagrans* strains were also investigated.

Materials and methods

Parasitic nematode

For induction we used *C. oncophora* L_3 instead of *O. ostertagi* L_3, which was the target organism in the first series of experiments. This made the experimentation easier, as *O. ostertagi* L_3 are more active and often escape from the Petri dishes, at temperatures above 10°C. *Cooperia oncophora* L_3, on the other hand, seldom escaped the agar, even though their potential to induce traps was

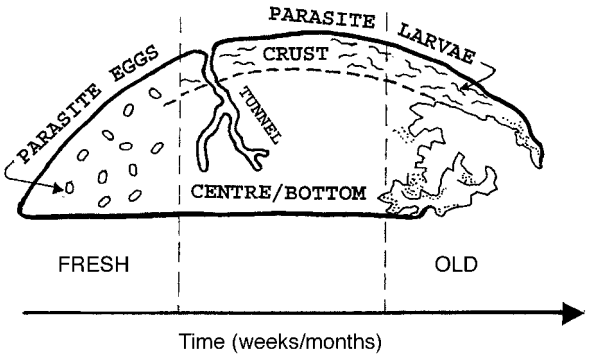

Fig. 1. An idealized cow pat is shown in cross section. During ageing (time), the pat will disintegrate due to the combined action of weathering and biotic factors such as microflora and the coprophilic fauna, i.e. flies, beetles and earthworms (Holter, 1979). Some of the bigger animals will open tunnels in the pat, especially in the surface layer. But tunnels may also originate from the underside of the pat, mainly by the action of earthworms and insect larvae. As water evaporates from the faecal surface, a firm crust layer with relatively low water content will be created. At the lower surface of the crust, a progressively expanding drying border may be found (broken line). Infective larvae (L_3) of e.g. *Ostertagia ostertagi* and *Cooperia oncophora* have a strong tendency to concentrate in the crust layer.

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Table 1. Average values of some important abiotic factors in the cow pat under Danish climatic conditions during summertime when cattle graze outdoors.

Abiotic factor	Crust layer	Centre/bottom
Temperature (°C)	10–40	15–35
Oxygen concentration (vol.%) (Holter, 1991)	10 → 15*	1 → (15)*
Light	Darkness, exept on the surface	Darkness
pH	7.0–8.5	7.0–8.5
Water content (% of fresh weight)	40–70	75–90

* The first figure is the average concentration in the fresh cow pat. After a period of 3–4 weeks, oxygen concentrations increase even at the centre/bottom of the disintegrating cow pat.

comparable to that found for *O. ostertagi*. Moreover, the developmental rate of *C. oncophora* is in accordance with the developmental rates of *O. ostertagi* larvae (Rose, 1963; Pandey, 1972).

Faeces containing eggs of *C. oncophora* Railliet 1898 were obtained from a calf carrying an experimental monospecific infection of this nematode. Infective third stage larvae (L_3) were developed in faecal cultures and subsequently extracted by a modified Baermann technique (Henriksen & Korsholm, 1983). The L_3 were not sterile, as they were extracted from cattle faeces. They were kept in shallow water at 10°C, until they were used in the induction experiments within 3 weeks.

Fungal material

The same three isolates 'CIII4', 'CI3' and 'Trol A' of *D. flagrans* Cooke 1969, as used in the first series of experiments (Grønvold *et al*., 1996), were used in the present series. The codes refer to our laboratory protocol.

Except for one experiment, performed in cattle faeces, all experiments, comprising mycelial growth rate and trap production, were carried out on diluted (1:10) corn meal agar (CMA) (Lysek & Nordbring-Hertz, 1981).

Mycelial growth rate under standard conditions

Growth rates were measured on diluted CMA (Lysek & Nordbring–Hertz, 1981) in Petri dishes (diameter 8.5 cm). Blocks of 3 × 3 mm agar from 2- to 4-week-old fungal cultures were placed at the periphery of the test dishes and the radial mycelial growth was measured.

Standard trap induction on standard Petri dishes

Induction was performed on diluted CMA (Lysek & Nordbring–Hertz, 1981) in small Petri dishes (10 cm^2; diameter 3.6 cm). Before inoculation with the fungus, as the CMA was still liquid, the Petri dishes, half filled with agar, were inclined 10° and turned around to create a thin agar film on the wall of the dishes. This prevented the nematodes from migrating down under the agar surface in crevices at the rim of the dishes.

Test Petri dishes were inoculated with 3 × 3 mm agar blocks cut from 2- to 4-week-old fungal cultures and placed at the centre of the test dishes. For the first 5 days, the Petri dishes were kept at 20°C without nematodes present, to allow the fungus to cover the whole agar surface. After 5 days, the 3 × 3 mm agar squares were removed, and the Petri dishes were then designated 'standard Petri dishes'.

As in the first series of experiments (Grønvold *et al*., 1996), standard trap induction on standard Petri dishes was established by placing 200 non-sterile *C. oncophora* L_3 in three drops of water for 2 days, i.e. 20 $L_3\,cm^{-2}$ for 2 days. The only difference from the first series of experiments was the use of a different parasite. At the end of the 2 days, three $0.5 \times 0.5\ cm^2$ squares were randomly marked at a distance of approximately 1 cm from the centre of each dish. The squares were scratched by a needle on the underside of the Petri dishes. This allowed microscopical inspection of the counting area from above on the agar surface and the number of traps could easily be enumerated. Only traps on the agar surface were counted.

General standard conditions

Experiments were performed at high relative humidity (95–100 RH%), pH 7, normal atmosphere (21 vol.% O_2) and in darkness. Only deviations from these conditions are mentioned in the following descriptions of single experiments. To keep the humidity high, all experiments on agar were done by placing the Petri dish cultures in closed transparent plastic boxes (6 × 22 × 30 cm).

Trap induction at different concentrations of parasite larvae

Trap induction of *D. flagrans* isolate CI3 on standard Petri dishes, containing diluted CMA, was performed at 20°C for 2 days with three replicates of each concentration of *C. oncophora* L_3: 0, 5, 10, 20, 50 and 100 $L_3\ cm^{-2}$. The water in which the parasitic larvae had been kept was used in the control Petri dishes without L_3.

Trap induction in different strains of Duddingtonia flagrans

Standard trap induction was performed on diluted CMA in standard Petri dishes. Three isolates (strains) of *D. flagrans* were examined: 'CIII4', 'CI3' and 'Trol A'. Each fungal strain was tested in three Petri dishes at 20°C. Differences in the trap–producing capacity was examined by an analysis of variance (Sokal & Rohlf, 1981).

Long term trap induction at different constant temperatures

Trap induction was performed on diluted CMA in standard Petri dishes with *D. flagrans* (CI3) by adding 200 L_3 which were kept in the Petri dish cultures from day 5 until day 15. During that period the concentration of trapping nets was measured at intervals. Three Petri dishes were placed permanently at 10°, 20° and 30°C, respectively.

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Growth rate and trap induction under anaerobic and microaerophilic conditions

Mycelial growth rate of *D. flagrans* (CI3) was measured in cultures growing on diluted CMA in an atmosphere with different oxygen content. Each of three cultures were placed in 2-litre containers, originally filled with a normal atmosphere, and in two experiments O_2 was more or less replaced by CO_2 using respectively Anaerocult^R A and C (Merck). In the first situation, the resultant atmosphere contained approximately 0 vol.% O_2 and 18 vol.% CO_2 (anaerobic) and, in the second situation, the atmosphere contained approximately 6 vol.% O_2 and 9 vol.% CO_2 (microaerophilic). As controls, three comparable cultures were placed in a normal (aerobic) atmosphere (21 vol.% O_2 and 0.03 vol.% CO_2). Except for the oxygen concentrations all parameters were standard. After 3 weeks, cultures from the anaerobic container were transferred to a normal atmosphere.

Trap induction, in *D. flagrans* (CI3) growing on diluted CMA, was performed in nine standard Petri dishes. When the L_3 had been supplied, the cultures were placed in a 2-litre container and an anaerobic atmosphere was created as described above. All other parameters were standard. After 1, 2 and 3 weeks three cultures were, each time, transferred to a normal atmosphere. At the time of transfer and again after 2 days in the normal atmosphere, the densities of traps were enumerated.

Growth rate and trap induction in light and darkness

Growth rate and trap induction in *D. flagrans* (CI3) were investigated using six fungal cultures in each case following the standard procedures, with the exceptions that half of the cultures, in each case, were placed in constant artificial light (giving 3000–3400 Lux on the agar surface, after passage of the boxes and the Petri dishes) while the other half were placed in constant darkness at a temperature of 24°C. Differences in growth rate and trap-producing capacity were examined by a statistical analysis of variance (Sokal & Rohlf, 1981).

Growth rate and trap induction at different pH-levels

Both growth rate and trap induction were investigated according to the standard procedure except for the different pH levels. Three fungal cultures of *D. flagrans* (CI3) were examined at each pH level for both growth rate and trap induction. Values of pH between 6 and 11 were created in diluted CMA using a relatively non-toxic buffer system containing a fixed concentration of piperazine dihydrochloride and glycylglycine and variable concentrations of NaOH (Smith & Smith, 1949).

The influence of water content in cattle faeces on the mycelial growth rate

Faeces from a housed calf were carefully stirred. Measurement of the initial water content (WC%) was based on the weight loss of two portions of 30 g of faeces after drying at 105°C for 2 days. Using this initial measurement, the WC% during the experimental period could be followed gravimetrically. The experiment was carried out in four Petri dishes (diameter 8.5 cm) each containing a 1 cm thick smooth faecal layer, weighing 30 g. On the surface in the centre of each faecal portion, ten seeds from a 3-week-old millet seed culture of *D. flagrans* (Trol A) were added. An unknown, but large, number of chlamydospores were adhering to each seed.

The four Petri dishes were placed at room temperature (20–22°C) in darkness and with an air humidity of 50–70% RH. The lids were kept closed on two Petri dish cultures for the 15 day experimental period. The two other Petri dishes were placed under exactly the same conditions but the lids were off during the initial 3 days. Then lids were placed also on these Petri dishes. Therefore the faecal WC% in the two first mentioned dishes was 84% dropping to 80% during the 15 days, while the faecal WC% in the last two dishes dropped from 84% to 19% during the first 3 days, and was kept on that level until the end of the 15 day period. The growth rate was determined by measuring mycelial growth on the faecal surface. Fungal growth inside the faeces could not be determined.

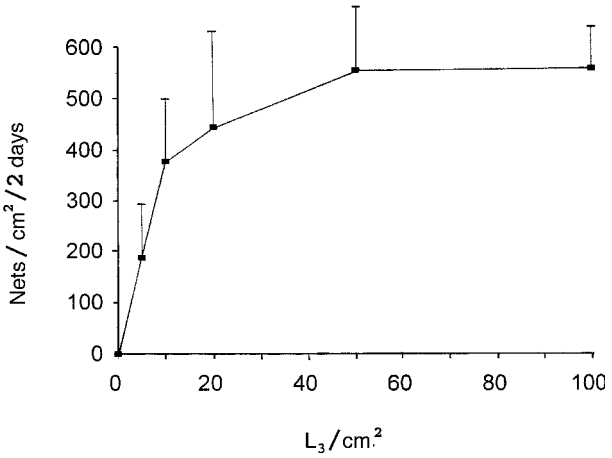

Fig. 2. The average numbers of trapping nets cm^{-2} made by *Duddingtonia flagrans* (CI3) when it was induced by increasing numbers of infective *Cooperia oncophora* larvae (L_3 cm^{-2}) in 2 days at 20°C. (+SE are indicated by vertical bars.)

Results

Trap induction at different concentrations of parasite larvae

On diluted CMA, *D. flagrans* (CI3) responds to increasing numbers of infective *C. oncophora* larvae by producing increasing numbers of trapping nets up to a maximum (fig. 2). Above a concentration of 50 L_3 cm^{-2} the fungus produces its maximum number, which in this case is between 500 and 600 nets cm^{-2} at 20°C in 2 days.

Trap induction in different strains of Duddingtonia flagrans

The three *D. flagrans* strains 'CIII4', 'CI3' and 'Trol A', when induced by 20 *C. oncophora* L_3 cm^{-2} in 2 days at 20°C, produced similar numbers of traps ($P > 0.05$) (fig. 3). A large number of trapping nets were produced in all fungal strains.

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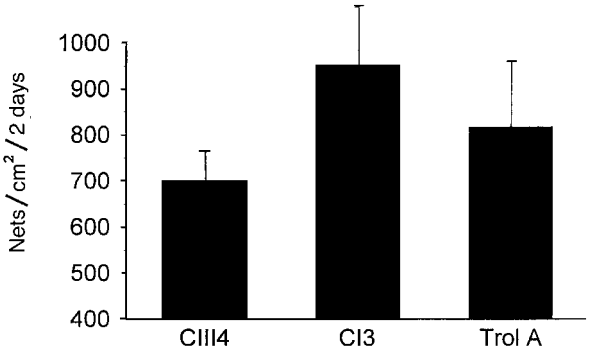


Fig. 3. The average numbers of trapping nets cm^{-2} created by three different strains (CIII4, CI3 and Trol A) of *Duddingtonia flagrans* when induced by 20 *Cooperia oncophora* L_3 cm^{-2} in 2 days at 20°C. (+SE are indicated by vertical bars.)

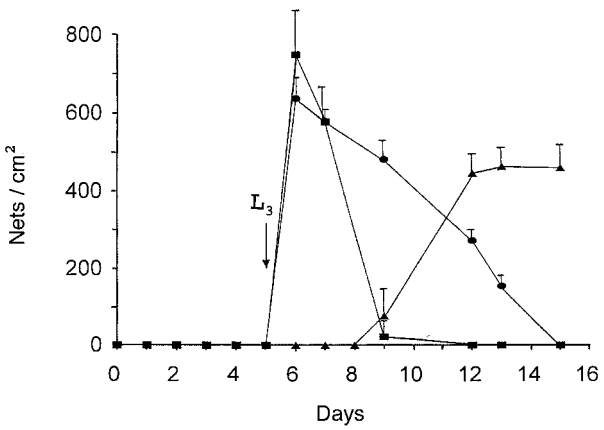
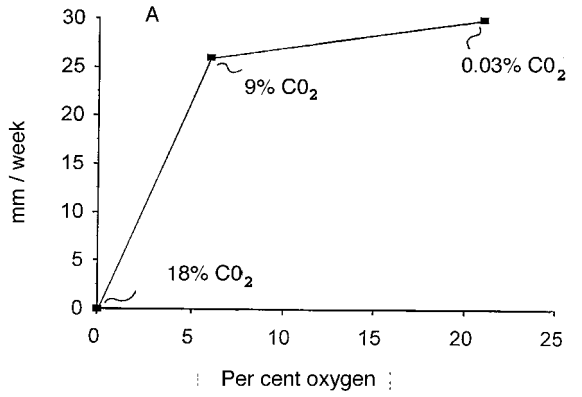


Fig. 4. Five-day-old cultures of *Duddingtonia flagrans* (CI3) were induced by 20 infective *Cooperia oncophora* L_3 cm^{-2} at 10°(▲), 20° (●) and 30°C (■). The average production of trapping nets cm^{-2} was measured at intervals from day 5 (the day when infective larvae were placed in contact with the fungus) to day 15. (+SE are indicated by vertical bars.)



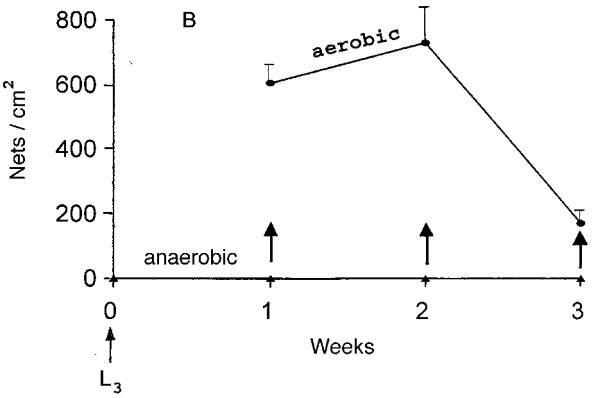


Fig. 5. A) The average growth rate (mm $week^{-1}$) for *Duddingtonia flagrans* (CI3) in an anaerobic (0 vol.% O_2), a microaerophilic (6 vol.% O_2) and an aerobic (21 vol.% O_2) atmosphere at 20°C. B) The average numbers of trapping nets cm^{-2} made by *D. flagrans* (CI3) were measured, when induced by 20 *Cooperia oncophora* L_3 cm^{-2} under anaerobic conditions for 1, 2 and 3 weeks. After 1, 2 and 3 weeks, cultures, still containing their *C. oncophora* larvae, were transferred (arrows) to a normal atmosphere for 2 days, and the average numbers of trapping nets cm^{-2} were measured again. The temperature was constantly 20°C. (The original data were mislaid in A, and therefore +SE are only indicated by vertical bars in B.)

Long term trap induction at different constant temperatures

Figure 4 shows the average production of trapping nets cm^{-2} in *D. flagrans* (CI3). The fungal cultures were 5 days old when induced by 20 infective *C. oncophora* L_3 cm^{-2}, at three different constant temperatures: 10°, 20° and 30°C . The fungus responded to L_3 very quickly at 30°C, with a maximum of nets formed 1 day after induction. A similarly quick response was found at 20°C. At 30°C nets disappeared almost completely 4 days after induction (on day 9) while at 20°C nets survived longer. At 10°C, trapping nets were produced slowly starting at day 4 after induction (on day 9) and continued over the following week to fairly high concentrations. The decrease in numbers of trapping nets at 20° and 30°C was a result of lysis of the mycelial cells. This was not the case at 10°C, because even after 14 days at 10°C, all trapping nets, created in surplus, i.e. which had not yet trapped any L_3, were in fact able to trap nematodes and kill them.

Growth rate and trap induction under anaerobic and microaerophilic conditions

Results from the growth rate experiment (fig. 5A) showed that *D. flagrans* (CI3) cannot grow in an anaerobic atmosphere containing 0 vol.% O_2 and 18 vol.% CO_2, but the mycelium did survive at least 3 weeks without oxygen. Under microaerophilic conditions (6 vol.% O_2 and 9 vol.% CO_2) the fungus has a growth rate comparable to the growth rate in a normal atmosphere (21 vol.% O_2 and 0.03 vol.% CO_2), (fig. 5A).

Results from the trap-induction experiment are shown in fig. 5B. *Duddingtonia flagrans* (CI3) did not produce

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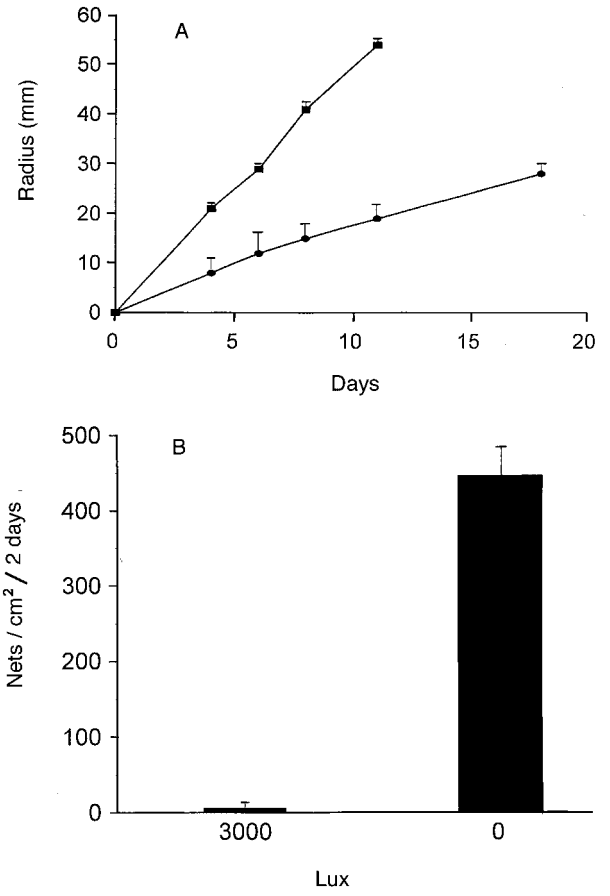

Fig. 6. A) The average *growth rate* (radius in mm) for *Duddingtonia flagrans* (CI3) at 24°C under artificial light (3400 Lux: ●) or in darkness (0 Lux: ■). B) The average numbers of trapping nets cm^{-2} made by *D. flagrans* (CI3) when induced at 24°C for 2 days by 20 *Cooperia oncophora* L_3 cm^{-2} under artificial light (3000 Lux) or in darkness. (+SE are indicated by vertical bars.)

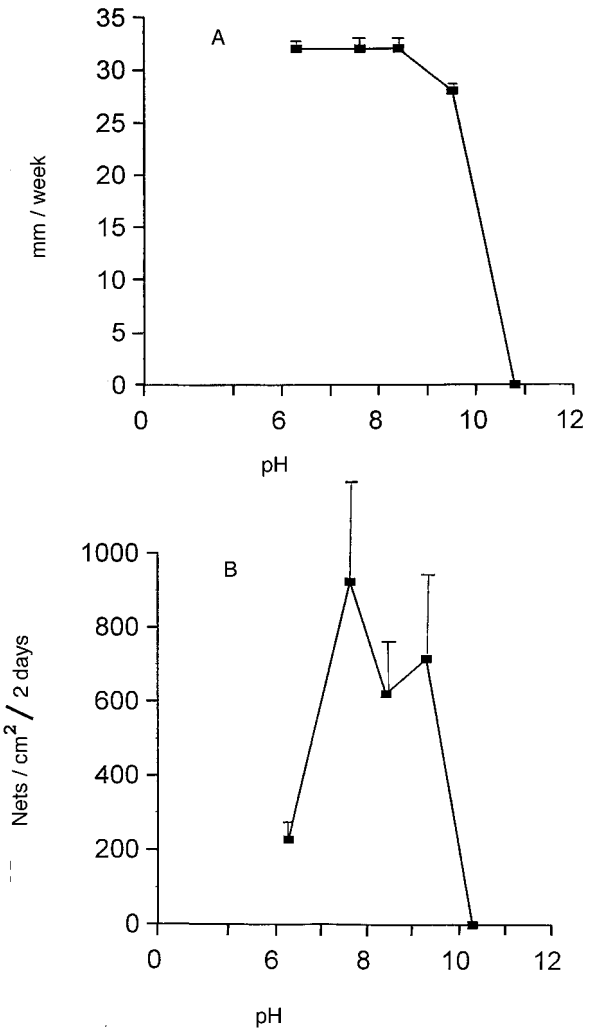

Fig. 7. A) The average growth rate (mm $week^{-1}$) for *Duddingtonia flagrans* (CI3) at 20°C and different pH values. B) The average numbers of trapping nets cm^{-2} made by *D. flagrans* (CI3) when induced at 20°C for 2 days by 20 *Cooperia oncophora* L_3 cm^{-2} at different pH values. (+SE are indicated by vertical bars.)

trapping nets in an anaerobic atmosphere consisting of 0 vol.% O_2 and 18 vol.% CO_2. When the fungal cultures were transferred to a normal aerobic atmosphere (21 vol.% O_2 and 0.03 vol.% CO_2), after 1 and 2 weeks under anaerobic conditions, the *C. oncophora* L_3 resumed their migration on the agar and, in response, *D. flagrans* produced trapping nets within the normal range of concentrations. After 3 weeks in an anaerobic atmosphere, the ability to produce trapping nets was reduced.

Under anaerobic conditions, the L_3 did not move. But direct observation showed that anaerobic conditions for at least 3 weeks did not harm the *C. oncophora* L_3, as they started migrating normally when transferred from an anaerobic to an aerobic atmosphere.

Growth rate and trap induction in light and darkness

Artificial light giving 3000–3400 Lux on the surface of the agar has a very significant ($P < 0.01$) depressing effect both on the growth rate (fig. 6A) and the production of trapping nets in *D. flagrans* (CI3) (fig. 6B). It is not known to which wavelengths the fungus is most sensitive.

Growth rate and trap induction at different pH levels

As the agar was liquid at pH 5.1, only pH values from 6.3 to 10.8 were included in the experiment, which was performed on the solid agar surface. *Duddingtonia flagrans* (CI3) grew and produced trapping nets in the pH interval from 6.3 to 9.3 (fig. 7A,B). Net-production has its optimum between pH 7 and 8 (fig. 7B).

The influence of water content in cattle faeces on the mycelial growth rate

The mycelial growth rate of *D. flagrans* (Trol A) was measured on the surface of: (i) dry cattle faeces where the

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WC% was reduced from 84% to 19% in 3 days and kept at this level during the following 12 days; and (ii) moist cattle faeces where the WC% was kept between 84% and 80%. On dry faeces the mycelial expansion was 7–10 mm during 15 days while on moist faeces the fungus expanded 15–20 mm during the same period.

Discussion

The results of the experiments performed on diluted CMA showed that the *D. flagrans* (CI3) has a high trapping capacity, as it produces an excess of trapping nets, at all tested levels of infective *C. oncophora* larvae, i.e. there were more nets created than L_3 added.

The results did not indicate any differences between the three tested strains of *D. flagrans* (CIII4, CI3 and Trol A) in their ability to produce trapping nets. However, direct observations did reveal some differences in the morphology of the nets. In that respect both 'CI3' and 'Trol A' had compact nets composed of thick arches of hyphae, while 'CIII4' created more open nets composed of more slender hyphae. It is unknown if the slender nets are more or less effective in trapping and killing nematodes. However most attention has been paid to strain number 'CI3', as it was selected for the first field trails in Denmark (Grønvold *et al*., 1993; Wolstrup *et al*., 1994), because it was the most effective candidate at that time.

It appears that *D. flagrans* (CI3) is able to produce nets at all temperatures between 10° and 30°C when induced by infective *C. oncophora* larvae. Under the non-sterile experimental conditions, at 20° and 30°C, the nets disappeared within 9 days. Moreover, nets are destroyed faster at 30°C than at 20°C. The most likely explanation is the higher microbial activity at 30°C, as direct observations clearly indicate that strong bacterial activity results in lysis of hyphae and nets. If 0.02% tetracycline chloride is added to the agar medium, the bacterial growth is suppressed and trapping nets of *Arthrobotrys oligospora* (another nematode-killing fungus) will maintain its trapping potential for 7 weeks, even at temperatures of 30°C (Grønvold, 1989). It would be interesting to know how long nets are able trap nematodes in cow pats, with its high microbial activity. At 10°C, bacterial activity is low and virtually no lysis occured. On the other hand, trapping nets are produced more slowly at this temperature. Under comparable conditions in the first series of experiments (Grønvold *et al*., 1996), long term induction by *O. ostertagia* L_3 only resulted in approximately 25 nets cm^{-2} in *D. flagrans* (CI3) at 10°C compared with 460 nets cm^{-2} here. This may be related to the behaviour of *O. ostertagi* L_3, which at 10°C tend to be inactive, roll up and remain in one place. This is not the case for *C. oncophora* L_3 which continue to migrate slowly also at 10°C, which induces the fungus to produce trapping nets.

The growth rate of soil fungi is usually unaffected until the oxygen concentration is lowered to about 4 vol.% O_2 in the gas phase (Griffin, 1972). *Duddingtonia flagrans* (CI3) grows at a normal rate at least down to an oxygen concentration of 6 vol.% O_2, and does not grow anaerobically. Moreover, *D. flagrans* may grow only in the upper 5 mm of an intact surface layer of fresh cattle faeces, because of the reduced oxygen tension inside the compact faecal material. On the other hand, it is known that during its disintegration, oxygen tension increases to above 10 vol.% oxygen gas even in the deeper layers of an 20- to 30-day-old cow pat (Holter, 1991), most likely giving *D. flagrans* a chance to be active also here. Under anaerobic conditions, *D. flagrans* (CI3) does not produce trapping nets, but it survives and retains its ability to produce nets for at least 3 weeks. The decrease in the ability to produce nets in an ageing mycelium placed anaerobically may primarily be a result of senility (or less likely, depletion of nutrients) rather than the lack of oxygen, because under sterile and aerobic conditions on agar at 10°, 20° and 30°C, *D. flagrans* loses its ability to produce traps after 2–3 weeks (Grønvold *et al*., 1996). Preliminary results have shown that *D. flagrans* (Trol A) does not produce trapping nets under microaerophilic conditions (6 vol.% O_2) even though it can grow under such conditions. The reason probably is that *C. oncophora* L_3 are immobile at this low oxygen tension. The fungus is not induced. However, as *C. oncophora* L_3 survive low oxygen tensions for several weeks, and as *D. flagrans* also survived low oxygen tensions and even grew under microaerophilic conditions (6 vol.% O_2), it must be expected that in the cow pat, the fungus will be ready to attack parasite larvae, when they start migrating under the influence of increasing oxygen tension created when the cow pat disintegrates.

As *D. flagrans* (CI3) is sensitive to light, it must be expected that *D. flagrans* is better adapted to conditions under the surface of a cow pat than on the very surface, where direct sunshine may expose it to more than 30,000 Lux in the daytime. The disintegration of the ageing cow pat may of course expose fungal hyphae inside the pat to detrimental radiation. The depressing effect of light has also been ascertained for *A. oligospora* (Grønvold, 1989).

Using a buffer system to evaluate the influence of pH, care has to be taken about conclusions drawn from negative results, as the fungus may react negatively to the hydrogen ion concentration as well as to the changing concentrations of the chemicals used in the buffer. It is also impossible to know which factor is the decisive one. As the present conclusion is based on positive results only, it is obvious, that *D. flagrans* (CI3) tolerates a pH range of between 6 and 9, values which are found within cow pats (table 1). It is interesting that *D. flagrans* (CI3) is able to be active at high pH values around and above pH 9.

Another potential problem concerning buffer systems is that the chemicals involved may affect the *C. oncophora* L_3, so that they become immobilized or die. If this happens, induction of trapping nets may be compromised. The present buffer system was composed of a basic solution constantly containing 1.591 g piperazine dihydrochloride + 1.321 g glycylglycine in 1 litre of water and adjusted with increasing amounts of 1 N NaOH for increasing pH values, e.g. 27.17 ml 1 N NaOH at a theoretical pH of 10. In a solution (without agar) at pH 10, the L_3 were very active when they were first placed in the solution. After 24 h in the solution, about 50% of the larvae were immobilized, presumably because piperazine dihydrochloride, which is a known anthelmintic, causes paralysis. Glycylglycine is a relatively non-toxic material which is a normal constituent of many proteins. However, even the problem with piperazine dihydrochloride

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cannot change the above conclusion, as a large number of nets were induced between pH 6 and 9. *Duddingtonia flagrans* (CI3) tolerates pH values between 6 and 9 and the fungus is induced to produce trapping nets by *C. oncophora* even though piperazine dihydrochloride most likely affects the migration of the nematodes at these values.

It is interesting that *D. flagrans* (Trol A) is able to grow even at very low water contents in cattle faeces. At least one field experiment has confirmed that the fungus is able to tolerate a dry period. In this field experiment (Wolstrup *et al*., 1994), cattle were fed *D. flagrans* (CI3) during a period of approximately 2 months with only 1 mm precipitation. Even in this extreme situation, it was clear the fungus had been very active in reducing the parasite concentrations in faeces after passage through the alimentary tract of cattle.

In conclusion, *D. flagrans* has a high nematode-trapping capacity and the fungus must be expected to be able to be especially active in the well aerated surface layer of a cow pat. In this part of the pat, which normally contains a high concentration of infective nematode parasitic larvae, the temperature can be high and the water content low. Based on the present results it therefore seems reasonable to characterize *D. flagrans* as 'coprophilic'.

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