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PREDATORY ACTIVITY OF *Arthrobotrys oligospora* AND *Duddingtonia flagrans* ON PRE-PARASITIC LARVAL STAGES OF CYATHOSTOMINAE UNDER DIFFERENT CONSTANT TEMPERATURES

ATIVIDADE PREDATÓRIA DE *Arthrobotrys oligospora* E *Duddingtonia flagrans* NOS ESTÁDIOS LARVARES PRÉ-PARASÍTICOS DE CYATHOSTOMINAE SOB DIFERENTES TEMPERATURAS CONSTANTES

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SUMMARY

*The effect of different temperatures on the predatory activity of **Arthrobotrys oligospora** and **Duddingtonia flagrans** on the free-living larval stages of cyathostomes were evaluated in an experiment where feces of horses containing the parasites' eggs were treated with these fungi and incubated under different constant temperatures (10°C, 15°C, 20°C, 25°C and 30°C). The results indicated that the optimum temperature for egg development was 25°C. At 10°C the number of L_3 recovered was practically zero, and at 15°C and 20°C, the percentage of larvae recovered was less than 3% of the total number of eggs per gram of feces. When these cultures subsequently were incubated for an additional period of 14 days at 27°C, they allowed the development of L_3. In all the cultures inoculated with fungi a significant reduction in the number of larvae was observed. When incubated at 25°C or 30°C, the fungi caused reductions above 90%, in the number of L_3. The samples cultivated at 10°C, 15°C, 20°C, 25°C and 30°C, when incubated for an additional period of 14 days at 27°C the reduction percentage of larvae was above 90% for **A. oligospora.** However, the same did not occur for **D. flagrans**. Here a reduction percentage between 47.5% and 41.8% was recorded when the cultures were incubated at 10°C and 20°C, respectively. The two species of fungi tested showed to be efficient in reducing the number of L_3 when mixed with equine feces and maintained at the same temperature for the development of larval pre-parasitic stages of cyathostomes.*

Key words: ***Arthrobotrys oligospora***, ***Duddingtonia flagrans***, *nematophagous fungi, cyathostominae, biological control.*

RESUMO

*A atividade predatória de fungos nematófagos e o desenvolvimento de estádios pré-parasitários de nematódeos estrongilídeos são influenciados pela temperatura. O efeito de diferentes temperaturas constantes na atividade predatória dos fungos **Arthrobotrys oligospora** e **Duddingtonia flagrans** sobre as fases larvares de vida livre dos nematódeos ciatostomíneos foi avaliado em um experimento onde fezes de cavalos contendo ovos de parasitos foram tratadas com fungos e incubadas em diferentes temperaturas constantes (10°C, 15°C, 20°C, 25°C e 30°C). Os resultados indicaram que a temperatura ótima de desenvolvimento de ovo até L_3 foi de 25°C. A 10°C o número de L_3 recuperado foi praticamente zero e a 15°C e 20°C os percentuais obtidos foram inferiores a 3% do total do número de ovos por grama de fezes. Quando estes cultivos foram incubados por um período adicional de 14 dias à 27°C permitiram o desenvolvimento até L_3. Em todos os cultivos inoculados com fungos, quando as larvas estavam presentes, houve redução significativa no número de larvas atribuído à ação predatória dos fungos. A 25°C e 30°C os fungos ocasionaram reduções acima de 90% no número de L_3. Os cultivos mantidos à 10°C, 15°C, 20°C, 25°C e 30°C quando incubados por um período adicional de 14 dias na temperatura controle, mantiveram percentuais de redução larvar superior a 90% para o fungo **A. oligospora**, o mesmo não ocorrendo para **D. flagrans**, que apresentou percentuais de 47,5% e 41,8% quando estimulado a 10°C e 20°C, respectivamente. Esses fungos demonstraram ser eficientes na redução do número de L_3 quando adicionados a fezes de eqüinos nas mesmas temperaturas ideais para o desenvolvimento dos estádios larvares pré-parasitários.*

Palavras-chave: ***Arthrobotrys oligospora, Duddingtonia flagrans*** *fungos nematófagos, cyathostominae, controle biológico.*

INTRODUCTION

Anthelminthic resistance in small strongyles in horses, primarily against

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benzimidazole, is well-documented and becoming common in several regions (BJØRN ***et al***., 1991; BOERSEMA ***et al***., 1991). Grazing practices and mechanical pasture hygiene have been recommended as an aid to reduce anthelmintic use to control cyathostomes (HERD, 1993). Biological control of free-living stages by the use of microfungi might be an alternative to integrate into future control practices of this horse helminthosis. The potential of this group of organisms to reduce free living stages of parasitic nematodes, which have similar cycle on pasture, has been well demonstrated at laboratory as well as field experiments (LARSEN, 1999).

The effect of nematophagous fungi against free-living stages of cysthostomes has been examined in a few studies. ***Arthrobotrys oligospora*** and ***Duddingtonia flagrans***, two nematophagous fungi which both produce hyphal traps significantly reduce the number of infective larvae in fecal cultures as well as in pasture (BIRD & HERD, 1995; CHARLES ***et al***., 1995, FERNÁNDEZ ***et al***., 1997). LARSEN ***et al***., (1995) demonstrated that a Danish isolate of ***D. flagrans*** was able to survive gut passage and subsequently trap larvae in horse feces. When tested in a field experiment, a daily dose of chlamydospores of ***D. flagrans*** mixed in a feed supplement and given to horses for a period of three months reduced significantly the transmission of larvae from fecal pats to herbage (LARSEN ***et al***., 1996). FERNÁNDEZ ***et al***., (1997) showed that when fungal spores were fed to horses for five consecutive days at a dose rate from 10^6 to 5 X 10^6 it gave a high and significant reduction in number of infective strongyle larvae.

For the Nematophagous fungi be used in biological control of horse nematodes, it has to act on fecal pats at different environmental conditions. Various morphogenic processes by nematophagous fungi, such us trap formation, are influenced by environmental conditions especially temperature (GRØNVOLD, 1989). The present paper focuses on the effect of different constant temperatures on the trapping efficacy of ***A. oligospora*** and ***D. flagrans*** against pre-parasitic larvae of cyathostomes in fecal culture.

MATERIALS AND METHODS

Cultures of ***A. oligospora*** (University of Guelph number 80) and ***D. flagrans*** (University of Guelph number 126, synonyms ***Trichothecium flagrans*** and ***Arthrobotrys flagrans***) used in this experiment were donated to our laboratory by Dr. Rupert Herd from Ohio State University. They were grown in corn meal agar (4% agar) at room temperature of 20°C- 27°C. When fungi covered the whole surface of the plates, conidia and mycelial fragments of ***A. oligospora*** were harvested by scraping the surface of the agar with the aid of a brush and by rinsing off the surface with distilled water. ***D. flagrans*** chlamydospores and mycelial fragments were recovered in the same manner as the conidia of ***A. oligospora***. However, to isolate the chlamydospores from the washed material, the suspension was mixed with a pipette, it was aspirated and released vigorously during many times in order to get a maximum isolation of chlamydospores. The concentration of fungal units in the suspension was adjusted after counting using of a haemacytometer chamber.

Feces were collected per rectum of horses carrying a natural infection of strongyles nematodes, which were known to be predominantly cyathostomes (98%) as determined through individual larval cultures. After thorough mixing and determination of number of egg per gram of feces (EPG), 90 aliquots of 4g each were distributed into disposable plastic cups with 60mℓ capacity. From those, 30 cultures were each treated with a 4mℓ suspension of 2000 conidia of ***A. oligospora*** in distilled water/ml, another 30 cultures were each treated with 4 ml of a suspension of chlamydospores of ***D. flagrans*** in distilled water/ml and the last 30 received 4mℓ of distilled water only. Subsequently, the cultures were individually homogenized. Out of the 30 samples submitted to each treatment, 10 of them were incubated at 27°C and the other 20 were incubated at the test temperature. Each group of samples was placed in a tray containing a thin layer of water in the bottom. The tray was covered with a plastic film to prevent loss of moisture during the 14 days incubation period. After the incubation, 10 samples incubated at the control temperature and 10 samples incubated at the test temperature were baermanized. The other 10 samples, which were incubated at the test temperature, were transferred to the control temperature for an additional incubation for 14 days after which they were also baermanized to recover the infective larvae (L_3). Recovered L_3 were fixed and preserved in formaline 10% for later quantification. This set-up was repeated five times, one for each of the five-test temperature studied: 10°C, 15°C, 20°C, 25°C and 30°C.

The number of larvae recovered from the cultures were analyzed after logarithmic transformation using the equation $X = \log_{10} (x + 1)$. In all the comparisons the Levine's test, analysis of variance and Tukey's test were used to the level of

significance of 5% (HENKEL, 1976; GLANTZ, 1992). For the analyses, the SPSS software, version 7.5 (SPSS, 1997) was used.

RESULTS AND DISCUSSIONS

A.oligospora and ***D. flagrans*** were both able to reduce the number of larvae in fecal cultures significantly. When added to feces and cultivated at 27°C a reduction of more than 90% in the number of infective larvae was observed (Table 1).

The number of infective larvae recovered at 10°C, 15°C and 20°C was significantly lower than the number recovered when samples were kept at 27°C (control temperature). Egg development up to infective stage at and below 20°C was less than 3% of the total number of EPG. Once cultures were reincubated at 27°C for an additional period of 14 days, development of infective larvae reached levels close to what was obtained at the control temperature (Table 1). Yields of infective larvae in temperatures below to 20°C were much lower as it was demonstrated in other studies (OGBOURNE, 1972; RUPASINGHE & OGBOURNE, 1978; ENGLISH, 1979; MFITILODZE & HUTCHINSON, 1987).

MFITILODZE & HUTCHINSON (1987), working with fecal samples of 5g and 10g observed that yields of infective larvae were much higher in samples with larger volume. They found that temperature and moisture interacted to affect both the rate of development and yields of infective larvae from horse feces. Larger fecal samples had protective effect in keeping the moisture contents and larval yields. In the present study, it was observed low percentage of egg, which developed up to infective stage in all cultures not treated with fungi (Table 1). This may relate to the low volume of fecal samples used. Since low numbers L_3 were obtained at 10°C, 15°C and 20°C, it was not possible to investigate the effect of temperature on the capacity of the fungi to act on the free living stages in the fecal environment, at least for a 14 day period. However, the results indicate that the maintenance of fungi at these temperatures for a 14 day period do not affect its predatory activity since they were, aside from ***D. flagrans*** kept at 10°C, capable of reducing significantly the number of larvae once samples were transferred to 27°C and incubated for an additional 14 days.

The number of larvae recovered from the cultures treated with ***D. flagrans*** at 10°C and 20°C and reincubated at 27°C where 47.5% and 41.8% of the number of larvae recovered from the samples treated only with distilled water (Table 1). Reductions in these temperatures due to the action of the fungi were expected to be higher, especially considering that reduction was almost 94% at 15°C. These results could have been due to interaction between the incubation period and the ratio of chlamydospore per egg. In these cultures the incubation period was 28 days and the number of chlamydospore per egg was below one. In previous experiment conducted with the same isolates,

Table 1 - Mean number of infective cyathostome larvae recovered from faecal cultures submitted to five constant temperatures (x ± s.d.) and the percentage of egg which developed up to infective stage in each treatment (in parenthesis).

Experiment	Initial EPG	Temperatures	Treatments		
			Distilled water	A. oligospora	D. flagrans
1	2165	CT	1074 ±506 (49.6)	42±50 (1.9)	37±20 (1.7)
		TT 10^0C	0 (0)	1±0.8 (0.05)	3±8 (0.1)
		TT+ reincubation CT	985±4 (45.5)	7±8 (0.7)	517±556 (23.9)
2	1033	CT	111±134 (10.7)	5±6 (0.5)	11±11 (1.1)
		TT 15^0C	2±3 (0.2)	4±5 (0.4)	0 (0)
		TT+ reincubation CT	161±65 (15.6)	7±6 (0.7)	10±11 (0.9)
3	3000	CT	1029±1004 (34.3)	30±25 (1.0)	29±21 (0.96)
		TT 20^0C	10±16 (0.3)	8±8 (0.26)	80±71 (2.7)
		TT+ reincubation CT	670±471 (22.3)	4±4 (0.13)	390±225 (13.0)
4	1200	CT	184±217 (15.3)	16±15 (1.3)	10±7 (0.8)
		TT 25^0C	364±161 (30.3)	2±3 (0.17)	20±11 (1.7)
		TT+ reincubation CT	263±177 (21.9)	11±9 (0.92)	12±12 (1.0)
5	1270	CT	910±389 (71.7)	20±16 (1.6)	25±19 (1.97)
		TT 30^0C	620±243 (48.8)	60±43 (4.7)	33±15 (2.6)
		TT+ reincubation CT	340±174 (26.8)	18±15 (1.4)	12±17 (0.95)

EPG - Eggs per gram
CT – Control temperature of 27ºC
TT – Test temperature in each experiment

significant reduction of infective cyathostome larva was observed only when a concentration equal or higher than one chlamydospores per egg was used (BIRD, 1995). In the experiment conducted here, the dosages of chlamydospores were determined by gram of feces. After converting it to chlamydopores per egg of cyathostome, only at 10°C and 20°C was the ratio below one (0.92 and 0.67, respectively). At the other test temperatures, the ratio chlamydospore: egg was above one (1.94, 1.67 and 1.57 at 15°C, 25°C and 30°C, respectively). The same ratio chlamydospore: egg was present at the control temperature (27°C) in the experiments run at 10°C and 20°C and the fungus was effective (reduction higher than 90%), however the incubation time for the control cultures was half the time used to the cultures reincubated at 10°C and 20°C. It may be possible that the low number at chlamydospores and the longer incubation period contributed low reduction of larval in those cultures. Besides, an experiment conducted in Denmark with seven isolates of ***D. flagrans*** concluded that this fungus grows slowly at temperatures lower than 25°C (LARSEN, 1991) and optimal growth is obtained at 30°C. When the isolates were studied at 10°C, 15°C and 20°C, radial growth of the seven isolates was smaller when compared to the growth of one isolate of ***A. oligospora*** and two isolates of ***Arthrobotrys superba***.

Both fungi tested species showed to be efficient in reducing the number of L_3 when mixed with equine feces and maintained at the same temperature for the development of larval pre-parasitic stages of cyathostomes.

The similarity of temperature requirements for the action of these fungi and the development of free-living stages of these nematodes in the fecal environment, as well as the capacity of the fungus to resume growth after being kept at lower temperatures, could facilitate the development of this alternative to classical control using anthelmintics.

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