Predacious activity of *Arthrobotrys spp* isolates on infective *Cooperia punctata* larvae*

CORRESPONDENCE TO:
Jackson Victor de Araújo
Departamento de Veterinária
Universidade Federal de Viçosa
36571-000 – Viçosa – MG
e-mail: jvictor@mail.ufv.br

1 - Departamento de Veterinária da Universidade Federal de Viçosa – MG

Atividade predatória de isolados de *Arthrobotrys spp* sobre larvas infectantes de *Cooperia punctata*

Jackson Victor de ARAÚJO[1]

SUMMARY

Laboratory experiments were performed in order to investigate the capacity of isolates from the predacious fungi *Arthrobotrys musiformis* (isolate 3), *A. conoides* (isolate A) and *A. robusta* (isolates B and E) to trap and kill infective *Cooperia punctata* larvae. Two groups were formed for each isolate: group 1, fungi and infective larvae and group 2, infective larvae (control). There were statistical differences ($p<0.05$) between the predacious activity of one isolate of *A. robusta* (isolate E) when compared with the other isolates of *Arthrobotrys spp* (isolates A, B and 3). No statistical difference ($p>0.05$) was found between the isolate E and the control. This indicates that there can be an existing variability within a fungus species or genus concerning the predation of infective *C. punctata* larvae.

UNITERMS: *Arthrobotrys spp*; Fungi; *Cooperia punctata*; Biological control.

INTRODUCTION

Cooperia is considered the most common genus of the nematode in bovine, particularly in tropical areas and included the species *C. punctata*[8].

A number of fungi are capable of trapping nematodes by means of specialized trapping devices. The predacious group, which includes the genus *Arthrobotrys*, produces an extensive system of hyphae in the environment. Along the hyphae there are organs that are able to capture living nematodes[5]. Performing laboratory tests, Araújo *et al.*[3,4], demonstrated the potential of isolates of *Arthrobotrys spp*, to reduce the amount of infective *Haemonchus placei* larvae.

This experiment was performed in order to compare the nematode-trapping potentials of different *Arthrobotrys spp* isolates on *Cooperia punctata* under laboratory conditions.

MATERIAL AND METHOD

Predacious fungi species from Brazil were maintained on a 2% potato dextrose agar at 4ºC in the dark. These predacious fungi species were denominated, according to Araújo *et al.*[3,4], *Arthrobotrys conoides* (strain A), *A. robusta* (strains B and E) and *A. musiformis* (strain 3). From the stock, cultures were transferred to Petri's dishes containing calf faecal agar made with 50 g macerated faeces, filtered through a 61 *u*m sieve. This filtrate was adjusted to 500 ml, added to 2% agar-agar and then autoclaved for 15 min. at 120ºC. To these dishes was added a 1 ml suspension with 1,000 *Panagrellus spp* (free living nematode) during a period of 2 days. In the 5th day, the dishes became completely overgrown by the different fungal isolates. Two ml of distilled water were added to each Petri's dish and conidia and mycelial fragments removed with a delicate brush and the isolated fungal material stored in 50 ml Erlenmeyer's flasks.

Infective *Cooperia punctata* larvae (L3) were kindly donated by Dr. M.C.R.Vieira-Bressan, Department of Parasitology, University of São Paulo. In order to remove bacteria and fungi, these nematodes and the free living nematodes were maintained in 15 ml tubes using a solution containing 0.05% streptomycin sulphate, 0.01% chloramphenicol and 0.05% B amphoterycin for a week. Later, the nematodes were washed 10 times in sterile distilled water and examined to check their viability.

The experiment was performed in Petri's dishes, measuring 8.5 cm in diameter, containing 2% water agar marked with fields of 4 mm in diameter according to Araújo *et al.*[3]. For each isolate, two groups were performed: group 1, consisted of 1,000 conidia of fungi and 10,000 *C. punctata* L3; and group 2 of 10,000 *C. punctata* L3 (control). Each group contained 3 replicates. Daily, for 20 days, 10 fields on the dishes from the groups were examined under an optical microscope. The number of freely migrating larvae from groups 1 and 2 were counted. On comparing the efficacy of each isolate, minimal significant test difference test was performed.

Twenty days later, the infective larvae from groups 1 and 2 were recovered by the Baermann's method and counted. The mean

* Financial support FINEP.

of 3 repetitions was calculated and minimal significant difference test ($p<0.05$) was performed.

RESULTS AND DISCUSSION

Fig. 1 shows the number of free infective *C. punctata* larvae in the 2% water agar medium in the Petri's dishes from group 1 of the isolates A, B, E and 3 and group 2 (control) during the experimental period. Isolate E showed less efficiency than isolates A, B and 3 ($p<0.05$) and it was not responsible for a significant reduction ($p>0.05$) in numbers when compared with the control group. This indicates that there is a variability within a fungal species or genus concerning the predation of infective *C. punctata* larvae. Araújo *et al.*[3] working in a laboratory experiment to investigate the capacity of different isolates of *Arthrobotrys* to trap and kill infective *Haemonchus placei* larvae concluded that the isolates A, B and E were the most efficient in destroying infective *H. placei* larvae. In the present experiment isolate E showed less predacious activity on infective *C. punctata* larvae, however the others isolates tested in both experiments, showed a similar activity.

Tab. 1 shows the number of infective *C. punctata* larvae recovered from Petri's dishes (groups 1 and 2) after 20 days of observation, using Baermann's apparatus. There was a significant difference ($p<0.05$) between all isolates and the control group. In a recent experiment Araújo *et al.*[2] selected nematode-trapping fungi to pass through the gastrointestinal tract of calves, after the oral administration to calves of four doses of 5×10^6 conidia of isolates of *Arthrobotrys spp*, every three hours, only an isolate of *A. robusta* (isolate E) passed through the gastrointestinal tract without loosing its viability to prey infective *Haemonchus placei* larvae, while no evidence was found in isolates B (*A. robusta*) and 3 (*A. musiformis*). More recently, Araújo[1] working in field tests with calves orally administered with 2 million conidia of isolate E of *A. robusta*, twice per week, during 4 months, compared it with the untreated animals, and found a reduction of 53.81% ($p<0.05$) in eggs of gastrointestinal parasite nematodes per gram of faeces and a reduction of 70.45% ($p<0.05$) in the number of helminths recovered at necropsy of the tracer calves.

According to Stirling[11] the loss of predatory capacity can occur in isolates when maintained for a long time in laboratory conditions without the supply of nematodes.

In vitro tests continue to be feasible in researches with nematophagous fungi, although with limitations. Normally they overestimate the activity of an agent, because they do not allow the nematode to escape. But it is an important technique to screen the nematophagous fungi activity against the helminths, since not all isolated fungi have necessarily, parasitic activity[3].

This is the first report using the nematode-trapping fungi *Arthrobotrys spp* and *C. punctata.* Gronvold *et al.*[6] showed that conidia of the predacious fungus *A. oligospora* resulted in a reduction of infective larvae of *Cooperia spp*, when added to faeces. Moreover, the trapping effect in faeces was shown to depend on the level of inoculation. Nansen *et al.*[10] working with *A. oligospora* and preparasitic and infective stages of *Cooperia*

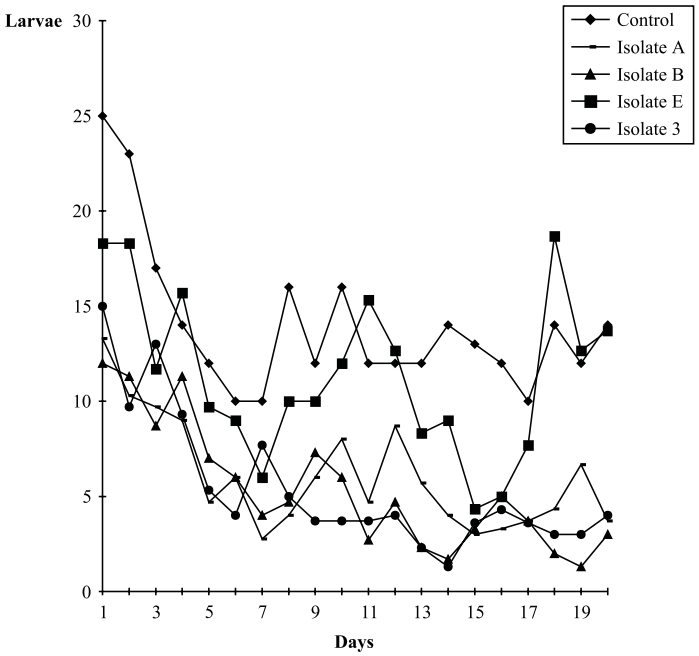

Figure 1

Numbers of migrating larvae of *Cooperia punctata* in contact with different isolates of *Arthrobotrys spp*, and in the control group, per microscopical field.

Table 1

Infective *Cooperia punctata* larvae (L3) recovered 20 days after contact with *Arthrobotrys spp*, Viçosa – MG, 1996.

Groups	Isolates	L3 ± standard deviations
1	A (*A. conoides*)	22 ± 6*
	B (*A. robusta*)	21 ± 8*
	E (*A. robusta*)	199 ± 22*
	3 (*A. musiformis*)	76 ± 12*
2	Control	645 ± 33

* Statistical difference ($p<0.05$).

oncophora observed that the first and second stages of *C. oncophora* were killed rapidly; in contrast L3 wriggled in the traps for many hours. This may be due to the difficulty for *A. oligospora* to penetrate the outer cuticle that protects the infective *C. oncophora* larvae. Nansen *et al.*[9] in an *in vitro* assay observed that *A. oligospora* is capable of destroying L3 of *C. oncophora* and *C. curticei*. Under field conditions, Hashmi; Connan[7] giving *A. oligospora* conidia in the food to calves, observed that the pasture grazed by those calves showed 62% less infective *C. oncophora* larvae.

It is important to test nematophagous fungi in different locations, since they can be more or less effective in different ecological niches. Future experiments may show if our isolates could be used for biological control of gastrointestinal nematode under natural conditions in grazing calves. According to Waller; Larsen[12] such control will never be a substitute for chemotherapy, where the primary purpose is worm removal from the host, but should be incorporated together with other options into integrated pest management systems.

RESUMO

Experimentos laboratoriais foram realizados para investigar a capacidade de isolados de fungos predadores das espécies *Arthrobotrys musiformis* (isolado 3), *A. conoides* (isolado A) e *A. robusta* (isolados B e E) de predar e matar larvas infectantes de *Cooperia punctata*. Dois grupos foram formados para o teste de cada isolado: grupo 1, fungos e larvas infectantes e grupo 2, larvas infectantes (controle). Houve diferença estatisticamente significativa ($p<0,05$) entre a atividade predatória do isolado E de *A. robusta*, quando comparado com todos os outros isolados de *Arthrobotrys spp* (isolados A, B e 3). Nenhuma diferença estatística ($p>0,05$) foi encontrada entre o isolado E e o grupo controle. Isto pode indicar uma variação existente dentro de uma mesma espécie de fungo ou gênero quanto à predação de larvas infectantes de *C. punctata*.

UNITERMOS: *Arthrobotrys*; Fungos; *Cooperia punctata*; Controle biológico.

REFERENCES

1- ARAÚJO, J.V. **Interação entre larvas infectantes de *Cooperia punctata* e fungos predadores do gênero *Arthrobotrys*, caracterização de isolados de *Arthrobotrys* e seu uso no controle biológico de nematódeos parasitos gastrintestinais de bovinos**. Belo Horizonte, 1996. 110p. Tese (Doutorado) – Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais.

2- ARAÚJO, J.V.; NETO, A.P.; AZEVEDO, M.H.F. Screening parasitic nematode-trapping fungi *Arthrobotrys* for passage through the gastrointestinal tract of calves. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.48, n.5, p.543-52, 1996.

3- ARAÚJO, J.V.; SANTOS, M.A.; FERRAZ, S.; MAIA, A.S. Antagonistic predacious *Arthrobotrys* fungi on infective *Haemonchus placei* larvae. **Journal of Helminthology**, v.67, n.3, p.136-8, 1993.

4- ARAÚJO, J.V.; SANTOS, M.A.; FERRAZ, S.; MAIA, A.S. Biological control in vitro of infective *Haemonchus placei* larvae by predacious fungi *Arthrobotrys musiformis*. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.46, n.3, p.197-204, 1994.

5- BARRON,G.L. **The Nematode-destroying fungi**. Ontario : Canadian Biological, 1977. p.140.

6- GRONVOLD, J.; KORSHOLM, L.; WOLSTRUP, J.; NANSEN, P.; HENRIKSEN, S.A. Laboratory experiments to evaluate the ability of *Arthrobotrys oligospora* to destroy larvae of *Cooperia* species, and to investigate the effect of physical factors on the growth of the fungus. **Journal of Helminthology**, v.59, n.4, p.119-25, 1985.

7- HASHMI, H.A.; CONNAN, R.M. Biological control of ruminant trichostrongylide by *Arthrobotrys oligospora*, a predacious fungus. **Parasitology Today**, v.5, n.2, p.28-30, 1989.

8- LIMA, W.S. **Dinâmica das populações de nematóides parasitos gastrintestinais em bovinos de corte, alguns aspectos da relação parasito hospedeiro e do comportamento dos estádios de vida-livre na região do vale do Rio Doce-MG**. Belo Horizonte, 1989. 178p. Tese (Doutorado) - Instituto de Ciências Biológicas, Universidade Federal de Minas gerais.

9- NANSEN, P.; GRONVOLD, J.; HENRIKSEN, S.A.; WOLSTRUP, J. Interactions between the predacious fungus *Arthrobotrys oligospora* and third-stage larvae of a series of animal-parasitic nematodes. **Veterinary Parasitology**, v.26, n.3/4, p.329-37, 1988.

10- NANSEN, P.; GRONVOLD, J.; HENRIKSEN, S.A.; WOLSTRUP, J. Predacious activity of the Nematode-destroying fungus, *Arthrobotrys oligospora* on preparasitic larvae of *Cooperia oncophora* and on soil nematodes. **Proceeding of Helminthological Society of Washington**, v.53, n.3, p.237-43, 1986.

11- STIRLING, G.R. **Biological control of plant parasitic nematodes**: progress, problems and prospects. Wallingford : Academic Press, 1991. p.282.

12- WALLER, P.J.; LARSEN, M. The role of nematophagous fungi in the biological control of nematode parasites of livestock. **International Journal of Parasitology**, v.23, n.4, p.539-46, 1993.

Received: 15/12/1995
Accepted: 16/04/1997