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Effect of spraying *Arthrobotrys conoides* conidia on pastures to control nematode infection in sheep

Efeito da pulverização de conídios de *Arthrobotrys conoides* na pastagem no controle da verminose em ovinos

Margarete Kimie Falbo[1*]; Vanete Thomaz Soccol[2]; Itacir Eloi Sandini[1]; Jaqueline Huzar Novakowiski[3]; Carlos Ricardo Soccol[2]

Abstract

The effect of spraying pastures with conidia of the fungus *Arthrobotrys conoides* (GenBank ID: JN191309) for the biological control of gastrointestinal nematode infection-pressure in lambs was assessed. A 12,000-m^2 area was divided into six 2,000-m^2 fenced areas. Two groups were formed: the treatment group comprised three fenced areas, where conidia were sprayed on the pasture weekly at 7.5 x 10^4 conidia m^{-2}; and the control group, also comprising three fenced areas, where conidia were not sprayed. The pastures included lopsided oat (*Avena strigosa* Schreb) and Italian ryegrass (*Lolium multiflorum* Lam.). Five naturally infected lambs, were placed between July and September in each fenced area. The effectiveness of biological control was assessed between May and September 2009 by counting the number of third-stage larvae (L3) in each pasture. Additionally, the egg output of the sentinel animals was monitored by counting the number of gastrointestinal nematode eggs per gram of faeces (EPG) and the average weight gain was measured. The negative impact on soil was assessed by counting the number of free-living nematodes and phytonematodes. The number of gastrointestinal nematode larvae in the treated pastures decreased. This was significant at two examination days (end August and end of September). At the end of the study, conidia treatment reduced gastrointestinal nematodes on pasture by 52.4% compared to the control group; this difference was statistically significant. Regarding the whole examination period the average reductions in EPG in treatment group was 49.1% compared to the control group. The most common genera of gastrointestinal nematodes were *Haemonchus* and *Trichostrongylus*. Animal weight gain and soil nematode counts did not differ significantly.
Key words: Biological control, nematophagous fungi, gastrointestinal nematodes, small ruminants

Resumo

Este experimento foi realizado para avaliar o efeito da pulverização de conídios do fungo *Arthrobotrys conoides* (GenBank ID: JN191309) sobre a pastagem visando o controle biológico da verminose em cordeiros. Uma área de 12.000 m^2 foi dividida em seis piquetes de 2.000 m^2. Dois grupos foram formados: grupo tratamento constituído por três piquetes em que foram pulverizados semanalmente conídios na pastagem na quantidade de 7,5 x 10^4 conídios m^{-2}. E o grupo controle, onde não foi pulverizado conídios na pastagem que era constituída por aveia preta (*Avena strigosa* Schreb) e azevém anual (*Lolium multiflorum* Lam.). Tanto nos piquetes controle quanto nos piquetes que receberam tratamento foram colocados cinco cordeiros naturalmente infectados por helmintos gastrintestinais. A avaliação do controle biológico foi realizada no período de maio a setembro de 2009, por meio da contagem de

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larvas infectantes (L3) na pastagem; taxa de infecção dos animais monitorados pela contagem de ovos de helmintos por grama de fezes (OPG) e o ganho de peso dos animais. O impacto negativo no solo foi avaliado pelo número de nematoides e fito nematoides de vida livre. O número de larvas infectantes(L3) nas pastagens tratadas reduziu significativamente no final dos meses de agosto e setembro. No final do experimento, houve redução de 52,4% de larvas infectantes na pastagem que foi pulverizado os conídios quando comparado ao grupo controle, diferindo estatisticamente entre si. Durante o período avaliado a redução média na contagem de OPG nos animais foi de 49,1% na pastagem tratada quando comparado ao controle. Os gêneros de helmintos predominantes foram *Haemonchus* e *Trichostrongylus*. O ganho de peso dos animais e o número de nematoides do solo não diferiram estatisticamente.
Palavras-chave: Controle biológico, fungos nematófagos, nematoide gastrointestinal, pequenos ruminantes

Introduction

Since the 1990s, several researchers have been warning that the intensive use of anthelmintics is the most important factor associated with the appearance of drug-resistant parasites in sheep (WALLER et al., 1995). Consequences of drug usage have been reported, including cases of resistance to different anthelmintics active compounds in several parts of the world (WALLER et al., 1995; THOMAZ-SOCCOL et al., 1996, 2004; VAN WYK et al., 1999; JACKSON; COOP, 2000; KAPLAN, 2004). The situation in Brazil is similar, with drug-resistant parasites in sheep being reported in the three states comprising the southern region of the country. In the state of Paraná, there are no longer any efficacious active compounds for the treatment of some herds (THOMAZ-SOCCOL et al., 1996, 2004). In Rio Grande do Sul, resistance to ivermectin and benzimidazoles affects 13% and 90% of farms, %, respectively (ECHEVARRIA et al., 1996). In Santa Catarina, parasites have exhibited resistance to multiple classes of anthelmintics (ROSALINSKI-MORAES et al., 2007).

This worrisome situation has led researchers from several areas of the world to search for alternative treatments and new control methods to reduce the use of anthelmintics. One of the alternatives currently being discussed is target selective treatment (TST), which consists of identifying only the animals requiring treatment on the grounds of indicators that vary depending on the parasite species infecting the herd (BATH; VAN WYK, 2009; KENYON et al., 2009). The FAMACHA method has been used since the beginning of the 2000s (VAN WYK; BATH, 2002). Diarrhea scoring, suggested by Cabaret et al. (2006), counting of eggs per gram of faeces (EPG) and production indices, such as weight gain, have also been proposed (STAFFORD; MORGAN; COLES, 2009).

Another alternative to anthelmintics use is biological control, which was developed in the 1940s (ROUBAUD and DESCAZEAUX, 1941 quoted by MOTA; CAMPOS; ARAÚJO, 2003). Studies of nematophagous fungi have shown their great potential as biological control agents against the free-living stage of ruminant gastrointestinal nematodes (PENA et al., 2002; FONTENOT et al., 2003; WALLER et al., 2004; LARSEN, 2006). Additionally, nematophagous fungi can be used together with other control strategies aimed at reducing pasture contamination and decreasing the population of nematodes that parasitize animals in commercial-scale farming systems with high population densities (WALLER; FAEDO, 1993).

Field studies have employed chlamydospores of the fungus *Duddingtonia flagrans*, administered via an oral route mixed with feed. Positive results, such as reduced numbers of infective larvae in pastures and decreased parasite burdens in sentinel animals, were observed by Fernandez et al. (1999), Faedo, Larsen and Thamsborg (2000) and Fontenot et al. (2003). However, other authors did not find any significant differences in the assessed parasitological parameters when using the same *D. flagrans* strain

(EYSKER et al., 2006; FAESSLER; TORGERSON; HERTZBERG, 2007; EPE et al., 2009). One of the factors that might contribute to negative results is the non-homogeneous distribution of spores throughout the pastures (WALLER; FAEDO, ELLIS, 2001; PENA et al., 2002). When spores are administered orally, it is difficult to confirm whether all of the animals actually ingested the same amount of spores, making homogeneous distribution in pastures difficult to achieve and confirm (EPE et al., 2009).

Therefore, the aim of this study was to assess the environmental impact and the effectiveness of spraying *Arthrobotrys conoides* (JN 191309) conidia in pastures as a method to control nematodiasis in sheep; the pastures contained lopsided oat (*Avena strigosa* Schreb) and Italian ryegrass (*Lolium multiflorum* Lam.).

Materials and Methods

Arthrobotrys conoides (GenBank ID: JN191309) *fungus*

The strain of *Arthrobotrys conoides* used was isolated in the central southern part of Paraná state. Sequence analysis of the internal transcribed spacer (ITS) region of the rDNA gene from the isolate using the BLAST algorithm, showed 97% identity with *A. conoides* strain 670. We chose to conduct the study with the isolated strain due to its superior adaptability to the local climactic conditions. *In vitro* testing with the *A. conoides* strain showed 96.35% efficiency after passage through the sheep gastrointestinal tract in decreasing the population of several nematode species in a pool containing 64% *Haemonchus* sp., 22% *Trichostrongylus* sp. and 14% *Strongyloides papillosus* (FALBO et al., 2013).

Conidia preparation for field testing and pasture spraying

Four-millimeter disks containing fungal material from isolated *A. conoides* (JN 191309) that were maintained on corn meal agar (CMA) (Difco® – 17 g^{-1}) at 4ºC in the dark, were transferred to 9-cm Petri dishes with potato dextrose agar (PDA) culture medium (Himédia®- 39 g/L^{-1}); the dishes were incubated in a BOD stove at 26ºC for six days. Then 4-mm culture fragments were transferred to 35 9-cm Petri dishes containing 15 mL of 1.5% agar with 5% wheat bran that were incubated at 26ºC in the dark for ten days. Next, the Petri dish surfaces were washed with 8 mL of distilled water with one drop of sterile Tween 80 and conidia were extracted with a sterile brush (ARAÚJO et al., 1993). The obtained solutions were placed in 500-mL Erlenmeyer flasks and homogenized utilizing a magnetic stirrer for 40 minutes. Next, the solutions were filtered through a folded gauze strainer twice to remove excess mycelia and to prevent the sprayer tip from clogging while applying the solution to the pasture. Conidia were counted in a Neubauer chamber using both sides of the chamber; counts were performed in triplicate according to the method described by Nielsen, Smyth and Greenfield (1991). Before and after spraying the conidia-solution, were tested in vitro for trapping capability. Spraying was performed weekly in fenced areas using a manual sprayer at a dose of 7.5×10^4 conidia per m^2. Application was always performed in the afternoon when the wind intensity was lowest; in cases of rain, spraying was postponed until the following day, because the rain could disperse the conidia in the pasture.

Study setting

This study was performed at the State University of Center West Paraná, located in the central southern area of the state, in the city of Guarapuava. According to Köppen's classification, this area has a subtropical, humid, mesothermal climate without a dry season and is characterized by cool summers

and moderate winters. The average temperature in the coldest month is below 18ºC, while the average temperature in the warmest month is below 22ºC. The annual precipitation varies between 1,400 and 2,000 mm, and precipitation is lowest in April, May and August. The altitude is approximately 1,100 m (MAACK, 1968).

In an area the integrated crop-livestock, 12,000 m^2 were divided into six 2,000-m^2 fenced areas. Corn or beans are grown in this area during the summer, and the pasture is grown for sheep production during the winter. Therefore, it was grazed by sheep during the previous winter from June 15, 2008 to November 15, 2008. Lopsided oat (*Avena strigosa* Schreb) and Italian ryegrass (*Lolium multiflorum* Lam.) were introduced in April 2009. Animals entered the fenced areas in May 2009, where they remained until September 2009.

Animals

Twenty-four ewes with their lambs, totaling 48 crossbred Ile de France and Corriedale animals, were distributed into pastures based on body weight. Four adults and four newborn lambs were placed in each of the six 2,000-m^2 fenced areas, and the lambs remained with their mothers from May 16 to June 9, 2009. There was a frost on June 3 that affected the pastures, causing the animals to be removed from the fenced areas on June 9; the animals were relocated for 20 days, until the pastures recovered. While the animals were away, the weekly spraying of conidia in the fenced areas and the collection of pasture samples to extract infective larvae (L3) were continued. On June 29, the animals were returned to the pasture, weaned and treated with moxidectin (Cydectin® , Ford Dogde, at a dose of 0.2 mg kg^{-1} live weight).

In this next phase, only were returned four weaned lambs and placed one more in each previous grazing group (3 males and 2 females, 10 weeks old); lambs were grouped based on their body weight.

Counting of eggs per gram of faeces (EPG), FAMACHA© and average weight gain

Animal fecal samples were collected every two weeks directly from the rectal ampulla and were processed utilizing the modified McMaster technique to establish the number of gastrointestinal nematodes EPG (GORDON; WHITLOCK, 1939; COLES et al., 1992). Fecal cultures were performed according to the methods described by Roberts and O'Sullivan (1950), and larvae identification was carried out utilizing criteria from Keith (1953) and Van Wyk, Cabaret and Michael (2004).

The lambs were weighed at the beginning of the study and then every 15 days. The FAMACHA© method of evaluation (VAN WYK; BATH, 2002) was applied to all animals beginning in August and then every two weeks until the end of October.

Pasture collection to recover and count infective larvae (L3) per dry mass kilogram

Prior to the entrance of animals into the fenced areas and then every 15 days, pasture samples were collected to extract L3. Collections were always made between 7:30 and 10 a.m. A total of eight samples were collected from each fenced area. Sample areas were selected by randomly throwing a 0.50 x 0.50 cm (0.25 m^2) frame; all pasture within this area was cut down to the soil with a Stanley knife and placed in plastic bags to be processed. When the square fell on an area full of faeces, it was thrown again. Samples were then mixed together and weighed; 200 grams were removed from the total amount, placed in paper bags and then placed in a stove at 72ºC for three days to obtain dry matter (DM) and fodder mass expressed in DM kg ha^{-1}. Infective larvae were extracted from 500 g pasture samples according to Baermann's method modified by Persson (1974). To remove finer soil particles, Kleenex® tissues were placed on the mesh to keep the pasture samples suspended (LARSSON et al., 2007). The samples were processed and brought to a final volume of 10 mL. For analysis, the tubes

were homogenized, and three 100-µL aliquots were removed to count and identify larvae (L3) (FONTENOT et al., 2003).

Soil sample collection

To assess the potential environmental impact of conidia, soil samples were collected monthly with a probe drill at depth of 0-5 cm to extract, count and classify free-living nematodes and phytonematodes. Ten soil samples were taken from each fenced area.

Extraction, counting and classification of soil nematodes

The 10 soil samples from each fenced area were mixed together, and 100 g was used to extract nematodes through a modified Baermann's funnel method (HOPPER, 1986) for 48 hours; samples were run in triplicate. The volume of the obtained solutions were brought to a final volume of 1 mL and homogenized. Next, three 100-µL aliquots were placed on a slide for the counting and classification of free-living nematodes and phytonematodes; criteria described by Bongers (1988) were used for identification.

Climate indices

Pluviometric indices and variations in ambient temperature were recorded daily during the study period at the meteorological station of State University of Center West Paraná, located 200 m from the experimental area. Pluviometric data were used to determine the climatological hydric balance using the "BHnorm" program, developed in Microsoft Excel spreadsheets by Rolim, Sentelhas and Barbieri (1998).

Statistical analysis

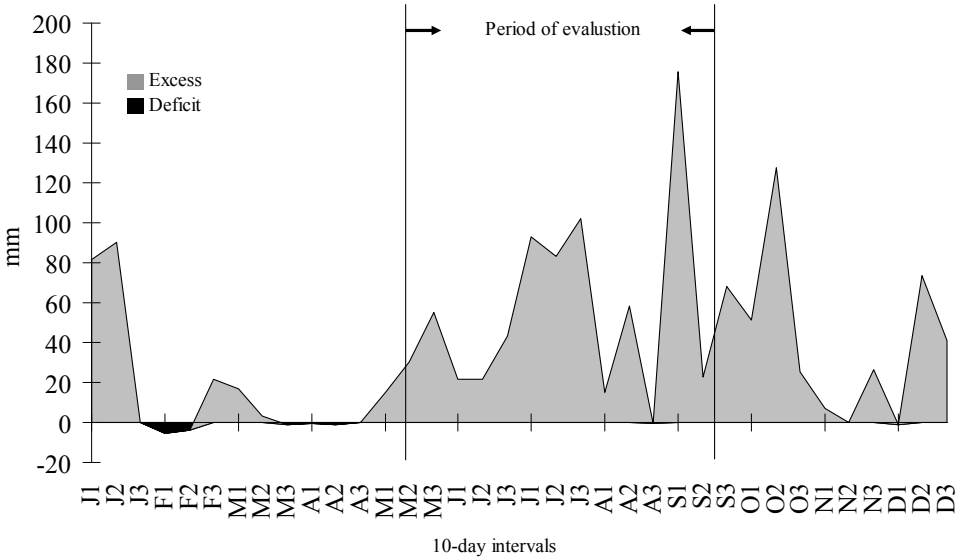
Data were subjected to analysis of variance, and means were compared using Tukey's test at 5% probability. SAS statistical software was used for all analyses.

Results

As shown in Figure 1, there was no water deficit, and the temperature varied between a minimum of –4ºC and a maximum of 28.2ºC; the minimum average temperature was 9.6ºC, and the maximum average was 19.8ºC (Figure 2). The lowest averages were seen in June and July, whereas the averages in the remaining months of May, August and September varied between 15.4ºC and 17ºC.

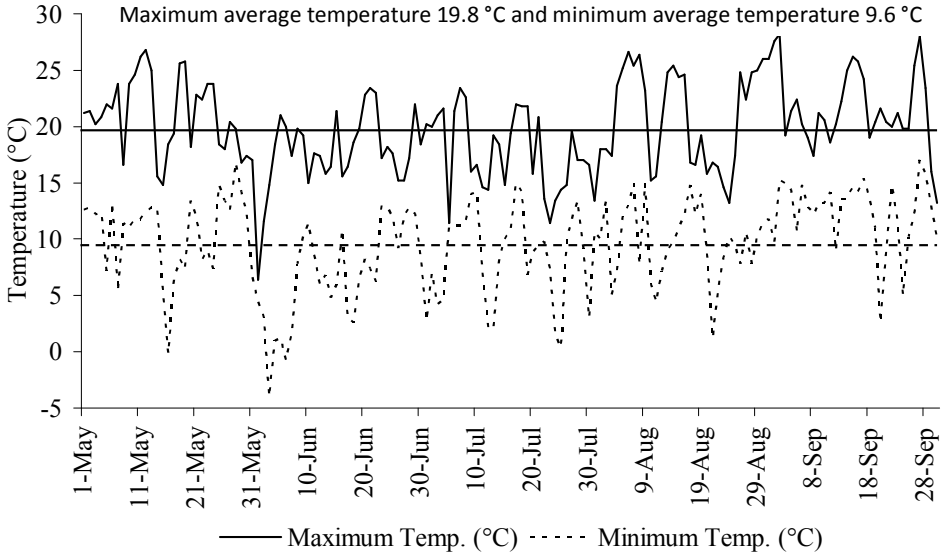
Prior to animal placement, there was an average of 228 and 430 larvae (L3) per kg DM on control and treatment pasture, respectively. End of June the L3 of the control pasture show lower counts than the treatment areas. At July, when the lambs were newly allocated to the experimental pastures, the average herbal L3 counts were equal between and the control and treatment areas. Of the following four sampling dates, two resolve significant differences and two show not. The average of all L3 count were greater in the control areas (2,035 L3 per kg DM) compared to the treated areas (969 L3 per kg DM); this 52.4% reduction was statistically significant (Table 1).

Figure 1. Rainfall recorded at 10-day intervals during 2009 in Guarapuava-Paraná, Brazil.



Source: Elaboration of the authors.

Figure 2. Maximum and minimum temperature from May to September 2009 in Guarapuava-Paraná, Brazil.



Source: Elaboration of the authors.

Table 1. Total number of gastrointestinal sheep nematode third-stage larvae (L3) per dry mass kilogram (DM) in the control and the *A. conoides* conidia-treated pastures from May to September 2009 in Guarapuava-PR, Brazil.

Groups	L3 Larvae per DM kg										
	May 15	May 30	Jun 14	Jun 29	Jul 14	Jul 29	Aug 13	Aug 28	Sep 12	Sep 27	Mean
Control group	228	1,000	2,852	351	3,584	1,535	1,042	3,246 a	758	5,752 a	2,035 b
Treatment group	430	510	1,436	1,355	985	1,658	1,067	187 b	539	1,526 b	969[1] a
VC (%)		52.46									

Means followed by different lower-case letters in columns and upper-case letters in rows differ according to Tukey's test at 5%.
[1] 52.4% reduction compared to control.
Source: Elaboration of the authors.

Animals in the treated areas exhibited reduced EPG counts compared to animals in the control areas. The overall mean EPG reduction was 49.1% (Table 2). No animal in any group required treatment for worms throughout the study. Although the EPG counts were high on August 24, we decided not to treat based on the good FAMACHA© evaluation (Table 3) and other clinical signs of worms, such as diarrhea and weight loss.

Animal evaluation using the FAMACHA© method showed that the lambs exhibited increasing FAMACHA values (average 1.11 the 2.44) over time. Animals in the conidia-treated areas exhibited constant values (1.8) during the last months of the study. Two and four weeks after spraying conidia finished the differences in FAMACHA values between animals in the control (2.3 and 2.4) and treated (1.8 and 1.8) pastures were statistically significant. However, analysis of the average animal weight gain revealed no significant differences during the study (Table 4).

The predominant genus of parasites in the fecal cultures was *Haemonchus* (91%), followed by *Trichostrongylus* (9%). However, between August 24 and September 21 2009, the percentages shifted to 62% and 38%, respectively.

Regarding the possible impact of conidia treatment on free-living soil nematodes and phytonematodes, there were no significant differences in the numbers of phytonematodes (Table 5) or free-living nematodes (Table 6) between the treated and untreated pastures.

Table 2. Eggs per gram of faeces (EPG) of animals in the control and the *A. conoides* conidia-treated pastures with percentage reduction in the treatment group compared to control group.

Date	EPG		Reduction
	Control group	Treatment group	(%)
Jul 29	531 b A	258 a A	51.4
Aug 12	905 b A	600 a A	33.7
Aug 24	2840 a A	1228 a A	56.7
Sep 21	921 ab A	596 a A	35.3
Mean	1355 A	689 A	49.1
VC (%)	22.0		

Same lowercase letters in columns and uppercase letters in rows do not differ according to Tukey's test at 5%.
Source: Elaboration of the authors.

Table 3. Average FAMACHA© evaluations of animals in the control and the *A. conoides* conidia-treated pastures between August and October 2009.

Treatment	Evaluation date (FAMACHA 1 to 5)					
	Aug 10	Aug 24	Sep 7	Sep 21	Oct 5	Oct 23
Control group	1.11 ns	1.50 ns	1.83 ns	1.89 ns	2.28 a	2.44 a
Treatment group	1.33	1.50	1.56	1.78	1.78 b	1.78 b
Mean	1.22	1.50	1.69	1.83	2.03	2.11
VC (%)	11.0	20.7	20.1	3.7	5.8	9.6

ns = non-significant. Means followed by different letters in columns differ according to Duncan's test ($p<0.05$).
Source: Elaboration of the authors.

Table 4. Initial and final live weight and daily average weight gain (DAG) of animals in the control and the *A. conoides* conidia-treated pastures.

Treatment	Live weight (kg)		DAG
	Initial	Final	(kg animal day)
Control group	17.0	37.1	0.161
Treatment group	16.3	36.2	0.159
Mean	16.6	36.6	0.160
VC (%)	17.8	15.0	16.8

Source: Elaboration of the authors.

Table 5. Number of phytonematodes per square meter of soil in fenced areas of the control and the *A. conoides* conidia-treated pastures from May to October 2009 in Guarapuava-PR, Brazil.

Treatment	Phytonematodes (number per square meter of soil x 10^4)						
	5/25/2009	6/26/2009	7/29/2009	8/26/2009	9/25/2009	10/28/2009	Mean
Control group	5.9	7.3	7.4	4.6	11.5	8.2	7.5
Treatment group	7.9	8.4	10.6	5.1	5.4	11.5	8.2
VC (%)	29.23						

Source: Elaboration of the authors.

Table 6. Number of free-living nematodes per square meter of soil in fenced areas of the control and the *A. conoides* conidia-treated pastures from May to October 2009 in Guarapuava-PR, Brazil.

Treatment	Free-living nematodes (number per square meter of soil x 10^4)						
	5/25/2009	6/26/2009	7/29/2009	8/26/2009	9/25/2009	10/28/2009	Mean
Control group	21.4	60.7	51.0	65.8	60.9	24.8	47.4
Treatment group	24.1	68.9	65.9	83.4	43.1	37.8	53.9
VC (%)	15.79						

Source: Elaboration of the authors.

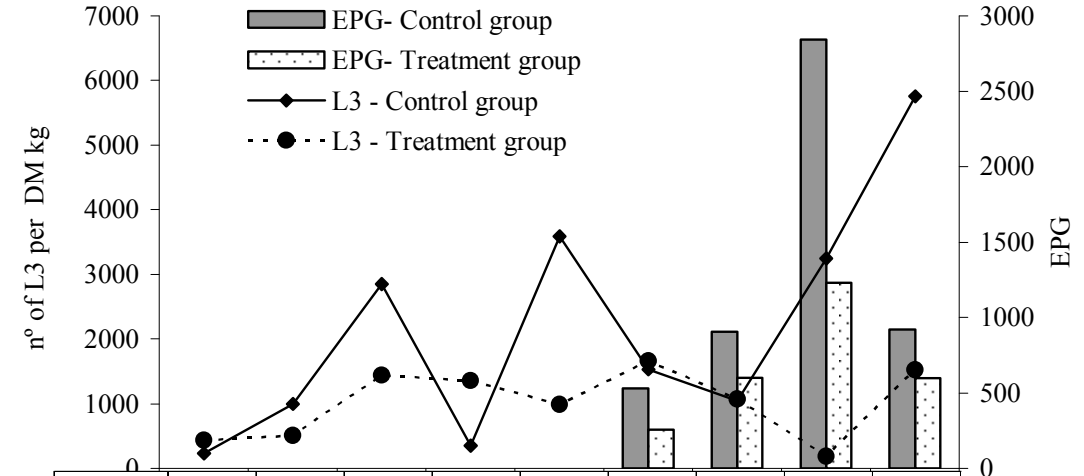
Discussion

Although the majority of the research work mentioned in the literature has been done with the fungus *D. flagra*ns, we opted to work with the fungus *A. conoides* (JN 191309). It happened because the results from *in vitro* tests after the passage by the gastrointestinal tract of lambs showed very promising data. In addition, the fungus was isolated from the same location where the field tests were performed, and according to Araújo et al. (2006) the fungal species from the same location should be preferred for controlling nematodes.

The ideal temperature for nematophagous fungi growth varies between 15ºC and 30ºC (PANDEY, 1973). Although the average temperature during this study remained at the minimum ideal limit for fungus growth, the high density of L3 seen in the pasture might also have stimulated their activity. According to Fontenot et al. (2003), variations in nematophagous fungal activity, both in fecal cultures and in pastures, might be associated with temperature and larval density. Additionally, as the ideal temperature for fungus growth is also the ideal temperature for the larval development of nematodes that parasitize ruminants, conditions that favor both fungus and parasite growth are likely.

Analysis of Figure 3 shows that there was little development of L3 after the decrease in temperature during the first two weeks of June. Conversely, increased temperatures favored L3 development and may have promoted fungal growth and increased nematophagous fungal efficacy. When comparing the control group to the conidia-treated group, there was a smaller number of L3, mainly during the last 45 days of the study; smaller EPG counts were also observed during this time.

Figure 3. Number of infective larvae stage (L3) in pasture, EPG and temperature variation over the course of the study.



Temp. °C	May 1st	May 2nd	Jun 1st	Jun 2nd	Jul 1st	Jul 2nd	Aug 1st	Aug 2nd	Set 2nd
Maximum	21.6	20.5	16.8	18.7	18.4	17.1	22.0	20.0	21.2
Minimum	11.0	10.2	4.5	9.1	8.1	8.9	9.7	9.8	11.7

Source: Elaboration of the authors.

Kahn et al. (2007) simulated environmental temperature variations to establish the best temperature range for the development and efficacy of *D. flagrans* fungus against *Haemonchus* infective larvae. They observed an 89% reduction in larvae number when the temperature varied between 6ºC and 19ºC and a 99.1% reduction when the temperature ranged between 9ºC and 25ºC. These findings suggest that there was fungal development and predatory activity even at the lowest temperatures.

In studies conducted in the Netherlands and Germany, Eysker et al. (2006) and Epe et al. (2009), respectively, observed reductions in EPG counts, number of L3 in pastures and parasite load in sentinel animals. These results were attributed to climatic conditions (high pluviometric index) and to the administration of chlamydospores by an oral route mixed with feed. However, it was difficult to confirm the amount of conidia ingested per animal, which interferes with the homogeneous distribution of spores throughout pastures.

Analysis of the number of infective larvae per kg^{-1} DM (Table 1), EPG counts (Table 2) and FAMACHA values (Table 3) revealed that the best reduction indices were observed at the end of the study, which suggests that longer periods of biological control might produce better results.

The strategy in this study was to spray conidia directly on the pasture, and two advantages were observed compared to oral administration. First, the distribution of spores was more homogeneous, as the actual amount of conidia excreted in stool is difficult to measure when an oral route is used. Additionally, direct spraying might allow for the determination of the ideal amount of conidia to be used in each area based on regional epidemiological studies. Studies carried out by Ojeda-Robertos et al. (2008a) suggest setting fungal dose based on the number of EPG, with a ratio of 5 chlamydospores per gram of faeces (CPG) per 10 EPG.

In this study, 7.5×10^4 conidia per m^2 were sprayed on the treated pastures weekly. As the oral dose administered in most studies is 10^6 per kg^{-1} live weight and the digestibility of chlamydospores is approximately 88 to 90%, (OJEDA-ROBERTOS et al., 2008b), the amount of sprayed conidia was similar to that used in oral administration studies.

Another point to consider is that spraying might make it possible for animals to ingest conidia during grazing and subsequently expel these *Arthrobotrys* conidia in their stools, contributing to the maintenance of conidia in the environment. Falbo et al. (2013) verified that the *A. conoides* strain (JN 191309) does not lose its predatory capacity even after the passage by the gastrointestinal tract of lambs.

Studies that administered the *D. flagrans* strain in feed reported different efficacies in bovines and sheep, which were attributed to differences in fecal structure that might influence fungal development or activity (FAESSLER; TORGERSON; HERTZBERG, 2007). Therefore, spraying conidia on pasture may be more feasible than oral administration, especially for small ruminants raised in commercial farms without supplements. However, is being necessary to perform experiments concerning the influence on humans, who inhale the conidia spray by mistake and about impact on humans working with conidia.

In regards to the environmental impact on soil nematodes, there was no difference between the groups. Yeates et al. (2007), in a study carried out in areas with faeces from groups of sheep that were given different anthelmintics treatments, observed that the fecal decomposition of animals treated with albendazole and ivermectin was slower compared to animals treated with *D. flagrans* fungus, suggesting a reduction in nematode activity. However, they also reported that there was no evidence that the persistence of *D. flagrans* in the field resulted in a negative environmental impact. According to Braga and Araújo (2014), cultural barriers must be broken down since the impact on the environment

is minimal, which becomes even more important to study the interaction mechanism of these organisms with their targets.

Faedo et al. (2002) also reported that it was unlikely that *D. flagrans* had a negative effect on nematodes that are beneficial to the soil. They observed no fungal predation on the nematode population, suggesting that the fungus exhibited minimal growth around in the fecal environment. According to Faedo et al. (2002), evaluation of predatory activity in the soil would be a useful indicator; unfortunately, there are no proper techniques yet available. Additionally this trial shows, that well observed lambs can stay quite healthy and perform enough without anthelmintic treatment even if the graze on contaminated pastures and develop high average EPGs.

The *Arthrobotrys* genus is an important group of parasitic fungus, which in practice should be considered for biological control (YANG et al., 2011). It is likely one of the most important and promising genus (BARON, 2003) and it has been mentioned that beyond the capacity of feeding from nematodes, it has a variety of other applications such as a source of nanoparticules (WANG et al., 2013).

Conclusions

Spraying of *A. conoides* (JN 191309) conidia on pastures seemed be an easy-to-administer and efficient alternative to anthelmintic treatment to reduce both larval environmental contamination and parasite infection in lambs. It reduced L3 in the pastures by 52.4% and the average EPG by 49.1%.

There was no evidence that application of the fungus *A. conoides* to pastures resulted in any predatory action on soil-dwelling free-living nematodes and phytonematodes.

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References

ARAÚJO, J. V.; SANTOS, M. A.; FERRAZ, S.; MAIA, A. S. Antagonistic effect of predacious *Arthrobotrys* fungi on infective *Haemonchus placei* larvae. *Journal of Helminthology***,** Cambridge, v. 67, n. 2, p. 136-138, 1993.

ARAÚJO, J. V.; ASSIS, R. C. L.; CAMPOS, A. K.; MOTA, M. A. Efeito antagônico de fungos predadores dos gêneros *Monacroporium, Arthrobotrys, Duddingtonia* sobre larvas infectantes de *Cooperia* sp e *Oesophagostomun* sp. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia,* Belo Horizonte, v. 58, n. 3, p. 373-380, 2006.

BARON, G. L. Predatory fungi, wood decay, and the carbon cycle. *Biodiversity,* London, v. 4, n. 1, p. 3-9, 2003.

BATH, G. F.; VAN WYK, J. A. The five points check for target selective treatment of internal parasites in small ruminants. *Small Ruminant Research*, Amsterdam, v. 86, n. 1-3, p. 6-13, 2009.

BONGERS, T. (Ed.). *De nematoden van Nederland*. The Netherlands: Pirola, Schoorl, 1988. 408 p.

BRAGA, F. R.; ARAÚJO, J. V. Nematophagous fungi for biological control for gastrointestinal nematodes in domestics animals. *Applied Microbiology Biotchenology,* Berlin, v. 98, n. 1, p. 79-82, 2014.

CABARET, J.; GONNORD, V.; CORTET, J.; SAUVÉ, C.; BALLET, J.; TOURNADRE, H.; BENOIT, M. Indicators for internal parasitic infections in organic flocks: the diarrhea score (disco) proposal for lambs. In: ORGANIC CONGRESS: ORGANIC FARMING AND EUROPEAN RURAL DEVELOPMENT, 2006, Odense. *Anais*... Odense: Organic Eprints, 2006. p. 552-553.

COLES, G. C.; BAUER, C.; BORGSTEEDE, F. H. M.; GEERTS, S.; KLEI, T. R.; TAYLOR, M. A.; WALLER, P. J. World Association for the advancement of veterinary parasitology (W. A. A. V. P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology*, Amsterdam, v. 44, n. 1-2, p. 35-44, 1992.

ECHEVARRIA, F.; BORBA, M. F. S.; PINHEIRO, A. C.; WALLER, P. J.; HANSEN, J. W. The prevalence of anthelmintic resistance in nematode parasites of sheep in Southern Latin America: Brazil. *Veterinary Parasitology*, Amsterdam, v. 62, n. 3-4, p. 199-206, 1996.

EPE, C.; HOLST, C.; KOOPMANN, R.; SCHNIEDER, T.; LARSEN, M.; VON SAMSON-HIMMELSTJERNA, G. Experiences with *Duddingtonia flagrans* administration to parasitized small ruminants. *Veterinary Parasitology*, Amsterdam, v. 159, n. 1, p. 86-90, 2009.

EYSKER, M.; BAKKER, N.; VAN DER HALL, Y. A.; VAN HECKE, I.; KOOYMAN, F. N.; VAN DER LINDEN, D.; SCHRAMA, C.; PLOEGER, H. W. The impact of daily *Duddingtonia flagrans* application to lactating ewes on gastrointestinal nematodes infections in their lambs in the Netherlands. *Veterinary Parasitology*, Amsterdam, v. 141, n. 1-2, p. 91-100, 2006.

FAEDO, M.; LARSEN, M.; THAMSBORG, S. 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*, Amsterdam, v. 94, n. 1-2, p. 55-65, 2000.

FAEDO M.; LARSEN, M.; DIMANDER, S. O.; YEATES, G. W.; HOGLUND, J.; WALLER, P. J. Growth of the fungus *Duddingtonia flagrans* in soil surrounding faeces deposited by cattle or sheep fed the fungus to control nematode parasites. *Biological Control*, San Diego, v. 23, n. 1, p. 64-70, 2002.

FAESSLER, H.; TORGERSON, P. R.; HERTZBERG, H. Failure of *Duddingtonia flagrans* to reduce gastrointestinal nematode infections in dairy ewes. *Veterinary Parasitology*, Amsterdam, v. 147, n. 1-2, p. 96-102, 2007.

FALBO, M. K.; THOMAZ-SOCCOL, V.; SANDINI, I. E.; VICENTE, V. A.; ROBL, D.; SOCCOL, C. R. Isolament and identification the nematophagous fungi *Arthrobotrys conoides*. *Parasitology Research*, Berlin, v. 112, n. 1, p. 177-185, 2013.

FERNANDEZ, A. S.; LARSEN, M.; HENNINGSEN, E.; NANSEN, P.; GRONVOLD, J.; BJORN, H.; WOLSTRUP, J. Effect of *Duddingtonia flagrans* against *Ostertagia ostertagi* in cattle grazing at different stocking rates. *Parasitology*, Cambridge, v. 119, n. 1, p. 105-111, 1999.

FONTENOT, M. E.; MILLER, J. E.; PEÑA, M. T.; LARSEN, M.; GILLESPIE, A. Efficiency of feeding *Duddingtonia flagrans* chlamydospores to grazing ewes on reducing availability of parasitic nematode larvae on pasture. *Veterinary Parasitology*, Amsterdam, v. 118, n. 3-4, p. 203-213, 2003.

GORDON, H.; WHITLOCK, H. N. A new technique for counting nematode eggs in sheep faeces. *Journal of the Council for Scientific and Industrial Research*, v. 12, n. 1, p. 50-52, 1939.

HOPPER, D. J. Extraction of free-living stages from soil. In: SOUTHEY, J. F. (Ed.). *Laboratory methods for work with plant and soil nematodes*. Ministry of agriculture, fisheries and food reference book. London: HMSO, 1986. p. 5-31.

JACKSON, F.; COOP, R. L. The development of anthelmintic resistance in sheep nematodes. *Parasitology*, Cambridge, v. 120, p. 95-107, 2000. Supplement.

KAHN, L. P.; NORMAN, T. M.; WALKDEN-BROWN, S. W.; CRAMPTON, A.; O'CONNOR, L. J. Trapping efficacy of *Duddingtonia flagrans* against *Haemonchus contortus* at temperatures existing at lambing in Australia. *Veterinary Parasitology*, Amsterdam, v. 146, n. 1-2, p. 83-89, 2007.

KAPLAN, R. M. Drug resistance in nematodes of veterinary importance: a status report. *Trends in Parasitology*, Philadelphia, v. 20, n. 10, p. 477-481, 2004.

KEITH, R. K. The differentiation of the infective larvae of some common nematode parasites of cattle. *Australian Journal of Zoology*, v. 1, n. 2, p. 223-235, 1953.

KENYON, F.; GREER, A. W.; COLES, G. C.; CRINGOLI, G.; PAPADOPOULOS, E.; CABARET, J.; BERRAG, B.; VARADY, M.; VAN WYK, J. A.; THOMAS, E.; VERCRUYSSE, J.; JACKSON, F. The role of targeted selective treatments in the development of refugia-based approaches to the control of gastrointestinal nematodes of small ruminants. *Veterinary Parasitology,* Amsterdam, v. 164, n. 1, p. 12-20, 2009.

LARSEN, M. Biological control of nematodes parasites in sheep. *Journal of Animal Science*, Champaign, v. 84, n. 13, p. 133-139, 2006.

LARSSON, A.; DIMANDER, S. O, RYDZIK, A.; UGGLA, A.; WALLER, P. J.; HOGLUND, J. A 3-year field evaluation of pasture rotation and supplementary feeding to control parasite infection in first-season grazing cattle: dynamics of pasture infectivity. *Veterinary Parasitology*, Amsterdam, v. 145, n. 1-2, p. 129-137, 2007.

MAACK, R. (Ed.). *Geografia física do Estado do Paraná*. Curitiba: Banco de Desenvolvimento do Estado do Paraná. Universidade Federal do Paraná, 1968. 350 p.

MOTA, M. A.; CAMPOS, A. K.; ARAÚJO, J. V. Controle biológico de helmintos parasitos de animais: estágio atual e perspectivas futuras. *Pesquisa Veterinária Brasileira*, Seropédica, v. 23, n. 3, p. 93-100, 2003.

NIELSEN, L. K.; SMYTH, G. K.; GREENFIELD, P. F. Hemocytometer cell count distributions: implications of non-Poisson behavior. *Biotechnology Progress*, New York, v. 7, n. 6, p. 560-563, 1991.

OJEDA-ROBERTOS, N. F.; TORRES-ACOSTA, J. F. J.; AGUILAR-CABALLERO, A. J.; AYALA-BURGOS, A.; COB-GALERA, L. A.; SANDOVAL-CASTRO, C. A.; BARRIENTOS-MEDINA, R. C.; MENDOZA DE GIVES, P. Assessing the efficacy of *Duddingtonia flagrans* chlamydospores per gram of faeces to control *Haemonchus contortus* larvae. *Veterinary Parasitology*, Amsterdam, v. 158, n. 4, p. 329-335, 2008a.

OJEDA-ROBERTOS, N. F.; TORRES-ACOSTA, J. F. J.; AYALA-BURGOS, A.; AGUILAR-CABALLERO, A. J.; COB-GALERA, L. A.; MENDOZA DE GIVES, P. A technique for the quantification of *Duddingtonia flagrans* chlamydospores in sheep faeces. *Veterinary Parasitology,* Amsterdam, v. 152, n. 3-4, p. 339-343, 2008b.

PANDEY, V. S. Predatory activity of nematode-trapping fungi against the larvae of *Trichostrongylus axei* and *Ostertagia ostertagia:* a possible method of biological control. *Journal of Helminthology*, Cambridge, v. 48, n. 1, p. 35-48, 1973.

PENA, M. T.; MILLER, J. E.; FONTENOT, M. E.; GILLESPIE, A.; LARSEN, M. Evaluation of *Duddingtonia flagrans* in reducing infective larvae of *Haemonchus contortus* in the faeces of sheep. *Veterinary Parasitology,* Amsterdam, v. 103, n. 3, p. 259-265, 2002.

PERSSON, L. A modified Baermann apparatus for the recovery of infective nematode larvae from herbage and manure. *Zentralbl Veterinarmed B*, New York, v. 21, n. 7, p. 483-488, 1974.

ROBERTS, F. H. S.; O'SULLIVAN, P. J. Methods for egg counts and larval cultures for strongyles infecting the gastro-intestinal tract of cattle. *Australian Journal of Agricultural Research*, v. 1, n. 1, p. 6-7, 1950.

ROLIM, G. S.; SENTELHAS, P. C.; BARBIERI, V. Planilhas no ambiente EXCEL para os cálculos de balanços hídricos: normal, seqüencial, de cultura e de produtividade real e potencial. *Revista Brasileira de Agrometeorologia,* Santa Maria, v. 6, n. 1, p. 133-137, 1998.

ROSALINSKI-MORAES, F.; MORETTO, L. M.; BRESOLIN, W. S.; GABRIELLI, I.; KAFER, L.; ZANCHET, I. K.; SONAGLIO, F.; THOMAZ-SOCCOL, V. Resistência anti-helmíntica em rebanhos ovinos da região da associação dos municípios do Alto Irani (Amai), oeste de Santa Catarina. *Ciência Animal Brasileira*, Goiânia, v. 8, n. 3, p. 559-565, 2007.

STAFFORD, K. A.; MORGAN, E. R.; COLES, G. C. Weight-based targeted selective treatment of gastrointestinal nematodes in a commercial sheep flock. *Veterinary Parasitology*, Amsterdam, v. 164, n. 1, p. 59-65, 2009.

THOMAZ-SOCCOL, V.; SOTOMAIOR, C.; SOUZA, F. P.; CASTRO, E. A. Occurrence of resistance to anthelmintics in sheep in Paraná State, Brazil. *Veterinary Record*, London, v. 139, n. 26, p. 421-422, 1996.

THOMAZ-SOCCOL, V.; SOUZA, F. P.; SOTOMAIOR, C.; CASTRO, E. A.; MILCZEWSKI, V.; PESSOA, M. C.; MOCELIN, G. Resistance of gastrointestinal nematodes to anthelmintics in sheep (*Ovies aries). Brazilian Archives Biological Technology*, Curitiba, v. 47, n. 1, p. 41-47, 2004.

VAN WYK, J. A.; STENSON, M. O.; VAN DER MERWE, J. S.; VORSTER, R. J.; VILJOEN, P. G. Anthelmintic resistance in South Africa: surveys indicate an extremely serious situation in sheep and goat farming. *Onderstepoort Journal of Veterinary Research*, Durbanville, v. 66, n. 3, p. 273-284, 1999.

VAN WYK, J. A.; BATH, G. F. The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. *Veterinary Research*, London, v. 33, n.5, p. 509-529, 2002.

VAN WYK, J. A.; CABARET, J.; MICHAEL, L. M. Morphological identification of nematode larvae of small ruminants and cattle simplified. *Veterinary Parasitology*, Amsterdam, v. 119, n. 2-3, p. 77-306, 2004.

WALLER, P. J.; FAEDO, M. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: screening studies. *Veterinary Parasitology,* Amsterdam, v. 49, n. 2-4, p. 285-297, 1993.

WALLER, P. J.; DASH, K. M.; BARGER, I. A.; LE JAMBRE, L. F.; PLANT, J. Anthelmintic resistance in nematode parasites of sheep: learning from the Australian experience. *Veterinary Record,* London, v. 136, n. 16, p. 411-413, 1995.

WALLER, P. J.; FAEDO, M.; ELLIS, K. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: towards the development of a fungal controlled release device. *Veterinary Parasitology*, Amsterdam, v. 102, n. 4, p. 299-308, 2001.

WALLER, P. J.; SCHWANN, O.; LJUNGSTRO, M. B. L.; RYDZIK, A.; YEATES, G. W. Evaluation of biological control of sheep parasites using *Duddingtonia flagrans* under commercial farming conditions on the Island of Gotland. *Veterinary Parasitology*, Amsterdam, v. 126, n. 3, p. 299-315, 2004.

WANG, Y.; SUN, L.; HUANG, Y.; LENAGHAN, S. C.; ZHANG, M. Naturally occurring nanoparticles from *Arthrobotrys oligospora* as a potencial imunostimulatory and antitumor agent. *Advanced Functional Materials*, Weinheim, v. 23, n.17, p. 2175- 2184, 2013.

YANG, J.; WANG, L.; JI, X.; FENG, Y.; LI, X.; ZOU, C.; XU, J.; REN, Y.; MI, Q.; LIU, S.; LIU, Y.; HUANG, X.; WANG, H.; NIU, X.; LI, J.; LIANG, L.; LUO, Y.; JI, K.; ZHOU, W.; YU, Z.; LI, G.; LIU, G.; LI, L.; QIAO, M.; FENG, L.; ZHANG, K. Genomic and proteomic analyses of the fungus Arthrobotrys oligospora provide insights into nematodes trap formation. *PLoS Pathogens*, v. 7, n. 9, p. 1-13, 2011.

YEATES, G. W.; SKIPP, R. A.; GRAY, R. A. J.; CHEN, L. Y.; WAGHORN, T. S. Impact on soil fauna of sheep faeces containing a range of parasite control agents. *Applied Soil Ecology*, Amsterdam, v. 35, n. 2, p. 380-389, 2007.