CAPTURE OF *COOPERIA PUNCTATA* INFECTIVE LARVAE BY NEMATODE-TRAPPING FUNGI *ARTHROBOTRYS*

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SUMMARY: This experiment was conducted to observe the behavior of infective *Cooperia punctata* larvae when captured by nematode-trapping fungi *Arthrobotrys*. Infective *C. punctata* larvae, previously treated with protease (Pronase E) at the first days of interaction, were less captured by three fungi isolates (one *A. musiformis* isolate, one *A. conoides* isolate and one *A. robusta* isolate), however, no statistical difference ($p>0.05$) was found when compared with untreated larvae (control group). A statistical difference ($p<0.05$) was found for the exsheathed larvae which presented higher capture rates by these three isolates when compared with non treated larvae. This indicates that surface receptors in exsheathed larvae could be different from those of intact larvae.

KEY WORDS: Biological control, nematodes, *Cooperia punctata*, nematode-trapping fungi, *Arthrobotrys*.

INTRODUCTION

The nematode-trapping fungi of the domestic animals nematode parasites free life stages are widely found in nature, occurring in plants, manure and soil (HASHMI & CONNAN, 1989). They attack live nematodes, using them as source of nutrients (NORDBRING-HERTZ, 1988). Their ability to kill nematodes is known for almost a century, and its biology has been hardly studied, mainly their use in the biological control of nematodes parasites. However, there are few studies regarding their effect on animal nematodes parasites (NANSEN *et alii*, 1988). These fungi produce a large system of hyphas and along them organs that capture and grab live nematodes are produced. Such organs are called trap.

According to NORDBRING-HERTZ (1982), BORREBAECK *et alii* (1984) and ROSEN *et alii* (1992), the interaction begin with the binding of a *Arthrobotrys oligospora* trap lectin to a carbohydrate in the cuticle of the nematode *Panagrellus redivivus*. This lectin has specificity for N-acetyl-D-galactosamina.

TUNLID *et alii* (1991) pointed that the adhesion between predacious fungi traps and nematodes are a complex process, that involves proteins and fungi surface polymers. Besides, different from pathogenic bacteria adhesion mechanisms, this process hasn't been characterized yet for fungi. According to BARRON (1977), although different aspects of taxonomy, morphology and physiology of nematode-trapping fungi have been studied for many years, the molecular basis of capture mechanism is not yet well understood. The present study was designed to observe the capture of *Cooperia punctata* infective larvae by nematode-trapping fungi *Arthrobotrys*.

MATERIALS AND METHODS

Organisms: *Cooperia punctata* Infective larvae (L_3) were obtained from faeces of calves experimentally infected with 1,000 L_3 per kg of live weight. The strain used was kindly supplied by Prof. Maria Cecília Reale Vieira Bressan, Department of Parasitology - São Paulo University. These larvae were kept in Griffin glasses with distilled water at room temperature.

Panagrellus (free living nematodes) were kept in Petri dishes with a medium made of moistened and crushed oat flakes. This strain was kindly supplied by Prof. Silamar Ferraz, Department of Phytopathology - Viçosa Federal

University. The worms were separated from culture medium using the Baermann apparatus, after 24 hours at room temperature. Then, they were kept in hemolysis test tubes.

In order to remove bacteria and fungi, the nematodes were washed ten times in sterile distilled water by centrifugation at 1,000 rpm, for five minutes. The supernatant was discarded at the end of each centrifugation. The nematodes were stocked at 4°C, for one week, in solution with 0.05% streptomycin sulfate, 0.05% chloramphenicol and 0.05% amphotericin B. After it they were washed in sterile distilled water as previously described. Afterwards their motility was observed under stereo microscope.

Seven isolates of nematode-trapping fungi *Arthrobotrys* were chopped every four months and kept in a test tube with potato-dextrose-agar 2% (PDA2%) at 4°C in the dark. These isolates, according to the keys of COOKE & GODFREY (1964), HAARD (1968) and VAN OORSCHOT (1985), were classified as two isolates of *A. conoides* (isolates A and D), one of *A. musiformis* (isolate 3) and two of *A. robusta* (isolates B and E). All samples were isolated from soils of different Brazilian localities. They were cultivated in Petri dishes (8.5 cm diameter) with water-agar 2% (WA2%), adding 4,000 *Panagrellus* to each dish in order to induce the production of trap (A+). All fungi cultures were kept at 25°C, in the dark, for seven days.

Capture tests: Infective larvae of *Cooperia punctata* were incubated in solution with 10 mg per ml of Pronase E in 10 mM of Tris/HCl buffer, pH 7.5, for one hour at 30°C, as described by JANSSON (1993). The control group was incubated only with Tris/HCl buffer. One thousand L_3 of *C. punctata* was spilled in each Petri dish (3 cm diameter) with the fungi isolates A+ grown in WA2%. Larvae were observed daily, during five days under light microscope (10-20 x). Three replicates of one hundred larvae each were observed to determine the percentage of capture. The results were transformed to archsine root of percentage to eliminate the average distortion and analyzed with the help of the Student T test ($p<0.05$),according to SNEDECOR & COCHRAM (1976).

L_3 of *Cooperia punctata* were incubated in solution of sodium hypochlorite at 0.2%, for 15 minutes, as described by COLES *et alii* (1980), to remove the external cuticle. The control group was incubated in sterile distilled water. Larvae were washed five times with sterile distilled water by centrifugation at 1,000 rpm, for five minutes. One thousand L_3 without external cuticle and one thousand intact were placed in Petri dishes (3 cm diameter) with the fungi isolates A+ grown in WA2% and observed daily during five days as described before. Statistical analysis of results were conducted as above described.

RESULTS

The results of capture tests with isolates 3, E, D, B and A after treatment of *C. punctata* L_3 with Pronase E are represented, respectively, in the figures 1, 2, 3, 4 and 5. No statistically significant differences ($p>0.05$) were found comparing all the Pronase E treated larvae with the controls. Nevertheless, for the isolates 3, E and D (Figures 1, 2 and 3), the capture of treated larvae was lower in relation to the control, in the first interaction days.

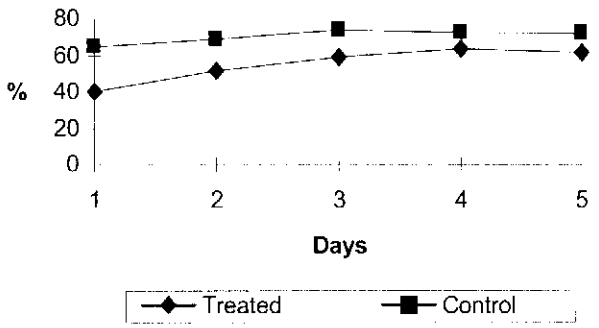

Fig. 1 - Percentage (%) of L_3 of *Cooperia punctata* captured with the isolate 3 after treatment with Pronase E. Average of three repetitions.

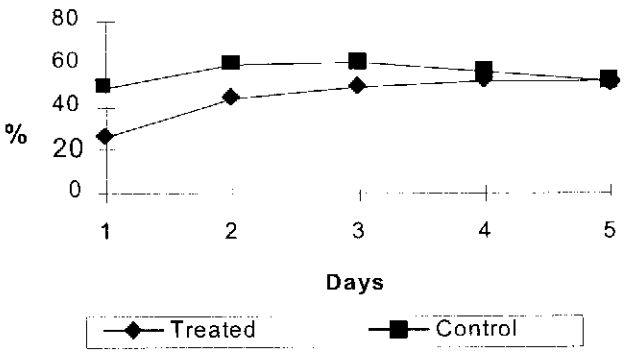

Fig. 2 - Percentage (%) of L_3 of *Cooperia punctata* captured with the isolate E after treatment with Pronase E. Average of three repetitions.

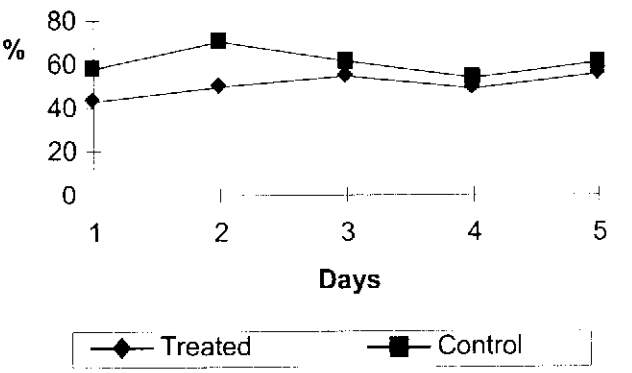

Fig. 3 - Percentage (%) of L_3 of *Cooperia punctata* captured with the isolate D after treatment with Pronase E. Average of three repetitions.

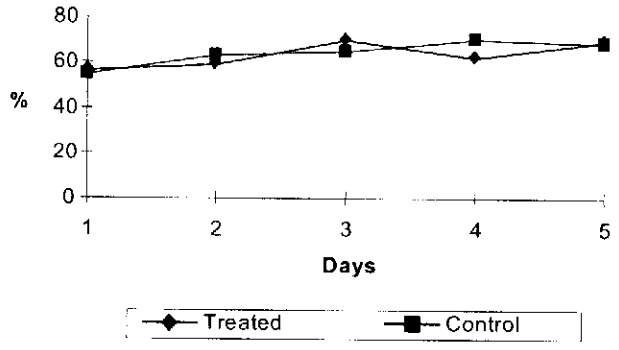

Fig. 4 - Percentage (%) of L_3 of *Cooperia punctata* captured with the isolate B after treatment with Pronase E. Average of three repetitions.

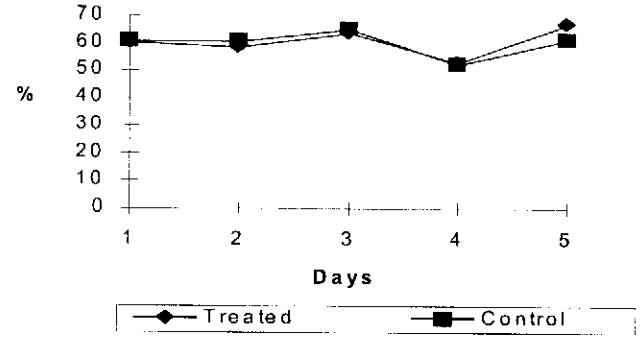

Fig. 5 - Percentage (%) of L_3 of *Cooperia punctata* captured with the isolate A after treatment with Pronase E. Average of three repetitions.

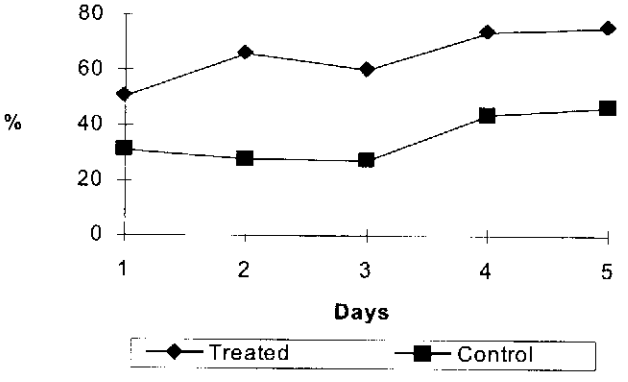

Fig. 6 - Percentage (%) of L_3 of *Cooperia punctata* captured with the isolate 3. Average of three repetitions.

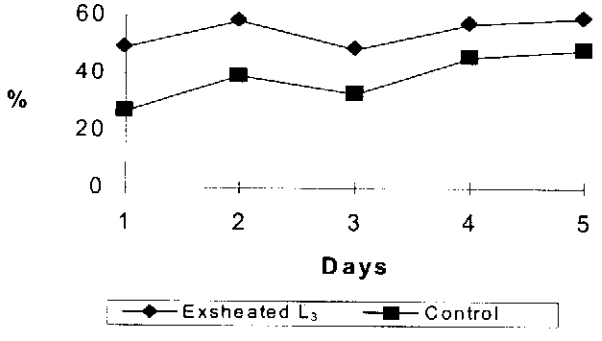

Fig. 7 - Percentage (%) of L_3 of *Cooperia punctata* captured with the isolateE. Average of three repetitions.

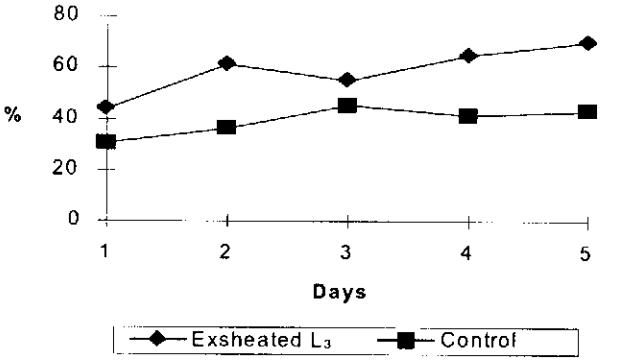

Fig. 8 - Percentage (%) of L_3 of *Cooperia punctata* captured with the isolate D. Average of three repetitions.

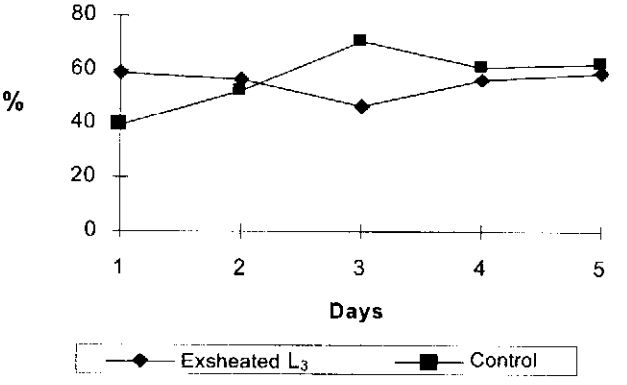

Fig. 9 - Percentage (%) of L_3 of *Cooperia punctata* captured with the isolate B. Average of three repetitions.

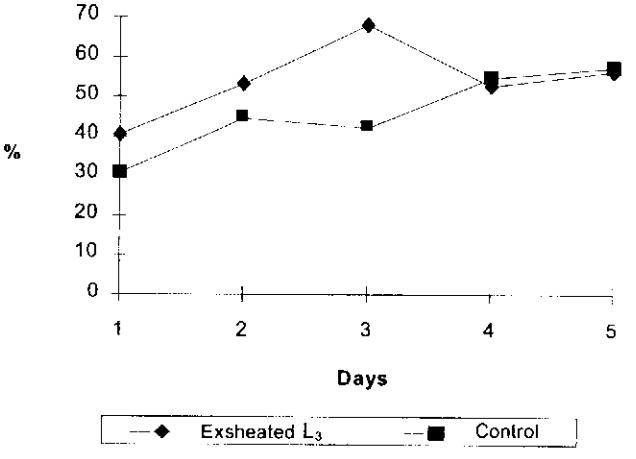

Fig. 10 - Percentage (%) of L_3 of *Cooperia punctata* captured with the isolate A. Average of three repetitions.

The results of capture tests done with exsheathed larvae and isolates 3, E, D, B and A, are represented, respectively, in the figures 6, 7, 8, 9 and 10. Isolates 3 of *A. musiformis*, E of *A. robusta* and D of *A. conoides* showed statistically significant ($p<0.05$) differences in relation to the control. The exsheathed larvae showed a higher percentage of capture.

DISCUSSION

The heterotrophic mode of nutrition, secretion of extracellular enzymes, absorption of nutrients through the cell wall and the apical growth are all typical of the fungi, which need a close contact with the substratum (JONES,1994). The capture of larvae treated with protease Pronase E (Figures 1, 2, 3, 4 and 5), was significantly lower for three isolates (3, E and D, respectively *A. musiformis*, *A. robusta* and *A. Conoides*), when compared with controls during the first days of interaction, although there was no statistically significant differences ($p>0,05$). In an experiment of JANSSON & NORDBRING-HERTZ (1984), the treatment of *Drechmeria coniospora* (DEUTEROMYCETES) conidia with trypsin reduced the adhesion of this endoparasitic fungus to *Panagrellus redivivus*. BIRD & ZUCKERMAN (1989) observed that in nematodes treated with proteolytic enzymes, the adhesion of Coriniformes bacteria to the surface of *Anguina agrostis* larvae was inhibited. The adhesion was recovered after 18 hours, suggesting that the nematodes secrete new glycoproteins involved in the adhesion. *Caenorhabiditis elegans* surface restoration was done 2 hours after the treatment with Pronase E, when adhesion of *D. coniospora* could occur (JANSSON,1994). This indicates that the nematodes recover their surface biochemical capacity.

The results of higher capture of exsheathed *C. punctata* larvae when compared to intact larvae ($p<0.05$) shown by isolates 3 of *A. musiformis*, E of *A. robusta* and D of *A. conoides* (Figures 6, 7 and 8) were different from the ones found by WHARTON & MURRAY (1990) working with *Trichostrongylus colubriformis* and *A. oligospora*, which showed lower capture for exsheathed larvae. JANSSON *et alii* (1985) observed fast infection of exsheathed *Nematospiroides dubius* L_3 by *D. coniospora*. Surface receptors and chemical composition of exsheathed larvae can be different from the intact larvae.

In order to better understand the interaction mechanisms between animal parasite nematodes and nematode-trapping fungi, further studies with other *Arthrobotrys* isolates and other nematodes species are necessary.

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SUMÁRIO

Este experimento foi realizado com o objetivo de observar o comportamento de larvas infectantes de *Cooperia punctata* quando capturadas pelo fungo nematófago *Arthrobotrys*. Larvas infectantes de *C. punctata* que receberam tratamento prévio com uma protease (Pronase E) nos primeiros dias de interação, sofreram uma menor captura por três isolados dos fungos *A. musiformis*, *A. conoides* e *A. robusta*. Contudo não existiu diferença estatística ($p>0.05$) na comparação com as larvas não tratadas (grupo controle). Diferença estatística ($p<0.05$) foi encontrada para as larvas desembainhadas, as quais apresentaram maiores taxas de capturas pelos isolados testados se comparadas com as larvas não tratadas. Isto indica que receptores de superfície das larvas desembainhadas são provavelmente diferentes dos existentes nas larvas intactas.

PALAVRAS-CHAVE: Controle biológico, nematóides, *Cooperia punctata*, fungo nematófago, *Arthrobotrys*.

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