

NEMATOPHAGOUS ACTIVITY ON MOSS AS CULTURAL SUBSTRATUM OF *Arthrobotrys tortor* Jarowaja ISOLATED IN ANTARCTICA

Actividad nematófaga de Arthrobotrys tortor Jarowaja aislado en la Antártica empleando musgos como sustrato

G. Caretta, G. del Frate & S. Tosi
Istituto di Micologia Medica,
Università degli Studi di Pavia
via S. Epifanio, 14, 27100, Pavia, Italia

Key Word: Nematophagous activity, *Arthrobotrys tortor*, mosses.

Palabras Clave: Actividad nematófaga, *Arthrobotrys tortor*, musgos.

SUMMARY

The predatory activity of *Arthrobotrys tortor* Jarowaja isolated in Antarctica was studied. Organs and way of capture of live nematodes have been studied on moss as cultural substratum. The study of the *A. tortor* ability to form nematode trapping-organs on this vegetals revealed new aspects in the relationships between the fungus nematodes and the moss.

INTRODUCTION

Mycological investigations of Antarctic material collected in localities of the Mid Victoria Land during the Italian antarctic expeditions (1988-1993) have indicated that the highest occurrence of fungi was recorded from mosses and soil when mosses were present. *Acremonium strictum* W. Gams, *Engyodontium album* (Limber) De Hoog, *Paecilomyces farinosus* (Holm) Brown & G. Sm., and *Arthrobotrys tortor* Jarowaja were only or mainly isolated from soil moss (Del Frate and Caretta, 1990). The moss was shown to be an optimal substratum suitable for the growth of these fungi particularly for *A. tortor*. In cultural laboratory experiments the growth of these fungus within 5 days at a range of temperatures between 5 and 25° C. This fact was previously reported by Duddington (1951); bryophytes, especially when moist, have proved to be the most fruitful material for supplying predacious fungi, as *A. oligospora* Fres. and *A. robusta* Duddington. *A. robusta* was recorded by (Gray et al., 1982; Gray & Smith 1984; Gray, 1985), as nematophagous fungus from the maritime antarctic; this species of *Arthrobotrys* was only isolated from bird-associated sites. In

RESUMEN

Se determinó la actividad predadora de *Arthrobotrys tortor* Jarowaja, aislado de suelos Antárticos. Se estudiaron los órganos y modalidades de captura de nemátodos vivos, empleando a los musgos como sustrato de cultivo. La habilidad de *A. tortor* para formar órganos de captura en estos vegetales, reveló nuevos aspectos en las relaciones entre el hongo, el nemátodo y los musgos.

Continental Antarctica, two species of *Arthrobotrys* were isolated, both from moss. *A. tortor* (Caretta et al., 1994) and *A. ferox* a new predaceous hyphomycete of the springtail *Gressittacantha terranova* (Onofri & Tosi, 1992). *A. tortor* was isolated originally by Jarowaja (1968) from sandy garden soil from Swider (Warsaw) and reported as a nematophagous fungus. In a subsequent survey of predaceous fungi in Poland (1970), seven strains of *A. tortor* were isolated by Jarowaja from loess soil; two of them were nematophagous, trapping nematodes by means of adhesive threedimensional nets.

In this work predatory activity of *A. tortor*, antarctic strains was studied. Organs and way of capture of live nematodes have been demonstrated on a plate culture containing the fungus, the nematodes feeding on bacteria and the moss as a natural substratum for the fungus.

MATERIAL AND METHODS

A strain of *A. tortor* isolated from moss samples collected in 1988 at Edmondson Point (Mid Victoria Land, Ross Sector 160-170° E) and cultured at 15° C on malt extract agar (MEA formula 1 Oxoid) was used. A

series of sterile plates has been prepared as follows: water agar plates (in 12 g of agar in 1 litre of water, sterilized and poured into 9 cm Petri dishes, 15 ml/plate) with the moss (*Brachytecium rutabulum* (Hedw.) Bruch et al.) previously sterilized and dispersed on the agar surface. Another series of plates was prepared, according to Duddington technique (1955), by using corn meal agar (Oxoid) as a medium to which the moss was added. Nematodes were collected from rabbit dung by means of the Baermann Funnel technique reported in Goodey (1949), and identified in the genus *Rhabditis* Dujardin; bacteria were always present in the plate, to provide food for the nematodes.

Fungus, nematodes and bacteria inoculations were completed in all plates of the two series by using these procedures. *A. tortor* was applied as a conidial suspension, and by micelial disks, both from a 72-hr-old culture of the fungus. A conidial suspension was prepared from conidia collected from cultures grown in Erlenmeyer flasks containing 20 ml of MEA. The suspension was washed in sterile tap water by centrifugation (1500 g) and adjusted to 1×10^6 conidia per millilitre and sprinkled on moss. Micelial disks from MEA culture were placed in the center of Petri plates. Nematodes and bacteria cultured on corn meal agar were transferred to the plates by using a sterilized spatula. The plates were incubated at 20°C.

RESULTS

Leaves and stems of *Brachythecium* were swiftly colonized by the fungus (fig. 1). On the moss, *A. tortor* developed and sporulated abundantly, both in the corn meal agar plates and in the water agar plates. It was possible to distinguish two different morphological aspects of the fungus: on the agar and on the moss. On the agar surface the fungus gave rise to three-dimensional adhesive networks and hyphal loops, generally in an upright position above the general level of the mycelium, according to the description of Jarowaja (1968, 1970). Abundant nematode-graveyards have been observed in areas with a lot of trapping organs. On moss the fungus formed strong and thick fasciculate aerial hyphae on which drops or sheath of sticky secretion were evident. All over the moss the aerial hyphae of the fungus was organized in a sort of spider's web.

Adhesive networks, developing by anastomosis of the recurved branch tips, were common, but simple adhesive loops were more frequent (fig. 2, 3, 4). Coiled hyphae arranged on one plane, were also born by the fasciculate hyphae. Nematodes crawling on the aerial mycelium were observed, and many of them have thrust their bodies into such a loop or have become tightly wedged inside it (fig. 5, 6, 7, 8, 10). Trapping organs were distributed all over the moss as well as on the thick and

strong fasciculate hyphae extending from one leaf moss to another. Drops of mucilaginous material along the hyphae and above all of the hyphal branches have been observed. Microscopical observation of the mucilaginous drops have revealed the presence of eggs (fig. 9), spawned at interval along the aerial hyphae, or young swimming nematodes.

DISCUSSION

Arthrobotrys Corda, is known to be one of the most interesting genus because of its many predaceous species. As mentioned by van Oorschot (1985), various types of trapping organs are produced by different species of *Arthrobotrys*; three species produce constricting rings and *A. entomopaga* Drechsler, *A. haptospora* Drechsler and *A. ferox*, produce adhesive knobs. Nematode traps are intimately connected with an adhesive phase that appears to be a prerequisite for organisms to penetrate and colonize the host in the more general parasitic-symbiotic relationships (Tunlid et al., 1992). Trapping organs do not develop in pure culture; the addition of nematodes or their extracts induces the development of them within 24-48 hr (Duddington, 1955; Pramer & Stollm, 1959; Nordbring-Herz, 1973). This fact shows that the series of interaction events between nematode and predaceous fungi may be initiated by a chemotactic/chemotropic response occurring at some distance from the host. Tunlid et al. (1992) reviewed several of the interaction steps in the nematode-nematophagous attachment of the fungi to nematodes and the biological and biochemical/molecular background to this adhesion. The above mentioned observations were conducted in cultural experiments on agar or liquid media. In our work the nematophagous activity of the fungus is studied on moss.

In the extreme dryness and low temperature conditions of Antarctica, the moss cushions are comfortable habitat. Moss can retain much water in its capillary spaces, formed between the partly overlapping leaves and the stems. These spaces possess considerable suction power and capacity (Overgaard, 1948). Moreover solar radiation on the water droplets in the moss may increase the temperature values. These facts can help to realize the life cycle of antarctic moss-fauna-mycobiota. *A. tortor* and nematodes, are two important components in the antarctic moss biotope and closely related to it. *Rhabditis* is a nematode genus previously recorded for Antarctica (Vinciguerra et al., 1989)

The study of the *A. tortor* ability to form nematode trapping organs on moss, revealed new aspects in the relationships between the fungus, nematodes and the moss. The architecture of the fungal mycelium and disposition of trapping organs on the moss have been

pointed out. *A. tortor*, captures nematodes not only by means of threedimensional adhesive nets and loops but also of the entire adhesive fasciculate aerial hyphae complex. It can be supposed the coiled hyphae, that are usually present in our strains, are a new kind of trapping organ, although nematodes have never been seen captured by such a structure. Moreover, the nematode is not only a prey for the fungus but it seems to utilize the fungus as a support for its eggs and larvae. It can be argued that eggs have more protection above the general soil level, being far from soil dangerous bacteria and other organisms. A possible equilibrium might be established between nematodes and the predacious fungus in this peculiar and limited habitat. Further researches will be carried out in order to study the econutritional relationships between *A. tortor* and nematodes in Antarctica.

Acknowledgments

This work was supported by a grant from the National Programme of Antarctic Research.

REFERENCES

- Caretta, G.; Del Frate, G.; Mangiarotti, A.M. (1994). A record of *Arthrobotrys tortor* Jarowaja and *Engyodontium album* (Limber) De Hoog from Antarctica. Boletín Micológico 9: 9-13
- Del Frate, G. & Caretta, G. (1990). Fungi isolated from Antarctic material. Polar Biol. 11: 1-7
- Duddington, C. L. (1951). The ecology of predacious fungi. I, Preliminary Survey. Trans. Brit. myc. Soc. 34: 322-331
- Duddington, C. L. (1955). Notes on the Technique of handling predacious fungi. Trans. Brit. myc. Soc. 38: 97-103
- Goodey, T. (1949). Laboratory methods for Work with Plant and Soil Nematodes. Ministry of Agriculture and Fisheries, Technical Bulletin N° 2, London: His Majesty's Stationery Office.
- Gray, N. F. (1985). Nematophagous fungi from the maritime antarctic: factor affecting distribution. Mycopathologia 90: 165-176
- Gray, N. F.; Wyborn, C. H. E.; Smith, R. I. L. (1982). Nematophagous fungi from the maritime Antarctic. Oikos 38: 194-201
- Gray, N. F. & Smith, R. I. L. (1984). The distribution of nematophagous fungi in the maritime Antarctic. Mycopathologia. 85: 81-92
- Jarowaja, N. (1968). *Arthrobotrys tortor* sp. nov. New predaceous nematode-killing fungus. Acta mycologica. 4: 241-247
- Jarowaja, N. (1970). The genus *Arthrobotrys*. Acta mycologica. 6: 337-406.
- Nordbring-Herz, B. (1973). Peptide-induced morphogenesis in the nematode-trapping fungus *Arthrobotrys oligospora*. Physiologia Plantarum. 29: 223-233
- Onofri, S. & Tosi, S. (1992). *Arthrobotrys ferox* sp. nov., a springtail capturing Hyphomycete from continental Antarctica. Mycotaxon 44: 445-451
- Oorschot, C.A.N. van (1985). Taxonomy of the *Dactylaria* complex. V. A review of *Arthrobotrys* and allied genera. Studies in Mycology 26: 61-95
- Overgaard, C. (1948). Studies on the soil microfauna. I. The moss inhabiting nematodes and rotifers. Publications de la Société des Sciences et des lettres d'Aarhus. 2, 1-131. Copenhagen
- Pramer, D. & Stoll, N. R. (1959). Nemin: a morphogenic substance causing trap formation by predaceous fungi. Science 129: 966-967
- Tunlid, A.; Jansson, H. B.; Nordbring-Hertz, B. (1992). Fungal attachment to nematodes. Mycol. Res. 96: 401-412
- Vinciguerra, M. T.; Binda, M. G.; Pilato, G. (1989). Faunistic research in Antarctica: first data on terrestrial and fresh-water nematodes and tordigrades. Atti del I Convegno di Biologia Antarctica, Roma 22-23 giugno; in Scienza e cultura, Ed. Universitarie Patavine: 111-113

Figures. - 1 to 9 *Arthobotrys tortor* growing on moss in nematodes infected cultures

Fig.1.- Strong fasciculate aerial hyphae extending from a moss leaf (bar 20µm). Fig 2,3,4.- Networks and coiled hyphae on aerial hyphae (Fig. 2, bar 48 µm; Fig. 3, bar 20 µm; Fig. 4, bar 48 µm). Fig 5,6.- Nematode captured by hyphal loop (Fig. 5, bar 48 µm; Fig. 6, bar 12 µm). Fig 7,8,10.- Nematodes captured on aerial hyphae (Fig. 7, bar 48 µm; Fig. 8 and 10, bar 12 µm). Fig 9.- Eggs and young nematodes in mucilaginous droplet (bar 12 µm).



