ORBILIACEOUS NEMATODE-TRAPPING FUNGI AND RELATED SPECIES IN WESTERN AUSTRALIA AND THEIR BIOLOGICAL ACTIVITIES

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Abstract

Twenty-nine isolates of orbiliaceous nematode-trapping fungi including 19 species of the genera *Arthrobotrys*, *Dactylellina*, and *Gamsylella* were obtained from various substrata ranging from wood chips, cultivated soils, organic matter, moss cushions and dung samples from Western Australia. In addition, eight taxonomically related species were isolated. Among the orbiliaceous nematode-trapping species, *A. amerospora*, *A. megalospora*, *A. multiformis*, *A. oligospora*, *and G. arcuata* are new for the Australian fungal flora. All isolates were screened for their biological activities including predacious, enzymatic and mycoparasitic activities and chemotaxis. Production of nematode-trapping organs was induced, where applicable, by axenic cultures of a free-living nematode, *Caenorhabditis elegans*. Although the secondary metabolites produced in pure culture by nematode-trapping fungi did not cause mortality of *C. elegans*, their predacious activity caused up to 90% mortality in *C. elegans* in Petri dishes between 0–4 days. Nematode-trapping fungi showed protease (63% of the isolates) and ligninase (11%) activity, none showed chitinase or cellulase activity on the solid media used.

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Introduction

A major group of nematode-trapping fungi are anamophic Orbiliaceae (Ascomycota). Teleomorphs, however, are unknown for the majority of species. Recently, Scholler *et al.* (1999) revised this group and proposed a new generic concept for anamorphs in which the trapping device is the main morphological criterion for delimitation of genera. Four genera are accepted by the authors, viz. *Arthrobotrys* Corda forming adhesive, nematode-induced networks, and three other which have a stronger tendency to form trapping organs spontaneously: *Drechslerella* Subram. forming constricting rings, *Dactylellina* M. Morelet forming stalked adhesive knobs only, or stalked knobs in combination with non-constricting rings, and *Gamsylella* Scholler *et al.* for species producing adhesive columns and unstalked knobs. We follow this concept and use the proposed species names in this article. Besides their predatory mode of nutrition, orbiliaceous nematode-trapping fungi are strong, fast growing (*Arthrobotrys*) to moderate and slowly growing (species of other genera) saprotrophs. Finally, a third, mycoparasitic mode of nutrition was observed by Tzean & Estey (1978) and Persson *et al.* (1985) for various orbiliaceous species. Most species are considered cosmopolitan, occurring in a variety of (mainly nutrient-rich) habitats including forest litter, rotting wood, dung, vegetable compost, moss cushions, coastal vegetation, and many other substrata (Barron 1977, 1982, Gray 1984).

Galper *et al.* (1995) evaluated the predatory activity and proneness to trap formation in view of selecting effective biological control agents. Quinn (1987) suggested that competition by common saprotrophic soil fungi could stimulate the predatory activity of nematode-trapping fungi, compensating for their low competitive saprotrophic ability. Rosenzweig & Ackroyd (1984) investigated the influence of soil micro-organisms on the trapping efficiency. Only one (*Saccharomyces cerevisiae* Meyen ex Hansen) of 29 organisms tested agglutinated

to traps of nematode-trapping fungi and prevented nematode capture. But the potential exists for a wide diversity of microbial species and strains to interfere with nematode capture in the soil.

In addition to predatory activity, some nematode-trapping fungi also produce nematicidal metabolites. For example, linoleic acid was isolated from submerged cultures of *Arthrobotrys conoides* Drechsler and pleurotin from species of the basidiomycete genus *Nematoctonus* (Anke *et al.* 1995). Increased production of nematicidal metabolites was observed when the formation of numerous nematode-trapping structures was induced in the medium either by the addition of a peptide or of nematodes (Jansson *et al.* 1997).

During the infection of nematodes and other small animals, nematode-trapping fungi produce hydrolytic enzymes such as proteases and chitinases. The proteases may be involved in the infection and immobilization of nematodes. Miller & Sands (1977) found that hydrolytic enzymes such as protease, lipase, collagenase and chitinases had nematicidal activities on plant-parasitic nematodes. Tunlid *et al.* (1994) purified and characterized an extracellular serine protease from *A. oligospora* Fresen. The hyphomycete *Dactylella oviparasitica* Stirling & Mankau (a nematode egg parasite) produced chitinase when grown on media containing colloidal chitin as a carbon source (Stirling & Mankau 1979). Although none of the chitinases from nematode-trapping fungi was isolated and characterized, the importance of the enzymes is indicated by the correlation between the virulence of fungal isolates and the level of chitinase activity (Dackman *et al.* 1989). In addition, production of cellulase and ligninase has been detected in certain orbiliaceous nematode-trapping fungi (Rubner 1996).

As mentioned above, some nematode-trapping fungi are mycoparasites. Their hyphae coil around those of the host fungus and restrict its growth without directly penetrating (Persson *et al.* 1985). *Arthrobotrys oligospora* coiled around hyphae of fungi belonging to various taxonomic groups tested including species of *Rhizoctonia*, *Trichoderma*, *Gliocladium* and *Penicillium* (Persson *et al.* 1985). These authors interpreted the behaviour of coiling as a competition for nutrients. Persson & Friman (1993) compared the proteolytic activities of mycelial extracts of coils from dual cultures of *A. oligospora* and *Rhizoctonia solani* Kühn with those of axenically grown hyphae. The coil extracts had significantly higher proteolytic activities than those of axenically grown hyphae, indicating that proteases are induced when these two organisms interact.

So far, the only studies on orbiliaceous nematode-trapping fungi in Australia were carried out by McCulloch (1977a, b) in Queensland. She isolated 57 species of nematophagous fungi species 25 of which are orbiliaceous ones. The most commonly recorded species were *Arthrobotrys conoides* Drechsler, *A. thaumasia* (Drechsler) S. Schenck *et al.*, *Dactyellina ellipsospora* (Preuss) M. Scholler *et al.*, *Drechslerella dactyoides* (Drechsler) M. Scholler *et al.* and *Gamsylella gephyropaga* (Drechsler) M. Scholler *et al.*

The present study was undertaken (1) to isolate and identify orbiliaceous nematode-trapping and related endoparasitic and non-nematophagous species from various substrata and sites in Western Australia, and (2) to compare their biological activities in relation to their nematophagous habit *in vitro*.

Materials and Methods

Sampling of materials

Nematode-trapping fungi were isolated from cultivated field soils in the Esperance District, from organic matter under a banana plantation at the University of Western Australia, from moss cushions and leaf litter in various aquatic environments around the metropolitan area of Perth, and from animal dung, mainly from cows and sheep in the wheat-belt of Western Australia. Sample sizes used for isolation of nematode-trapping fungi were not uniform.

Isolation of nematode-trapping fungi (sprinkled-plate and Baermann funnel technique)

The sprinkled-plate technique (described in Duddington 1955) was used to isolate nematode-trapping fungi. From the cultivated soils and organic matter, a sample of approximately 1 g was sprinkled onto a plate with well-dried 2% water agar (WA). Leaf litter material was chopped into small pieces and placed in a sieve (5 cm diam.) in a mist chamber that was operated at intervals of 3 min. on and 3 min. off for two days to wash off dirt and surface contaminants. The washed material (*c.* 20 pieces) was dried on a sterile filter paper and sprinkled onto WA. Moss cushions and dung samples, were chopped into small pieces and were plated onto WA.

The Baermann funnel method (Barron 1978) is based on the assumption that, in any natural population of nematodes, a proportion is infected by fungal parasites but the infected hosts are still sufficiently mobile to be recovered by this procedure. Soil (20 g) was wrapped in tissue paper and placed in a Baermann funnel for one day. The collecting tube was then removed, the supernatant removed, and the 1 ml of nematode suspension remaining in the bottom of the tube directly plated out onto well-dried WA.

A suspension (*c.* 5000 individuals) of axenic nematodes *Caenorhabditis elegans* Maupas was added to the plates as bait. The nematodes were rinsed three times with sterile water before plating. *Caenorhabditis elegans* was maintained axenically in 250 ml flasks containing 50 ml of haemoglobin medium (Vanfleteren 1978) on a shaker (100 rpm, 20°C). The plates were kept at room temperature and examined every day after the first three to four days using a dissecting microscope at × 30. A flamed glass needle was used to select single conidia of sporulating nematode-trapping fungi, which were then transferred to cornmeal agar Difco (CMA) to be cultured and preserved.

Identification of nematophagous fungi

All isolates were cultivated on half strength cornmeal agar Difco (1/2 CMA: CMA 8.5 g and agar 8 g) for microscopic observation. After incubation at 25°C, they were examined repeatedly for up to one month for the presence of chlamydospores. All isolates were studied and identified using a light microspore (Olympus BH-2) after cultivating isolates on WA and adding *C. elegans* (*c.* 500 individuals per plate). Measurements were carried out in a water mount at a magnification of × 400 or × 1000, using the keys provided by Barron (1977), Liu & Zhang (1994), van Oorschot (1985) and Rubner (1996).

Predacious activities on agar

Caenorhabditis elegans (*c.* 500 individuals) were added to each isolate on WA after four days of growth for fast-growing isolates, and after seven days for slow-growing ones. The plates were incubated at 25°C and examined every 12 h. The LT_{90} was determined, *i.e.* the time needed for 90% of the nematodes to succumb to the predators.

Nematicidal activities (mortality)

Each fungal isolate was grown in conical flasks containing 20 ml of potato dextrose broth (PDB) with or without *C. elegans* for seven days at 25°C. Culture filtrates of each isolate were obtained by filtering the culture broth through a 0.2 μm Millipore filter and transferring 200 μl of filtrate into each well (capacity 300 μl) of a 96-well flat-bottomed microtitre plate. A 10 μl suspension of axenic *C. elegans* containing *c.* 50 nematodes was transferred into each well, which was then sealed with parafilm to prevent evaporation. The nematodes were examined every 12 h. for two days with an inverted microscope at × 10. The nematodes were rated for mortality with determination of the LT_{50}, *i.e.* the time needed for 50% mortality of the nematode population, while their behaviour on PDB served as a control (Park *et al.* 2001).

Attraction and repulsion

Attraction and repulsion of nematode-trapping fungi were studied using the agar plate method modified by Jansson (1982). Each of the nematode-trapping fungi was grown on dilute cornmeal agar Difco (1/10 CMA: CMA 1.7 g and agar 9.6 g) at 25°C for seven days. Two agar plugs from actively growing fungal colonies of each isolate were placed in each quadrant of a 9 cm diam. Petri dish containing WA. Control discs (1/10 CMA) were placed between the test fungi in the Petri dish. After 24 h., a drop of the nematode suspension (*c.* 500) was added to the middle of the plates. To determine nematode migration, the dishes were examined every three h. for 24 h. Attraction or repulsion is recorded when more than 50% of the observed nematodes had migrated towards or away from a certain isolate. This experiment was repeated twice.

Enzymatic activities

Protease, chitinase, cellulase and ligninase activities were examined by determining the ability of isolates of the nematode-trapping fungi to grow on specific substrates in defined and semi-defined solid media. An agar plug of the fungi were placed on Petri plate of the media containing 1% gelatine (Kunert *et al.* 1987), 0.2% colloidal chitin (Dackman *et al.* 1989), 4% cellulose power (Paterson & Bridge 1994) or 0.02% Poly R-478 dye (Sigma) (Paterson & Bridge 1994) for determination of protease, chitinase, cellulase or ligninase, respectively. A clear zone (halo) around a colony of an isolate in the appropriate medium was taken as a sign of enzymatic activity. Protease activity was assessed after three d. incubation at 25°C, whereas chitinase, cellulase and ligninase activities were assessed in seven-days intervals for three weeks at 25°C.

Table 1. Trapping organ formation in the presence of the nematode *Caenorhabditis elegans* and enzymatic and predacious activities of orbiliaceous nematode-trapping fungi and related species.

Fungus species*	Isolate	Trapping organs	Predacious activity(LT_{90})†	Coiling‡	Enzymatic activity# (protease & ligninase)		Origin!
Gamsylella arcuata **(WAC10282)**	8077	Hyphal branches	1	-	-	-	LL
Gamsylella gephyropaga **(WAC10284)**	4612	and/or unstalked	0	-	+	-	AF
G. gephyropaga **(WAC10286)**	4623	knobs	4	-	-	-	CS
G. gephyropaga **(WAC10287)**	8097	"	2	-	+	-	LL
G. gephyropaga **(WAC10288)**	8098	"	0	-	+	-	LL
G. gephyropaga **(WAC10289)**	8102	"	2	-	+	-	LL
G. robusta **(CBS110125)**	8132	"	2	-	-	-	LL
Gamsylella sp. **(WAC10291)**	8178	"	4	-	+	-	LL
Dactylellina haptotyla **(WAC10292)**	4601	Stalked knobs with	4	-	+	-	BM
Dactylellina sp. **(WAC10293)**	4606	or without non-	0	-	+	-	WC
Dactylellina sp. **(WAC10294)**	8071	constricting rings	1	-	+	-	LL
Da. haptotyla **(WAC10295)**	8072	"	1	-	+	-	LL
Da. cf. *leptospora* **(WAC10296)**	8076	"	na	-	-	-	LL
Dactylellina sp. **(WAC10297)**	8162	"	2	-	+	-	LL
Arthrobotrys oligospora **(WAC10298)**	4602	Networks	1	+	+	-	BM
A. musiformis **(WAC10299)**	4603	"	1	+	+	-	MC
Arthrobotrys sp. **(WAC10300)**	4605	"	2	+	+	-	AF
A. oligospora **(WAC10301)**	4610	"	1	-	-	-	BM
A. oligospora **(WAC10302)**	4615	"	2	+	+	-	MC
A. amerospora **(WAC10303)**	4616	"	3	-	+	-	BM
Arthrobotrys sp. **(WAC10304)**	4618	"	1	+	-	+	BM
A. thaumasia **(WAC10305)**	4619	"	1	+	-	-	CS
A. cf. *salina* **(CBS110117)**	4620	"	1	+	-	-	CS
A. thaumasia **(WAC10307)**	4621	"	1	+	-	-	CS
A. thaumasia **(WAC10308)**	4622	"	1	+	-	-	CS
A. megalospora **(WAC10309)**	8070	"	1	+	+	-	LL
A. musiformis **(WAC10310)**	8080	"	1	+	+	+	LL
A. eudermata **(CBS110116)**	8082	"	1	+	-	-	LL
A. multiformis **(WAC10312)**	8193	"	3	+	-	-	LL
Harposporium leptospira **(CBS109331)**	4613	Endoparasite	1	na	+	-	AF
Dactylella oxyspora **(WAC10314)**	4608	Non-predacious	ne	-	+	-	AF
Dactylella sp. **(WAC10315)**	4609	fungi	ne	-	+	-	WC
D. oxyspora **(WAC10316)**	4617	"	ne	-	+	-	WC
D. cylindrospora **(WAC10317)**	8007	"	ne	-	+	-	LL
Dactylella sp. **(WAC10318)**	8074	"	ne	-	-	-	LL
Dactylella sp. **(WAC10319)**	8120	"	ne	-	-	-	LL
D. cylindrospora **(WAC10320)**	8137	"	ne	-	+	+	LL
Dactylella sp. **(WAC10321)**	8179	"	ne	-	+	+	LL

* A = *Arthrobotrys*; Da = *Dactylellina*; D = *Dactylella*; G = *Gamsylella*
WAC = Western Australia Collection assess no.; CBS = Centraalbureau voor Schimmelcultures assess no.

† Predacious activity: days needed to reach 90% mortality of the nematode population in a Petri dish.

‡ Coiling on *R. solani*: + = positive reaction; - = negative reaction.

Enzymatic activity: + = positive reaction; - = negative reaction. Activities of cellulase and chitinase are not presented since all isolates showed negative reaction.

! AF = Animal faeces; BM = Banana plantation mulch; CS = Cultivated soil; LL = Leaf litter; MC = Moss cushion; WC = Wood chips.

na = not applicable; ne = not evident.

Hyphal coiling

To examine the interaction between the nematode-trapping fungi and *Rhizoctonia solani* Kühn, dual cultures were prepared. The isolates of the nematode-trapping fungi and *R. solani* (AG8) were placed approximately 4 cm apart on half-strength potato-dextrose agar (1/2 PDA: PDA 19.5 g and agar 2.5 g) and 2% WA. The times of inoculation and incubation were adjusted according to the growth rate of each fungus, allowing the cultures to meet in the middle of the Petri dish at 25°C. The plates were examined every day after the two fungi had made contact.

Results

Species of nematode-trapping fungi isolated and their abundance in Western Australia

A total of 38 isolates representing 26 species (including 19 orbiliaceous nematode-trapping, one endoparasitic, and six non-predacious species) were obtained from the various samples collected (Table 1). From leaf litter samples taken in aquatic environments, 19 isolates were obtained and purified. A further 19 isolates included in this study originated from wood chips, cultivated soils, organic matter, moss cushion or dung samples.

Orbiliaceous nematode-trapping species are *Arthrobotrys amerospora* S. Schenck *et al.*, *A. eudermata* (Drechsler) Scholler *et al.*, *A. megalospora* (Drechsler) M. Scholler *et al.*, *A. multiformis* (Dowsett, J. Reid & Kalkat) M. Scholler *et al.*, *A. musiformis* Drechsler, *A. oligospora* Fresen., *A.* cf. *salina* (R.C. Cooke & C.H. Dickinson) M. Scholler *et al.*, *A. thaumasia* (Drechsler) Schenck *et al.*, two unidentified *Arthrobotrys* spp., *Gamsylella arcuata* (Scheuer & Webster) M. Scholler *et al.*, *G. gephyropaga* (Drechsler) Scholler *et al.*, *G. robusta* (R.S. McCulloch) M. Scholler *et al.*, one unidentified *Gamsylella* sp., *Dactylellina haptotyla* (Drechsler) M. Scholler *et al.*, *Dactylellina* cf. *leptospora* (Drechsler) M. Morelet, and three unidentified *Dactylellina* spp. Furthermore one endoparasitic species, *Harposporium leptospira* Drechsler and six non-nematophagous species of the genus *Dactylella* were found. These non-nematophagous species are *Dactylella cylindrospora* (R.C. Cooke) A. Rubner, *D. oxyspora* (Sacc. & Marchal) Matsush., and four unidentified *Dactylella* spp. *Gamsylella gephyropaga* is the most common species and has been isolated five times (for numbers of isolates of other species see Table 1).

Formation of chlamydospores

Seven isolates of *Arthrobotrys* species [*A. musiformis* (#4603, #8080), *A. oligospora* (#4615), *A. amerospora* (#4616), *A.* cf. *salina* (#4620), and *A. thaumasia* (#4621, #4622)] produced chlamydospores after a month on CMA. That is all species except for *A. megalospora* and *A. eudermata*.

Predacious activities

Three isolates [*Dactylellina* sp. (#4606) and *G. gephyropaga* (#4612, #8098)] formed their traps in pure cultures even in the absence of nematodes, and killed 90% of nematodes within 12 h. after the nematodes were introduced. With the exception of the nine isolates that did not form any trapping organs under the *in vitro* conditions provided, the time needed for trap formation by nematode-trapping fungi following addition of nematodes varied from three h. to two d. The overall predacious activity of nematode-trapping fungi was also different, ranging from one to four days needed to kill more than 90% of the nematodes (Table 1).

Enzymatic activities

None of the isolates showed detectable levels of chitinase or cellulase activity even after one-month incubation at 25°C. Four isolates [*Arthrobotrys* species (#4618), *A. musiformis* (#8080), *D. cylindrospora* (#8137) and *Dactylella* species (#8179)] showed lignolytic activity on the medium. Protease activity was observed for 23 of 38 isolates without presenting any specific patterns in relation to types of traps or species (Table 1).

Hyphal coiling

All isolates of *Arthrobotrys* species except *A. oligospora* (#4610) and *A. amerospora* (#4616) were found to coil around *R. solani*. No hyphal coiling was observed in the isolates belonging to other genera (Table 1).

Attraction and repulsion

Depending on migration ability of *C. elegans* towards the nematode-trapping fungi, only two isolates, *Dactylellina haptotyla* (#4601) and *Dactylella* species (#8120), showed strong repulsion of the nematodes. Nine [*Dactylellina* species (#4606, #8071), *Dactylella* oxyspora (#4608, #4617), *Arthrobotrys* species (#4618), *A. megaspora* (#8070), *Dactylella* species (#8074), *A. eudermata* (#8082) and *D. cylindrospora* (#8137)], caused no effect on the nematodes which were scattered over the surface of agar. The remaining 27 isolates showed

some degree of attraction of the nematodes. Of these, six [*A. musiformis* (#4603), *Arthrobotrys* species (#4605), *A. oligospora* (#4610), *D. cylindrospora* (#8007), *Da. haptotyla* (#8072) and *G. gephyropaga* (#8098)], showed strong attraction with more than 90% of the nematodes being lured to the agar plug after 12 h. incubation.

Discussion

This is the first published record of nematode-trapping fungi isolated from various substrata in Western Australia. *Arthrobotrys amerospora*, *A. megalospora*, *A. multiformis*, *A. oligospora*, *G. arcuata* and the endoparasite *Harposporium leptospira* are new records in Australia. Interestingly, no species of the genus *Drechslerella* (the fourth genus within orbiliaceous nematode-trapping fungi) was isolated, although most species of this genus are common, with worldwide distribution, and two species were isolated by McCulloch (1977a) in Queensland. Six of the isolates of orbiliaceous nematode-trapping fungi of this study could not be identified to species level. As far as we know, there is no record of a *Dactylella* species from Australia so far. Therefore *D. cylindrospora* and *D. oxyspora* have to be considered new species for Australia as well.

The biological activities of these fungi were studied in relation to their nematophagous habit. Nematode-trapping fungi have often been studied for their potential as biological control agents of plant-parasitic nematodes (see, *e.g.*, the review by Stirling 1991). Predacious activity has invariably been regarded as criterion in the selection of potential biocontrol isolates. Galper *et al.* (1995) screened the predacious activity of 18 isolates of nematode-trapping fungi and found that more than 90% of *C. elegans* were trapped by efficient species. In our study, the network-forming isolates (species of the genus *Arthrobotrys*) varied in the rate of predacious activity, requiring one to three days, whereas the isolates with hyphal branches or non-constricting rings required from zero to four days to trap more than 90% of *C. elegans*. Some evidence of host specificity was suggested implying that trapping organs are produced only after contact with certain groups of nematodes. Drechsler (1937) and Scholler & Rubner (1999) found that species with stalked knobs (genus *Dactylellina*) may be host-specific. In our observation, *Dactylellina* cf. *leptospora* (#8076) did not trap *C. elegans* while it does trap *Panagrellus redivivus* L.

Chlamydospores, resting spores permitting survival under adverse environmental conditions, are found in many nematode-trapping fungi. Production of chlamydospores is affected by cultural conditions such as incubation time, temperature and media used (*e.g.* Rubner 1996). Cooke & Satchuthananthavale (1968) suggested that the production of chlamydospores was not a reliable taxonomic criterion to discriminate between species of nematode-trapping fungi. Scholler *et al.* (1999), however, list chlamydospore formation only for species of the genera *Drechslerella* and *Arthrobotrys*. Our observations support Scholler *et al.* (1999) since we observed chlamydospores only in isolates of *Arthrobotrys* spp. The three isolates obtained from cultivated soils [*A.* cf. *salina* (#4620) and *A. thaumasia* (#4621, #4622)] produced chlamydospores in culture. This suggests that isolates growing under periodically dry conditions may have an advantage in survival by their chlamydospores.

The metabolites of the isolates used in this study did not show any nematicidal activities against the test nematode, *C. elegans*. Anke *et al.* (1995) found some nematicidal activities from strains of nematode-trapping fungi such as *A. oligospora*. Of the compounds they tested, linoleic acid showed strong activity towards *C. elegans*. In addition, the production of the acid increased with the number of nematode-trapping structures that was induced in the medium either by adding chemicals such as peptides (Rosenzweig 1984) or living nematodes (Jansson *et al.* 1997).

Dackman *et al.* (1989) investigated the production of enzymes by using skim-milk agar. They found that an isolate of *A. oligospora* did not produce any detectable levels of enzymatic activities on solid media. In our study, the isolates showed various levels of protease activity on solid media containing 1% gelatine as a protein substrate. Unlike nematode endoparasites such as *Pochonia chlamydosporia* (Gams & Zare 2001, Zare *et al.* 2001) and *Paecilomyces lilacinus* which show different degrees of chitinase activity, none of the nematode-trapping fungi tested here produced detectable levels of chitinases. Rubner (1996) used a phenoloxidase test to detect ligninolytic abilities of 30 strains of nematode-trapping fungi and found that only two (*Dactylella oviparasitica* Stirling & Mankau and *Drechslerella effusa* (Jarow.) Scholler *et al.*) reacted positively and three (*Dactylella oxyspora*, *G. arcuata* and *G. phymatopaga* (Drechsler) Scholler *et al.*) revealed only weak activities. Many nematode-trapping fungi are known to occur on rotting wood and would presumably be capable of cellulolytic activity to varying degrees (Dixon 1952). Barron (1992) also emphasized the importance of lignolytic and cellulolytic activities produced by nematode-trapping fungi, suggesting that the nematode-trapping

habit of nematophagous fungi is an evolutionary response by cellulolytic or lignolytic fungi to nutrient deficiencies in N-limited habitats. This theory has been proven wrong for *A. oligospora* in *in-vitro* experiments by Scholler & Rubner (1994). The authors found that this species performs very low predatory activity when grown in nitrogen-poor agar media whereas predatory activity was high when the carbon source was in short supply.

Hyphal coils and mycoparasitic activity have been reported in *A. oligospora* (Persson *et al.* 1985, Tzean & Estey 1978), in *A. superba* (Persson *et al.* 1985, Saxena *et al.* 1989), in *Dactylellina entomopaga* (Drechsler) Scholler *et al.*, and in *Dactylellina haptotyla* (Persson *et al.* 1985). With the exception of two isolates (*A. oligospora* and *A. amerospora*), all *Arthrobotrys* spp. tested in this study coiled around *R. solani*, but we could not confirm hyphal coiling in species belonging to genera other than *Arthrobotrys*. Probably, this feature is restricted to the genus *Arthrobotrys*, because the coiling of the two *Dactylellina* spp. mentioned by Persson *et al.* (1985) was more like normal hyphae than coils in *A.oligospora* and *A. superba* (B. Nordbring-Hertz, personal communication).

Jansson (1982) found a correlation between predacity in nematode-trapping fungi and their capacity to attract nematodes. In cultures of *A. oligospora*, the presence of traps appeared to increase the ability to attract nematodes. In our study, no consistent relationship between predacity and attraction of *C. elegans* by nematode-trapping fungi was observed. But the six isolates that attracted more than 90% of the nematodes showed strong predacious activity (LT_{90} = 0-2 days). Interestingly, the non-predacious isolate of *D. cylindrospora* strongly attracted *C. elegans*. One of the isolates that showed repulsion (*Dactylellina haptotyla #4601*) showed relatively weak predacious activity.

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