# Nematophagous Fungi

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# Nematophagous Fungi

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Nematophagous fungi are microfungi that can capture, kill and digest nematodes. They use special mycelial structures, the so-called traps, or spores to trap vermiform nematodes or hyphal tips to attack nematode eggs and cysts before penetration of the nematode cuticle, invasion and digestion.

Advanced article

Article Contents



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## Introduction

Nematophagous fungi are natural enemies of nematodes. They comprise three main groups of fungi: the nematode-trapping and the endoparasitic fungi that attack vermiform living nematodes by using specialized structures, and the egg- and cyst-parasitic fungi that attack these stages with their hyphal tips. The reason for the continuing interest in these fungi is, in part, their potential as biocontrol agents against plant- and animal-parasitic nematodes. From this point of view especially, the egg- and cyst-parasitic fungi have been investigated in depth because of the promise of these fungi as biocontrol agents. Another reason for the continued fascination in nematophagous fungi is the remarkable morphological adaptations and the dramatic capturing of nematodes by both nematode-trapping and endoparasitic fungi. In addition, both fungi and nematodes can be grown in the laboratory fairly easily, providing an excellent model system for interaction studies. **See also**: Biological control by microorganisms; Nematoda (round worms)

The nematode-trapping and endoparasitic fungi are found in all major taxonomic groups of fungi, and they occur in all sorts of soil environments where they survive mainly as saprophytes. The ability to use nematodes as an additional nutrient source provides them with a nutritional advantage. The fungi enter their parasitic stage when they change their morphology and traps or mature spores are formed. The development of infection structures is a prerequisite for the trapping of nematodes. The mechanisms behind this development and the mechanisms behind the capture process, including attraction, adhesion, penetration and digestion of nematodes, are the main topics of this article.

## Overview of Nematophagous Fungi

Nematophagous (nematode-destroying) fungi comprise more than 200 species of taxonomically diverse fungi that all share the ability to attack living nematodes (juveniles, adults and eggs) and use them as nutrients. The fungi differ in their saprophytic/parasitic ability. While many of the trap-forming and egg-parasitic fungi can survive in soil saprophytically, the endoparasites are mostly more dependent on nematodes as nutrient (obligate parasites). **See also**: Parasitism: the variety of parasites

The ability to capture nematodes is connected with a specific developmental phase of the fungal mycelium (**Table 1**, **Figure 1**). The trapping (predatory) fungi have developed sophisticated hyphal structures, such as hyphal nets, knobs, branches or rings, in which nematodes are captured by adhesion or mechanically. The endoparasites, on the other hand, attack nematodes with their spores, which either adhere to the surface of nematodes or are swallowed by them. Irrespective of the infection method, the result is always the same: the death of the nematode. Examples of the first group (**Table 1**) are *Arthrobotrys* spp., such as *A. oligospora, A. conoides, A. musiformis* and *A. superba*, which all form three-dimensional adhesive nets, whereas *A. dactyloides* uses constricting rings to capture nematodes mechanically by the swelling of the ring cells. Adhesive branches and adhesive knobs appear in the genus *Monacrosporium*. *M. haptotylum* (*Dactylaria candida*) produces both adhesive knobs and nonconstricting rings. **See also**: Basidiomycota; Deuteromycetes (Fungi Imperfecti); Hyphae

Among the endoparasites, *Drechmeria coniospora*, *Hirsutella rhossoliensis*, *Haptoglossa dickii* and *Catenaria anguillulae* infect nematodes with their spores and spend their vegetative lives inside infected nematodes. The genus *Nematoctonus* captures nematodes with both adhesive traps and adhesive spores and thus constitutes a link between the two groups (**Table 1**). A further mechanism of trapping nematodes is evident in the wood-decomposing oyster mushroom, *Pleurotus ostreatus*. The oyster mushroom immobilizes the nematode host by a toxin produced on

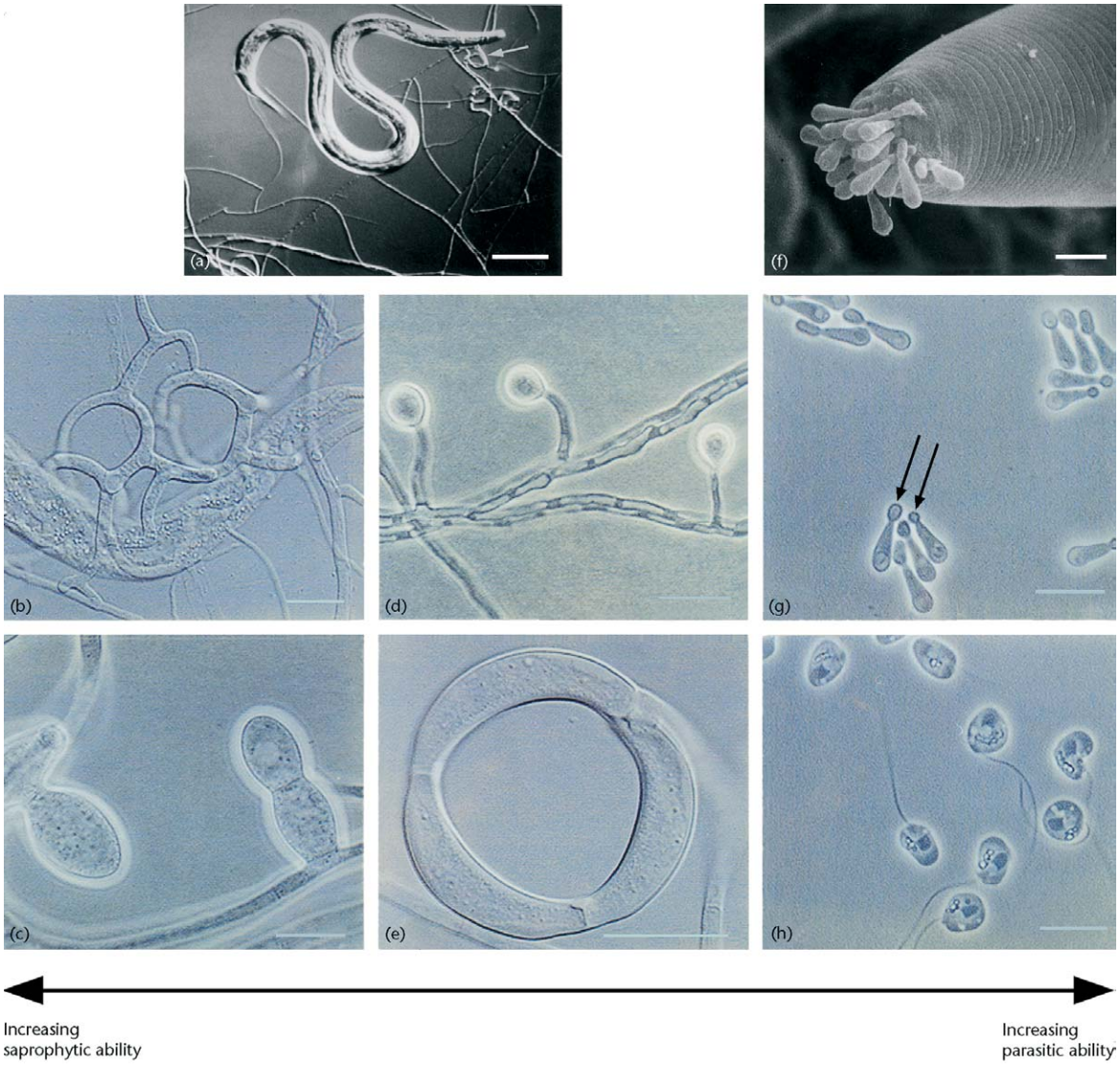


**Figure 1** Diversity of trapping structures in nematophagous fungi. (a) Nematode trapped (arrow) by *A. oligospora*. Bar, 100 μm. Reproduced from Nordbring-Hertz B, Zunke U, Wyss U and Veenhuis M (1986) *Trap Formation and Capture of Nematodes by Arthrobotrys oligospora*. Film No C1622. Courtesy of Institut für den Wissenschaftlichen Film, Göttingen. (b) Adhesive network of *A. oligospora*, developed from digested nematode. Bar, 20 μm. (c) Adhesive branches of *M. gephyropagum*. Bar, 10 μm. (d) Adhesive knobs of *M. haptotylum*. Bar, 10 μm. (e) Constricting ring of *A. brochopaga*. Bar, 5 μm. (b–e) Reproduced from Nordbring-Hertz *et al.* (1995a). Courtesy of Institut für den Wissenschaftlichen Film, Göttingen. (f) Nematode infected by conidiospores of *D. coniospora*. Bar, 5 μm. Reproduced from Jansson H-B (1982) Attraction of nematodes to endoparasitic fungi. *Transactions of the British Mycological Society* **79**: 25–29. Courtesy of the British Mycological Society. (g) *D. coniospora* spores with adhesive buds (arrows). Bar, 10 μm. (h) Zoospores of *Catenaria anguillulae*. Bar, 10 μm. (g–h) reproduced from Nordbring-Hertz *et al.* (1995a). Courtesy of Institut für den Wissenschaftlichen Film, Göttingen.

specialized hyphal stalks and the hyphal tips grow chemotropically through the mouth of their victims and digest the contents. The egg-parasitic fungi, e.g. *Pochonia chlamydosporia* (previously *Verticillium chlamydosporium*), use appressoria to penetrate nematode eggshells. Several stages of all these fungi are described in a film that illustrates the different strategies used by the fungi (Nordbring-Hertz *et al.*, 1995a). **See also**: Mushrooms and mushroom cultivation

## Diversity of Infection Structures

### Nematode-trapping fungi

As shown above, nematophagous fungi present a high diversity not only in respect of taxonomic distribution but also in respect of the trapping structures formed. The type of nematode-trapping structures formed depends on species or even strains of species as well as on environmental

**Table 1** Typical infection structures of some nematophagous fungi

| Infection structure | Species | Taxonomic classification |
|---|---|---|
| Adhesive nets | *Arthrobotrys oligospora* | Deuteromycetes |
| | *A. conoides* | |
| | *A. musiformis* | |
| | *A. superba* | |
| | *Duddingtonia flagrans* | |
| Adhesive branches | *Monacrosporium gephyropagum* | Deuteromycetes |
| Adhesive knobs | *M. ellipsosporum* | Deuteromycetes |
| | *M. haptotylum* | |
| Constricting rings | *A. dactyloides* | Deuteromycetes |
| | *A. brochopaga* | |
| Adhesive knobs and adhesive spores | *Nematoctonus concurrens* | Basidiomycetes |
| Adhesive spores | *N. leiosporus* | Basidiomycetes |
| | *Drechmeria coniospora* | Deuteromycetes |
| | *Hirsutella rhossoliensis* | |
| Ingested spores | *Harposporium anguillulae* | Deuteromycetes |
| Zoospores | *Catenaria anguillulae* | Chytridiomycetes |
| | *Haptoglossa dickii* | Oomycetes |
| Adhesive hyphae | *Stylopage hadra* | Zygomycetes |
| | *Cystopage cladospora* | |
| Toxic droplets | *Pleurotus ostreatus* | Basidiomycetes |
| Appressoria | *Pochonia chlamydosporia* | Deuteromycetes |

conditions, both biotic and abiotic. The most important biotic factor is living nematodes, which not only induce the formation of trapping structures by touching the mycelium but also serve as a food source for the fungi after they have been invaded by the fungi. Thus, the relationship to nematodes is 2-fold: first, nematodes may induce the formation of the structures in which they are later captured; and, second, after invasion of the nematodes by the fungus they serve as an additional food source.

**Figure 1** shows that *Arthrobotrys* spp. generally are more saprophytic than the endoparasites. Many of the *Arthrobotrys* spp. do not form traps spontaneously but the fungi are dependent on environmental conditions, especially the presence of nematodes for induction of traps. Trapping structures of other fungi, such as branches, knobs and constricting rings, may be formed spontaneously, indicating the greater need of these fungi for nematodes as nutrient source.

While the endoparasites and the spontaneous trap-formers (**Figure 1**) present a high parasitic ability, the more saprophytic trap-formers, such as *Arthrobotrys* spp., have a unique ability to change their morphology to increase their parasitic ability. As mentioned above, external stimuli, such as nematodes, induce the formation of adhesive traps in all trap-forming fungi. In *A. oligospora*, small peptides with a high proportion of nonpolar and aromatic amino acids or their amino acid constituents in combination with a low-nutrient status induce trap formation in both solid and liquid media. **Figure 2a** shows a peptide-induced trap of *A. oligospora*. Based on this knowledge, a growth technique has been developed where the fungus may be studied both in its saprophytic and its parasitic phase.

As seen in **Table 1**, most *Arthrobotrys* spp. are characterized by the adhesive network trap. This trap may consist of a single ring or a fully developed three-dimensional network. Under certain conditions, *A. superba*, for example, may not develop complete nets but captures nematodes by adhesive branches. Adhesive branches are regularly formed spontaneously in *Monacrosporium gephyropagum*. Occasionally, such branches may combine to form simple rings. Adhesive knobs are formed on delicate stalks on the mycelium of *M. haptotylum*. This species also produces nonconstricting rings on delicate stalks. Both knobs and rings may detach from the underlying mycelium and be carried away by the nematodes.

Less than fully developed traps may be efficient in trapping nematodes. Some species (e.g. *A. superba*) may capture nematodes on initials (branches) of adhesive nets, or even on adhesive hyphae, as in *Stylopage* and *Cystopage* spp. Furthermore, traps may be formed directly on germination of conidia (spores) to form the so-called conidial traps. This developmental pattern occurs in practically all trap-forming species when conidia are allowed to germinate in natural substrates, such as cow dung or rhizosphere soil (Persmark and Nordbring-Hertz, 1997). A mutant of *A. oligospora* not only forms conidial traps on its conidia when still on upright conidiophores; it also produces large amounts of normal traps on the mycelium. Such examples may indicate

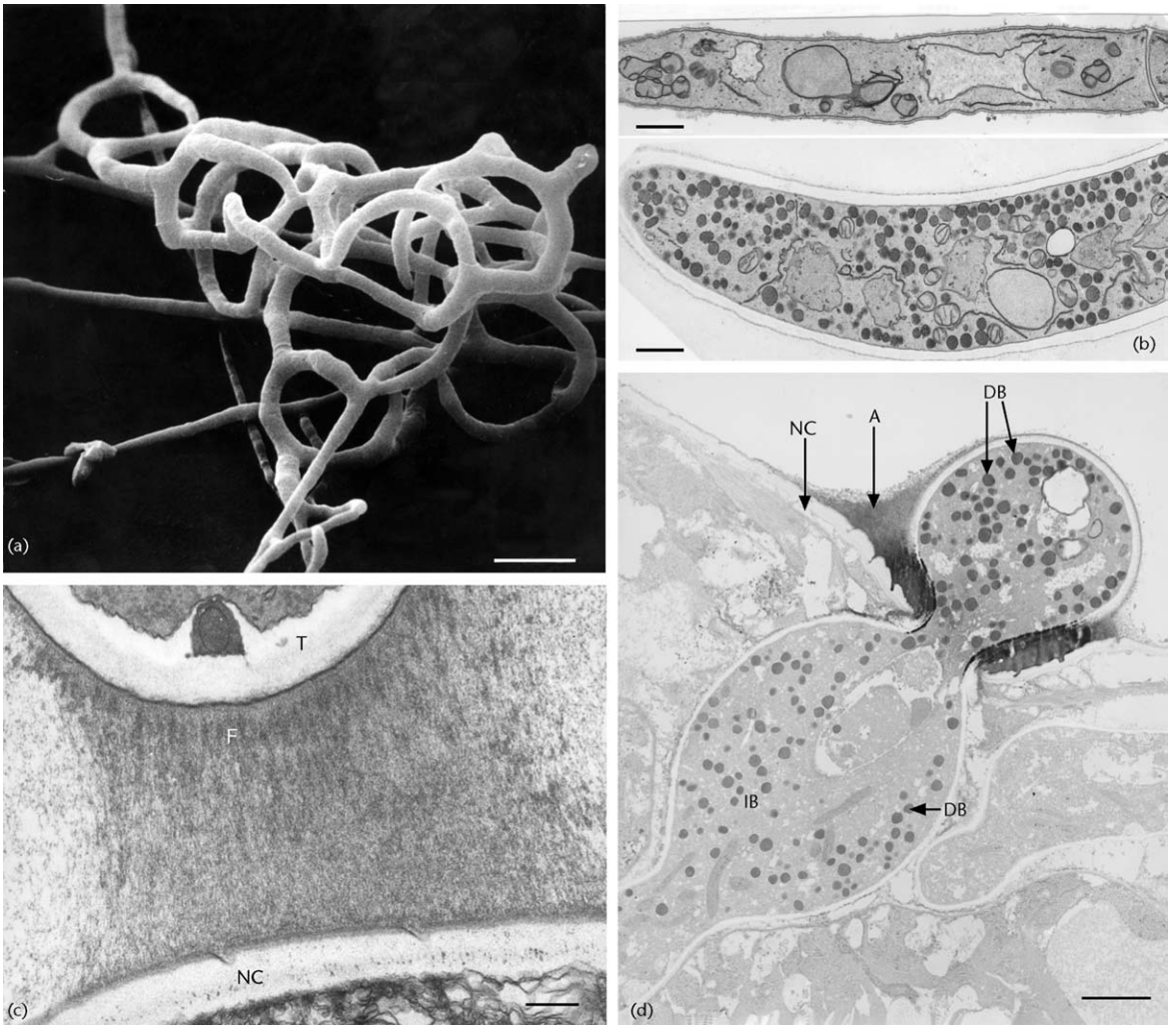


**Figure 2** Trapping of nematodes by *A. oligospora*. (a) Scanning electron micrograph (SEM) of peptide-induced adhesive network. Bar, 10 μm. Reproduced from Lysek G and Nordbring-Hertz B (1983) Die Biologie der nematodenfangender Pilze. *Forum Mikrobiologie* **6**: 201–208. Courtesy of G-I-T Verlag Ernst Giebeler, Darmstadt. (b) Transmission electron micrograph (TEM) of vegetative hypha (upper panel) and a trap cell. Bar, 1 μm. Note dense bodies only in the trap cell. Reproduced from Nordbring-Hertz B (1984) Mycelial development and lectin–carbohydrate interactions in nematode-trapping fungi. In: Jennings DH and Rayner ADM (eds) *The Ecology and Physiology of the Fungal Mycelium*, pp. 419–432. Courtesy of Cambridge University Press. (c) TEM of adhesive *A. oligospora*. After capture of the nematode the fibrils ( F) of the adhesive becomes directed from the trap (T) towards the nematode cuticle (NC). Bar, 1 μm. (d) TEM of penetration of nematode cuticle by *A. oligospora*. Note electron dense bodies (DB), adhesive coating (A), nematode cuticle (NC) and infection bulb (IB). Bar, 1 μm. (c and d) Reproduced from Veenhuis M, Nordbring-Hertz B and Harder W (1985) An electron microscopical analysis of capture and initial stages of penetration of nematodes by *A. oligospora*. *Antonie van Leeuwenhoek* **51**: 385–398. Courtesy of Kluwer Academic Publishers, Dordrecht.

an increased efficiency of these fungi to decrease nematode numbers in the environment. Another morphological adaptation of the mycelium of *A. oligospora* is the response to the presence of other fungi: *A. oligospora* may coil around their hyphae and consume the contents of these cells (mycoparasitism). Furthermore, *A. oligospora* may form appressoria in response to plant roots. Both the coiling of hyphae and appressoria in the rhizosphere are examples of the diversity of ways in which nematode-trapping fungi can cope with varying environmental conditions. All these adaptations point to an extensive plasticity of infection structures in nematode-trapping fungi. **See also**: Fungal spores

## Endoparasites

A similar diversity also exists among the endoparasites. *D. coniospora* forms large numbers of conidia in comparison to production of hyphal material. In a single infected

nematode, *D. coniospora* may produce as many as 10 000 conidia while the endoparasite *H. rhossoliensis,* which sporulates singly, produces 100–1000 conidia per infected nematode. Both fungi develop an adhesive bud (**Figure 1g**) on their conidia with which they infect the nematode. The genus *Harposporium* contains fungi that produce spores with special shapes, which are ingested by the nematodes. Because of their shapes, the spores get stuck in the oesophagus and from there initiate infection of the nematodes. *C. anguillulae* infects nematodes with their motile zoospores which encyst on and adhere to the nematode. Finally, in the genus *Haptoglossa* the spores form an infection 'gun cell' which forcibly injects the infective principle into the nematode host.

### Egg-parasitic fungi

The fungi that parasitize the nonmotile stages of nematodes, i.e. eggs, use a different strategy. Hyphae of *P. chlamydospora* and other fungi grow towards the eggs and appressoria are formed on the hyphal tips which penetrate the eggshell. The fungi then digest the contents of the egg, both immature and mature (containing juveniles) eggs.

### Ultrastructure

The development of infection structures has been followed by light and video-'enhanced' contrast microscopy and by scanning and transmission electron microscopy (SEM and TEM). TEM revealed a common feature observed in all traps of trap-forming fungi: the presence of numerous cytosolic organelles, the so-called dense bodies that are formed directly on initiation of the trap. Normal vegetative hyphae invariably lack dense bodies (**Figure 2b**). The dense bodies develop from specialized regions of the endoplasmic reticulum and exhibit catalase and amino acid oxidase activity and thus are peroxisomal in nature. They are supposed to be involved in penetration and digestion of the nematode. **See also**: Electron microscopy; Transmission electron microscopy: preparation of specimens

In contrast to traps, the conidia of endoparasitic fungi do not contain dense bodies. This difference is clearly emphasized in the case of the conidial traps of trap-forming fungi. These infection structures are similar to those of the endoparasitic fungi, in that they are free entities and may be carried away by a captured nematode. However, it is perfectly clear that the conidial traps of *A. oligospora* belong to the trapping fungi, as they contain numerous dense bodies, present also in the conidial cell from which they are formed (Nordbring-Hertz *et al.*,1995b).

### Gene expression

The global patterns of gene expression has been studied in traps and mycelium of *M. haptotylum* (Ahrén *et al.*, 2005). The advantage of using *M. haptotylum* is that during growth in liquid cultures with heavy aeration, the connection between the traps (knobs) and mycelium can be broken easily and the knobs can be separated from the mycelium by filtration. The isolated knobs retain their function as infection structures, i.e. they can 'capture' and infect nematodes. Ribonucleic acid (RNA) was isolated from traps and mycelium and hybridized to a deoxyribonucleic acid (DNA) microarray containing probes for 2 822 putative genes. Despite the fact that the knobs and mycelium were grown in the same medium, there were substantial differences in the patterns of genes expressed in the two cell types. In total, 23.3% of the putative genes were differentially expressed in knobs versus mycelium. Several of these genes displayed sequence similarities to genes known to be involved in regulating morphogenesis and cell polarity in fungi. Several homologues to genes involved in stress response, protein synthesis and protein degradation, transcription and carbon metabolism were also differentially expressed. Regulated were also genes known to be involved in the proliferation of peroxisomes. Interestingly, a number of the genes that were differentially expressed in trap cells are also known to be regulated during the development of appressoria formed by plant pathogenic fungi.

## Taxonomy and Evolution of Nematophagous Fungi

Nematophagous fungi are found in all major groups of fungi, including lower (oomycetes, chytridiomycetes, zygomycetes) and higher fungi (ascomycetes, basidiomycetes and deuteromycetes) (**Table 1**). Most nematophagous fungi, including both nematode-trapping and endoparasitic species, are deuteromycetes (asexual fungi). **See also**: Deuteromycetes (Fungi Imperfecti); Fungi and the history of mycology

The taxonomic position of some of these species has been clarified by the discovery of the corresponding sexual stages of the fungus (Pfister, 1997). For example, the sexual stages (teleomorphs) of a number of *Arthrobotrys*, *Monacrosporium* and *Dactylella* species (anamorphs) have been identified as *Orbilia* spp. belonging to the discomycetes (Ascomycetes). Species of the genus *Nematoctonus* are distinguished from all other nematode-trapping deuteromycetes, not only by being both nematode-trapping and endoparasitic but also by having hyphae with clamp connections, typical for basidiomycetes. Consequently, several isolates of *Nematoctonus* have been shown to produce fruit bodies of a gilled mushroom (*Hohenbuehelia* spp.).

### Molecular phylogeny

The fact that species of nematode-trapping fungi are found in all major groups of fungi indicates that nematode

parasitism has evolved independently several times. Molecular methods offer new possibilities to examine the evolutionary origin and the relationships of nematode-trapping fungi in more detail. Analysis of ribosomal DNA (rDNA) sequences have proven to be particularly valuable for reconstructing phylogenetical relationships of fungi. Analysis of the 18S rDNA region have recently shown that a number of the common species of nematode-trapping fungi, including species of the genera *Arthrobotrys*, *Dactylaria* and *Monacrosporium*, form a monophyletic group (clade) (Liou and Tzean, 1997; Ahrén *et al*., 1998) (**Figure 3**). Notably, the phylogenetic patterns within this clade were not concordant with the morphology of the conidia and the conidiophores according to traditional classification but rather with the morphology of the infection structures. Three lineages of species were identified within the clade of nematode-trapping fungi. One lineage contains species having constricting rings, a second lineage includes nonparasitic species of the closely related genus *Dactylella*, and a third lineage has various adhesive structures (nets, hyphae and knobs) to infect nematodes. The separation of species forming constricting rings and adhesive trapping devices is well supported by their differences in morphology and trapping mechanisms (see below). Further studies are needed to position the identified clade of nematode-trapping fungi within the ascomycetes.

The above analyses suggest that trapping devices provide the most relevant morphological features for taxonomic classification of predatory anamorphic *Orbiliaceae*. Accordingly, Scholler *et al*. (1999) suggested that these fungi should be divided into four genera: *Arthrobotrys* forming adhesive networks, *Drechslerella* forming constricting rings, *Dactylellina* forming stalked adhesive knobs and *Gamsylella* species producing adhesive columns and unstalked knobs.

## Evolution

There is a growing body of evidence to suggest that the parasitic habit of nematode-trapping fungi has evolved among cellulolytic or lignolytic fungi as a response to nutrient deficiencies in nitrogen-limiting habitats (Barron, 1992). In such environments (like soils) with a high carbon:nitrogen ratio, nematodes might serve as an important source of nitrogen during growth on carbohydrate-containing substrates. Many nematode-trapping deuteromycetes are indeed good saprophytes and can utilize cellulose and other polysaccharides as carbon sources. Notably, the saprophytic ability varies among nematode-trapping fungi and is correlated with their parasitic activity. Species with high parasitic activity grow more slowly and

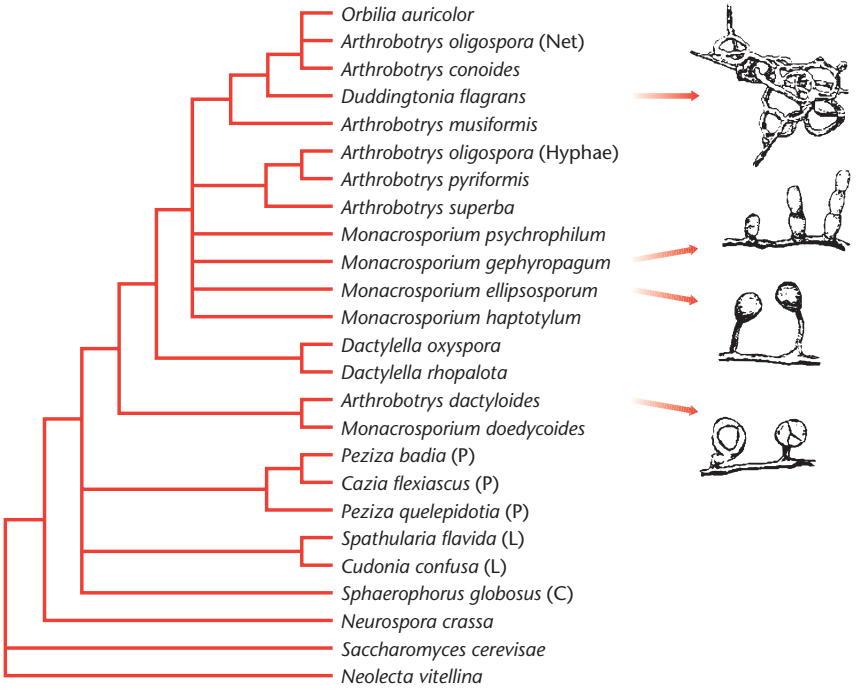


**Figure 3** A phylogenetic tree based on the sequences of 18S rDNA showing the relationships among the nematode-trapping fungi and the position of this clade among species from the Pezizales (P), Leotiales (L) and Calciales (C). *Neolecta vitellina* was used as an outgroup for the analyses. Note that the phylogenetic pattern is concordant with the structure of the trapping devices. *Orbilia auricolor* is the teleomorph (sexual stage) of *A. oligospora*. After Ahrén *et al.* (1998).

have more special nutrient requirements than species with low parasitic activity (cf. **Figure 1**). Thus, it appears that over evolutionary time, the more specialized parasitic species have lost some of the activity of the enzymes involved in saprophytic metabolism. The fact that several of the identified teleomorphs of nematode-trapping deuteromycetes are wood decomposers also supports the hypothesis that nematode-trapping fungi have evolved from cellulolytic or lignolytic fungi. **See also**: Coevolution: host–parasite

The phylogenetic relationships of other nematophagous fungi, including the endoparasites, is still virtually unknown. A different evolutionary history is expected within the endoparasitic fungi, which are mostly more dependent on nematodes.

## Ecology of Nematophagous Fungi

### Occurrence

Nematophagous fungi have been found in all regions of the world, from the tropics to Antarctica. They have been reported from agricultural, garden and forest soils, and are especially abundant in soils rich in organic material. A simple method of obtaining nematophagous fungi is to use the so-called soil sprinkling technique, where approximately 1 g of soil is sprinkled on the surface of a water agar plate together with a suspension of nematodes added as a bait. The plates are observed for 5–6 weeks under a microscope at low magnification and examined for trapped nematodes, trapping organs and conidia of nematophagous fungi. **See also**: Fungal ecology

Many soils contain 10–15 different species of nematophagous fungi. *Arthrobotrys* spp. appear to be common in most soils, with *A. oligospora* found most frequently in temperate regions and *A. musiformis* in tropical areas, although both species occur abundantly and ubiquitously. Among the lower fungi, the zygomycetes *Stylopage* spp. and *Cystopage* spp., and the chytridiomycete *Catenaria anguillulae* are often found.

In agricultural soils in temperate regions the nematode-trapping fungi follow a seasonal variation, with highest densities and number of species in late summer and autumn, possibly due to the higher soil temperature and increased input of organic debris. The fungi are most frequent in the upper 20 cm of the soil and appear to be almost absent below 40 cm (Persmark *et al*., 1996). A strict correlation between number of propagules of nematophagous fungi and number of nematodes is difficult to obtain, although in some soils a correlation exists between number of species of nematophagous fungi and the number of nematodes. This raises the question whether the parasitism of nematophagous fungi can regulate the population size of soil nematodes. Experiments in soil microcosms using the endoparasitic fungus *H. rhossoliensis* and plant parasitic nematodes have shown that the level of fungal parasitism is dependent on the nematode density, although there is a relatively long time lag in the response of the fungal population to changes in the number of nematodes (Jaffee *et al*., 1992). **See also**: Parasitism: the variety of parasites

Mostly, plant-parasitic nematodes attack plant roots and, therefore, the ability of the nematophagous fungi to grow in the rhizosphere is of great importance for their capacity to control these nematodes. Many nematode-trapping fungi have been found to occur more frequently in the rhizospheres of several plants, especially leguminous plants, e.g. soybean and pea, than in root-free soil. This effect could possibly be due to increased or changed root exudation in these plants. To evaluate whether trapping structures and consequently trapping of nematodes are actually more abundant in rhizosphere soil, new techniques have to be developed to examine the activity of nematophagous fungi *in situ*. **See also**: Rhizosphere

### Interactions with other fungi and plants

Apart from attacking nematodes nematophagous fungi also have the capacity to infect other fungi (act as mycoparasites). Nematode-trapping fungi such as *A. oligospora* attack their host fungi by coiling of the hyphae of the nematode-trapping fungi around the host hyphae, which results in disintegration of the host cell cytoplasm without penetration of the host. It was shown that nutrient transfer took place between the nematode-trapping fungus *A. oligospora* and its host *R. solani* using radioactive phosphorous tracing (Olsson and Persson, 1994).

*A. oligospora*, *P. chlamydosporia* and other nematophagous fungi have the capacity to colonize plant roots (Bordallo *et al*., 2002). The fungi grow inter- and intracellularly and form appressoria when penetrating plant cell walls of epidermis and cortex cells, but never enter vascular tissues. Histochemical stains show plant defence reactions, e.g. papillae, lignitubers and other cell wall appositions induced by nematophagous fungi, but these never prevented root colonization. The growth of the nematophagous fungi in plant roots is endophytic, i.e. the host remains asymptomatic. Endophytic growth of *P. chlamydosporia* in barley and wheat roots appeared to increase plant growth and reduce growth of the plant parasitic take-all fungus *Gaeumannomyces graminis* var. *tritici* (Monfort *et al*., 2005).

Mycoparasitism and plant endophytism may be important issues for extension of the biological control potential of the nematophagous fungi.

### Biological control

One important aspect of nematophagous fungi is the possibility of using them for biological control of plant- and animal-parasitic nematodes. Plant-parasitic nematodes,

e.g. root-knot and cyst nematodes, are global pests in agriculture and horticulture, causing severe yield losses. Owing to the ban of many nematicides, e.g. methyl bromide, because of health and environmental concerns, new alternatives for nematode control are therefore needed. Biological control may be such an alternative. **See also**: Biological control by microorganisms

There are two general ways of applying biological control of nematodes using nematophagous fungi: addition of large amounts of fungi to the soil; or stimulation of the activity of the existing fungi using various amendments. For plant-parasitic nematodes the early experiments concentrated on using nematode-trapping fungi, e.g. *Arthrobotrys* or *Monacrosporium* species, and later shifted towards endoparasitic fungi, e.g. *H. rhossoliensis* and *D. coniospora*, and egg-parasitic fungi, e.g. *P. chlamydosporia*. The performances of these biological control agents have varied and, so far, no commercial products are available. **See also**: Biological control

There is a renewed interest in using nematode-trapping fungi, partly due to an increased knowledge on the biology of these fungi and partly due to better methods of formulating and applying fungal biocontrol agents to soil. One way to improve the control potential of nematophagous fungi would be to use genetic engineering to increase the pathogenicity and survival of the introduced fungus. Using genetic transformation, it was possible to generate mutants of the nematode-trapping fungus *A. oligospora* overexpressing a protease gene (*PII*). Mutants containing additional copies of the *PII* gene developed a higher number of infection structures and had an increased speed of capturing and killing nematodes (Åhman *et al*., 2002). Furthermore, it was recently reported that formulations of the nematode-trapping fungus *A. dactyloides* were able to reduce infection on tomato by root-knot nematodes in field experiments. A similar reduction was not shown with the egg-parasite *P. chlamydosporia* in the same experiment. A major problem of adding nematophagous and other biocontrol fungi to soil is their low ability to establish in the complex soil environment. It has been suggested by Bourne *et al*. (1996) that rhizosphere colonization is necessary for successful establishment, and therefore screening for rhizosphere-competent strains of nematophagous fungi is of paramount importance.

Animal-parasitic nematodes cause illness and severe weight loss in livestock all over the world. The chemicals presently used to control these nematodes, anthelmintics, have been shown to develop resistance in the parasitic nematode fauna. A promising approach has been presented in feeding the grazing animals with fungal mycelium containing chlamydospores of nematode-trapping fungi, e.g. *Duddingtonia flagrans*. By allowing the spores to be transported through the animal guts, and grow and produce traps in the faeces and surrounding grass, the fungus then captures newly hatched juveniles of the parasites and reduces the nematode burden in the fields. The population structure of nematophagous fungi is largely unknown. Such knowledge is of importance for evaluating the fate and risk of an unwanted spread of an applied biological control agent. Using various genetic markers, it was recently shown that the genetic variation in a worldwide collection of the nematode-trapping fungus *D. flagrans* was very low (Ahrén *et al*., 2004). The data show that *D. flagrans* is mainly clonal and no recombination could be detected not even within the same country. Thus it is unlikely that a mass applied strain of *D. flagrans* will recombine with local isolates.

Although not considered as traditional biological control, another promising approach by which nematophagous fungi, as well as other soil fungi, can be used for developing new means to control animal- and plant-parasitic nematodes is to use the antagonists as a source for isolating new compounds with nematicidal activity (Anke *et al*., 1995).

## Nematode–Fungus Interaction Mechanisms

### Recognition and host specificity

The question of how nematophagous fungi recognize their prey is complex. No simple host specificity has been found in any of the nematode-trapping species, while experiments with the endoparasite *D. coniospora* have revealed somewhat higher host specificity. Nevertheless, it appears that there are recognition events in the cell–cell communication at several steps of the interaction between fungus and nematode, which might elicit a defined biochemical, physiological or morphological response. Nematodes are attracted to the mycelia of the fungi in which they may induce trap formation and they are attracted even more to fully developed traps and spores. This is followed by a 'short-range' or contact communication: adhesion. This step may involve an interaction between a carbohydrate-binding protein (lectin) in the fungus and a carbohydrate receptor on the nematode. Recognition of the host is probably also important for the subsequent steps of the infection, including penetration of the nematode cuticle. **See also**: Lectins; Parasitism: variety of parasites

## Attraction

Nematodes are attracted by compounds released from the mycelium and traps of nematode-trapping fungi, and the spores of endoparasites. Both the morphology and consequently the saprophytic/parasitic ability strongly influence the attractiveness of the fungi. Fungi that are more parasitic appear to have a stronger attraction than the more saprophytic ones; that is, the endoparasitic species infecting nematodes with conidia are more effective in attracting

nematodes than the more saprophytic species with different kinds of trapping devices (cf. **Figure 1**).

## Adhesion

The contact and adhesion of nematodes to the traps and spores of nematophagous fungi can be observed in the electron microscope. In *A. oligospora* the three-dimensional nets are surrounded by a layer of extracellular fibrils even before the interaction with the nematodes. After contact, these fibrils become directed perpendicularly to the host surface, probably to facilitate the anchoring and further fungal invasion of the nematode (**Figure 2c**). The endoparasite *D. coniospora* shows a completely different type of adhesive that seems to be composed of radiating fibrils irrespective of whether contact with the nematode has been established or not. Furthermore, the spores of *D. coniospora* adhere specifically to the sensory organs at the tip of the head of the nematode, thereby blocking nematode attraction (**Figure 1f**). The chemical composition of the surface fibrils of nematophagous fungi is not known in detail but they do contain both proteins and carbohydrate-containing polymers. **See also**: Fungal spores

## Penetration

The adhesion of the traps to the nematode results in a differentiation of the fungi. In *A. oligospora,* a penetration tube forms and pierces the nematode cuticle (**Figure 2d**). This step probably involves both the activity of hydrolytic enzymes solubilizing the macromolecules of the cuticle and the activity of a mechanical pressure generated by the penetrating growing fungus. The nematode cuticle is composed mainly of proteins including collagen, and several proteases have been isolated from nematophagous fungi that can hydrolyse proteins of the cuticle. In all cases these proteases belong to the family of serine proteases, and after obtaining data from sequencing, it has been demonstrated that they have a high homology to the subtilisin-type of serine proteases (Åhman *et al*., 1996; Bonants *et al*., 1995). In the endoparasite *D. coniospora*, a chymoptrypsin-like protease appears to be involved in the penetration process **See also**: Proteases.

More detailed studies of the subtilisin PII produced by *A. oligospora* have indicated that this type of proteases can have a number of different functions (Åhman *et al*., 2002). Thus, apart from being involved in penetration and digestion of the cuticle and tissues of infected nematodes, PII appears to have a nematotoxic activity.

## Digestion and Storage of Nutrients

Following penetration, the nematode is digested by the infecting fungus. Once inside the nematode, the penetration tube of *A. oligospora* swells to form a large infection bulb (**Figure 2d**). The development of the bulb and trophic hyphae occurs in parallel with dramatic changes in the ultrastructure and physiology of the fungus. The dense bodies are degraded in the trap cells and in the bulb. The bulb and the trophic hyphae typically contain normal cell organelles, endoplasmatic reticulum being particularly well developed. At later stages, lipid droplets accumulate in the trophic hyphae, which are probably involved in the assimilation and storage of nutrients obtained from the infected nematode. In contrast to the trap-forming fungi, the endoparasite *D. coniospora* does not form an infection bulb upon penetration and does not have dense bodies, which are typical for the trap-forming fungi. Along with formation of lipid droplets, another way for *A. oligospora* to store nutrients derived from the host is to produce large amounts of a lectin in the cytoplasm (Rosén *et al*., 1997). This protein (designated *Arthrobotrys oligospora* lectin, AOL) is a member of a novel family of low molecular weight lectins, sharing similar primary sequences and binding properties, which have so far only been identified in a few filamentous fungi (Rosén *et al*., 1996). During the infection of nematodes, AOL is rapidly synthesized in *A. oligospora* once nematodes have been penetrated and digestion has started. Large amounts of AOL are accumulated in the trophic hyphae growing inside the nematode. Later, the lectin is transported from the infected nematode to other parts of the mycelium, where it can be degraded and support the growth of the fungus. Although the mechanisms are not known, it has been suggested that AOL, like other lectins, is involved in a recognition event during the interaction with the nematodes. The fact that the AOL family of lectins binds to sugar structures that are typical of animal glycoproteins including nematodes, but not found in fungi, supports this hypothesis. **See also**: Fungal physiology; Lectins

## Constricting Rings

Although the patterns of nematode infection of other predatory fungi, which use adhesive layers for capturing nematodes (nets, hyphae or knobs), are less thoroughly studied, they appear to be largely similar to those described for *A. oligospora*. In contrast, the trapping mechanism of constricting rings is completely different. When a nematode moves into the ring, it triggers a response such that the three cells composing the ring rapidly swell inward and close around the nematode (**Figure 4**). Other stimuli, such as touch by a needle of the inside (luminal) surface of a ring, or heat, can also trigger the closure of the trap. The reaction is rapid (0.1 s), irreversible, and is accompanied by a large increase in cell volume leading to an almost complete closure of the aperture of the trap. Following capture, the fungus produces a penetration tube that pierces the

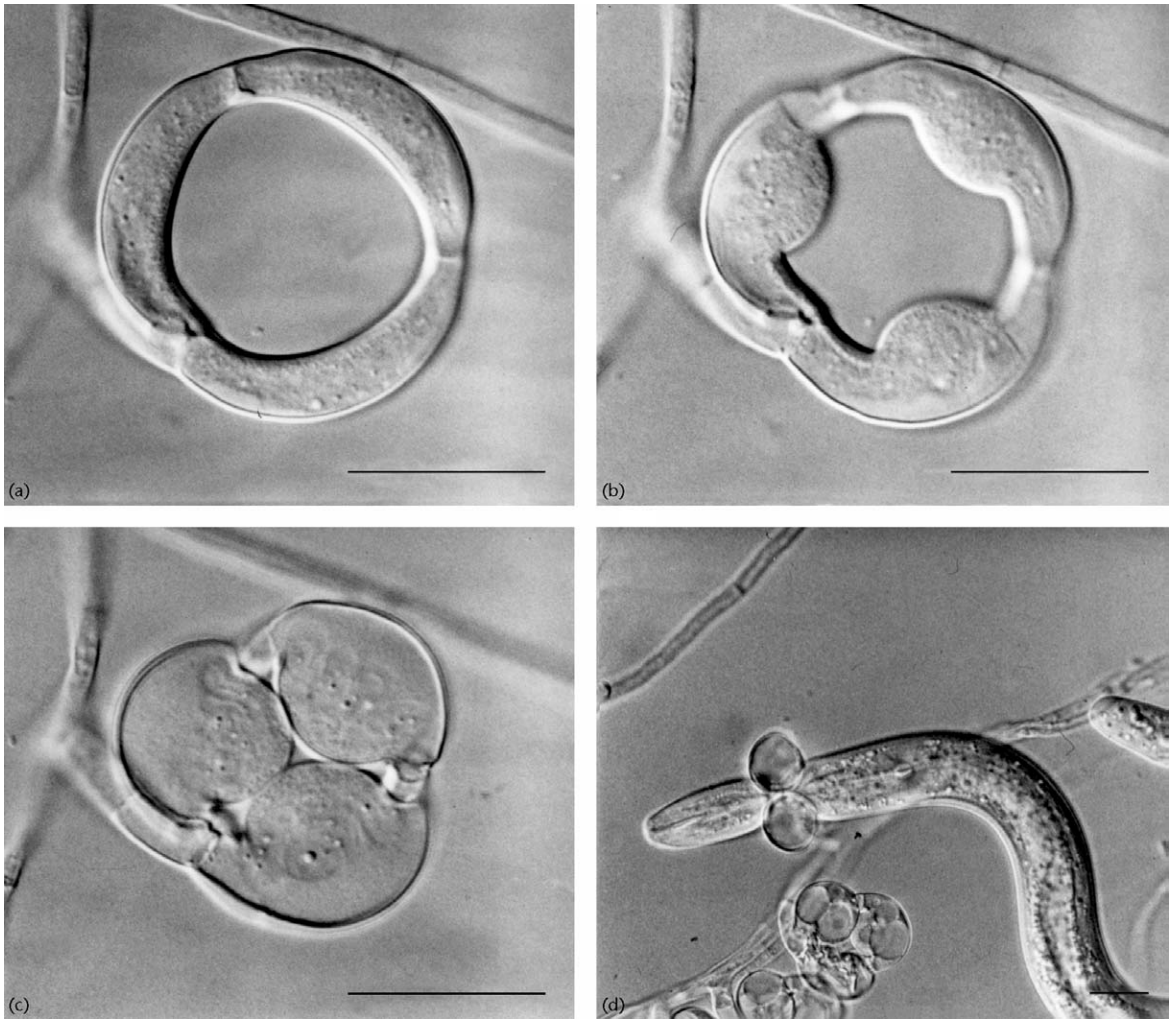


**Figure 4** Trapping mechanism of constricting rings of *A. brochopaga*. (a–c) Closure of a ring triggered by applying heat to the trap. The closure is rapid (0.1 s), irreversible and is accompanied by a large increase in cell volume leading to an almost complete closure of the aperture of the trap. Bars, 5 μm. (d) Nematode firmly captured in a ring. Bar, 10 μm. Reproduced from Nordbring-Hertz *et al*. (1995a). Courtesy by Institut für den Wissenschaftlichen Film, Göttingen.

nematode cuticle. Inside the nematode a small infection bulb is formed from which trophic hyphae develop.

The mechanism by which the constricting rings are closed is not known in detail. Electron microscopy has shown that during the ring-cell expansion, the outer cell wall of the ring cells is ruptured along a defined line on the inner surface of the ring. It has been suggested that this release of wall pressure will lead to a rapid uptake of water, followed by an expansion of the elastic inner wall of the ring cells. The signal transduction pathway involved in the inflation of the ring cells has been examined in *A. dactyloides* (Chen *et al*., 2001). In this fungus it appears that the pressure exerted by a nematode on the ring activates G-proteins in the ring cells. The activation leads to an increase in cytoplasmic $Ca^{2+}$, activation of calmodulin and finally the opening of water channels. The ring cells expand to constrict the ring and thus immobilize the nematode.

## References

Åhman J, Ek B, Rask L and Tunlid A (1996) Sequence analysis and regulation of a cuticle degrading serine protease from the nematophagous fungus *Arthrobotrys oligospora*. *Microbiology* **142**: 1605–1616.

Åhman J, Olsson M, Johansson T *et al*. (2002) Improving the pathogenicity of a nematode-trapping fungus by genetic engineering of subtilisin with nematotoxic activity. *Applied and Environmental Microbiology* **68**: 3408–3415.

Ahren D, Faedo M, Rajashekar B and Tunlid A (2004) Low genetic diversity among isolates of the nematode-trapping fungus *Duddingtonia flagrans* – evidence for a recent worldwide dispersion from a single common ancestor. *Mycological Research* **108**: 1205–1214.

Ahrén D, Tholander M, Fekete C *et al*. (2005) Comparison of gene expression in trap cells and vegetative hyphae of the nematophagous fungus *Monacrosporium haptotylum*. *Microbiology* **151**: 789–803.

Ahrén D, Ursing BM and Tunlid A (1998) Phylogeny of nematode-trapping fungi based on 18S rDNA sequences. *FEMS Microbiology Letters* **158**: 179–184.

Anke H, Stadler M, Mayer A and Sterner O (1995) Secondary metabolites with nematicidal and antimicrobial activity from nematophagous fungi and Ascomycetes. *Canadian Journal of Botany* **73**(suppl. 1): S932–S939.

Barron GL (1992) Lignolytic and cellulolytic fungi as predators and parasites. In: Carroll GC and Wicklow DT (eds) *The Fungal Community, Its Organization and Role in the Ecosystems,* pp. 311–326. New York: Marcel Dekker.

Bonants PJM, Fitters PF L, Thijs H *et al*. (1995) A basic serine protease from *Paecilomyces lilacinus* with biological activity against *Meloidogyne hapla* eggs. *Microbiology* **141**: 775–784.

Bordallo JJ, Lopez-Llorca LV, Jansson H-B *et al*. (2002) Effects of egg-parasitic and nematode-trapping fungi on plant roots. *New Phytologist* **154**: 491–499.

Bourne JM, Kerry BR and De Leij FAAM (1996) The importance of the host plant on the interaction between root-knot nematodes (*Meloidogyne* spp.) and the nematophagous fungus, *Verticillium chlamydosporium* Goddard. *Biocontrol Science and Technology* **6**: 539–548.

Chen T-H, Hsu C-S, Tsai P-J, Ho Y-F and Lin N-S (2001) Heterotrimeric G-protein and signal transduction in the nematode-trapping fungus *Arthrobotrys dactyloides*. *Planta (Berlin)* **212**: 858–863.

Jaffee B, Phillips R, Muldoon A and Mangel M (1992) Density-dependent host–pathogen dynamics in soil microcosms. *Ecology* **73**: 495–506.

Liou GY and Tzean SS (1997) Phylogeny of the genus *Arthrobotrys* and allied nematode-trapping fungi based on rDNA sequences. *Mycologia* **89**: 876–884.

Monfort E, Lopez-Llorca LV, Jansson H-B *et al*. (2005) Colonisation of seminal roots of wheat and barley by egg-parasitic nematophagous fungi and their effects on *Gaeumannomyces graminis* var. *tritici* and development of root-rot. *Soil Biology and Biochemistry* **37**: 1229–1235.

Nordbring-Hertz B, Jansson H-B, Friman E *et al*. (1995a) *Nematophagous Fungi. Film No C1851* Göttingen, Germany: Institut für den Wissenschaftlichen Film.

Nordbring-Hertz B, Neumeister H, Sjollema K and Veenhuis M (1995b) A conidial trap-forming mutant of *Arthrobotrys oligospora*. *Mycological Research* **99**: 1395–1398.

Olsson S and Persson Y (1994) Transfer of phosphorus from *Rhizoctonia solani* to the mycoparasite *Arthrobotrys oligospora*. *Mycological Research* **98**: 1065–1068.

Persmark L, Banck A and Jansson H-B (1996) Population dynamics of nematophagous fungi and nematodes in an arable soil: vertical and seasonal fluctuations. *Soil Biology and Biochemistry* **28**: 1005–1014.

Persmark L and Nordbring-Hertz B (1997) Conidial trap formation of nematode-trapping fungi in soil and soil extracts. *FEMS Microbiology Ecology* **22**: 313–324.

Pfister DH (1997) Castor, Pollux and life histories of fungi. *Mycologia* **89**: 1–23.

Rosén S, Kata M, Persson Y *et al*. (1996) Molecular characterization of a saline-soluble lectin from a parasitic fungus. Extensive sequence similarity between fungal lectins. *European Journal of Biochemistry* **238**: 822–829.

Rosén S, Sjollema K, Veenhuis M and Tunlid A (1997) A cytoplasmic lectin produced by the fungus *Arthrobotrys oligospora* functions as a storage protein during saprophytic and parasitic growth. *Microbiology* **143**: 2593–2604.

Scholler M, Hagedorn G and Rubner A (1999) A reevaluation of predatory orbiliaceous fungi II A new generic concept. *Sydowia* **51**(1): 89–113.

## Further Reading

Barron GL (1977) *The Nematode-destroying Fungi*. Guelph, Ontario: Canadian Biological Publications.

Dijksterhuis J, Veenhuis M, Harder W and Nordbring-Hertz B (1994) Nematophagous fungi: physiological aspects and structure–function relationships. *Advances in Microbial Physiology* **36**: 111–143.

Duddington CL (1962) Predacious fungi and the control of eelworms. In: Carthy JD and Duddington CL (eds) *Viewpoints in Biology*, pp. 151–200. London: Butterworth.

Glockling SL and Beakes GW (2000) A review of the taxonomy, biology and infection strategies of 'biflagellate holocarpic' parasites of nematodes. *Fungal Diversity* **4**: 1–20.

Jansson H-B, Tunlid A and Nordbring-Hertz B (1997) Biological control: nematodes. In: Anke T (ed.) *Fungal Biotechnology*, pp. 38–50. Weinheim, Germany: Chapman & Hall.

Jansson H-B and Lopez-Llorca LV (2001) Biology of nematophagous fungi. In: Misra JK & Horn BW (eds) *Mycology: Trichomycetes, other Fungal Groups and Mushrooms*, pp. 145–173. Enfield: Science Publishers.

Jansson, H-B and Lopez-Llorca LV (2004) Control of nematodes by fungi. In: Arora DK (ed.) *Fungal Biotechnology in Agriculture, Food, and Environmental Applications*, pp. 205–215. New York: Marcel Dekker.

Morton CO, Hirsch PR and Kerry BR (2004) Infection of plant-parasitic nematodes by nematophagous fungi – a review of the application of molecular biology to understand infection processes and to improve biological control. *Nematology* **6**: 161–170.

Nordbring-Hertz B (1988) Ecology and recognition in the nematode-nematophagous fungus system. In: Marshall KC (ed.) *Advances in Microbial Ecology*, vol. 10, pp. 81–114. New York: Plenum Press.

Nordbring-Hertz B (2004) Morphogenesis in the nematode-trapping fungus *Arthrobotrys oligospora* – an extensive plasticity of infection structures. *Mycologist* **18**:125–133.

Pramer D (1964) Nematode-trapping fungi. *Science* **144**: 382–388.

Rubner A (1996) Revision of predacious hyphomycetes in the *Dactylella–Monacrosporium* complex. *Studies in Mycology* **39**: 1–134.

Stirling GR (1991) *Biological Control of Plant Parasitic Nematodes Progress, Problems and Prospects*. Wallingford, UK: CAB International.

Tunlid A, Jansson H-B and Nordbring-Hertz B (1992) Fungal attachment to nematodes. *Mycological Research* **96**: 401–412.