NEMATOPHAGOUS FUNGI

Anyone who has grown tomatoes in their home garden will be aware of the damage caused by plant-parasitic nematodes. The plants establish well but then become chlorotic and begin to wilt, and struggle to produce fruit. When they are removed from the soil and discarded, the cause becomes obvious: the root system has been badly damaged by root-knot nematode (Fig. 1). This small, worm-like organism has invaded the roots, modified the cells so it can feed on them, and produced galls that restrict the root system and limit the uptake of water and nutrients.

Fig. 1. Root-knot nematode damage on tomato roots

Vegetable growers will also know that root-knot nematode is an important pest, as it often causes yield losses, particularly if crops are grown in light-textured sandy soils. Tomato, eggplant, capsicum, beans, and cucurbits are some of the many crops affected. Losses on crops such as carrot, potato and sweetpotato can be very high because the underground roots and tubers cannot be sold due to severe nematode damage. And losses are not just restricted to vegetables. Grapevine, peach, almond, and pineapple are some of the many perennial crops that are attacked by the nematode.

Although root-knot nematode is Australia's most damaging nematode pest, plant-parasitic nematodes are a widespread problem and most agricultural crops are affected. Many different genera and species cause losses and the most important are listed in Table 1. Although these microscopic worms vary in shape and size, all are armed with a spear that is very much like a hypodermic needle. When a nematode is feeding on the root system, the spear is inserted into a cell and its contents are removed (Fig. 2).

Table 1. Australia's most important plant-parasitic nematodes

Common name	Genus	Crops damaged by the nematode
Root-knot nematode	*Meloidogyne*	Most vegetables, many perennial horticultural crops, and legumes such as lucerne, clover and soybean
Cyst nematode	*Heterodera*	Wheat, barley, oats
Cyst nematode	*Globodera*	Potato
Root-lesion nematode	*Pratylenchus*	Cereals, canola, sorghum, maize, peanut, chickpea, potato, apple, banana, sugarcane
Burrowing nematode	*Radopholus*	Banana
Reniform nematode	*Rotylenchulus*	Cotton, pineapple, banana
Citrus nematode	*Tylenchulus*	Citrus, grape
Sting nematode	*Ibipora*	Turfgrasses

Fig. 2. A root-lesion nematode female (about 0.7 mm long), and its head end showing the feeding spear

Although plant-parasitic nematodes are an insidious problem in agriculture, they do not cause damage in natural systems such as forests and grasslands, and rarely cause problems in relatively undisturbed landscapes under pasture. In these environments there is an active and diverse soil biological community which contains organisms that regulate nematode populations and keep pest nematodes under control.

Many different soil organisms parasitise or prey on plant-parasitic nematodes including bacteria in the genus *Pasteuria*, mesostigmatid mites and a group of relatively large nematodes that consume other nematodes. However, the most important natural enemies of nematodes are fungi.

The fungi which attack nematodes are abundant in natural soils, where they play a significant role in maintaining the balance of microbial life. They are also found in agricultural soils and are readily isolated from decaying organic materials. Hundreds of species have been described and they occupy many taxonomic groups within the fungal kingdom. This article focuses on the most widely studied groups but also mentions the oomycetes, as they are often thought of as fungi due to similarities in their mycelial growth habits and modes of nutrition.

Nematode-trapping fungi

Several groups of fungi produce specialised trapping devices to capture nematodes, but genera in the order Orbiliales are by far the most common and widely studied. Usually referred to as nematode-trapping fungi, they respond to the presence of nematodes by producing mycelial traps that capture and kill their prey. Five types of trapping device are produced and they are depicted in Fig. 3.

Adhesive networks. This is the most common trapping mechanism. Lateral branches grow from the vegetative mycelium and then curve around to form a loop. In most species, multiple loops are formed, resulting in a two or three-dimensional network of traps that are covered with adhesive material.

Adhesive knobs. The trapping device is usually a spherical adhesive knob that is borne on a short, slender, erect stalk. A nematode is usually caught by the knobs, but if it breaks free with knobs still attached, the fungus will still parasitise the nematode.

Constricting rings. This type of trap is the most sophisticated trapping device. A three-celled ring with an inside diameter of about 20µm is formed on a short, stout stalk. When a nematode moves into the ring, the cells in the ring inflate instantaneously and hold the victim securely. Assimilative hyphae then fill the body of the prey and consume its contents.

Non-constricting rings. These traps are similar to constricting rings, but the cells do not inflate when a nematode moves into the ring. Also, the support stalk is longer and often breaks when a nematode is wedged in the ring.

Adhesive branches. These erect trapping structures are covered with adhesive and arise from short laterals that grow from prostrate hyphae.

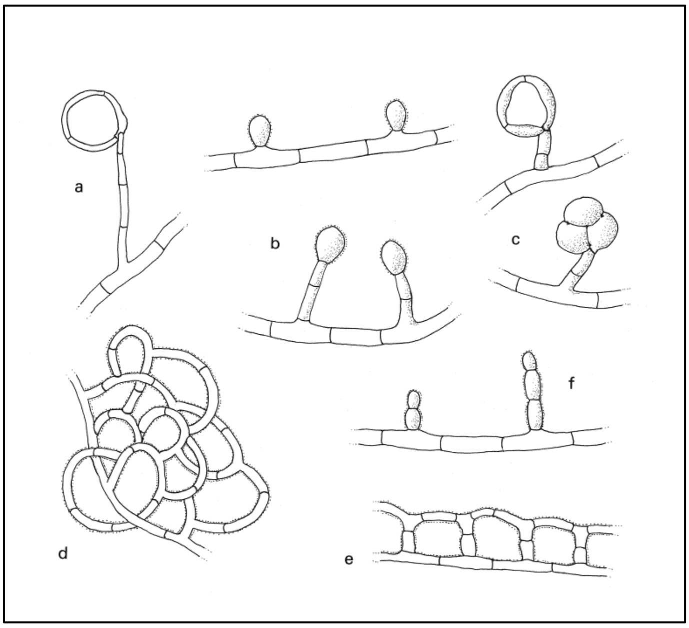


Fig. 3. Trapping devices used by nematode-trapping Orbiliales to capture nematodes. a) non-constricting rings; b) adhesive knobs; c) constricting rings; d) three-dimensional adhesive network; e) two-dimensional adhesive net; f) adhesive branches.

Traditionally, the nematode-trapping fungi were assigned to three genera (*Arthrobotrys*, *Dactylella* and *Monacrosporium*) on the basis of the morphology of their conidia and conidiophores (Fig 4). However, molecular studies have shown that the type of trapping device is a more useful taxonomic character. Consequently, these fungi have now been assigned to three genera: *Arthrobotrys* for adhesive networks, *Drechslerella* for constricting rings and *Dactylellina* for stalked adhesive knobs and non-constricting rings.

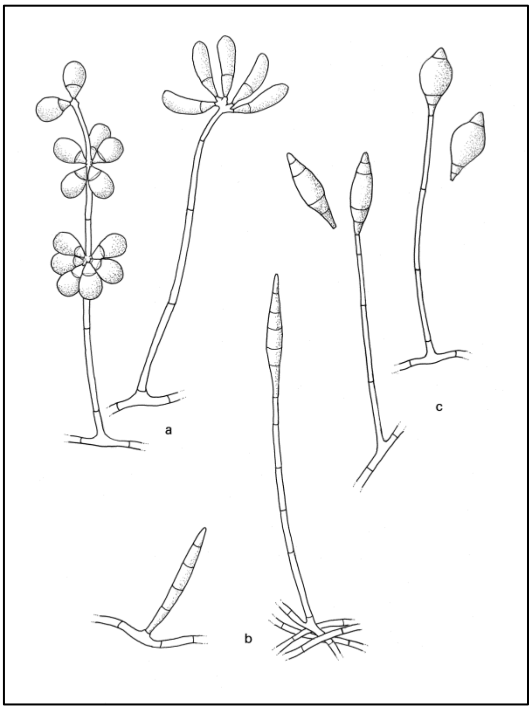


Fig. 4. Features of conidial and conidiophore morphology traditionally used to differentiate various genera of nematode-trapping fungi a) *Arthrobotrys*; b) *Dactylella*; and c) *Monacrosporium.*

The nematode-trapping fungi reproduce asexually but also have sexual stages in the genus *Orbilia* (family Orbiliaceae), a fungus that is often found on the surface of logs, fallen tree trunks and decayed bark in forested areas. When nematodes are added to cultures derived from ascospores produced by these fungi, typical nematode-trapping organs are produced by some species.

The oyster mushroom and its relatives

The oyster mushroom (*Pleurotus ostreatus*) is a gilled fungus that grows in many places but is widespread in temperate and subtropical forests, where is acts as a primary decomposer of wood (Fig 5). As the fungus can be cultivated on straw, sawdust and other substrates, the oyster mushroom is easy to obtain and is widely used for culinary purposes. It is also one of the few carnivorous mushrooms.

Studies have shown that the oyster mushroom produces adhesive knobs that are used to capture nematodes. A potent toxin is released to inactivate the nematode and then hyphae grow towards the body orifices of the immobilized nematode and penetrate, colonise, and digest the prey. Other species of *Pleurotus* also use nematodes as a food source, while two other gilled mushroom genera (*Hohenbuehelia* and *Resupinatus*) are known to attack and digest nematodes.

The asexual state of *Hohenbuehelia* is *Nematoctonus* and this fungus has long been known to capture nematodes. Some *Nematoctonus* species produce extensive hyphal systems, with adhesive hour-glass knobs being formed directly on their hyphae. Once the knobs attach to a nematode, the fungus penetrates and the body is colonised. In other species, the organs of capture do not remain on the hyphae. Instead, cigar-shaped conidia are detached and each fallen conidium then produces a terminal adhesive knob that attaches to the nematode (Fig. 6).

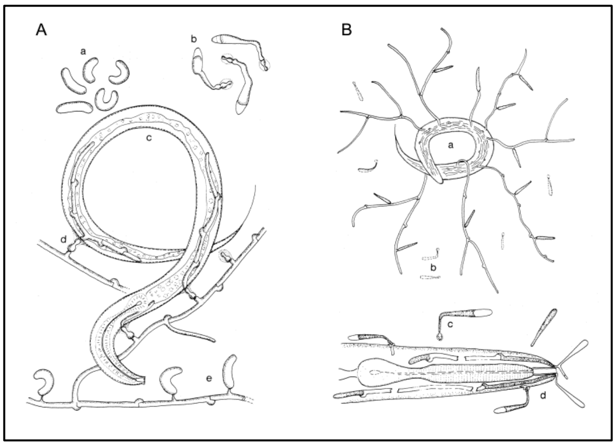


Fig. 5. Oyster mushroom (*Pleurotus ostreatus*). **Fig. 6.** Modes of infection by different species of *Nematoctonus*. A. A predatory species showing a) conidia; b) germinating conidia producing an adhesive knob; c) nematode caught by adhesive knobs on hyphae; d) hour-glass-shaped adhesive knob; and e) conidia being produced on hypha. B. An endoparasitic species showing a) an infected host with conidiiferous hyphae; b) and c) an erect outgrowth with terminal adhesive bud produced by fallen conidia; and d) infected host with conidia attached to the cuticle. (From Barron, 1977)

Endoparasitic nematode-destroying fungi

A wide range of endoparasitic fungi can parasitise nematodes and they differ from nematode trappers in that they have no extensive hyphal development outside the body of their host. Instead, they exist primarily as spores that remain viable but dormant in the environment. Eventually the spores are either ingested by the host or adhere to its cuticle. In some cases, flagellate spores may be produced that encyst directly on the body of the nematode.

Many different endoparasites are found in soil and it is impossible to discuss all of them here. *Catenaria anguillulae*, a member of a small family of zoosporic fungi formerly categorised as chytrids is one example. Its zoospores have a single whiplash flagellum and they differentiate within zoosporangia inside the host body, escape through the tip of a solitary exit tube and swim towards their nematode prey. On reaching a host, the spores encyst and germ tubes either enter the body through orifices or penetrate directly through the cuticle to initiate a new infection (Fig. 7). Opinions vary as to whether *C. anguillulae* is a strong or weak parasite of nematodes but because water is required for zoospore movement, this fungus is only likely to be effective in relatively moist environments.

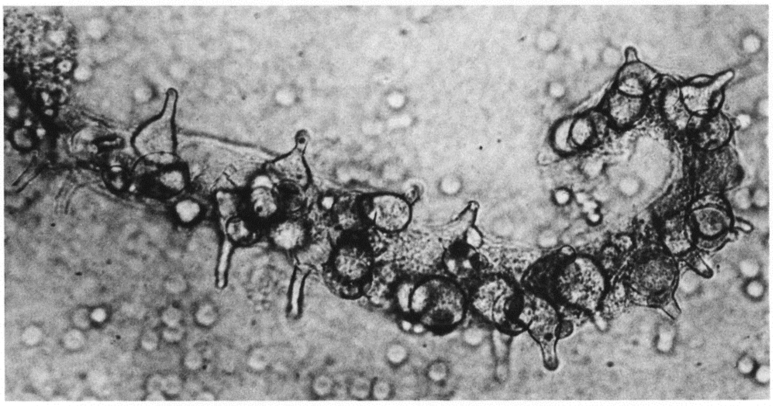


Fig. 7. Zoosporangia of *Catenaria anguillulae* inside an infected nematode, with exit tubes being produced to the exterior (From Barron, 1977)

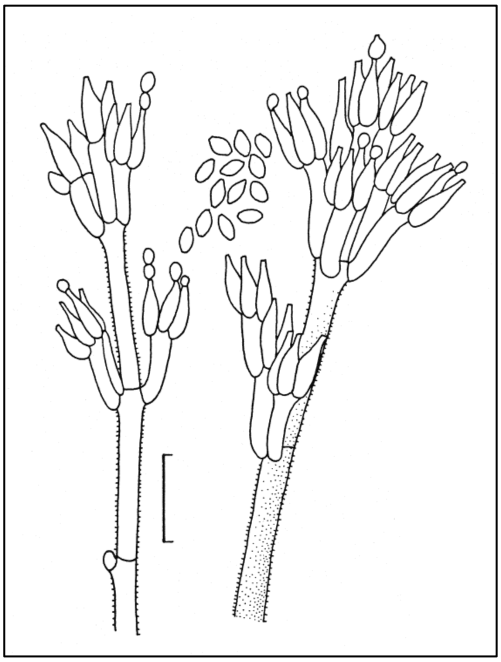
One zoosporic fungus that is an excellent biocontrol agent is the oomycete *Nematophthora gynophila*. It is very common in England where it kills large numbers of cereal cyst nematode, a major pest of wheat, barley, and oats. This nematode establishes a feeding site in the root and then begins to swell, with the posterior end of the developing female eventually bursting out of the root and becoming visible on the root surface. When the soil is wet, these immature females are attacked by zoospores of *N. gynophila*. Once the nematode is infected, more zoospores are formed within sporangia and they are then released to initiate new infections. Thick-walled resting spores (oospores) are eventually produced, with about 3,000 oospores usually found in spore masses within each infected nematode. *N. gynophila* is not a good saprophyte (i.e. it is not capable of surviving on dead or decaying organic matter) and in nature is almost certainly an obligate parasite, capable of growing only within the body of its nematode host.

Egg-parasitic fungi

Root-knot and cyst nematode are the world's most damaging nematode pests and one characteristic they have in common is that female nematodes produce large numbers of eggs. The eggs of root-knot nematode are clustered in an egg mass on the root surface whereas cyst nematode eggs are retained within the body of the female. Given the widespread distribution of these nematodes, it is not surprising that fungi have been found that are able to parasitise their eggs.

Purpureocillium lilacinum (syn. *Paecilomyces lilacinus*) is the most widely researched biological control agent of nematodes (Fig. 8). In soil, the fungus appears to be a relatively good saprophyte, as it thrives on a wide range of organic materials and will grow and sporulate on numerous carbon sources, including substrates that are commonly found in soil. However, *P. lilacinum* is also able to parasitise nematode eggs, with studies of the colonisation and infection process showing that hyphae grow over the surface of the egg and then rapidly penetrate the eggshell. Occasionally appressoria are formed but more commonly individual hyphae simply penetrate the shell. Strains of *P. lilacinus* are registered as bionematicides in many countries but one concern with this fungus is that it sometimes causes eye infections, particularly in patients who have compromised immune systems or intraocular lens implants.

Pochonia chlamydosporia (syn. *Verticillium chlamydosporium*) is a widely distributed fungus that can survive saprophytically in soil and parasitise both nematode and mollusc eggs. It also infects oospores of *Phytophthora*, an important plant pathogen. When the fungus parasitises nematode eggs it uses root exudates as a food source, colonises the rhizosphere and then grows into the eggs. Once the eggs have been destroyed, thick-walled chlamydospores are produced as a survival mechanism (Fig. 9).



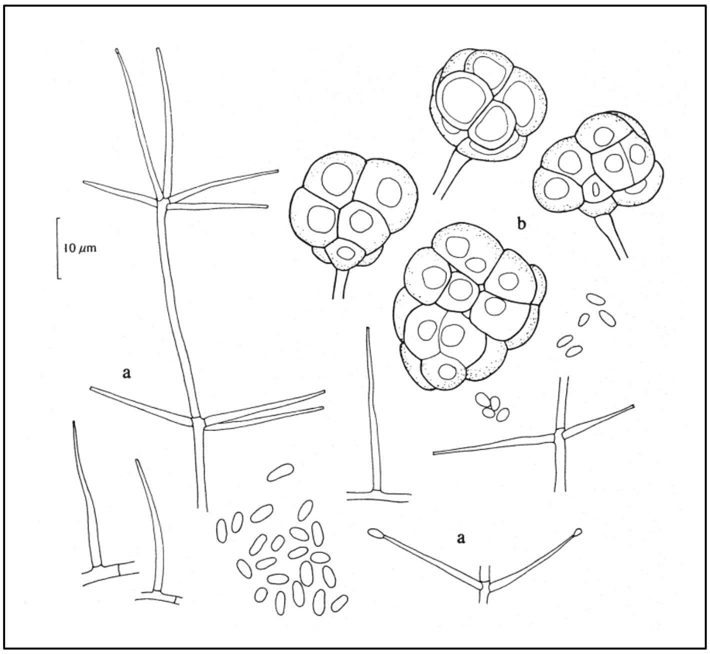


Fig. 8. Conidiophores and conidia of *Purpureocillium lilacinum*.
Fig. 9. *Pochonia chlamydosporia* showing a) conidia and condiophores and b) chlamydospores.

The genus *Hyalorbilia* contains a group of slow-growing fungi that are highly dependent on nematodes for nutrition. These fungi are relatively specialised parasites of nematode eggs (Fig. 10) and are closely related to the nematode-trapping fungi discussed earlier. The first species to be described (*Dactylella oviparasitica* syn. *Brachyphoris oviparasitica)* is now *H. oviparasitica*, and it was initially found in a Californian peach orchard where root-knot nematode populations were relatively low. Almost all the eggs in each egg mass were parasitised by the fungus and as the nematode did not produce large numbers of eggs on peach, the fungus was thought to be responsible for suppressing the pest. Later, the same fungus was found in a field that had very few cyst nematodes and follow-on studies showed that it was the primary suppressive agent in that location. However, *H. oviparasitica* is quite specialised and relies on its host nematode for nutrition, and so the challenge of the future is to understand how to manipulate nematode populations to favour the fungus, and then utilize this knowledge in integrated nematode management programmes.

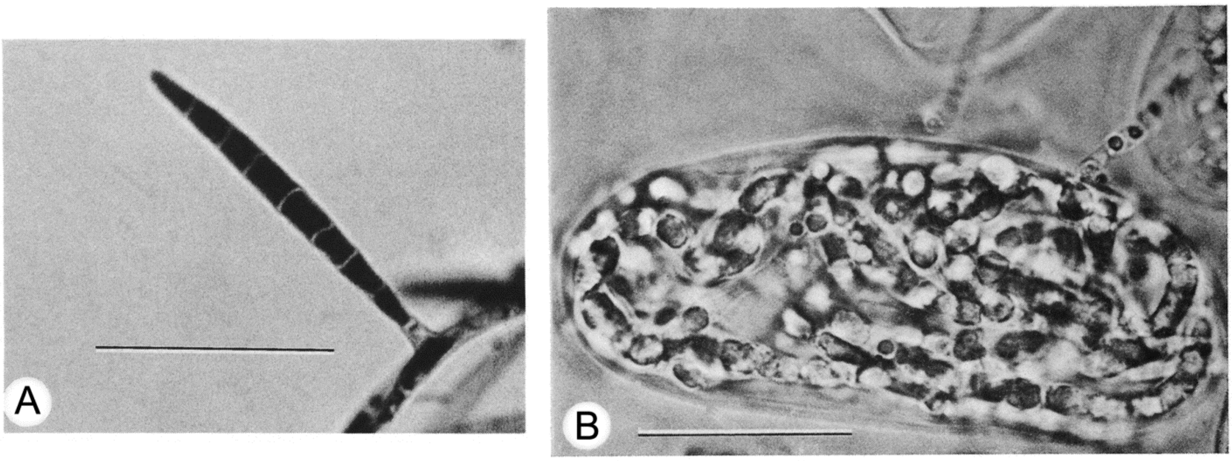


Fig. 10. A) Mature conidium *of Brachyphoris oviparasitica*; and B) a root-knot nematode egg parasitised by the fungus.

Why do some fungi capture nematodes?

The reason some fungi have evolved to capture nematodes has always fascinated scientists. Several hypotheses have been proposed to explain the phenomenon but one of the most likely reasons some fungi produce traps and become carnivores is that it enables them to obtain nitrogen in low-nitrogen environments.

It has long been known that many of the fungi which capture nematodes live on rotted logs and dead or decomposing wood and bark that is commonly found in natural environments. Since wood is a nitrogen-poor substrate (C: N ratio usually >350), the ability to capture nematodes appears to be an evolutionary response that enables fungi capable of digesting cellulose and lignin to overcome the nutrient stresses associated with their nitrogen-limited habitat. Nematodes are an excellent source of nitrogen as their C:N ratio usually ranges from 6:1 to 12:1.

It is important to recognise that the nematodes being captured in decomposing wood and plant residues are not plant parasites. Bacterial-feeding and fungal-feeding nematodes dominate the nematode community in such environments and they will be the main food source for the fungi. However, when decomposition is occurring in the soil or on the soil surface, plant-parasites that are feeding on plant roots will be present and they will also be consumed.

The loss of nematophagous fungi from agricultural soils

Fungi capable of capturing nematodes can certainly be found in agricultural soils but numbers are much lower than in natural soils and there is also much less diversity.

- The organic matter content of soils used for agriculture is usually 50-80% lower than it was when the land was first cleared. As most fungi (including the nematophagous fungi) rely on organic matter to obtain carbon and other nutrients, their population densities have declined in soils that have been cropped for many years
- Most agricultural soils are regularly tilled and this breaks up fungal mycelium into fragments. Thus, fungi are continually having to use their nutritional resources to restore their mycelial state
- Nitrogen is commonly applied to agricultural soils and when it is readily available, nematophagous fungi may not need to resort to carnivory to obtain nitrogen
- Some agricultural soils, particularly those used for vegetable production, are fumigated before the crop is planted. As soil fumigants are broad-spectrum biocides, they have disastrous effects on nematophagous fungi and other beneficial soil organisms.

This article began with a comment that plant-parasitic nematodes are an insidious problem in agriculture. As nematophagous fungi play a key role in regulating nematode populations, their loss from agricultural soils is certainly one of the reasons plant-parasitic nematodes are now major pests. Thus, the best way of reducing losses from plant-parasitic nematodes is to adopt practices that enhance rather than deplete a group of fungi that are capable of parasitising or preying on nematodes. This means integrating the three principal components of conservation agriculture into the farming system: crop rotation, minimum tillage, and retention of crop residues on the soil surface as mulch. When this is done, the fungal community will begin to re-establish because rotation and cover crops provide the carbon that fungi use as a food source; hyphal networks are no longer being disrupted by tillage; and mulch reduces moisture loss from the soil, dampens temperature fluctuations, and therefore provides an ideal habitat for fungi. Although the benefits of making these changes will not be seen immediately, one of the long-term effects will be a reduction in losses caused by plant-parasitic nematodes.

Another way of minimising damage caused by plant-parasitic nematodes is to use nematophagous fungi as a biological nematicide. Commercial products containing *Purpureocillium lilacinum* and *Pochonia chlamydosporia* are available and formulations containing other fungi will almost certainly be produced in future. Such products should be trialled on-farm to see whether they provide worthwhile nematode control.

Literature cited and Further reading

Askary TH, Martinelli PRP (2015) Biocontrol Agents of Phytonematodes. CAB International, Chapters 3- 8 (pages 81-213)

Barron GL (1977) The nematode-destroying fungi. Lancaster Press, Inc., Lancaster, Pennsylvania, 140 pp.

Stirling GR (2014). Biological Control of Plant-parasitic Nematodes. 2nd edition. Soil Ecosystem Management in Sustainable Agriculture. CAB International, Chapter 5, pages 101-156 and Chapter 12, pages 351-370.

Taxonomic information

The taxonomy of one of the main groups of nematophagous fungi (the Orbiliaceae) has undergone many changes in the last 30 years. These changes are discussed in the following paper.

Baral H-O, Weber E, Gams W, Hagedorn G et al. (2018) Generic names in the Orbiliaceae (Orbiliomycetes) and recommendations on which names should be protected or suppressed. *Mycological Progress* 17, 5-31.

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