TWO FUNGI PARASITIC ON FEMALES OF CYST-NEMATODES (HETERODERA SPP.)

BY B. R. KERRY AND D. H. CRUMP
Rothamsted Experimental Station, Harpenden, Herts.

A nematode parasitic fungus, *Nematophthora gynophila* gen. et sp.nov. which attacks females of cyst-nematodes (*Heterodera* spp.) is described and placed in the Leptolegniellaceae Dick. Parasitized female nematodes fail to form cysts as the fungus destroys the body wall and cuticle and eventually replaces the body contents with a mass of resting spores. A Lagenidiaceous fungus is reported which also kills female cyst-nematodes. The release of zoospores by both fungi is described, and their significance in biological control is discussed.

Kerry (1974) first reported an 'Entomophthora-like fungus' killing females of the cereal cyst-nematode, *Heterodera avenae* Woll. The fungus was so-called because hyphal morphology and resting spore production were like that in the genus *Entomophthora*. Subsequently Kerry & Crump (1977) found that the fungus produced spores which were released through discharge tubes thus making an association with the Entomophthoraceae impossible. Recently biflagellate spores were observed emerging from the discharge tubes and the fungus is now placed in the Oomycetes, being described here as *Nematophthora gynophila* gen. et sp.nov. in the Leptolegniellaceae Dick.

During studies on zoospore release from diseased nematodes, a second fungus was observed whose biflagellate spores matured in a vesicle formed at the tip of the discharge tube. This fungus is a member of the Lagenidiaceae, an order containing *Lagenidium* and *Myzocytium*, which are also endoparasitic in nematodes. Separation of these genera is difficult and Barron (1976) described a nematophagous fungus, *Myzocytium intermedium*, having characters of both. Until the sexual structures leading to the production of resting spores have been observed, it is impossible to refer the second fungus to one or other of these genera.

**METHODS**

Diseased female nematodes were collected from the roots of oats cv. Milford and barley cv. Athos grown in Petri dish observation chambers (Crump & Kerry, 1977). Soil from Butt Close, Woburn Experimental Farm, was the source of both fungi, and nematodes were added either by mixing with equal volumes of soil containing large numbers of *H. avenae* cysts or by adding second-stage juveniles hatched *in vitro*. The dishes were kept in the glasshouse at approximately 14 °C in 14 h daylength and swollen female nematodes ruptured the root cortex and were exposed in the soil after 8–12 weeks. Diseased females were picked off the root surface and studies were made on fresh material and permanent preparations made in 0.5% Trypan blue in lactophenol. Females whose cuticle was penetrated by discharge tubes were placed in annular open slides (Doncaster, 1975) and spore release in sterilized soil water observed under an inverted microscope.

**NEMATOPHTHORA**

*Nematophthora* gen.nov.


Sp. typ.: *N. gynophila*.

*Mycelium* filamentous, holocarpic, mainly intramatrical, *hyphae* irregular and much branched. *Extramatrical hyphae* narrow, rarely branched and at maturity function as sporangia. *Zoosporae* laterally biflagellate, completing development within the sporangia, encysting inside or outside zoosporangia. *Oosporae* are produced laterally on undifferentiated *hyphae* which are septate at sporogenesis, one *spore* produced on each *hyphal* segment. Morphologically distinct gametangia absent. Oospore containing reserve globules in granular cytoplasm. Oospore wall very thick, two-layered with a separable endospore membrane.
Nematophthora gynophila sp. nov.


Mycelium holocarpic, broad, (8–) 12 (–15) μm diam, frequently branched, mainly intramatrical and eventually occupying most of the substratum; hyphae are thin-walled, contain dense granular protoplasm and often disarticulate into short segments of varying shape and size. Extramatrical hyphae narrow, 6 μm diam, rarely branched up to 350 μm long functioning as discharge tubes which are not separated from the intramatrical hypha by a septum. Zoosporae 7 × 11 μm, vary considerably in shape, laterally biflagellate, uniseriate in sporangia but may be 2 or 3 ranks distally, about 75 in number (20–120). Zoosporae, 7 μm diam, may encyst within or outside sporangium. Occasionally diploidi.

Oosporae produced laterally on undifferentiated hyphal segments c. 35 μm long, one spore per segment. Oospore spherical or subspherical, 20 μm diam, sometimes irregular in shape, having a wall 4 μm thick, which is evenly thickened containing pits. Spherical central to subcentral reserve body about 9 μm diam. Endospore with a distinct separable membrane.

Parasitic on females of cyst-nematodes (Heterodera spp.) widely distributed in England and Wales particularly in soils infested with the cereal cyst-nematode, H. avenae.

This fungus is placed in the Leptolegniellaceae Dick, because of the arrangement of reserve globules in the oospore (Fig. 1H), the oospore wall morphology (Fig. 2A–B), the delimitation of the zoospore within the sporangium which lacks a basal septum and the encysted zoospore diameter, all of which are similar to those described for the family by Dick (1971). The family contains four genera, Aphanodictyon Huneyc., Aphanomycopsis Scheffel, Brevilegniella Dick and Leptolegniella Huneyc., none of which includes species parasitic on nematodes. Nematophthora is established primarily on the basis of the lateral production of oospores on undifferentiated hyphal segments. The fungus cannot be placed in Aphanodictyon which has a eucarpic mycelium and discrete gametangia and although the other three genera are holocarpic and lack discrete gametangia the oospores are produced within the hyphal segments. Spore release in Nematophthora differs from Brevilegniella where the spores are non-motile, from Aphanomycopsis where they encyst at the mouth of the sporangia, but not from Leptolegniella where they are free swimming on discharge. Thus the genus Nematophthora is closest to Leptolegniella in many characters except in oospore production and the nematode-parasitic habit.

Adult cyst-nematode females or developing juveniles exposed in the soil may be attacked by the fungus but juveniles within roots are not parasitized. Infection has not been observed but probably takes place through the cuticle which is eventually totally destroyed. Recently infected nematodes are flaccid and contain the broad vegetative hyphae which are aseptate and frequently branched (Fig. 1A). The intramatrical hyphae become septate and may disarticulate into a number of segments (Fig. 1B) which give rise to the sporangia whose discharge tubes (extramatrical hyphae) penetrate the disrupted cuticle and pro- trude about 50 μm into the soil (Fig. 1C). Occasionally the discharge tubes are much longer and branched. Cleavage of the zoosporae within the sporangia has not been observed. Soon after zoospore development is complete the tip of the discharge tube swells, ruptures and the spores are rapidly released (Fig. 2C–F). The production of zoospores takes 1–2 h in the laboratory and their release takes less than a minute. The laterally biflagellate zoospores (Fig. 2I–K) may move away rapidly or remain in a group swimming weakly. The activity of spores after discharge is variable; some spores encyst within a few minutes, others remain motile for almost an hour. Each nematode may give rise to more than a hundred sporangia (x = 50 ± 14) and each sporangium to 20–120
Nematophthora gynophila gen. et sp. nov.
(60 ± 11) zoospores. All the protoplasmic contents of the hyphal segment and the discharge tube are cleaved into zoospores (Fig. 1D). Occasionally empty encysted spores and a second motile spore were observed, suggesting this fungus is diplanetic (Fig. 2G–H). In the experimental conditions, only a few spores germinated to produce a germ-tube, even in the presence of healthy nematodes. Spores which encysted within the sporangia were not seen to germinate or produce another motile stage.

Each remaining hypha is divided into a number of segments by septa, each segment giving rise to a single oospore (Fig. 1E); the entire protoplasm is withdrawn from the hypha. The infected nematode is eventually filled with c. 3000 spores (Fig. 1F). Usually the nematode cuticle is completely destroyed and the characteristic cysts are not formed, but occasionally oospores have been found within cysts (Fig. 1G) presumably after late infection of the female.

Although they contain only a few hyphae, diseased females can be easily distinguished by their lack of turgor. From this stage to being completely filled with resting spores takes approximately 4 days at 13°C. Such fragile spore masses are readily dispersed by other soil organisms (Crump & Kerry, 1977).

**The Lagenidiaceous fungus**

This fungus was rarely recovered from soil and was found in only a few nematodes. Like *Nematophthora gynophila*, it attacked living females of the cereal cyst-nematode on roots resulting in the disruption of the cuticle and the prevention of cyst formation. Within the nematode, the lagenidiaceous fungus produced an extensive hyphal network which disarticulated into short segments, 9 μm diam, that were often lobed but only occasionally branched (Fig. 3A). The intramatrical hyphae which function as sporangia, may give rise to one or more discharge tubes, 4 × 66 μm. Cleavage and maturation of the zoospores takes place in a vesicle at the mouth of the tube. Before release, the tip of the sporangium swells and the undifferentiated protoplasm rapidly flows out (c. 50 sec) from the hyphal segment (Fig. 3B–D) and tube to produce the vesicle, 35 μm diam. After about 5 min the cleavage into separate spores is apparent and after 9 min they move independently within the vesicle (Fig. 3E–H). Activity within the vesicle increases as the flagella develop and, after about 16 min, the spores swim rapidly away. Approximately 20 zoospores were produced in each vesicle. The biflagellate spores remained motile for up to an hour and were stronger swimmers than those of *N. gynophila*. The zoospores encyst and after a few hours about 10% germinated forming a germ-tube (Fig. 3I). There is no evidence of diplanetism.

Oospores, 16 μm diam, are produced within swollen segments of the thallus (Fig. 3J). The spore is thick-walled and there may be 1–4 per segment. Unlike *N. gynophila* this fungus will attack nematode eggs (Fig. 3K).

Seven species of *Myzocytium* and *Lagenidium* have been found in nematodes, *L. parthenosporium* being isolated from a *Heterodera* sp. (Karling, 1944). However, only *L. caudatum* produces a well-developed vesicle in which the zoospores mature and in this species resting spores are unknown.

**Discussion**

In a recent survey, most soils in southern Britain that were infested with the cereal cyst-nematode also contained *N. gynophila* (Kerry & Crump, 1977). So far it has not been possible to germinate the oospores or culture the fungus on artificial media. Hence the fungus can only be isolated by inoculating field soil with nematodes and growing a cereal host on which the females develop and may become parasitized. Using such a technique *N. gynophila* has been isolated only from soils containing *H. avenae*, although Tribe (1977a) has found a fungus believed to be the same in *H. schachtii* females from Sweden. In tests in the glasshouse, Kerry & Crump (1977) infected *H. carota*, *H. cruciferae*, *H. goettingiana*, *H. schachtii* and *H. trifolii* but not the potato cyst-nematode, *Globodera rostochiensis*. The lagenidiaceous fungus has been found at two sites only, Butt Close Field, Woburn Experimental Farm, and Pennings Field, Stoke Hill Farm, Erlestoke, Devizes. This fungus infected *H. avenae* and *H. schachtii* females in laboratory tests.

A third fungus *Tarichium auxiliare* Kuhn attacking females of cyst-nematodes was found by Kuhn (1877) in females of *H. schachtii*. It was redescribed by Tribe (1977b) as *Catenaria...*
Fig. 3. The Lagenidiaceous fungus. (A) Vegetative hyphae bearing exit tubes (20 μm); (B–D) formation of vesicle at tip of sporangium (10 μm); (E–H) maturation of zoospores in vesicle (10 μm); (I) encysted zoospore (arrowed) and germinating spore (10 μm); (J) oospores formed in swollen segment of hypha (5 μm); (K) oospores within nematode egg (20 μm). Figures in parentheses refer to scale bars.
auxiliaris (Kuhn) Tribe after he found it had motile zoospores. Catenaria auxiliaris occurs in soils in Europe and North America that contain the beet cyst-nematode (Tribe, 1977a) and has been found parasitizing females of H. avenae (Kerry, 1975). It is the only one of these fungi found in the potato cyst-nematode, G. pallida (Kerry, Jenkinson & Crump, 1975). Thus, several groups of fungi with motile zoospores contain species with the ability to destroy females of cyst-nematodes on roots.

The cereal cyst-nematode often fails to multiply in soils where cereal host plants are grown intensively and it is a characteristic of these soils that large numbers of the females produced on the roots fail to form cysts full of eggs (Kerry, 1975). Nematophagous fungi attacking females and eggs, of which N. gynophila is the most important, are responsible for this control in a wide range of soils.

These fungi increase in soils where cereals are grown frequently and where female cyst-nematodes are produced in numbers each year. The situation has been modelled by Perry (1978). Eventually an equilibrium population of the nematode is established at about 10 eggs/g soil or fewer which causes little yield loss. This is the only known example of natural agents giving effective, long-term control of a cyst-nematode. These fungi can be cultured artificially and introduced to soil where they are absent then other important cyst-nematodes, which are susceptible in laboratory tests, may be controlled.

We thank Mr C. C. Doncaster for help in filming the release of zoospores, and Mrs L. A. Mullen for technical assistance. We also thank Dr M. W. Dick, Botany Department, Reading University, for helpful discussions, reading the script, and writing the Latin diagnoses.

REFERENCES


(Received for publication 10 February 1979)