Biocontrol: Fungal Parasites of Female Cyst Nematodes[1]

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Abstract: Three species of fungi, *Catenaria auxiliaris* (Kühn) Tribe, *Nematophthora gynophila* Kerry and Crump, and a Lagenidiaceous fungus have been found attacking female cyst nematodes. All are zoosporic fungi which parasitize females on the root surface, cause the breakdown of the nematode cuticle, and prevent cyst formation. Their identification and some aspects of their biology are reviewed. *N. gynophila* is widespread in Britain and reduces populations of the cereal cyst nematode, *Heterodera avenae* Woll., to nondamaging levels. The potential of these nematophagous fungi as biocontrol agents is discussed. *Key Words: Catenaria auxiliaris, Nematophthora gynophila,* Lagenidiaceous fungus, *Heterodera* spp., "decline phenomenon," biocontrol.

Parasitism of female cyst nematodes by a fungus was first recorded by Kühn in Germany in 1877. He identified *Tarichium auxiliare* in the beet cyst nematode, *Heterodera schachtii* Schmidt (13). A century later the fungus was redescribed as *Catenaria auxiliaris* (Kühn) Tribe after it was found to produce posteriorly uniflagellate zoospores (18). Interest in fungi attacking female cyst nematodes has increased because such parasites can reduce populations of the cereal cyst nematode, *H. avenae* Wollenweber, to nondamagaing levels despite continuous cropping with its cereal hosts (8,9). A number of fungal parasites also have been found attacking the eggs of *H. avenae* and may help to control this nematode.

When a female cyst nematode dies, its body wall tans to form a characteristic cyst enclosing the eggs. Some fungi isolated from cysts (14) are not parasitic but derive their nutrition from mucilage surrounding the eggs. When cysts are examined for possible parasites, only fungi isolated from eggs should be considered. Fungi attacking cyst nematodes have been reviewed recently (19). This paper is confined to those fungi that parasitize females on roots before they form cysts. Some aspects of their biology are reviewed, and the importance of one of these fungi, *Nematophthora gynophila* Kerry and Crump, in reducing the numbers of *H. avenae* is discussed. *Verticillium chlamydosporium* Goddard, previously considered as a parasite of nematode eggs, has been recovered from virgin females of *H. avenae* before egg production has begun (Kerry, unpublished). The conditions which favour infection of the female are not known, but cyst formation is not prevented, and this fungus is not discussed further here.

Taxonomy and life history: Only three species of fungi have been isolated from females of cyst nematodes. All are zoosporic fungi which kill the females, cause the breakdown of the nematode cuticle, and prevent cyst formation (Table 1). *C. auxiliaris* is a Chytrid in the order Blastocladiales, whereas *N. gynophila* and the undescribed Lagenidiaceous fungus are Oomycetes in the orders Saprolegniales and Lagenidiales, respectively. The main characters which separate these species are summarized in Table 2.

The vegetative thallus (rhizomycelium) of *C. auxiliaris* consists of a chain of swollen cells delimited by septa. Narrow hyphae between the cells are absent, but the rhizomycelium bears rhizoids. At maturity the swollen cells form precursor sporangia

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Table 1. Species of Phycomycetous fungi, in the subdivision Mastigomycotina, parasitic on females of cyst nematodes.

Class	Order	Species	Reference
Chytridiomycetes	Blastocladiales	*Catenaria auxiliaris* (Kühn) Tribe	18
Oomycetes	Saprolegniales	*Nematophthora gynophila* Kerry and Crump	10
	Lagenidiales	'Lagenidiaceous fungus'	10

Table 2. Diagnostic characteristics of three species of Phycomycetous fungi parasitic on females of cyst nematodes.

Character	Species		
	C. auxiliaris	*N. gynophila*	'Lagenidiaceous fungus'
Hyphae	Rhizomycelium	Extensive, branching mycelium (d 12 μm)	Short, lobed hyphal segments (d 9 μm)
Zoosporangium	Spherical (d 25–45 μm) papillae but no discharge tubes	Hyphal segment with long discharge tube	As in *N. gynophila*
Zoosporogenesis	Within sporangia	Within sporangia	Within vesicle at tip of discharge tube
Zoospore	Posteriorly uniflagellate (d 3 μm)	Laterally biflagellate (d 7 μm)	Laterally biflagellate (d 8 μm)
Secondary zoospore	Absent	Present	Absent
Resting spore production	Within resting sporangia, one/sporangium	Laterally on vegetative hypha, one/segment	Within swollen hyphal segments, 1–4/segment
Resting spore	Spherical (d 25–45 μm) wall reticulate	Spherical/subspherical (d 20 μm) wall with pits	Spherical (d 16 μm) wall smooth

which may give rise to a zoosporangium or a resting sporangium. The cytoplasm within the zoosporangia divides into spores which vary in shape and have a single posteriorly directed flagellum. The zoospores are released through several papillae; elongate discharge tubes are lacking. These spores released within the female body must reach the outside to infect other females, either by passing through her natural openings or through the disrupted cuticle. The resting sporangium produces a single reticulate resting spore. We cannot predict whether a precursor sporangium will produce zoospores or a resting spore, but the latter are more common in parasitized females. *C. anguillulae* Sorokin has been found in the males of the potato cyst nematode, *Globodera rostochiensis* Wollenweber, but not in females (4).

N. gynophila produces an extensive, branching mycelium which becomes septate and often disarticulates into short segments. These segments of vegetative hyphae may give rise to long discharge tubes and form sporangia which penetrate the disrupted nematode cuticle and protrude into the soil. The cytoplasm within sporangia divides into zoospores which at maturity are released rapidly after the tip of the discharge tube ruptures. The zoospores are biflagellate. After a short active period (3–60 min) they encyst, eventually producing either a germ tube or a second motile stage. Each nematode can give rise to 200 sporangia, and each sporangium may produce up to 120 zoospores. The remaining hyphae give rise to the resting spores which are produced laterally, one spore per hyphal segment. The infected female is eventually filled with about 3,000 thick-walled resting spores.

The mycelium of the Lagenidiaceous fungus is only occasionally branched, but the short hyphal segments may be lobed and give rise to one or more narrow discharge tubes which, as with *N. gynophila*, penetrate the disrupted nematode cuticle. Undifferentiated cytoplasm is released into a vesicle formed at the tip of the discharge tube. Within 15 min at laboratory temperatures (ca. 18 C) the cytoplasm divides into about 20 biflagellate spores. The mature zoospores rupture the boundary membrane around the vesicle and swim away, remaining active for up to 1 h before they encyst. The encysted spores produce a germ tube; there is no evidence of a second motile stage. Thick-walled resting spores are produced within swollen segments of the thallus. The gametangia that produce the resting spore have not been observed, and it is not possible to refer this fungus to a genus. The Lagenidiales contains two genera, *Lagenidium* and *Myzocytium*, which have species endoparasitic in nematodes. Separation of the two genera is difficult, and *M. inter-*

medium Barron has characters of both (1). One species, *L. parthenosporum* Karling, has been recovered from a *Heterodera* sp. in Brazil but differs from the fungus described above in the method of zoospore production and in the development of stellate resting spores (7).

Disease symptoms and infection: Recently infected females are flaccid, but early stages of infection can be determined reliably only after squashing the nematode and examining for the presence of a mycelium. When females are filled with resting spores, their cuticles are usually completely destroyed and the fragile spore masses are readily dispersed by other soil organisms (2). *N. gynophila* can infect females of the cereal cyst nematode within 2 d of their emergence on roots, and after 7 d at 13 C the nematode is completely destroyed (2). Using zoospores of *C. auxiliaris* to infect females of *H. schachtii,* a rhizomycelium could be detected within the nematode in 3–4 days, but observations were not continued until the fungus completed its life cycle (18).

Female nematodes become infected after they rupture the root cortex and are exposed in soil. Juveniles within roots are not parasitized, but those developing semi-endoparasitically (2,17) may become infected. The ability of the zoospores of *N. gynophila* to encyst and later give rise to another motile stage will increase the chances of infecting female nematodes, which continue to emerge on roots for many weeks. The zoospores of *C. auxiliaris* and the Lagenidiaceous fungus do not form secondary motile spores, and they must find suitable hosts before their energy sources are exhausted. It is not known how long encysted zoospores can survive in soil.

The mobility of zoospores of *N. gynophila* is reduced, and fewer females of *H. avenae* become infected, when soils become dry. The amount of rainfall during summer (June to August), when females rupture the root cortex and are exposed in soil to fungal attack, was correlated with the number of infected females on the roots of barley cv. Julia on a sandy loam soil at Woburn, Bedfordshire, U.K. (Fig. 1). In 1978 irrigation was used in June and July when rainfall fell below average. At the same site, during a prolonged drought in June, July, and early August 1976, $< 1\%$ of females were parasitized by *N. gynophila.*

The zoospores of *N. gynophila* and the Lagenidiaceous fungus remained active for up to 1 h in soil water at laboratory temperatures (10). Zoospores move only small distances (1–3 cm) in soil and may be stimulated to encyst by repeated contact with soil particles (3). However, the movement of water in cracks in soil and in root clefts could greatly increase the distribution of motile and nonmotile spores. The movement of zoospores (d 10–15 μm) of *Phytophthora cryptogea* Pethybridge and Lafferty is limited to pores > 60 μm in diameter (3), and as these pores would need to be filled with water, the movement of zoospores in

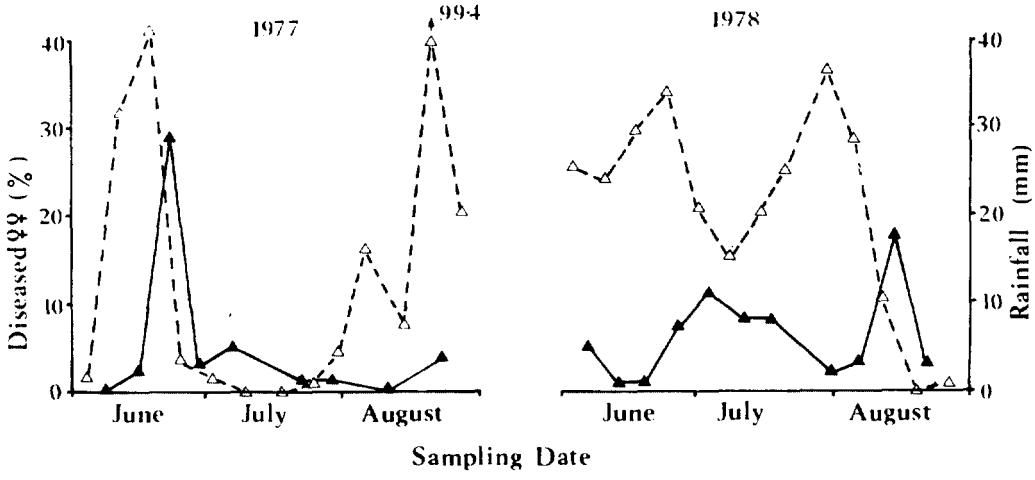

Fig. 1. Relationship between rainfall and infection of females of *H. avenae* by *N. gynophila* in 2 yr at Woburn. ▲ Diseased females; Δ rainfall, and irrigation in 1978.

soil is likely to be much more restricted than that of nematodes. However, *N. gynophila* is effective in limiting populations of *H. avenae* in close-textured soils.

The infection of a nematode population is a two-stage process: the overwintering resting spores give rise to primary infections which are then spread by zoospores. This situation has been modelled (16). As zoospore movement is limited in soil and the distance between females often large, compared with the distances over which spores can infect, resting spores must be numerous and well distributed in soil to ensure infection of large numbers of females and a reduction in nematode numbers.

Distribution and host range: There are no techniques presently available for isolating these parasitic fungi from soil, and their occurrence can be detected only by the presence of diseased females. About 100 soil samples from Southern Britain have been collected from cereal fields infested with the cereal cyst nematode and examined for the presence of parasitic fungi, using the methods of Kerry (8). Second-stage juveniles were sometimes added to the soil in pots where field populations were low. *N. gynophila* is widespread on a range of soils and was found in more than 90% of the samples. This fungus has not been found in the absence of *H. avenae* in Britain, but parasitized females of *H. schachtii* have been reported from Sweden (19). The Lagenidiaceous fungus has been recovered from two widely separated sites, one a sandy loam and the other a calcareous silty loam, and may be more widespread than this suggests.

C. auxiliaris was more common in soils where cereal cyst nematode populations failed to build up but was found attacking few females and considered not very important (5,9). About 15% of soils infested with *H. avenae* also contain *C. auxiliaris,* but the fungus is more widespread in fields infested with *H. schachtii* (18).

Females of a number of cyst nematode species are parasitized (Table 3). *N. gynophila* has been isolated from females of *H. schachtii* and *H. avenae* in the field, whereas the other *Heterodera* spp. were infected in laboratory tests (9), but *G. rostochiensis* was not a host. *C. auxiliaris* is the only fungus so far identified which attacks females of potato cyst nematodes, but this was found in a laboratory culture of *G. pallida* Stone and not in the field (12). The Lagenidiaceous fungus has been recovered from females of *H. avenae* in the field and from *H. schachtii* in pot tests.

Table 3. Host range of Phycomycetes parasitizing females of cyst nematodes.

Parasite	Host	Reference
C. auxiliaris	*H. schachtii*	18
	H. avenae	8
	G. pallida	12
N. gynophila	*H. avenae*	8
	H. cruciferae,	
	H. goettingiana	
	H. trifolii,	
	H. carotae	
	H. schachtii	9
'Lagenidiaceous fungus'	*H. avenae,*	10
	H. schachtii	

Estimation of kill: Parasitized females are fragile and difficult to extract from roots and soil using standard wet sieving techniques. *N. gynophila* can destroy its host in < 7 days, so frequent sampling (at least weekly) is necessary to make accurate estimates of parasitism. Female nematodes are readily dislodged from roots during sampling, and root and soil samples should be extracted to determine total nematode numbers, which can be expressed as numbers/100g soil (Fig. 2). In soils where fungi are

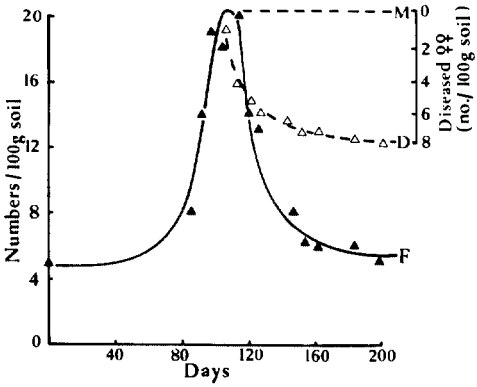

Fig. 2. Changes in numbers of females and full cysts of *H. avenae* and parasitism by *N. gynophila,* through a growing season on barley cv. Julia. ▲ numbers of females and cysts with contents; Δ accumulated numbers of females destroyed by *N. gynophila.*

active, female numbers increase to a maximum in late June and then decline rapidly. The number of nematodes killed is the difference between the maximum number of females and full cysts (M) and the number of full cysts post harvest (F). Where parasitic fungi are absent or inactive, most females survive to form cysts and M ≏ F. As diseased females are rapidly destroyed, the same infected nematode is unlikely to survive and be counted on more than one sampling occasion, and so the numbers parasitized on each weekly sampling are accumulated (D). Parasitism before females reach their maximum numbers is ignored. Approximately 50% of the kill (M − F) can be accounted for by parasitized females recovered from roots and soil. Because of the difficulties in extraction, this is an underestimate, and parasitic fungi probably account for most of the losses of females observed.

Effect of parasitic fungi on the numbers of H. avenae: Populations of the cereal cyst nematode often fail to multiply when host crops are grown intensively. Such a "decline phenomenon" has been reported from Great Britain, Germany, Holland, and Denmark on a wide range of soils whether autumn or spring cereals are sown (5,6,15). Poor nematode multiplication has been associated with high levels of fungal parasites attacking females and eggs (9). Williams (20) demonstrated that soil drenches of formalin (38% formaldehyde) at 3,000 l/ha resulted in increased *H. avenae* populations in soil where numbers were in decline: formalin reduces the numbers of parasitic fungi in soil, more females survive to become cysts, and nematode numbers increase (11). In soils where fungi were active formalin increased post-harvest cyst and egg numbers of *H. avenae* by about 95%, whereas the survival of females was not affected by formalin where *N. gynophila* was absent or inactive (Table 4). Limiting fungal activity by reducing the amount of water added to pots during the period when females were on the root surface also resulted in increased nematode populations. If fungi were killed by formalin, reducing the amount of water had no effect on nematode numbers (11).

On a sandy loam site at Woburn Experimental Farm, formalin applied 4 wk before drilling increased nematode multiplication on spring barley cv. Julia and reduced fungal parasitism (Fig. 3). In plots which had received formalin in the previous year only, nematode numbers increased less markedly and parasitism was greater than in plots which were treated before sowing. Fungal infection was greatest and final nematode populations smallest in untreated soil. The multiplication of *H. avenae* was greater in 1977 than in 1978 because much of the infection occurred in wet conditions in mid-June 1977 when few females were exposed on roots. In 1978 infection continued throughout the wet summer.

Table 4. Survival (%) of females of *Heterodera avenae* in soil treated with formalin in the presence and absence of *N. gynophila*.*

Treatment	*N. gynophila* Present	Absent
Formalin (3,000 l/ha)	104	128
Untreated	41	125

*Survival estimated from the maximum numbers of females produced and the new cyst numbers in soil after harvest (see Fig. 2).

In all our studies in pots and in the field, there has been a negative correlation between the activity of parasitic fungi, particularly *N. gynophila,* and the multiplication of *H. avenae.* Evidently such parasites can limit populations of *H. avenae* in the field. This is the first well-documented case of long-term effective biological control of a plant parasitic nematode.

Potential as biological control agents: Cyst nematode populations consist of many juveniles and, because of mortality during development, few surviving adults (Fig. 4). On barley cv. Proctor growing in pots of soil infested with *H. avenae,* approximately 90% of the population failed to invade or become established in the roots and only 8% survived to adulthood. These losses vary considerably with different hosts and nematode densities, but to ensure survival of the nematode, adults need to produce many eggs. Biological control agents, such as nematode-trapping fungi, must kill most of the juveniles to decrease significantly the number of adults. Fungal parasites, such as those discussed in this paper, that kill the

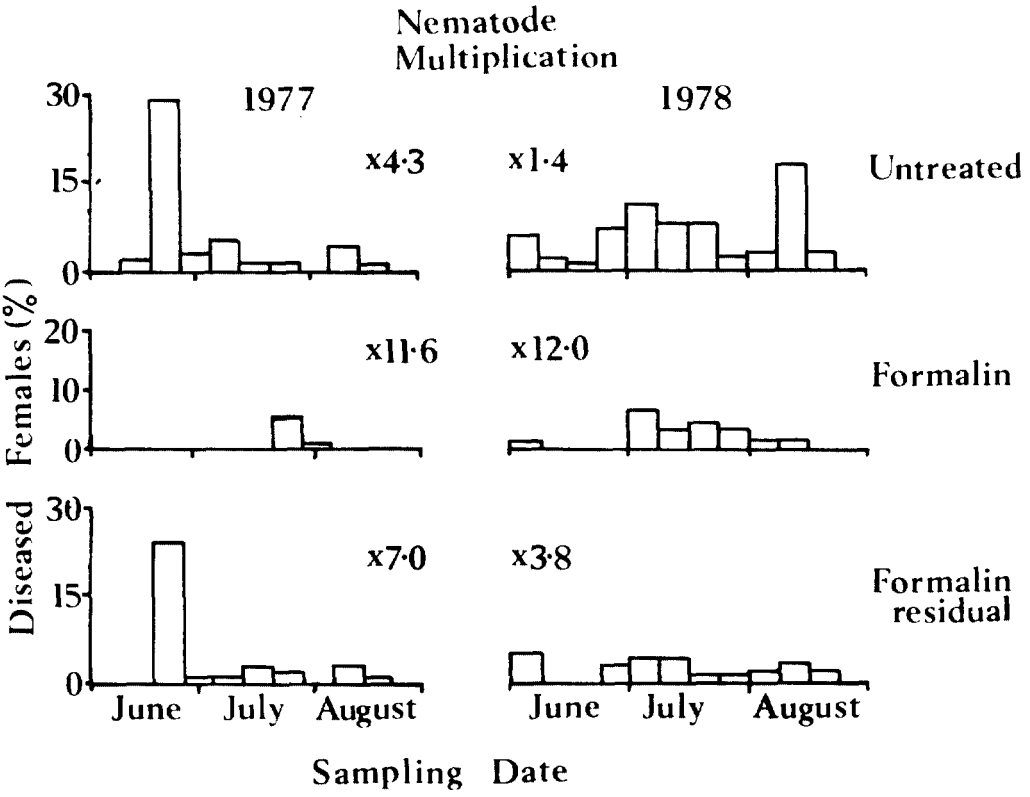

Fig. 3. Effect of formalin soil drenches on the degree of fungal parasitism of females of *H. avenae* and on nematode multiplication in 2 yr on barley cv. Julia at Woburn.

egg-producing adult stage are likely to be more effective in reducing nematode populations than those attacking juveniles.

Fungal parasites are unlikely to eradicate cyst nematodes, but they do lower the equilibrium population. For *H. avenae* in Britain, the result is a population between 5 and 10 eggs/g soil. Such an infestation causes no apparent yield losses in our cool, damp summers. In drier and hotter conditions abroad, infestations would have to be controlled at lower levels if damage was to be avoided. Most parasitism is density dependent, and it is not clear how effective these fungi might be in limiting small nematode populations.

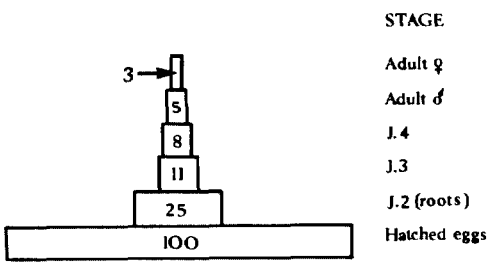

Fig. 4. Mortality (%) at different stages in the life cycle of *H. avenae* on barley cv. Proctor.

Even if these parasitic fungi can be cultured artificially and added to soil where they are absent, it may take a few years for them to increase enough to control nematode numbers. As the fungi may be specific to females of cyst nematodes, damage to crops during this period may be severe and growers would have to use tolerant varieties or nematicides. Once estabilshed in soil, however, such fungal parasites are as effective as a resistant cultivar or an efficient nematicide in limiting nematode numbers.

Although the effectiveness of these fungi is related to the rainfall during the period when female nematodes rupture the root cortex and are exposed in soil, moisture is adequate on most soils in most years in Britain. The resting spores of *N. gynophila* can survive in soil for at least 2 yr and could be used to control cyst-nematode pests on crops which demand a rotation.

Crop rotation has been the main method of limiting cyst nematode populations, but

resistant varieties or nematicides may allow growers to intensify production. Because the fungi discussed here attack a number of cyst nematode pests, and once established in soil would require no handling by the grower, their potential as control agents should be fully examined.

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