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Fungal Parasites: A Weapon Against Cyst Nematodes

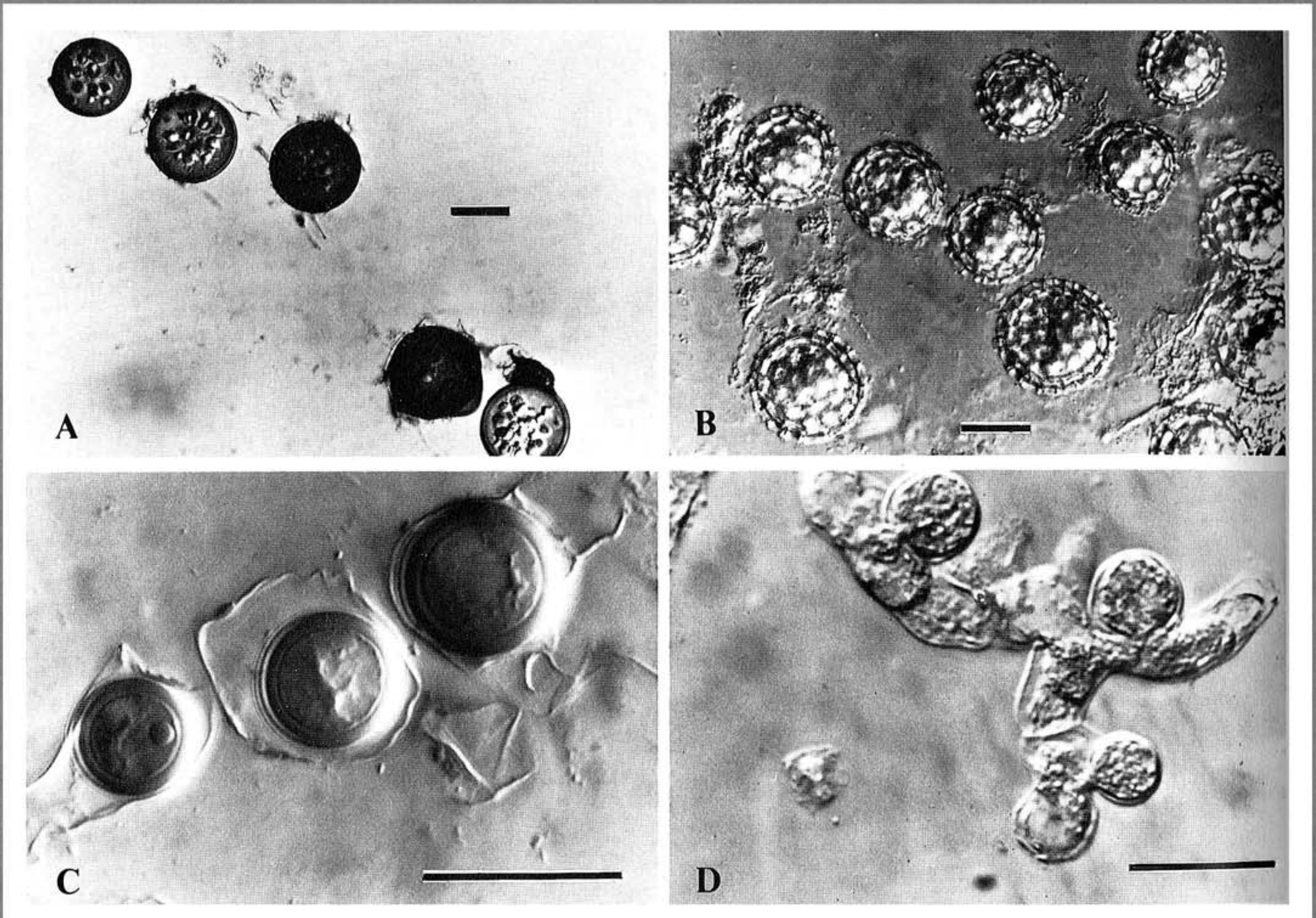

Fig. 1. Fungal spores from females of *Heterodera avenae*: (A) zoosporangia of *Catenaria auxiliaris*; (B) reticulate resting spores of *C. auxiliaris*; (C) resting spores of a lagenidiaceous fungus in oogonia; (D) resting spore production of *Nematophthora gynophila*. Scale bars = 25 μm.

Cyst nematodes (Heteroderinae) are the most important plant-parasitic nematodes in the temperate regions of the world, reducing yields in a wide range of crops. The minute (<1 mm) nematodes do not create their own living space in the large bulk of soil (2,000 m^3/ha to plow depth) and are confined to the macropores between aggregates. Although the soil pore system constrains their movement to less than 1 m annually, the nematodes may be widely spread by man's activities, eg, in soil adhering to machinery or in planting material. Cyst nematodes tend to multiply slowly; populations rarely increase more than 50 times in a season, and in northern Europe there are only one or two generations. In susceptible crops, however, densities of over 100 eggs per gram of soil may be reached, causing severe yield losses.

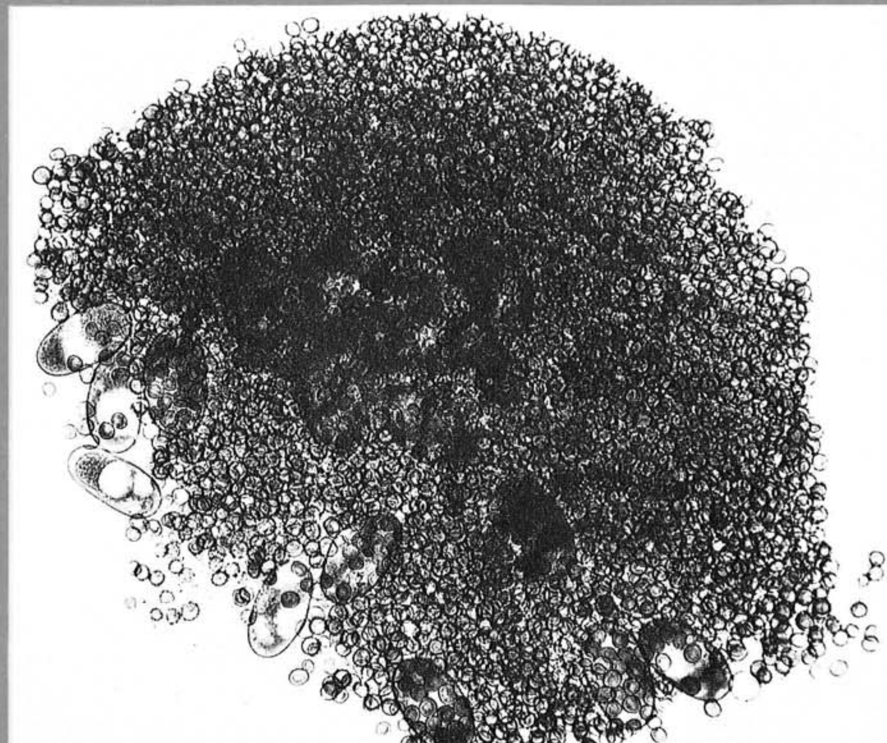

Fig. 2. In female cyst nematode killed by *Nematophthora gynophila*, cuticle is completely destroyed and thick-walled resting spores fill the body cavity.

Difficulties of Control

No known control method eradicates nematodes. A measure must be very efficient just to reduce the population; a 10-fold multiplication rate after a 90% kill would restore the population to pretreatment density. Crop rotation is commonly used but loses efficiency with species that have a wide host range, including common weeds. After heavy infestations, nonhost cropping for 9 years or longer may be necessary to reduce nematode numbers to nondamaging levels. Effective nematicides are available, but the application rates needed to kill nematodes in soil are often many times higher than those used to kill aboveground insects or fungi. Nematicides are short-lived and apparently do not accumulate in soil, but chemical treatments tend to be expensive and are restricted to high-value crops. Cultivars resistant to some cyst nematode species successfully reduce infestations but select aggressive pathotypes, and when such pathotypes are in the soil the cultivars should be used in rotation with nonhost crops or nematicides.

Integrating these control measures may prevent nematode populations from increasing to damaging levels (1). At present, the natural enemies of cyst nematodes are not used in this integrated approach because we know too little about them and because those that have been tried, such as the predacious fungi, have proved ineffective. Recently, however, the cyst nematode *Heterodera avenae* Woll. was shown to be controlled on susceptible cereal crops by parasitic fungi that kill female nematodes and eggs.

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Life Cycle of Cyst Nematodes

When a female cyst nematode dies, the cuticle tans to form a resistant envelope around the eggs in her body, and the eggs within this protective cyst may remain viable for many years. Each cyst is about 0.5 mm in diameter and contains up to 600 eggs surrounded by mucilage. Parasites can spread rapidly in such an environment, and a number of fungi have been isolated from nematode eggs (5). Predatory nematodes are occasionally found within cysts and may feed on eggs (Table 1).

When eggs hatch, often after stimulation from host roots, the second-stage juveniles leave the cyst and move through soil to invade the plant. Cyst nematodes are obligate parasites, and the juveniles must find a host root or starve in soil. Although this active phase in the nematode's life cycle is relatively short, juveniles invading the root system are the major cause of damage, and most research on control has concentrated on killing them and preventing yield losses.

Theoretically, with sexually reproducing species whose females produce an average of 200 eggs each, only 1% of the juveniles need survive to maintain populations. Some nematicides can prevent nematode multiplication by stopping juveniles from invading roots, but no natural agent has been as effective. The predacious fungi that occur in many soils and ensnare active nematodes on sticky hyphae or in ring traps have been studied most. These fungi are nonselective and

Table 1. Natural enemies of cyst nematodes

Stage in life cycle	Habitat	Parasites	Predators
Eggs	Females and cysts	Rickettsias; fungi	Nematodes; mites; Collembola
Second-stage juveniles and adult males	Soil	Rickettsias; trapping and endoparasitic fungi	Protozoa; nematodes; tardigrades; enchytraeids
Developing juveniles	Roots	Competition from root-infecting fungi	Competition from root-infecting fungi
Females	Roots and soil	Rickettsias; endoparasitic fungi	Collembola

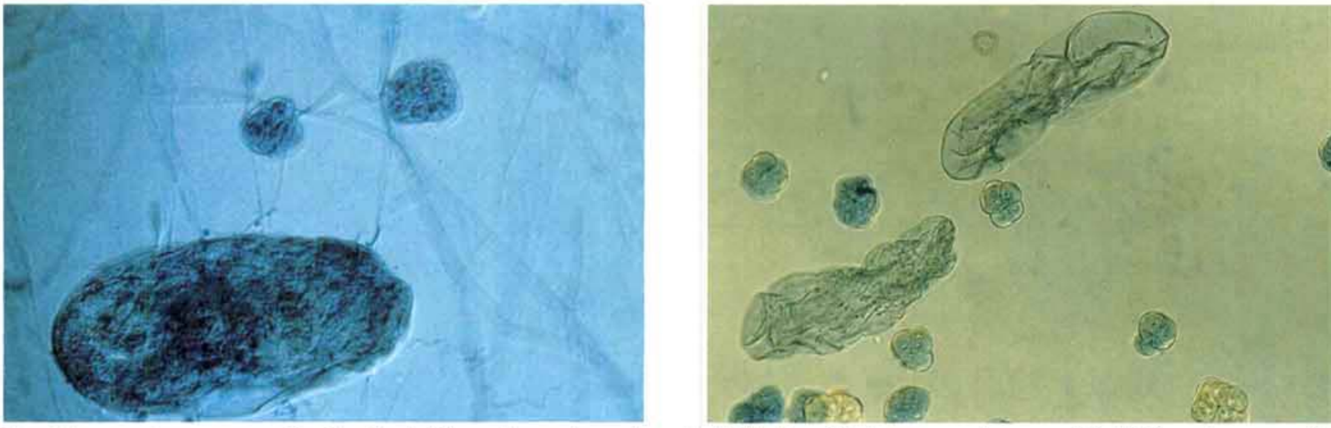

Fig. 3. Nematode eggs parasitized by *Verticillium chlamydosporium*: (Left) Hyphae-filled egg on water agar. (Right) Shrunken eggs after destruction of contents, with dictyochlamydospores.

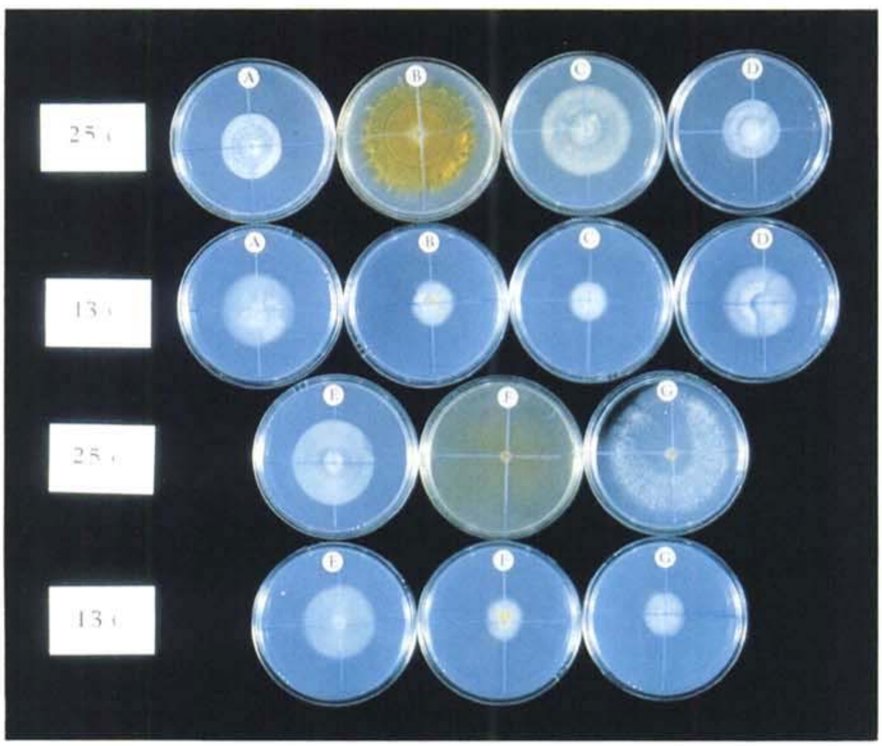

Fig. 4. Variability in growth and sporulation among isolates (A–G) of *Verticillium chlamydosporium* growing on cornmeal agar at 13 and 25 C.

will "capture" most types of nematode and, in addition, can survive saprophytically. Adding large quantities of organic matter to soil increases their numbers, but nematode infestation is reduced only temporarily. Adult males emerging from roots to fertilize females are also exposed to parasitism and predation. Predators that feed voraciously on nematodes in the laboratory—where they have no choice of food and the nematodes have no escape—are ineffective in soil.

Once inside the root, cyst nematode juveniles become sedentary, establishing the specialized feeding cells essential for further development. Root-infecting fungi can penetrate these cells, causing death of the nematode. Within the roots, however, the nematode is protected from predators and parasites; no fungi are known to attack juveniles within roots. Juveniles developing on the surface of fine lateral roots may be parasitized, however.

Females greatly increase in size, rupture the root cortex, and are exposed in soil for several weeks while feeding and producing eggs before forming a cyst. In many fields in Great Britain and in other parts of Europe, large numbers of cereal cyst nematode females can be found on the roots of susceptible cereal grains in June, July, and August, but few survive and form cysts full of eggs.

The Cereal Cyst Nematode and the Fungal Parasites

The cereal cyst nematode is native to Great Britain and parasitizes wild grasses as well as maize, oats, wheat, and barley. Numbers of *H. avenae* under grass are small but increase rapidly when pastures are plowed and sowed to cereal crops. Populations may then decline to nondamaging densities despite intensive or continuous cropping with cereal hosts. The nematode fails to multiply on oats, wheat, and barley sowed in the autumn or spring in a wide range of soils.

In field trials at the Rothamsted Experimental Station in England, partial soil sterilization with drenches of Formalin (38% formaldehyde) at 3,000 L/ha resulted in increased numbers of nematodes. Formalin removed a competitor or parasite from the soil that had been inhibiting *H. avenae* reproduction. In 1974, a fungal parasite attacking female nematodes on roots was found in fields where cereals were grown intensively. Further investigation revealed that most cereal cyst nematode populations were affected by fungi that parasitized and killed the females and eggs.

The three species of these phycomycetous fungi are *Catenaria auxiliaris* (Kühn) Tribe, *Nematophthora gynophila* Kerry and Crump, and an undescribed lagenidiaceous fungus (Fig. 1); their taxonomic differences have been summarized elsewhere (2). All three cause similar symptoms in the nematode. Females infected early lose turgidity and contain mycelium that rapidly destroys the nematode cuticle. Zoospores are released and the remaining mycelium produces thick-walled resting spores that fill the body cavity (Fig. 2). The spore masses are fragile and readily dispersed by other soil organisms. *N. gynophila* is the most common parasite and has been recovered from females in about 90% of fields infested with *H. avenae*. Females are parasitized within a few days of exposure on the root surface and are destroyed in about 4 days at 13 C.

The resting spores of *N. gynophila* can be extracted from soil, and densities of about 200 spores per gram of soil have been recorded. Although these spores have not germinated in culture, they presumably cause the initial infection within the nematode population, and motile zoospores spread the disease. Little rainfall in June, July, and August, when females on the roots of cereals are exposed to parasitism, greatly reduces the infection rate. Presumably, low moisture levels inhibit activity of the zoospores. In most years, however, moisture is sufficient for the fungi to be effective even on free-draining soil overlying chalk.

Female nematodes continue to appear on the roots for many weeks. Zoospores of *N. gynophila* may encyst after an active period of about an hour and later go through another motile stage, thus extending the time during which females may become infected. Although not definitely known, the longevity of encysted spores is at least 5 days. The zoospores of *C. auxiliaris* and the lagenidiaceous fungus encyst and germinate readily in soil water; they must find a suitable host before they starve.

Little is known of the life history of these fungi in soil. They have not been found in the absence of cyst nematodes and may be obligate parasites. If so, the fungi will not increase in soil with no female cyst nematodes and their numbers will depend on the intensity of cropping with nematode hosts.

The use of fungal parasites for biological control must be integrated with other methods to minimize damage while resting spore numbers increase. Once established in soil, the spores can survive at least 2 years in sufficient numbers to control nematodes whose host crops are grown in rotation. To shorten the time for numbers to increase to effective densities, methods of culturing the fungi on artificial media and of adding spores to soil are needed.

N. gynophila and *C. auxiliaris* have been recovered from females of the beet cyst nematode (*H. schachtii* Schmidt), and the lagenidiaceous fungus has parasitized the nematode in field samples and pot tests. In similar tests, *N. gynophila* parasitized *H. trifolii* Goffart, *H. goettingiana* Liebscher, *H. cruciferae* Franklin, and *H. carotae* Jones but not *Globodera rostochiensis* Wollenweber. *C. auxiliaris* infected females of *G. pallida* Stone. These fungi therefore could be useful against a number of important cyst nematode pests.

Verticillium chlamydosporium Goddard is mainly an egg parasite but will attack virgin females before egg laying has begun. Eggs within females in roots and within cysts in soil are parasitized. The hyphae can penetrate the cyst wall and destroy the contents of the eggs at all stages of development (Fig. 3). The characteristic dictyochlamydospores are not produced within the egg but may be found within or on the surface of the cyst. *V. chlamydosporium* can be cultured on artificial media, but growth and sporulation among isolates vary greatly (Fig. 4). Conidia have not been found in nematodes but are readily produced in cultures.

V. chlamydosporium is a general parasite that has been recovered from terrestrial mollusc eggs and from soils without cyst nematodes. Eggs of all cyst nematode species tested are susceptible. Parasitized females form small cysts containing few eggs, which are also infected.

Effect of Fungal Parasitism on Nematode Numbers in Soil

We have attempted to recover diseased nematodes from soil and host roots and to relate infection to nematode multiplication. Because females are destroyed rapidly, sampling must be done at least once a week to obtain accurate estimates of kill. Such work can be very time-consuming, especially since the early stages of infection can be reliably determined only by dissecting each nematode. Infection continues over a number of weeks. Standard wet sieving techniques are not very efficient for extracting diseased females with destroyed cuticles; about 50% of killed females have been recovered and fungal parasites identified. Rates of fungal parasitism in encysted eggs can be readily determined. In pot tests and field samples, high rates of parasitism and poor nematode multiplication are significantly correlated (2,3).

Soil drenching with Formalin has also been used to investigate the importance of fungal parasitism. To overcome the nematicidal effects of Formalin, second-stage juveniles were added to pots 4 weeks after treatment. This allowed similar numbers of females to develop in treated and untreated soil and the effects of Formalin on fungal parasitism to be studied. The sterilant almost eliminated the fungal parasites, and most female nematodes survived to form cysts full of eggs. In untreated soil, 95% of the females were killed. Formalin had no effect on nematode multiplication in soils with no fungal parasites or in dried soil with inactive parasites (4). Field results have been similar. The nematicidal action of Formalin that reduces juvenile invasion is outweighed by the sterilant's fungicidal effect that reduces parasitism; because more females survive in untreated plots, nematode populations increase.

Further Possibilities

By attacking females and eggs, the fungal parasites control *H. avenae* populations and allow farmers to grow susceptible crops continuously on nematode-infested land. This is the first known case of effective biological control of a cyst nematode. Further research is necessary to determine if such fungi can be used as preventatives against *H. avenae* in soils where they are absent or against other cyst nematodes on other crops. As the intensification of agriculture continues, these fungi may prove to be important weapons against nematode pests.

Literature Cited

1. Jones, F. G. W., and Kempton, R. A. 1978. Population dynamics, population models and integrated control. Pages 333-361 in: J. F. Southey, ed. Plant Nematology. MAFF/ADAS GD1. Her Majesty's Stationery Office, London. 440 pp.
2. Kerry, B. R. 1980. Fungal parasites of female cyst-nematodes. J. Nematol. 12:253-259.
3. Kerry, B. R., and Crump, D. H. 1977. Observations on fungal parasites of females and eggs of the cereal cyst-nematode, *Heterodera avenae*, and other cyst nematodes. Nematologica 23:193-201.
4. Kerry, B. R., Crump, D. H., and Mullen, L. A. 1980. Parasitic fungi, soil moisture, and the multiplication of the cereal cyst-nematode, *Heterodera avenae*. Nematologica. 26:57-68.
5. Tribe, H. J. 1977. Pathology of cyst-nematodes. Biol. Rev. 55:477-507.

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Dr. Kerry received his Ph.D. at the University of Reading, England, in 1973. Since then, he has worked in the Nematology Department at Rothamsted Experimental Station at Harpenden, where he is currently investigating the use of parasitic fungi to control nematode pests.