Effect of time sampling, method of isolation and age of nematode on the species of fungi isolated from females of *Heterodera schachtii* and *H. avenae*

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SUMMARY

Fungi were isolated from infected females of *Heterodera schachtii* and *H. avenae*, and from roots of sugar beet, on different occasions in two soils. The three most common fungi in both soils were *Verticillium chlamydosporium*, *Fusarium oxysporum* and *Cylindrocarpon destructans*, but the most active species changed with different sampling occasions. Spreading individual females across the agar and taking three sub-cultures rather than taking a single sub-culture from an intact female, resulted in up to three fungal species being isolated from some females. *Nematophthora gynophila* infected significantly more females of *H. avenae* than *H. schachtii*, but infection rates by other fungi were similar in the two nematodes. Infection by all fungi except *N. gynophila* caused premature tanning of the female cuticle. Similar fungal species and numbers of fungi per female were recovered from females of four different ages.

RÉSUMÉ

*Effet du moment de l'échantillonnage, de la méthode d'isolement et de l'âge du nématode sur les espèces de champignons isolées de femelles d'*Heterodera schachtii *et* H. avenae

Des champignons ont été isolés de femelles infestées d'*Heterodera schachtii* et *H. avenae*, ainsi que de racines de betterave à sucre, à plusieurs reprises dans deux sols différents. Les champignons les plus communs dans ces deux sols sont *Verticillium chlamydosporium*, *Fusarium oxysporum* et *Cylindrocarpon destructans*, mais les espèces les plus actives varient avec le prélèvement. L'étalement de femelles sur les plaques d'agar et la constitution de trois sous-cultures (plutôt que d'une seule sous-culture à partir d'une femelle intacte) conduit à l'isolement possible de trois espèces de champignons à partir d'une seule femelle. *Nematophthora gynophila* infeste significativement plus de femelles d'*H. avenae* que de *H. schachtii*, mais les taux d'infestation sont similaires dans le cas des autres champignons. Sauf *N. gynophila*, tous les champignons provoquent un tannage prématuré de la cuticule de la femelle. Les mêmes espèces de champignons, et à des taux semblables, ont été isolées de quatre lots de femelles ayant des âges différents.

In most surveys for fungal parasites of cyst nematodes, single species of fungi have been isolated from eggs or whole cysts on one occasion; such surveys have a number of limitations. Crump (1985) found parasites of females to be more important than parasites of eggs in the natural control of *Heterodera schachtii* Schmidt. Bursnall and Tribe (1974) reported more than one fungal species in some eggs of *H. schachtii*. As females mature their tissues break down and are accessible through the females' natural openings to a range of fungi in the soil or root. Hence, fast growing saprophytic fungi may be isolated in preference to slow growing parasitic species if the female or cyst is placed intact on agar. The species of fungi isolated from females could also vary with the age of the female and at different times in the growing season (Gintis, Morgan-Jones & Rodriguez-Kabana, 1983).

Fungi such as *Cylindrocarpon destructans* (Zins.) Scholten, which colonise the root cortex, may kill females by infecting the syncytial cell on which the nematode is feeding (Crump & Kerry, 1987). For this reason, fungi should be isolated from roots as well as females in any study of the interaction between nematodes and fungi.

In the first experiment reported here fungi were isolated from infected females of *H. schachtii* placed intact on agar, from females broken open and spread across the agar and from roots at four different intervals during the growing season. In the second experiment (different soil and growing season) the fungi isolated from females of different ages were compared for *H. schachtii* and *H. avenae* Woll. on one occasion and *H. schachtii* alone on a second occasion.

Materials and methods

ISOLATION OF FUNGI FROM INTACT AND SPREAD FEMALES OF *H. SCHACHTII*, AND ROOTS OF SUGAR BEET, ON FOUR OCCASIONS

Three sugar beet plants, together with the surrounding soil, were collected on 8th September, 10th, 28th October and 22nd November from four replicate plots in which sugar beet had been grown continuously for 15 years at Broom's Barn Experimental Station. Females were washed off the roots and extracted from the soil using a fluidising column (Trudgill, Evans & Faulkner, 1972) as described by Kerry (1975). Individual females were dissected and examined for fungal mycelia or spores. Phycomycetous fungi, such as *Nematophthora gynophila* Kerry & Crump, can be easily identified in this way, but other fungi that do not produce characteristic structures in nematodes need to be cultured on a nutrient agar to encourage sporulation. Infection of females could usually be detected without dissection due to premature tanning of the cuticle, and 20 per plot were selected. The sub-crystalline layers and any debris adhering to the cuticle were removed, and the females washed in sterilised distilled water. Each was plated onto 0.8 % water agar containing 0.05 g/litre each of the antibiotics streptomycin sulphate, aureomycin and chloramphenicol.

Ten females per plot were left intact on the agar and ten were cut into several pieces and spread across the agar, apart from the first sampling occasion when insufficient infected females were recovered for the latter treatment. The plates were observed daily for fungal growth and small sections of developing colonies were transferred aseptically onto 1.7 % cornmeal agar (containing the same antibiotics) to aid sporulation and identification. One sub-culture was taken from each of the intact females and three from each of the spread females.

Thirty 0.5 cm lengths of lateral root per plot were removed from the tap roots and surface sterilised by immersion in 2 % sodium hypochlorite solution for three minutes, followed by washing in sterile distilled water. Ten discs (1 cm diam.) of agar were cut from Petri dishes (9 cm diam.) containing 0.8 % water agar and antibiotics. The Petri dishes were inverted and the agar discs placed on the inside of the lid; one of the surface sterilised root pieces was put on each of these discs. This method allowed ten root pieces to be plated onto every Petri dish without the fungal colonies growing into each other. The agar remaining in the Petri dish base helped to maintain high humidity and prevented the discs from drying out. The plates were incubated at 20° and fungi growing from the roots were aseptically sub-cultured onto 1.7 % cornmeal agar to obtain pure cultures for identification.

Fungi which occured regularly but could not be identified were sent to the Commonwealth Mycological Institute, Kew, for identification.

ISOLATION OF FUNGI FROM *H. AVENAE* AND *H. SCHACHTII* FEMALES OF DIFFERENT AGES ON ONE AND TWO OCCASIONS, RESPECTIVELY

Females of *H. schachtii* were collected from sugar beet roots on 19th July and 11th September and females of *H. avenae* from barley roots on the first date only, from Woburn Experimental Farm. The numbers of females and proportions infected with fungi were estimated, and 25 infected females per plot were spread across agar plates as before. The females were categorised according to the stage of development they had reached :

1. Immature, before egg production.
2. Few eggs, egg production not completed.
3. Egg production almost complete, but not all eggs fully differentiated.
4. Fully mature.

Table 1

Fungal species identified in intact females, spread females and roots, and the percentages of the infected females or roots containing these species, in Broom's Barn soil (means of four replicates ± S.E.)

Fungal species	Sampling occasion	Intact females	Spread females	Roots
Fusarium oxysporum	1	87 ± 2.4	—	44 ± 3.5
	2	37 ± 15.0	33 ± 13.0	7 ± 2.5
	3	30 ± 13.5	41 ± 4.4	4 ± 2.8
	4	15 ± 2.9	23 ± 4.8	3 ± 1.7
Cylindrocarpon destructans	1	0 ± 0	—	3 ± 0.9
	2	3 ± 2.5	3 ± 2.5	17 ± 6.9
	3	0 ± 0	3 ± 7.5	20 ± 1.6
	4	5 ± 2.9	15 ± 9.6	9 ± 2.3
Verticillium chlamydosporium	1	0 ± 0	—	0 ± 0
	2	10 ± 4.1	23 ± 4.8	0 ± 0
	3	5 ± 2.9	7 ± 7.3	0 ± 0
	4	8 ± 2.5	8 ± 2.5	0 ± 0
Paecilomyces lilacinus	1	0 ± 0	—	4 ± 0.3
	2	8 ± 4.8	8 ± 7.5	4 ± 2.8
	3	5 ± 2.9	13 ± 6.0	4 ± 2.8
	4	8 ± 2.5	3 ± 2.5	3 ± 2.1
Phoma medicaginis	1	3 ± 2.5	—	13 ± 2.2
	2	8 ± 2.5	10 ± 5.8	0 ± 0
	3	8 ± 4.8	7 ± 7.3	8 ± 2.9
	4	15 ± 5.0	15 ± 6.5	4 ± 1.4
Exophiala mansonii	1	3 ± 2.5	—	0 ± 0
	2	5 ± 5.0	10 ± 4.1	0 ± 0
	3	0 ± 0	0 ± 0	0 ± 0
	4	8 ± 4.8	8 ± 7.5	0 ± 0

Results

ISOLATION OF FUNGI FROM INTACT AND SPREAD FEMALES OF *H. SCHACHTII*, AND ROOTS OF SUGAR BEET, ON FOUR OCCASIONS

Of the fungal species identified, *Fusarium oxysporum* Schlecht occurred the most frequently, in both females and roots (Tab. 1). Some unidentified species were separated on the appearance of their colony growth, and eight and eleven such species were isolated from females and roots respectively, six of which occurred in both. A fungus with dark mycelia was isolated from 30, 3 and 13 % of roots, intact and spread females respectively, and a fungus with slow growing light coloured mycelia from 4, 7 and 18 %, respectively. All other unidentified species occurred in small proportions (< 5 %) of the infected females and roots.

Fusarium oxysporum was isolated from a greater proportion of intact females ($P < 0.001$) and roots ($P < 0.001$) on the first sampling occasion (first generation) than on subsequent ones (second generation), whereas *C. destructans* was isolated from a smaller ($P < 0.05$) proportion of roots on the first sampling occasion than on the second and third. Few females were infected with *C. destructans* and there were no significant differences between sampling occasions.

Spreading the females across the agar rather than plating out intact resulted in only one extra fungal species (unidentified) being isolated, but the identified species were all isolated from more females. Although over half the spread females contained only one fungal species, the remainder contained two or even three species, with little variation between sampling occasions (Tab. 2).

Table 2

Percentages of spread females containing one, two or three fungal species in Broom's Barn soil (means of four replicates)

Number of fungal species	*Sampling occasion* 2	3	4	*Mean*
1	65	57	54	59
2	22	31	26	26
3	14	11	21	15

ISOLATION OF FUNGI FROM *H. AVENAE* and *H. SCHACHTII* FEMALES OF DIFFERENT AGES ON ONE AND TWO OCCASIONS, RESPECTIVELY

Nematophthora gynophila infected more ($P < 0.001$) females of *H. avenae* (44 %) than *H. schachtii* (0.9 %) on sampling occasion 1. *Verticillium chlamydosporium* Goddard, *C. destructans* and *F. oxysporum* were the three main fungi isolated by plating the females onto agar (Tab. 3). *Verticillium chlamydosporium* occurred most frequently in both nematode species on sampling occasion 1, but its occurrence decreased ($P < 0.05$) on sampling occasion 2. It was more common ($P < 0.001$) than *C. destructans* and *F. oxysporum* on the first sampling occasion, but less common ($P < 0.01$) than these fungi on the second. The proportions of *H. schachtii* females infected with *C. destructans* and *F. oxysporum* increased ($P < 0.05$) from the first to the second sampling occasion.

The fungi that did not sporulate and could not therefore be identified, were grouped according to their colony growth, and all occurred in small proportions (< 4 %) of the total infected females.

Table 3

Fungal species identified in spread females of *H. avenae* and *H. schachtii* and percentages of infected females containing these species, in Woburn soil (means of four replicates ± S.E.)

Fungal species	*Sampling 1* H. avenae	H. schachtii	*Sampling 2* H. schachtii
Verticillium chlamydosporium	47 ± 7.5	42 ± 6.2	14 ± 4.8
Cylindrocarpon destructans	2 ± 1.2	15 ± 3.0	30 ± 7.4
Fusarium oxysporum	0 ± 0	0 ± 0	29 ± 16.0
Paecilomyces lilacinus	3 ± 2.1	1 ± 1.0	0 ± 0
Volutella ciliata	6 ± 4.8	8 ± 3.3	0 ± 0
Mortierella elongata	11 ± 11.0	0 ± 0	0 ± 0
Exophiala mansonii	0 ± 0	0 ± 0	11 ± 3.4
Pyrenochaeta terrestris	4 ± 1.8	3 ± 1.9	3 ± 3.0
Cladosporium herbarum	2 ± 1.2	6 ± 2.6	0 ± 0
Fusarium equiseti	5 ± 5.0	0 ± 0	0 ± 0
Fusarium culmorum	2 ± 1.2	0 ± 0	1 ± 1.0
Fusarium flocciferum	0 ± 0	0 ± 0	1 ± 1.0
Cylindrocarpon olidium	2 ± 2.0	0 ± 0	1 ± 1.0

Table 4

Percentages of infected females containing one, two or three fungal species in Woburn soil (means of four replicates)

Number of	*Sampling 1*		*Sampling 2*	
fungal species	H. avenae	H. schachtii	H. schachtii	*mean*
1	78	65	56	66
2	18	30	36	28
3	4	5	8	6

The mean proportions of infected females containing one, two or three fungal species (Tab. 4) were similar to those in the first experiment.

All infected females were tanned regardless of their stage of development. Although fewer category 4 (mature) females were recovered than younger categories, the stage of development had no significant effect on the occurrence of *V. chlamydosporium*, *C. destructans* or *F. oxysporum*, or on the numbers of fungal species recovered per female.

Discussion

Although *F. oxysporum* was the most common fungus isolated from infected females and roots in the Broom's Barn soil, this is the first report of it infecting *H. schachtii* in Britain. It was considered important in the natural control of *H. schachtii* populations in California (Nigh, Thomason & Van Gundy, 1980*a*; Roberts, Thomason & McKinney, 1981) and has been isolated from *H. schachtii* in Czechoslovakia (Vinduska, 1982). Apart from Gintis Morgan-Jones and Rodriguez-Kabana (1983) who isolated it from young females of *H. glycines* Ichinohe, it has always been considered a parasite of eggs rather than females. *Cylindrocarpon destructans* is a common soil fungus and has been isolated from the rhizosphere of sugar beet and wheat roots (Van Emden, 1972). Although it is widespread in cyst nematode populations, it was described by Tribe (1977) as a minor pathogen since it only occurred in small proportions of eggs. However, it significantly reduced the number of *H. avenae* Woll. infected wheat plants (Kondakova & Tikhonova, 1981), and was one of the most important fungal parasites in the decline of *H. schachtii* populations during 17 years of sugar beet monoculture (Heijbroek, 1983). *Paecilomyces lilacinus* (Thom.) Samson and *Phoma medicaginis* Malbr. and Roum. were also recovered from infected females and roots, the first report of these fungi in nematodes in Britain. Morgan-Jones, White and Rodriguez-Kabana (1984) considered that of all the fungi they had encountered to date, *P. lilacinus* had the greatest potential for application as a biological control agent of phytonematodes in subtropical and tropical agricultural soils. *Verticillium chlamydosporium* and *Exophiala mansonii* (Castell.) de Hoog were not recovered from surface sterilised roots, suggesting that they invaded the females directly from the soil or from the rhizosphere. Some isolates of *V. chlamydosporium* are known to be capable of colonising the rhizosphere (Kerry, Simon & Rovira, 1984). This fungus is widespread in cyst nematode populations and has been described as the most important egg parasite of both *H. avenae* and *H. schachtii* (Kerry & Crump, 1977; Tribe, 1977). All these fungi, except *P. medicaginis*, were also isolated from infected females in the Woburn soil, together with several other species (Tab. 3), of which only *Cladosporium herbarum* (Pers.) Link has previously been reported from *H. schachtii* (Vinduska, 1982). It is likely, therefore, that the main fungal species isolated in these two soils occur consistently in cyst nematode populations, but that the list of minor species would be extended with more work of this type.

Nematophthora gynophila infected more females of *H. avenae* than *H. schachtii* in the Woburn soil. This is one of the main fungi involved in the natural control of *H. avenae* populations and although Crump (1985) found it to be widespread in *H. schachtii* infested soils, its occurrence was greater in the presence of *H. avenae*. It is likely, therefore, that *H. avenae* is a better host for this fungus than *H. schachtii*.

A change in the occurrence of certain fungal species occurred in both experiments, especially in the Woburn soil when *V. chlamydosporium* was the main species on the first sampling occasion with *C. destructans* and *F. oxysporum* commoner on the second. Hence, timing of sampling affects which fungal species is recovered most frequently. Although *F. oxysporum* was the most common species in the Broom's Barn soil, in a pot experiment using soil from the same plot the previous year *C. destructans* was the most common species on two occasions, one in the first and one in the second generation (Crump & Kerry, 1987).

The occurrence of more than one fungal species in some of the infected females means that methods used could determine which fungi are isolated. A fungus isolated from a single colony growing from an intact female may not necessarily be the fungus that first parasitised it. Gintis, Morgan-Jones and Rodriguez-Kabana (1983) reported an increase in numbers of fungal species with an increase in the age of *H. glycines*, from young females inside the root to cysts. However, their results are based on populations of the nematode whereas my work, which suggested that age had no significant effect, involved isolating fungi from individual females.

It is likely that more than one fungus is important in the natural control of nematodes at a particular site, and

environmental factors probably determine which species is most active at a particular time. Nigh, Thomason and Van Gundy (1980*b*) found that *Acremonium strictum* Gams infected more eggs of *H. schachtii* in saturated soils, while *F. oxysporum* was more active in dry soils. This variation in fungal species with time makes interpretation of results from a single sampling occasion difficult, and much work is needed to obtain an accurate picture of the natural control operating in a given soil during a growing season.

ACKNOWLEDGEMENT

I am grateful to the director and staff at the Commonwealth Mycological Institute, Kew, for their help in identifying some species of fungi

REFERENCES

BURNSALL, L. A. & TRIBE, H. T. (1974). Fungal parasitism in cysts of *Heterodera*. II. Egg parasites of *H. schachtii*. *Trans. Br. mycol. Soc.*, 62 : 595-601.

CRUMP, D. H. (1985). *Fungal parasites of the beet cyst nematode*. Ph. D. Thes., Univ. London, 231 p.

CRUMP, D. H. & KERRY, B. R. (1987). Studies on the population dynamics and fungal parasitism of *Heterodera schachtii* in soil from a sugar beet monoculture. *Crop Protection*, 6, 49-55.

GINTIS, B. O., MORGAN-JONES, G. & RODRIGUEZ-KABANA, R. (1983). Fungi associated with several developmental stages of *Heterodera glycines* from an Alabama field soil. *Nematropica*, 13 : 181-200.

HEIJBROEK, W. (1983). Some effects of fungal parasites on the population development of the beet cyst nematode (*Heterodera schachtii* Schm.). *Meded. Fac. Landbwet. Rijksuniv. Gent*, 48 : 433-439.

KERRY, B. R. (1975). The extraction of cysts of the cereal cyst nematode, *Heterodera avenae*, from soil. *Nematologica*, 21 : 163-168.

KERRY, B. R. & 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.

KERRY, B. R., SIMON, A. & ROVIRA, A. D. (1984). Observations on the introduction of *Verticillium chlamydosporium* and other parasitic fungi into soil for control of the cereal cyst nematode *Heterodera avenae*. *Ann. appl. Biol.*, 105 : 509-516.

KONDAKOVA, E. I. & TIKHONOVA, L. V. (1981). The effect of parasitic fungi on the infection of wheat with *Heterodera avenae Woll. Byull. Vses. Inst. gel'mint. K. I. Skryabina*, 31 : 33-35.

MORGAN-JONES, G., WHITE, J. F. & RODRIGUEZ-KABANA, R. (1984). Phytonematode pathology ultrastructural studies. 2. Parasitism of *Meloidogyne arenaria* eggs and larvae by *Paecilomyces lilacinus*. *Nematropica*, 14 : 57-71.

NIGH, E. A., THOMASON, I. J. & VAN GUNDY, S. D. (1980*a*). Identification and distribution of fungal parasites of *Heterodera schachtii* eggs in California. *Phytopathology*, 70 : 884-889.

NIGH, E. A., THOMASON, I. J. & VAN GUNDY, S. D. (1980*b*). Effect of temperature and moisture on parasitism of *Heterodera schachtii* eggs by *Acremonium strictum* and *Fusarium oxysporum*. *Phytopathology*, 70 : 889-891.

ROBERTS, P. A., THOMASON, I. J. & MCKINNEY, H. E. (1981). Influence of non-hosts, crucifers and fungal parasites on field populations of *Heterodera schachtii*. *J. Nematol.*, 13 : 164-171.

TRIBE, H. T. (1977). Pathology of cyst nematodes. *Biol. Rev.*, 52 : 477-507.

TRUDGILL, D. L., EVANS, K. & FAULKNER, G. (1972). A fluidising column for extracting nematodes from soil. *Nematologica*, 18 : 469-475.

VAN EMDEN, J. H. (1972). Soil mycoflora in relation to some crop plants. *E.P.P.O. Bull.*, 7 : 17-26.

VINDUSKA, L. (1982). Fungal parasitism of cysts of the beet eelworm *Heterodera schachtii*. *Rostlinna Vyroba*, 28 : 257-262.

Accepté pour publication le 26 mars 1987.

Erratum

In the following publication :

BILGRAMI, A. L., AHMAD, I. & JAIRAJPURI, M. S. (1986). A study of the intestinal contents of some mononchs. *Revue Nématol.*, 9 (2) : 191-194 :

— Page 191 (authors), " Shamin " should read as " Shamim ".

— Page 192 (right column) line - 7 : " nematode genera were most commonly found in " should read as " intestine of *I. monohystera*. The least active *I. basidontus* ".

— Page 193 (discussion) line - 4 : " signicant " should read as " significant ".