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Champignons associés aux kystes de *Globodera rostochiensis, G. pallida*, et *Heterodera schachtii*, ainsi qu'aux masses d'œufs et aux femelles du *Meloidogyne hapla*

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Article abstract

Cysts of *Heterodera schachtii* from sugar-beet (*Beta vulgaris*) fields and cysts of *Globodera rostochiensis* and *G. pallida* from potato (*Solanum tuberosum*) fields in northern Belgium, as well as egg masses and females of *Meloidogyne hapla* from a tomato (*Lycopersicon esculentum*) field in the Flemish-Brabant province, Belgium, were collected and examined for the presence of fungi. Of the total of 374 cysts of *H. schachtii*, 57.7% were colonized by one or more of 18 different species of fungi, all of which were from the genra *Acremonium, Chaetomium, Cylindrocarpon, Fusarium, Gliocladium, Humicola, Mariannaea, Nematophthora, Periconia, Phoma,* and *Verticillium*, and 45.3% of the 726 cysts of *Globodera spp*. were colonized by one or more of 18 different species, from the same gene. Of the 160 egg masses of *M. hapla*, 32% were colonized by one or more of 18 species of the genra *Arthrobotrys, Cylindrocarpon, Fusarium, Monacrosporium, Paecilomyces, Phoma, Plectosphaerella,* and *Verticillium*, while 31% of the 160 females were colonized by 12 species, from the same gene except *Paecilomyces* and *Plectosphaerella*. *Fusarium oxysporum* was by far the predominant species in both the cyst and root-knot nematodes. A black yeast-like fungus was found in cysts.

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Fungi associated with cysts of *Globodera rostochiensis, G. pallida,* and *Heterodera schachtii;* and egg masses and females of *Meloidogyne hapla* in Belgium

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Cysts of *Heterodera schachtii* from sugar-beet (*Beta vulgaris*) fields and cysts of *Globodera rostochiensis* and *G. pallida* from potato (*Solanum tuberosum*) fields in northern Belgium, as well as egg masses and females of *Meloidogyne hapla* from a tomato (*Lycopersicon esculentum*) field in the Flemish-Brabant province, Belgium, were collected and examined for the presence of fungi. Of the total of 374 cysts of *H. schachtii*, 57.7% were colonized by one or more of 18 different species of fungi, all of which were from the genera *Acremonium, Chaetomium, Cylindrocarpon, Fusarium, Gliocladium, Humicola, Mariannaea, Nematophthora, Periconia, Phoma,* and *Verticillium,* and 45.3% of the 726 cysts of *Globodera* spp. were colonized by one or more of 18 different species, from the same genera. Of the 160 egg masses of *M. hapla*, 32% were colonized by one or more of 18 species of the genera *Arthrobotrys, Cylindrocarpon, Fusarium, Monacrosporium, Paecilomyces, Phoma, Plectosphaerella,* and *Verticillium,* while 31% of the 160 females were colonized by 12 species, from the same genera except *Paecilomyces* and *Plectosphaerella. Fusarium oxysporum* was by far the predominant species in both the cyst and root-knot nematodes. A black yeast-like fungus was found in cysts.

[Champignons associés aux kystes de *Globodera rostochiensis, G. pallida,* et *Heterodera schachtii,* ainsi qu'aux masses d'œufs et aux femelles du *Meloidogyne hapla* en Belgique]

Des kystes du *Heterodera schachtii* provenant de champs de betteraves à sucre (*Beta vulgaris*), des kystes du *Globodera rostochiensis* et du *G. pallida* provenant de champs de pommes de terre (*Solanum tuberosum*) du nord de la Belgique, ainsi que des masses d'œufs et des femelles du *Meloidogyne hapla* provenant d'un champ de tomates (*Lycopersicon esculentum*) dans la province de Flemish-Brabant, Belgique, furent collectés et examinés pour la présence de champignons. Des 374 kystes du *H. schachtii* examinés, 57,7 % étaient colonisés par une ou plus des 18 espèces différentes de champignons, toutes appartenant aux genres *Acremonium, Chaetomium, Cylindrocarpon, Fusarium, Gliocladium, Humicola, Mariannaea, Nema-*

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tophthora, Periconia, Phoma ou *Verticillium*; 45,3 % des 726 kystes des *Globodera* spp. étaient colonisés par une ou plus des 18 espèces différentes des mêmes genres. Des 160 masses d'œufs examinées de *M. hapla*, 32 % étaient colonisées par une ou plus des 18 espèces appartenant aux genres *Arthrobotrys, Cylindrocarpon, Fusarium, Monacrosporium, Paecilomyces, Phoma, Plectosphaerella* ou *Verticillium*, alors que 31 % des 160 femelles étaient colonisées par 12 espèces des mêmes genres, sauf *Paecilomyces* et *Plectosphaerella*. Le *Fusarium oxysporum* était de loin l'espèce prédominante dans les kystes et les nématodes des nodosités. Un champignon noir, de type levure, fut aussi trouvé dans les kystes.

INTRODUCTION

Biological control of plant-parasitic nematodes using nematophagous fungi has received considerable attention recently, because of the urgent need for alternatives to replace synthetic nematicides that are being phased out due to environmental concerns (Kerry 1990). The beet cyst nematode (BCN), *Heterodera schachtii* Schmidt; the potato cyst nematodes (PCN), *Globodera rostochiensis* Wollenweber and *Globodera pallida* Stone; and a root-knot nematode (RKN), *Meloidogyne hapla* Chitwood, are some of the most important plant-parasitic nematodes in the world. Since nematophagous fungi were first discovered in soil in 1852 (Fresenius 1852), more than 150 species of fungi have been identified as colonizers of cysts, females, and eggs of eight species of cyst nematodes in soil, including BCN and PCN (Kerry 1988). Less than 20 species of fungi were found colonizing eggs of *Meloidogyne* spp. (Chen *et al.* 1996; Gaspard *et al.* 1990; Rodriguez-Kabana and Morgan-Jones 1988; Stirling and Mankau 1978). Relatively fewer species of fungi have been identified as colonizers of females of *Meloidogyne* spp. (Jatala 1986). The most common fungal genera included *Cylindrocarpon, Fusarium, Gliocladium, Paecilomyces, Phoma*, and *Verticillium* (Bursnall and Tribe 1974; Chen *et al.* 1996; Clovis and Nolan 1983). The percentages of cysts, eggs, and females of cyst nematodes colonized by fungi in agricultural soil ranged from 10 - 90%, with about 50% being the most common (Clovis and Nolan 1983; Tribe 1980). The percentage of eggs of RKN colonized by fungi ranged from 29% to 50% (Chen *et al.* 1996; Morgan-Jones *et al.* 1984). Two possible routes for biological management of plant parasitic nematodes have been proposed: 1) to mass-produce an effective nematode-destroying fungus in the laboratory, and then apply it to soil (Coosemans 1991); and 2) to enhance the natural nematophagous fungal populations in soil by altering their surrounding conditions (for example, by adding organic amendments or changing cultural practices). So far, commercial success of these approaches has been limited, however, there are encouraging reports on reducing nematode populations by adding certain kinds of amendments, such as chitin and green manure crops to soil (Schlang *et al.* 1988; Spiegel *et al.* 1987).

The objective of this study was to investigate the species and frequencies of fungi colonizing cysts of BCN, PCN, and egg masses and females of RKN collected from northern Belgium.

MATERIALS AND METHODS

Fungal isolation from cysts of BCN and PCN

Soil samples were collected from sugar-beet (*Beta vulgaris* L.) fields naturally infested with BCN, and from potato (*Solanum tuberosum* L.) fields infested with PCN in several areas of northern Belgium. The soil was air-dried overnight and the cysts were extracted by the Fenwick can method (Fenwick 1952). A total of 374 cysts of BCN, and 726 of PCN were collected. Cysts were hand-picked under a stereoscopic microscope

at 15 x magnification and transferred consecutively into a 10% sodium hypochlorite solution for 5 min, 100 μL L^{-1} streptomycin for 15 min, 20 μL L^{-1} malachite green for 10 min, and sterilized water for surface disinfestation. The cysts were partially air-dried afterwards. Four surface-dried, disinfested cysts were placed onto the corners at a sterilized square cover glass which was on potato dextrose agar in a petri dish under sterile conditions. The petri dishes were sealed with paraffin film and incubated at 25°C. Fungi growing from the cysts were examined visually or with a microscope at low magnification to determine the sites from which the fungi grew. The fungal colonies emerging from cysts were transferred once they reached the agar under the cover glass. Identifications of the fungi were made from these subcultures. If needed, nematodes were added to fungal cultures to induce sporulation for identification.

Fungal isolation from egg masses and females of RKN

A soil infested with *M. hapla* was collected from a tomato field in the province Flemish-Brabant, Belgium (lat. 50°53' N, long. 4°42' E). Tomato (*Lycopersicon esculentum* Mill. cv. Moneymaker) was planted in the collected soil on a greenhouse bench. This nematode culture had been maintained in the greenhouse for 3 yr previously. Root pieces with galls were collected and washed in running tap water for 24 h for cleaning and softening the root tissues. The egg masses were first separated from the galls and the females were separated from roots using sharp pointed forceps. Caution was taken to avoid breaking the eggs or females. A total of 160 egg masses and 160 females were placed into separate Bureau of Plant Industry watch glasses, and were surface-sterilized as above. Females were divided into two groups according to their colours: white (healthy) and grey (abnormal). Fungi associated with the egg masses and females were isolated by the same procedures used for isolation of fungi from cysts.

RESULTS

Fungi associated with cysts of BCN

Of the 374 cysts of BCN examined, 210 or 57.7% were colonized by fungi (Table 1). Seventeen species of fungi were identified, representing 11 different

Table 1. Fungal species associated with cysts of *Heterodera schachtii* from northern Belgium

Fungal species	Cysts colonized by fungi	
	Number	Percentage
Acremonium strictum	5	1.3
Chaetomium gracile	3	0.8
Cylindrocarpon destructans	23	6.1
Cylindrocarpon gracile	3	0.8
Cylindrocarpon lucidum	4	1.1
Fusarium heterosporium	2	0.6
Fusarium moniliforme	3	0.8
Fusarium oxysporum	111	29.0
Fusarium solani	7	1.9
Fusarium tabacinum	6	1.6
Gliocladium roseum	2	0.6
Humicola fascoatra	4	1.1
Mariannaea elegans	3	0.8
Nematophthora gynophila	5	1.3
Periconia macrospinosa	6	1.6
Phoma eupyrana	7	1.9
Verticillium coccosporium	4	1.1
Unidentified fungus	12	3.3
Total cysts colonized[a]	210	57.7

[a] A total of 374 cysts were examined.

genera. Of the fungi identified, most were species of *Fusarium* or *Cylindrocarpon*. *Fusarium oxysporum* Schlechtendahl was found to be associated with 111 cysts (29%) and *Cylindrocarpon destructans* (Zinser) Scholten colonized 6.1% of the cysts. *Acremonium strictum* W. Gams, *Chaetomium gracile* Udagawa, *Gliocladium roseum* Bainier, *Humicola fuscoatra* Traeen, *Mariannaea elegans* (Corda) Arnaud *et* Samson, *Nematophthora gynophila* Kerry *et* Crump, *Periconia macrospinosa* Lefebver *et* Johnson, *Phoma eupyrena* Saccardo, and *Verticillium coccosporium* W. Gams were infrequently associated with cysts (Table 1). One species of an unidentified black yeast-like fungus was associated with 3.3% of the cysts. Most fungi isolated emerged from anywhere on the cyst surface whereas *C. destructans* emerged only from the vulva of the cysts.

Fungi associated with cysts of PCN

Of the 726 cysts of PCN examined, 339 (45.3%) were colonized by fungi (Table 2). Seventeen species of fungi were isolated and identified. Most were the same species of fungi that were isolated from cysts of BCN. The frequencies of association of these fungi with cysts of PCN were similar to those of BCN. Most fungi associated with cysts of PCN were species of either *Fusarium* or *Cylindrocarpon*. *Fusarium oxysporum* was associated with 190 cysts (26.1%), and *C. destructans* and *F. heterosporum* Nees each colonized 4% of the cysts. All other species from other genera occurred at relatively low frequency (ca. 1%) (Table 2). The unidentified fungus appeared to be the same species as the unidentified fungus from cysts of BCN.

Fungi associated with egg masses and females of RKN

Of the total of 160 egg masses and 160 females of *M. hapla* examined, 32% of the egg masses and 31% of the females were colonized by fungi (Table 3). Eighteen species of fungi were isolated and identified from egg masses, while only 12 species were isolated from females. Eleven of the 12 species were also isolated from egg masses. *Cylindrocarpon destructans*, *Fusarium semitectum* Berkeley *et* Ravenel, *Fusarium sporotrichioides* Sherbakoff, *Fusarium stoveri* Booth, *Fusarium* sp., and *Paecilomyces*

Table 2. Fungal species associated with cysts of *Globodera rostochiensis* and *Globodera pallida* from northern Belgium

Fungal species	Cysts colonized by fungi	
	Number	Percentage
Alternaria alternata	8	1.1
Chaetomium gracile	5	0.7
Cylindrocarpon destructans	29	4.0
Cylindrocarpon sp.	1	0.1
Fusarium heterosporum	29	4.0
Fusarium moniliforme	6	0.8
Fusarium oxysporum	190	26.1
Fusarium semitectum	4	0.6
Fusarium solani	7	0.9
Fusarium stoveri	1	0.1
Gliocladium roseum	8	1.1
Mariannaea elegans	7	0.9
Penicillium simplicissium	7	0.9
Periconia macrospinosa	4	0.6
Phoma medicaginis	2	0.3
Trichocladium asperum	3	0.4
Verticillium coccosporum	5	0.7
Unidentified fungus	12	1.6
Total cysts colonized[a]	339	45.3

[a] A total of 726 cysts were examined.

Table 3. Fungal species associated with egg masses and females of *Meloidogyne hapla* from a tomato field in northern Belgium

Fungal species	Egg masses		Females	
	Number	Percentage	Number	Percentage
Arthrobotrys oligospora	4	2.5	6	3.8
Cylindrocarpon destructans	2	1.3	–[a]	–
Cylindrocarpon didymum	2	1.3	3	1.9
Cylindrocarpon effusum	1	0.6	1	0.6
Cylindrocarpon ianthothele	1	0.6	1	0.6
Cylindrocarpon olidum	2	1.3	3	1.9
Fusarium oxysporum	11	6.8	10	6.5
Fusarium semitectum	1	0.6	–	–
Fusarium sponotrichioides	3	1.9	–	–
Fusarium sp.	2	1.3	–	–
Fusarium stoveri	1	0.6	–	–
Monacrosporium psychrophilum	2	1.3	3	1.9
Paecilomyces lilacinus	1	0.6	–	–
Phoma medicaginis	2	1.3	3	1.9
Plectosphaerella cucumerina	8	5.0	–	–
Rhizoctonia solani	–	–	9	5.6
Verticillium chlamydosporium	3	1.9	5	3.1
Verticillium gonioides	1	0.6	3	1.9
Verticillium suchlasporium	1	0.6	2	1.3
Total colonized by fungi[b]	52	32.0	50	31.0

[a] Dash (-) indicates none colonized by this fungus.
[b] A total of 160 egg masses and 160 females were examined.

lilacinus (Thom) Samson were isolated only from egg masses. *Rhizoctonia solani* Kuhn was the only fungal species to be associated only with females. *Arthrobotrys oligospora* Fresenius and *Monacrosporium psychrophilum* (Drechsler) R. Cook *et* Dickinson, two nematode-trapping fungi, were associated with both egg masses and females of RKN. *Arthrobotrys oligospora* was isolated from 2.5% of the egg masses and from 3.8% of the females, whereas *M. psychrophilum* was isolated from 1.3% of the egg masses and from 1.9% of the females of the RKN. Three species of *Verticillium* were isolated from both egg masses and females. *Verticillium chlamydosporium* Goddard was isolated from 1.9% of the egg masses and from 3.1% of the females.

The fungi most frequently isolated from egg masses were species of *Fusarium* and *Cylindrocarpon*. *Fusarium oxysporum* was isolated from 6.8% of the egg masses, *Cylindrocarpon* spp. from 5.1%, and *Plectosphaerella cucumerina* (Lindf.) W. Gams from 5%. Other species had relatively low frequencies of association with egg masses.

The fungi most frequently isolated from females were *F. oxysporum* (6.5% frequency) and *Rhizoctonia solani* (5.6%). Females that were grey-coloured often yielded fungi, whereas the white, creamy-coloured females did not.

DISCUSSION

This survey showed that numerous fungi were associated with cysts of BCN and PCN, and with egg masses and females of RKN in Belgium. The fungal genera from BCN, PCN, and RKN were similar, especially those found in BCN and PCN. These fungi associated with nematodes may represent a distinct mycoflora in the soil. Agricultural soil generally contains hundreds of species of fungi belonging to 170 genera (Domsch *et al.* 1980). At the species level, 11 of 18 fungal species were isolated from cysts of both BCN and PCN, whereas only *F. oxysporum* and *C. destructans* were associated with BCN, PCN, and RKN. This suggests that the mycofloras of the cyst nematodes may be dif-

ferent from the mycoflora of root-knot nematodes, which is probably due to the fact that the cyst is not a living stage of a nematode. It is not surprising that most fungi associated with females were associated with egg masses as well, since females and egg masses are in close contact within the galls. More fungal species were identified as colonizers of egg masses than of females. This result is in agreement with the findings of others mentioned in the introduction. Fungi encountered in females are generally parasites, while many species of fungi isolated from egg masses are saprophytic because they can use the non-live substrate (gelatinous matrix) in the egg masses. *Cylindrocarpon destructans* has been reported as an egg parasite of several cyst nematodes (Dackman and Nordbring-Hertz 1985, Nigh *et al.* 1980); *Paecilomyces lilacinus* is a well-known egg parasite of *Meloidogyne* spp. (Dube and Smart 1987, Jatala 1986). A *Fusarium* species associated with egg masses but not females has been reported to be an egg parasite (Morgan-Jones *et al.* 1984). *Plectosphaerella cucumerina* has been reported to be associated with cysts of *H. glycines* (Carris *et al.* 1989), but whether it is an egg parasite or not needs further study. The fact that *R. solani* colonized only females, but neither egg masses nor cysts of BCN and PCN, suggests that this fungus did not come from egg masses but from the host plant. *Rhizoctonia solani* is a well-known endotrophic mycorrhizal fungus (Harley 1968), which suggest that this fungus colonizes females of RKN through plant cells, rather than through egg masses. The study shows that females of RKN buried in the plant are still vulnerable to attack by soil fungi.

All the fungi isolated from BCN, PCN, and RKN have been reported to be associated with plant-parasitic nematodes, especially cyst-forming nematodes. A few of them have been proven obligate parasites of nematodes but most of them are opportunistic parasites and saprophytes. For those obligate parasites, their effectiveness in destroying nematodes *in vitro* has not led them to be successful bio-control agents of plant-parasitic nematodes. However, there are reports which indicate that viability of nematodes was greatly reduced after being colonized by some of these opportunistic fungal parasites in laboratory (Nigh *et al.* 1980). In soil, the populations of these opportunistic fungi associated with nematodes can be significantly greater than populations of obligate parasites (Yu 1989). The suppressiveness of suppressive soils against plant-parasitic nematodes has been reported to be positively related to the population of all the fungal parasites, including the opportunists (Kerry 1988). Although a great deal of knowledge is lacking on the mode of action and population dynamics of these opportunistic fungal parasites, their importance in future integrated management of plant-parasitic nematodes should not be underestimated.

The association of *A. oligospora* and *M. psychrophilum*, two nematode-trapping fungi, with egg masses and females of RKN is not surprising, because these fungi are saprophytic. However, no nematode-trapping fungi were previously reported from cysts of BCN and PCN.

The unidentified fungus is probably the black yeast-like fungus reported to be associated with cysts of *H. glycines* in Arkansas, Missouri, and Mississippi by Morgan-Jones *et al.* (1981), and in Florida by Chen *et al.* (1994).

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