



Fungi isolated from cysts of the beet cyst nematode parasitized its eggs and counterbalanced root damages

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Abstract

Finding beneficial fungi antagonistic toward nematodes is important for developing more sustainable agricultural practices. We isolated abundant fungi from cysts of the beet cyst nematode *Heterodera schachtii* and tested their interaction with nematodes and sugar beet. By molecular screening of fungal communities colonizing five field populations of *H. schachtii* using DGGE and Sanger sequencing, three strains identified as *Exophiala* sp., *Pochonia chlamydosporia* and *Pyrenochaeta* sp. were frequently found in infected cysts and then specifically isolated (in pure culture). The three isolates were able to re-infect the cysts and colonize the eggs of *H. schachtii* in vitro fulfilling Koch's postulates. In greenhouse trials, the root weight of sugar beet plants grown in substrates inoculated with nematodes and the fungal isolates was significantly higher compared to plants inoculated with nematodes only. The number of cysts propagated on roots was lower in substrates inoculated with *Pyrenochaeta* sp. than those in substrates inoculated with the other two fungi. However, cyst numbers did not significantly differ from the control because the propagation rate of the nematode increased with the root weight. The proportion of infected eggs per cyst was higher under the fungal treatments than the control, and it increased with the number of propagated cysts. The results showed that the here-studied strains of *Exophiala* sp., *Pochonia chlamydosporia* and *Pyrenochaeta* sp. were frequently found colonizing the nematode cysts of different populations of *H. schachtii* and were efficient in parasitizing the eggs of the nematode.

Keywords Beet cyst nematode · *Exophiala* · *Heterodera schachtii* · Nematophagous fungi · *Pochonia chlamydosporia* · *Pyrenochaeta*

Key message

- Screening of the beet cyst nematode *Heterodera schachtii* for egg-parasitic fungi resulted in the isolation of three fungal strains of *Exophiala* sp. *Pochonia chlamydosporia*

and *Pyrenochaeta* sp. as the most frequent nematophagous fungi parasitizing the eggs of different populations of *H. schachtii* in North Rhine-Westphalia, Germany.

- The parasitism of isolates could be reproduced in in vitro and greenhouse studies.
- Inoculation of sugar beet plants with these fungi resulted in a significant plant development in nematode-infected plants suggesting further studies to investigate endophytic interaction between these strains with sugar beet within a tripartite interaction system including fungus–plant–plant parasitic nematode.

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Introduction

The beet cyst nematode (BCN) *Heterodera schachtii* is a cyst-forming plant parasitic nematode causing considerable economic losses in sugar beet (*Beta vulgaris*) production (Müller 1999). Wide non-host rotations and the introduction

of resistant cover crops besides the use of resistant sugar beet cultivars are the most common approaches to manage BCN (Müller 1999). These strategies might be either uneconomic or the resistance efficiency could be lost over time because the parasitism genes of nematodes are highly diverse and plant nematode resistance genes are still elusive (Griffin 1982). Population dynamics of plant parasitic nematodes (PPNs) are not only affected by changes in abiotic factors such as temperature and moisture but also by nematode antagonists living in the soil (Trivedi 2003). Cyst nematode populations can be suppressed by several antagonistic fungi, which are taxonomically diverse and are found throughout the kingdom of fungi. This group of fungi is capable of capturing and parasitizing nematodes (Nordbring-Hertz et al. 2011). A sub-group of these fungi named as egg parasites attacks the eggs and females of sedentary PPNs including cyst-forming nematodes and infects their host through individual hyphae or specialized infection structures like appressoria (Ashrafi et al. 2017; Lopez-Llorca et al. 2002; Morgan-Jones et al. 1983, 1984).

The females of cyst nematodes are potential target to be attacked by nematophagous fungi. During nematode development, an immature female ruptures the roots, develops and becomes gravid, and later dies and becomes a cyst that can remain infective in soil for several years in the absence of a host plant. This process causes a long-term exposure of female and cyst nematodes to different egg-parasitic fungi (Dijksterhuis et al. 1994; Jansson and Lopez-Llorca 2004; Ashrafi et al. 2017). The most intensively studied egg-parasitic fungi are *Pochonia chlamydosporia*, *Purpureocillium lilacinum*, and several species within the genera of *Metarhizium* and *Trichoderma* (Kerry 1980; Siddiqui and Mahmood 1996; Stirling 2014).

Several of these fungi have been reported to have a multifunctional lifestyle within a tripartite interaction, in which the fungus lives as an endophyte of the host plant and a pathogen of nematodes or insects (Barelli et al. 2016; Schouten 2016). For example, some nematophagous fungi (e.g. representatives of *Arthrobotrys*, *Lecanicillium lecanii*, *Pochonia chlamydosporia*, and *Purpureocillium lilacinum*) had been reported to colonize the plant roots endophytically (Bordallo et al. 2002; Lopez-Llorca et al. 2002; Schouten 2016), and it had been shown that an endophytic isolate of *P. chlamydosporia* produces plant hormones, which promote plant growth and subsequently increase the tolerance level to nematodes (Schouten 2016).

In addition to the above-mentioned fungi, several other fungal species were reported to parasitize nematode eggs: strains of *Exophiala pisciphila* and *Pyrenochaeta terrestris* (Syn *Setophoma terrestris*) were frequently found colonizing eggs of *Globodera rostochiensis* (potato cyst nematode), *Heterodera avenae* (cereal cyst nematode) and *Heterodera glycines* (soybean cyst nematode) (Chen et al. 1996; Chen

and Chen 2002; Dackman 1990; Dackman and Nordbring-Hertz 1985). The genus *Exophiala* is diverse in ecology, and it includes several species frequently isolated from different hosts and natural environments, e.g. plant tissues, soil and water (Addy et al. 2005; Maciá-Vicente et al. 2016; Najafzadeh et al. 2013). The genus also accommodates species reported as plant endophytes. Wang et al. (2013) reported an endophytic strain of *Exophiala salmonis* isolated from the roots of *Paris polyphylla*. Recently, Maciá-Vicente et al. (2016) described the endophytic *Exophiala radialis* isolated from the roots of *Microthlaspi perfoliatum*.

In a previous study (Nuaima et al. 2019), the denaturing gradient gel electrophoresis (DGGE) analysis of the fungal community infecting the cysts of a field population showed the frequency of two fungi, *Exophiala* sp. and *P. chlamydosporia*. In the present study, during a molecular screening based on DGGE conducted to analyze the fungal community infecting the cysts of different field populations of *H. schachtii* in Germany, several fungal isolates were frequently found colonizing the cysts of *H. schachtii*. The fungi were isolated, and their antagonistic potential toward the nematode eggs was evaluated in in vitro and in greenhouse experiments. The objectives of the present study were therefore addressed to: (1) isolate the most frequent fungal strains colonizing different field populations of *H. schachtii* and (2) examine the biocontrol potential of the isolated fungi on *H. schachtii* in vitro and greenhouse trials.

Materials and methods

Nematode collection and materials examined

Cysts of the BCN *H. schachtii* were collected from soils of five sugar beet fields naturally infected with BCN, located in Lower Saxony and North Rhine-Westphalia in Germany (Table 1). The soil samples were taken at 0–30 cm depth from each of the sugar beet fields. The nematode cysts were extracted from the soil samples using a centrifugal floatation technique with $MgSO_4$ solution of 1.28 g/cm^3 (Müller 1980). Representatives of cyst samples collected from all

Table 1 Coordinates of sugar beet fields located in North Rhine-Westphalia, Germany that represent the origin of *H. schachtii* populations processed in this study

Sugar beet fields	Coordinates	Number of samples
Hottorf	50.593005 N, 6.205978 E	29
Kerpen-Blatzheim	50.859300 N, 6.627100 E	4
Euskirchen-Dom-Esch	50.685500 N, 6.860600 E	6
Titz-Kalrath	50.975300 N, 6.450200 E	4
Swisttal-Miel	50.667000 N, 6.943200 E	6

fields were screened for nematode infection by fungi. The symptomatic cyst samples collected from “Hottorf” were additionally used for fungal isolation.

DNA extraction, PCR amplification, sequencing and DGGE analyses

To extract genomic DNA of fungal strains colonizing cysts of *H. schachtii*, ten cysts of each soil sample were hand-picked and collected in 1.5 ml tubes containing 30 µl of water. Cysts were crushed, and DNA was then extracted using the cell lysis buffer as described in detail by Nuaima et al. (2018). For culture-dependent species identification, fungal mycelium was obtained from the pure cultures grown on potato dextrose agar (PDA) and transferred to 1.5 ml tubes. Fungal cells were mechanically disrupted and processed as described above. DNA was purified using the DNA Clean & Concentrator Kit (Zymo Research, Freiburg, Germany) and used as a template for PCR amplification.

The internal transcribed spacers (ITS 1 and 2 region) including the 5.8S rDNA of the ribosomal gene cluster was amplified with primer sets ITS1f & ITS4 (White et al. 1990) and ITS1f-GC & ITS2 with a GC tail specific for denaturing gradient gel electrophoresis (DGGE) according to Rungis (2015). The long PCR-amplified ITS fragments with sizes of 450–750 bp were purified using a High Pure PCR Product Purification Kit (Merck, Darmstadt, Germany) following the manufacturer’s instructions, and sequenced by Macrogen Europe (Amsterdam, The Netherlands) with primers ITS1f and ITS4. The obtained sequences were assembled with Sequencher 5.4.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and deposited in GenBank under the following accession numbers: MN537134–MN537137. The sequences generated were compared with the publically available sequences in GenBank using a BLASTn search for a preliminary DNA-based identification.

The short ITS1 fragments amplified with ITS1f-GC and ITS2 with about 250 bp were used for DGGE as recently described (Nuaima et al. 2019). The ITS1 fragments were PCR-amplified from the cloned and sequenced ITS fragments of *Exophiala* sp. and *P. chlamydosporia* (Nuaima et al. 2019) and used as references in DGGE analysis.

Isolation of fungi from symptomatic cysts of *H. schachtii*

Field-collected cyst samples (Hottorf Germany) were examined under an Olympus SZX 12 dissecting microscope to handpick the cysts displaying symptoms of fungal infection like discernible fungal hyphae or discolored eggs. The selected symptomatic cysts were surface sterilized in 0.5% sodium hypochlorite (NaOCl) for 10 min following three washes with distilled water. The surface-sterilized cysts

were individually placed onto potato dextrose agar (PDA, Carl Roth GmbH, Germany) supplemented with penicillin G (240 mg/L) and streptomycin sulfate (200 mg/L). Plates were incubated at room conditions and monitored regularly. To purify the obtained isolates, fungal mycelia growing from the cultured cysts were subcultured on new PDA plates. Pure cultures obtained were initially grouped based on their morphological criteria and later were also processed for DNA-based identification.

In vitro pathogenicity tests of the fungal isolates toward nematode eggs

Healthy-looking gravid females and cysts extracted from a greenhouse-propagated population (Gross Munzel, Germany) were surface sterilized as described above. The fungal isolates of interest (Ex007, Pc001, Py004) obtained in this study were grown on PDA for up to 1 month in five replicates. Ten surface-sterilized healthy cysts were placed on top of each of the fungal colonies and incubated at room conditions. Incubated cysts were microscopically monitored at regular intervals for fungal infection. Cysts placed on blank PDA were considered as the control treatment.

To examine pathogenicity of fungal isolates of interest toward nematode females, 6 weeks-old gravid females were extracted from roots, washed with sterilized distilled water, and incubated on fungal colonies. A sterility check was done prior to incubation by imprinting the females into PDA.

Pathogenicity tests against *H. schachtii* in vivo

Pot experiments were conducted to assess the biocontrol potential of the fungal strains against *H. schachtii*. Autoclaved-sterilized loess soil (Müller and Rumpfenhorst 2000) enriched with slow-release fertilizer (Osmocote Exact Standard®) at a rate of 1.25 g/kg was used as the substrate. Two-month-old PDA cultures of the fungal isolates were used for the experiments. Pots (500 ml) were inoculated with 15 plugs (5 mm in diam.) of either one fungal isolate or a mixture of two isolates. In total, the treatments were as follows: (1) isolate Ex007, (2) isolate Pc001, (3) isolate Py004, (4) isolates Ex007 + Pc001, and (5) untreated control. Each treatment was replicated 15 times. Control treatments consisted of pots inoculated with plugs of non-fungal colonized PDA. Pots were placed in a greenhouse and incubated for 1 week to allow the fungi to establish in the soil. Pots were then sown with a nematode susceptible sugar beet cultivar “Beretta.” Two weeks later, each plant was inoculated with 2500 freshly hatched second-stage juveniles (J2) from the greenhouse-propagated cysts of *H. schachtii*. The plants were grown at 16/20 °C with a 16-h photoperiod for 8 months to obtain four nematode generations.

Fresh shoots were removed, and the roots were left for 2 weeks more in the pots to allow full development/maturation of the cysts. Roots were gently removed from the soil and rinsed until no soil debris was left. The fresh weight of each root sample was recorded. Nematode cysts were extracted by washing the entire soil of each pot through a 250- μm sieve and counted under a stereo microscope. To record the number of fungal infected eggs, the cysts of each treatment were pooled and a sub-sample consisting of 1000 cysts was processed. The cysts were squashed using a tissue grinder for 30 s (IKA[®]-Werke GmbH & Co. KG, Staufen, Germany) to collect the eggs. Eggs were counted, and the mean numbers of eggs per cyst were calculated and reported. The infected eggs and cysts were photographed using light microscopy as described by Ashrafi et al. (2017).

Data analysis

Using the R software, the data describing the root weight, the propagated cysts and the infected eggs were analyzed by generalized linear models.

Results

Frequency of cyst colonizing fungi

The presence of fungi in cysts was assessed for cyst samples collected from five sugar beet fields located in North Rhine-Westphalia. From the field site “Hottorf,” 29 PCR-amplified ITS fragments, each representing the fungi colonizing cysts of *H. schachtii* was analyzed using DGGE. Each of the resolved patterns was unique, and thus the fungal communities infecting the cysts were different among the 29 ITS patterns. Nevertheless, four ITS bands were highly frequent among all 29 patterns, and two out of the four were identical to the fungal clones of *Exophiala* sp. and *P. chlamydosporia* in their electrophoretic mobility in DGGE (Supplementary Fig. 1). The ITS patterns for the fungi infecting the cysts extracted from the other four field populations of *H. schachtii* were different within and among the nematode populations. The most frequent two ITS bands were identical in their electrophoretic mobility to the ITS clones of *Exophiala* sp. and *P. chlamydosporia* (Supplementary Fig. 2).

Fungal isolation

Four fungal strains were isolated from the cyst samples collected from Hottorf. The BLASTn search of the ITS sequences of the isolates revealed relatedness of the obtained fungal strains to the genera *Exophiala*, *Fusarium*, *Pochonia* and *Pyrenochaeta*: The fungal strains EX007 and Py004 showed high sequence similarity with 99% and 100% to the

endophytic strains reported from the genera of *Exophiala* and *Pyrenochaeta*, respectively. The strain Fu010 was identified/grouped as a *Fusarium* species and was not included in further experiments. The strain Pc001 was identified as *P. chlamydosporia*, which was also supported by morphological examinations of the pure cultures (Supplementary Fig. 3).

In vitro parasitism of fungal strains toward nematode eggs

The fungal strains Ex007, Pc001, and Py004 were capable of colonizing the cysts and infecting the eggs of *H. schachtii* in vitro. The strains Ex007 (*Exophiala* sp.) and Py004 (*Pyrenochaeta* sp.) rendered incubated cysts discolored. The symptomatic cysts contained eggs, which were fully colonized and rendered light to dark brownish appearance (Fig. 1d, g). The fungal isolate Ex007 parasitized and discolored gravid females 1 week after incubation (Fig. 1b). Strain Pc001 (*P. chlamydosporia*) infected the nematode eggs and destroyed the embryonic juveniles (Fig. 1i); however, neither the infected cysts nor the colonized eggs had a discolored appearance. The sterility check revealed no fungal growth from the examined cysts.

Greenhouse experiments

The effect of fungi on plant growth

The plant roots treated with the here-studied fungal strains developed significantly better in comparison with the control treatment (non-fungal treated) in the presence of the nematodes (Fig. 2a–e, f). Among the four fungal treatments, the root weight did not differ significantly (Fig. 2f).

Correlation between number of propagated cysts and root weight

For all treatments, the population density of propagated cysts was positively correlated with the root weight (Fig. 3a). When the root weights in the treatments of Ex007 (*Exophiala* sp.), Pc001 (*P. chlamydosporia*), and Ex007 + Pc001 (*Exophiala* sp. plus *P. chlamydosporia*) were significantly higher than the control, the number of cysts was also significantly higher in these fungal treatments than in the control (Fig. 3b). However, the number of cysts did not significantly differ between the treatments of Py004 (*Pyrenochaeta* sp.) and control (Fig. 3b).

Parasitic potential toward nematode eggs

All fungal isolates could parasitize nematode eggs in the greenhouse. The number of infected eggs per cyst was

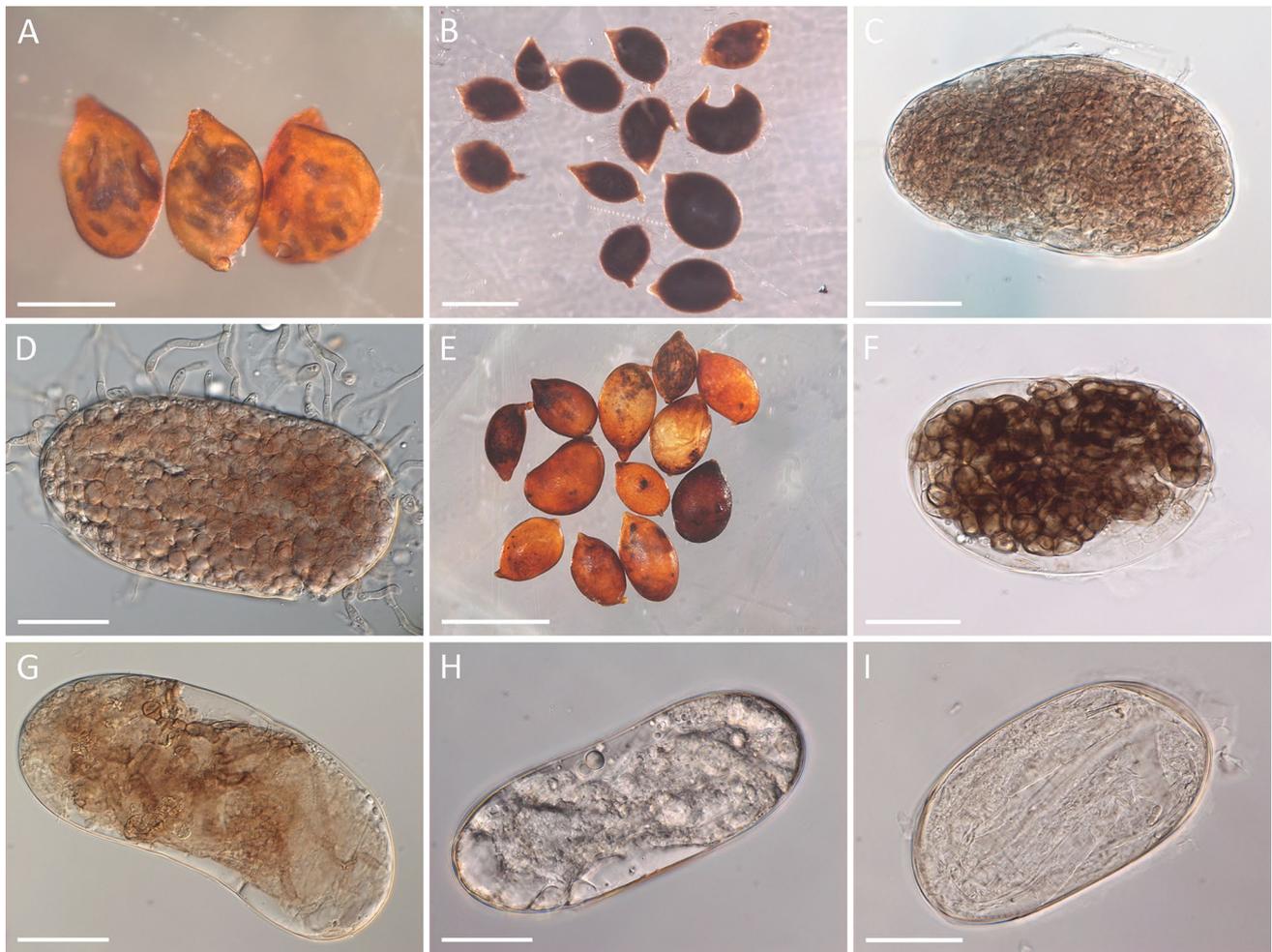


Fig. 1 The isolates of *Exophiala* sp., *Pyrenochaeta* sp., and *Pochonia chlamydosporia* infected cysts and colonized eggs of *H. schachtii* in laboratory and greenhouse trials. **a** Infected cysts with *Exophiala* sp. from greenhouse trials. **b** Infected gravid females with *Exophiala* sp. from in vitro trials. **c** Infected egg with *Exophiala* sp. from greenhouse trials. **d** Egg infected by *Exophiala* sp. from in vitro trials. **e**

Cysts infected with *Pyrenochaeta* sp. from greenhouse trials. **f** Egg infected with *Pyrenochaeta* sp. from greenhouse trials. **g** Egg infected by *Pyrenochaeta* sp. from in vitro trials. **h** Egg infected with *P. chlamydosporia* from greenhouse trials. **i** Egg infected with *P. chlamydosporia* from in vitro trials

similar among the four fungal treatments (Fig. 4a). Under the fungal treatments of Ex007, Pc001, and Py004, high proportions of infected eggs were isolated (Fig. 1c, f, h). The infected cysts extracted from the fungal treatments of Ex007 and Py004 displayed a discolored appearance (Fig. 1a, e). The number of infected eggs per cyst was similar among the four fungal treatments (Fig. 4a). The microscopic examination and the data analysis showed that the proportion of infected/discolored eggs was higher under the fungal treatments than in the control (Fig. 4a). A significant positive correlation was apparent between the number of infected eggs and the number of cysts under the experimental treatments (Fig. 4b).

Discussion

The fungal isolates *Exophiala* sp. (strains Ex007), *P. chlamydosporia* (strain Pc001), and *Pyrenochaeta* sp. (strain Py004) were found to frequently parasitize the eggs of *H. schachtii* during this study. The ability of *Exophiala* and *Pochonia* species to parasitize the eggs of *H. schachtii* (Chen and Chen 2003; Kerry 2000) could explain the high prevalence of these colonizers inside the cysts originating from different populations in this study (see Supplementary Figs. 1 and 2). The fungal strain of *Pyrenochaeta* sp. was also frequently found colonizing the nematode cysts,

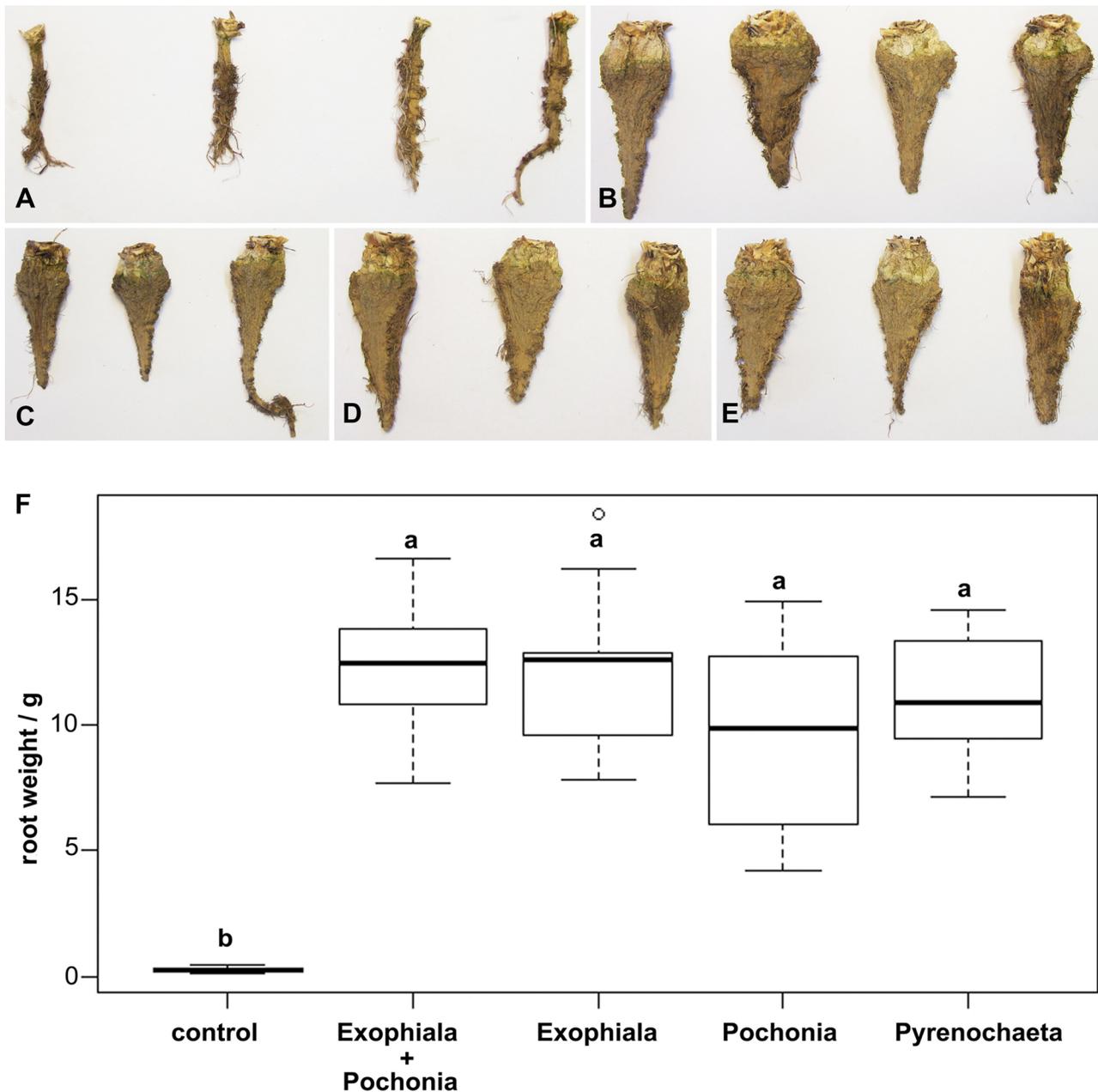


Fig. 2 Effects of fungal strains on plant growth in the presence of nematode. **a** Sugar beet roots grown in substrates inoculated only with nematodes under the control treatment. **b** Sugar beet roots grown in substrates inoculated with nematodes and *Exophiala* sp. and *Pochonia chlamydosporia*. **c** Sugar beet roots grown in substrates inoculated with nematodes and *Exophiala* sp. **d** Sugar beet roots grown in substrates inoculated with nematodes and *P. chlamydosporia*.

ria. **e** Sugar beet roots grown in substrates inoculated with nematodes and *Pyrenochaeta* sp. **f** Box plots calculated for root weight of sugar beet planted for 8 months to obtain four nematode generations under four fungal and a control treatments. Different letters indicate significant differences in root weight among treatments (Dunn test, $P < 0.05$, $n = 15$)

where four out of ten isolates extracted from the cysts of *H. schachtii* were identified as *Pyrenochaeta* sp. suggesting a high parasitic potential of this strain toward *H. schachtii*. Parasitism of *Pyrenochaeta* against other plant parasitic nematodes was already reported for *P. terrestris*

isolated from the soybean cyst nematode, *Heterodera glycines* showing a high rate of egg parasitism (Chen et al. 1996). The pathogenicity tests revealed that these fungi parasitized the females of the nematode. The parasitic ability of the fungi might be higher on the gravid females than

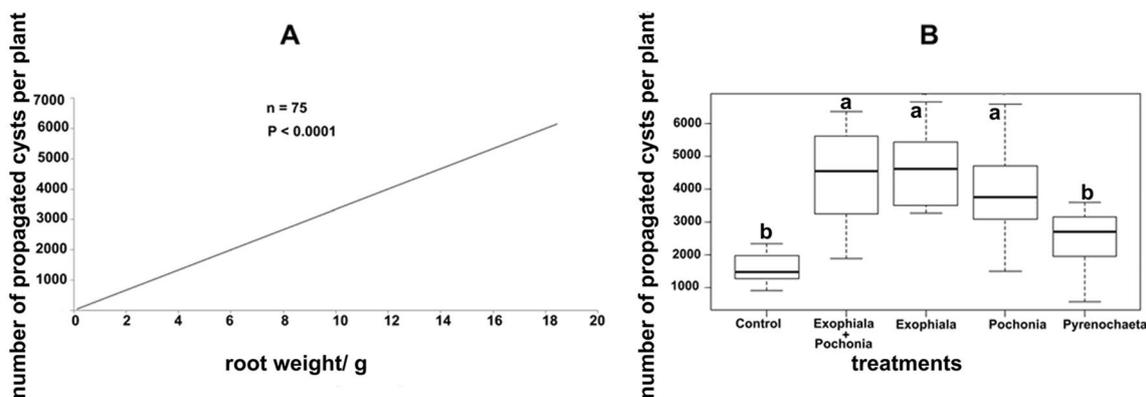


Fig. 3 Correlation between number of propagated cysts and root weight. **a** Regression lines of propagated cysts of *Heterodera schachtii* with respect to the root weight of sugar beet planted under fungal and control treatments (*T*-Test, *P*-values calculated at 95% confidence level, number of pairwise combination is indicated by *n*).

b Box plots for numbers of cysts of *H. schachtii* at Gross Munzel that propagated for four generations on sugar beet under the fungi and control treatments. Different letters indicate significant differences in numbers of propagated cysts among treatments (Dunn test, *P* < 0.05, *n* = 15)

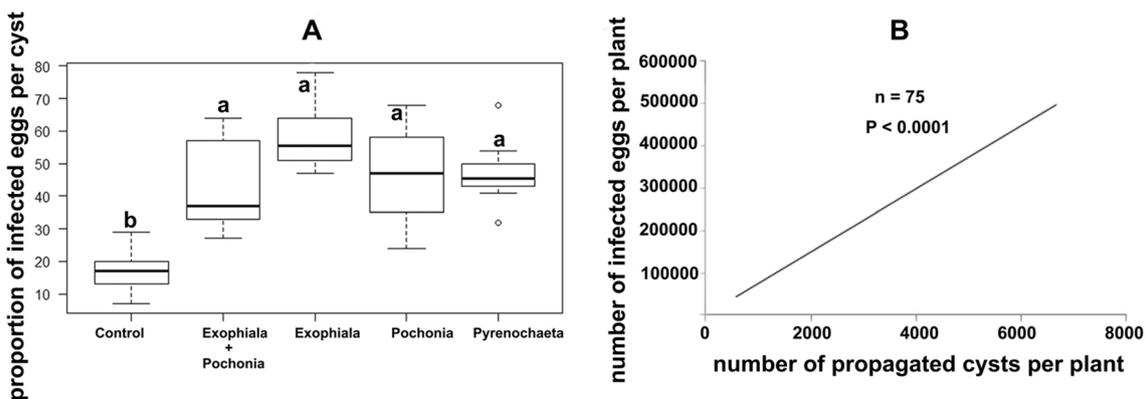


Fig. 4 Parasitic potential of fungal strains toward nematode eggs. **a** Box plots for the proportion of infected eggs per cyst of *Heterodera schachtii* at Gross Munzel that propagated for four generations on sugar beet under fungal and control treatments. Different letters indicate significant differences in numbers of propagated cysts among

treatments (Dunn test, *P* < 0.05, *n* = 15). **b** Regression line of infected eggs of *H. schachtii* with respect to the number of propagated cysts (*T*-Test, *P*-values calculated at 95% confidence level, number of pairwise combination is indicated by *n*)

on old cysts because the body wall of the gravid females is not hardened enough to protect the eggs. This can give the opportunity for the fungal parasites to attack the eggs faster (Cayrol et al. 1982; Kerry and Irving 1986).

In this experiment, cysts were not used as nematode inoculum because of the relatively high variability of egg numbers and hatching rate. The initial infection of the cysts by the fungi of interest is expected to affect the nematode hatching process, which could consequently increase the variability of the number of juveniles hatched from the infected cysts. This would then affect the final evaluation of the nematicidal activity of the target isolates.

Surprisingly, the results of this research showed that the roots of sugar beet infected with *H. schachtii* were significantly larger in fungal-treated plants compared to the control

without fungi. These effects might have been caused directly by the fungi reducing the nematode numbers in the early phases of root growth, thus reducing their negative impact on the development of the roots. Indirect effects like plant growth promotion or inducing the plant immune defense against the nematodes might have also played a role in case the fungi are able to colonize the plant roots endophytically, but were not studied here.

Nevertheless, the ITS sequences of the isolates of *Exophiala* sp. and *Pyrenochaeta* sp. were highly similar to the endophytic fungi isolated from *Paris polyphylla* and *Microthlaspi* spp., respectively (Glynou et al. 2016; Wang et al. 2013). In culture, they developed darkly pigmented and septate hyphae, which are characteristic features for dark septate endophytes (DSE). Dark septate

endophytes are ubiquitous fungi reported from the roots of a wide range of host plants and habitats (Jumpponen and Trappe 1998; Rodriguez et al. 2009). Recently, Ashrafi et al. (2018) for the first time reported a DSE parasitizing eggs of a plant parasitic nematode, namely the cereal cyst nematode *Heterodera filipjevi*. The ecological role that this group of fungi plays is not well understood. However, their mutualistic interaction with the plant hosts has been frequently reported (Mandyam et al. 2010; Newsham et al. 2009).

Some endophytic fungi were shown to produce plant hormones, which can mediate plant growth (Aly et al. 2011; Tan and Zou 2001). For example, the auxins produced by the endophytes *Trichoderma virens* or *T. atroviride* increased the biomass and accelerated the root development of *Arabidopsis thaliana* seedlings (Contreras-Cornejo et al. 2009). Recently, an endophytic isolate of the nematophagous *P. chlamydosporia* was reported to produce indol acetic acid (IAA) that promoted root growth (Schouten 2016). The fungal strains EX007 and Py004 need to be studied for further identification, but given their sequence similarity to reported endophytic strains they might play a role as endophytic fungi in enhancing plant growth and increasing the biomass. It can be hypothesized that these strains might produce a set of metabolites, which could be involved in plant growth via growth mediation, induced resistance or nematicidal activity.

The number of propagated cysts of *H. schachtii* increased with increasing biomass of plant roots. In fact, the increase of the root branching leads to an increase of potential infection sites (Curtis et al. 2009; Schouteden et al. 2015). On the other hand, the increase in root growth leads to an increase of nutrient uptake, which compensates the reduction in the root growth caused by nematode infection (Schouteden et al. 2015). This might explain the correlation between the number of cysts and root biomass we observed during this study. Cyst formation on the root colonized by endophytic nematode parasitic fungi may provide nutrition sources for those fungi (Jansson and Lopez-Llorca 2004). Therefore, the limitation in the numbers of females will limit the presence of fungal parasites (Kerry et al. 1982; Lopez-Llorca and Boag 1993). This concept could explain the positive correlation between the ratio of egg infection and the number of propagated cysts.

Under the fungal treatments in this study, fungal inoculated plants developed larger roots. This could potentially provide more feeding sites for the nematodes, which might lead to reproduction of more consecutive generations and subsequently lead to higher final nematode populations. However, the density of infected eggs increased by increasing nematode numbers. The same observations were made by Lopez-Llorca and Boag (1993), who found higher numbers of *H. avenae* juveniles in roots inoculated with *P. chlamydosporia* than in non-treated controls.

Fungi parasitic to plant parasitic nematodes may in future considerably contribute to a more sustainable agriculture. Screening for beneficial fungi antagonistic to plant parasitic nematodes should consider the ability for mass production of these fungi (Schouten 2016). The three isolated fungal strains infecting populations of *H. schachtii* were culturable and efficiently parasitized the eggs of *H. schachtii*. Consequently, the fungal isolates of *Exophiala*, *P. chlamydosporia* and *Pyrenochaeta* could be promising candidates to reduce the impact of *H. schachtii* on sugar beet.

To increase the efficiency of the fungal parasitism toward plant parasitic nematodes, the variety of the host plant has to be taken into account (Stirling 2014). On susceptible varieties, the rate of nematode reproduction will be high, especially for those species having a life cycle with several generations per season like *H. schachtii*, which can produce 3–4 generations in a growing season. This high multiplication rate tends to negate the effects of antagonists, as high levels of parasitism and predation may remove surplus nematodes but do not diminish the final nematode numbers (Stirling 2014). Using tolerant plant cultivars besides the biological control agent is therefore of importance to have a sustainable management of the plant parasitic nematodes (Stirling 2014). In this respect, identifying fungi that parasitize the eggs of *H. schachtii* and have beneficial on the root growth might be especially promising to develop a sustainable management of this plant parasitic nematode.

Author contribution

RHN: Performing the molecular assay, laboratory and greenhouse trials, analyzing and interpreting the data of the work, writing the original draft. SA: Contribution to the design and the laboratory work, reviewing and editing the writing. WM: reviewing and editing the writing. HH: Substantial contributions to the conception and design of the work, reviewing and editing the writing.

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Data availability statement All data and fungal strains of this study are available on request.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical approval This research does not contain any studies with human participants and/or animals.

Informed consent Informed consent was obtained from all co-authors included in this study.

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