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ORIGINAL ARTICLE

Effect of ectomycorrhizal fungal species on population growth and food preference of a fungivorous nematode

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Abstract

Fungivorous nematodes can use ectomycorrhizal (ECM) fungi as food resources in forest soils, and they may establish close predator–prey relationships in forest ecosystems. However, the effect of ECM fungal species on the growth of fungivorous nematodes is poorly studied. To identify fungivorous nematode propagation and preference for ECM fungi, we investigated the in vitro population growth and food attraction of the fungivorous nematode *Aphelenchoides* sp. on media with four ECM fungal species: *Cenococcum geophilum*, *Pisolithus tinctorius*, *Rhizopogon roseolus* and *Suillus granulatus*. Individual nematodes were fed on hyphae of all four ECM fungal species grown on modified Melin-Norkrans agar media. Nematode numbers were significantly lower on *P. tinctorius* than on all other fungal species. The other three species produced similar population growth rates, with *S. granulatus* producing the greatest number of nematodes at 2, 3 and 4 weeks and *C. geophilum* and *R. roseolus* producing the largest number after 8 weeks. In the histogram for nematode length classes, a unimodal pattern was fitted for *P. tinctorius* and *R. roseolus*, but a bimodal pattern was fitted for *C. geophilum* and *S. granulatus* by the Silverman test. The attraction of nematodes to *S. granulatus* was significantly higher than that to other ECM fungi. Our findings suggest that the propagation and body size of nematodes are ECM fungal species dependent. Predator–prey relationships between fungivorous nematodes and ECM fungi may accelerate nutrient cycles in forest ecosystems.

Keywords *Aphelenchoides* sp. · Body size · Ectomycorrhizal fungal isolates · Food attraction · Foraging observation · Silverman test

Introduction

Nematodes are one of the most abundant animals on Earth and a major group of soil microfauna (van den Hoogen et al. 2019). They play crucial roles in nutrient cycling, particularly of carbon and nitrogen compounds, and occupy key positions in the soil food web, contributing to ecosystem function and health (Ingham et al. 1985; Ferris 2010). Nematodes from soil communities have been classified according to their feeding habits into fungivorous, bacteriovorus, herbivorous, omnivorous and predatory species (Yeates et al. 1993). Among these, grazing by fungivorous and bacteriovorus nematodes has been suggested to enhance nitrogen mineralization, benefiting plant growth (Ingham et al. 1985; Okada and Ferris 2001; Irshad et al. 2011).

Fungivorous nematodes feed on a wide range of soil fungal species, including saprophytic, pathogenic and mycorrhizal fungi by penetrating the hyphal walls with their oral spear followed by the consumption of the cellular contents (Sutherland and Fortin 1968; Giannakis and Sanders 1990; Ruess and Dighton 1996; Ruess et al. 2000; Okada and Kadota 2003; Hasna et al. 2007). Some ectomycorrhizal (ECM) fungi are used as food resources by fungivorous nematodes (Ruess and Dighton 1996). In fact, ECM fungal species such as *Amanita rubescens* and *Suillus granulatus* have been shown to increase nematode populations more than 1000 times compared to initial inoculated numbers (Sutherland and Fortin 1968; Giannakis and Sanders 1990; Ruess et al. 2000). Food quality was suggested to affect nematode body size and thus was also a good predictor for food preference because differences in nutrient content among foods influenced the developmental level of nematodes (Yeates 1998; Liu et al. 2021). For example, nematode body size and fecundity decreased in unfavourable food

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resource environments (Liu et al. 2021). The foraging activities of nematodes depend on the intensity, concentration and composition of food sources and on the neurosensory abilities of the nematodes (Huettel 1986; Bilgrami et al. 2001). In addition, more than one fungal species can be available to a nematode in the soil environment, and the respiration and exudation from extramatrical fungal hyphae can be decisive for nematodes' feeding intensity (Cairney 2012). Thus, fungivorous nematodes commonly use ECM fungi as food resources, and these two groups may establish close predator–prey relationships.

In eastern Asian coastal regions, monocultures of Japanese black pine (*Pinus thunbergii*) forests have been established for protection against tidal intrusion and salt-bearing winds to maintain sand dune stabilization and to provide recreational opportunities (Forest Agency of Japan 2020). Most *P. thunbergii* roots in coastal forests are colonised by ECM fungi such as *Cenococcum geophilum*, which is a dominant species in the coastal pine forests (Matsuda et al. 2009; Obase et al. 2009). This colonization by ECM fungi is essential for obtaining nutrients and reducing saline stress in coastal pine forests (Obase et al. 2010; Matsuda et al. 2017).

Fungivorous nematodes such as *Aphelenchoides* spp. dominate in the coastal *P. thunbergii* forest (Kitagami et al. 2018), and ECM fungi might provide a food resource and a suited habitat for them (Ruess and Dighton 1996; Háněl 1998; Kudrin et al. 2021). However, to the best of our knowledge, little information is available on the effect of nematode feeding on ECM fungi. The crucial interactions are nematodes grazing on ECM fungi, which may restrict mycorrhizal development (Riffle 1975; Giannakis and Sanders 1990; Thakur and Geisen 2019) or promote nutrient cycling in soil systems through increased nitrogen mineralization (Ingham et al. 1985; Chen and Ferris 1999; Okada and Ferris 2001). Thus, since the direct feeding of nematodes affects the establishment of plant-fungal symbionts, knowledge on the degree of utilization and preference of nematodes for different species of ECM fungi requires in-depth studies. In this respect, the effects of ECM fungal species on the growth of fungivorous nematodes are still unclear.

The present study aimed to evaluate the preference and propagation of a fungivorous nematode feeding on various ECM fungal species that are mycobionts of *P. thunbergii*. For this, we investigated the in vitro population growth and food attraction of an *Aphelenchoides* sp. in media with four ECM fungal species. We hypothesized that (1) fungivorous nematodes can feed on *C. geophilum* as a constantly accessible food resource as *C. geophilum* is a predominant fungus in the coastal pine forests (Matsuda et al. 2009; Obase et al. 2009, 2011). Moreover, a previous study showed that the population of fungivorous nematodes was propagated in *C. geophilum* media (Ruess et al. 2000). Hypothesis: (2) The distribution of nematode body sizes differs depending on ECM fungal species similarly to what was observed for saprophytic fungal species by Yeates (1998) and Okada and Kadota (2003). Hypothesis: (3) The fungi that enhance population growth most have the highest attraction on the nematodes.

Materials and methods

Fungal and nematode isolates

The *Cenococcum geophilum* isolate (Cg_YK0120) was obtained from ECM roots from an 18-year-old coastal *P. thunbergii* forest in Machiya, Mie Prefecture, Japan (34°44 N, 136°31E) in July 2020, following Matsuda et al. (2017). Healthy and turgid ECM roots with relatively few soil particles were selected under a stereomicroscope (SZX 16, Olympus, Tokyo, Japan) at magnifications of up to 115×. The roots that were morphologically identified as *C. geophilum* were immersed in 30% H_2O_2 for 10 s and then rinsed twice with sterilised distilled water in Petri dishes. The excess water was then removed by blotting the roots dry with sterilised filter paper. ECM roots were placed on Melin-Norkrans (MMN) agar media (Marx 1969). All plate cultures were incubated at 25 °C in the dark for 3–4 weeks. Mycelia that emerged from the roots were transferred onto a new MMN agar medium. In addition, *Pisolithus tinctorius* (Pt_YK0620), *Rhizopogon roseolus* (Rr_YK0620) and *Suillus granulatus* (Sg_YK0620) isolates were obtained from fruiting bodies occurring in the coastal *P. thunbergii* forest in July 2020. Internal fungal blocks (10 mm^3) of the fruiting bodies were placed on MMN agar medium. All plates were incubated at 25 °C in the dark for 3–4 weeks, and the mycelia that emerged from the blocks were transferred onto a new MMN agar medium.

A fungivorous nematode isolate, *Aphelenchoides* sp. (Ap_YK0620), was obtained from sandy soils at a 20-cm depth in a coastal *P. thunbergii* forest in July 2020. Briefly, nematodes were extracted from 100 g of fresh soil using the modified Baermann funnel method (Ruess 1995). From these, one *Aphelenchoides* sp. culture was originally established with a single female and propagated on colonies of a pathogenic fungus, *Botrytis cinerea*, on potato dextrose agar (PDA) medium (Eiken Chemical, Tokyo, Japan).

Observation of nematode foraging on ectomycorrhizal fungal hyphae

Four fungal isolates were pre-grown on PDA medium at 25 °C in the dark. The medium was cut into 1-cm squares and placed on a glass slide. Five juveniles of *Aphelenchoides* sp. were transferred to the medium. A cover glass was then placed on the medium, and the slide was examined under a light microscope (BX53, Olympus, Tokyo, Japan; 100–400× magnification) to observe the foraging behaviour of nematodes for 5 min per individual. The

nematodes feeding on the hyphae of four ECM fungal species were recorded as image (TIFF 1920 × 1080) and video (MP4, 10 FPS, 1280 × 720) files using a digital camera (DP74, Olympus, Japan) and Olympus CellSens Standard 1.6 (Olympus Soft Imaging Solutions GmbH, Münster, Germany). The duration of hyphal feeding trials of 17 nematodes (four individuals at *C. geophilum*, five at *P. tinctorius*, five at *R. roseolus* and four at *S. granulatus*) was measured for 5 min per individual.

Population growth test

Population growth tests of *Aphelenchoides* sp. were conducted with four species of ECM fungal isolates grown on 1/10 strength PDA media containing 3.9 g PDA and agar (30 g/L). A 1-cm cork borer was used to obtain fungal inocula from the edges of two-month-old pre-cultured fungal colonies on PDA media. The inocula were then transferred into 9-cm plastic culture plates, each containing 20 mL of PDA medium. The plates were incubated in the dark for 30 days at 20 °C. After 30 days of incubation, 15 juvenile nematodes of *Aphelenchoides* sp. with body length < 500 µm were transferred near the edge of the mycelia using an insect needle under a stereomicroscope (up to 115 × magnification; SZX16, Olympus, Tokyo, Japan). Previous studies suggested that the appropriate temperature for nematode propagation was around 20 to 24 °C (Rössner and Nagel 1984; Okada and Ferris 2001). In addition, we successfully maintained the *Aphelenchoides* sp. population at 20 °C for 3 months on 1/10 strength PDA media based on our preliminary test (data not shown). Thus, we considered that cultural hyphae were able to maintain the nematode population for at least 2 months, and nematode population density and mycelial growth were monitored after 1, 2, 3, 4 and 8 weeks. At week 8, mycelial growth was measured as the mean of the largest diameter and the diameter at a right angle to that diameter; then, the initial diameter (10 mm) was subtracted to determine the net radial growth. The PDA medium was chopped into 1-cm fragments, and the nematodes were extracted using the modified Baermann funnel technique for 24 h at 25 °C (Ruess 1995). The extracted nematodes were transferred into Petri dishes filled with distilled water and counted under a stereomicroscope. There were five replicates of each fungus species per harvest. After the third week, the number of nematodes increased by more than 1000 individuals, so the samples were diluted 10- or 100-fold with distilled water before counting.

Measurement of nematode body size

After 4 weeks of incubation of the nematode population, the body size of specimens was measured. Nematodes from all five replicates were pooled to obtain a sufficient number of individuals. Some nematodes were then transferred onto a glass slide with vertical lines and killed by heat shock. The first 150 individuals, irrespective of age (juveniles and adults), encountered in each sample were measured (body length and width, in µm) under a light microscope with a polyline function implemented in Olympus CellSens Standard 1.6 (Olympus Soft Imaging Solutions GmbH, Münster, Germany). We chose size classes of nematode body length based on the Sturges' formula (Sturges 1926).

$$k = log_2 N + 1$$

where k means number of classes and N means sample size, i.e., 150 nematode individuals.

Based on the formula, nematode body lengths were divided into eight classes between 100 and 899 µm. Moreover, through the observation of nematodes, 100 to 399 µm individuals were clearly defined as sexually immature, whereas 600 to 899 µm ones were found to be clearly mature. The 400–599-µm ones were assigned into a middle class which are probably not sexually matured.

Attraction of nematodes to four ectomycorrhizal fungi

The attraction of *Aphelenchoides* sp. to the four ECM fungi was tested on 3% water agar media (9-cm diameter), following Hasna et al. (2007). All fungi were pre-grown in PDA medium at 25 °C in the dark. One-centimetre PDA discs containing mycelia from each of the four fungal cultures were placed on water agar medium, and a PDA disc without ECM fungus was used as control. Thus, five discs were placed on a water agar medium in a ring 2 cm from the central hole. Then, 100 µL of sterile water containing nematodes (242 ± 9, mean ± SE) was immediately applied in the centre of water ager medium (Fig. S1). These media were incubated at 20 °C in the dark for 24 h. Afterward, 1.7-cm-diameter discs were punched with a cork borer to retrieve individual PDA discs. The punched discs were separately used for the extraction of nematodes using the modified Baermann funnel method for 24 h at 25 °C (Ruess 1995). The agar media remaining on each plate was used to extract nematodes using the same process to retrieve the inoculated nematodes. The retrieved fraction of nematodes and the attractivity of the four fungal species were calculated as follows:

$$\begin{aligned} Received\ rate(\%) =& (total\ number\ of\ nematodes\ on\ agar\ medium) \\ & /(initially\ applied\ number\ of\ nematodes\ ,i.e.,\ 242) \times 100 \end{aligned}$$

$$\begin{aligned} Attraction(\%) =& (number\ of\ nematodes\ on\ a\ certain\ fungal\ disc) \\ & /(total\ number\ of\ nematodes\ on\ agar\ medium) \times 100 \end{aligned}$$

Statistical analyses

To compare the number of nematodes and the attraction to the four fungal species, the Steel–Dwass test was performed using the *NSM3* package (Schneider et al. 2021) in R software version 4.1.0 (R Development Core Team 2021). Moreover, the Silverman test (Silverman 1981) was performed to compare the histogram patterns of nematode body sizes among the fungal species using the *silvermantest* package (Schwaiger and Holzmann 2021) in R. To detect multimodality of histogram patterns, the test was conducted according to the null hypothesis that the underlying density has at most k modes. For all analyses, the significance level was set at $p < 0.05$, unless otherwise stated.

Results

Observation of nematodes foraging on four ectomycorrhizal fungi

Foraging of nematodes on hyphae was visually observed for all four ECM fungi (Fig. 1; Videos 1–4). When the nematodes reached the hyphal cells, they penetrated the hyphal walls with

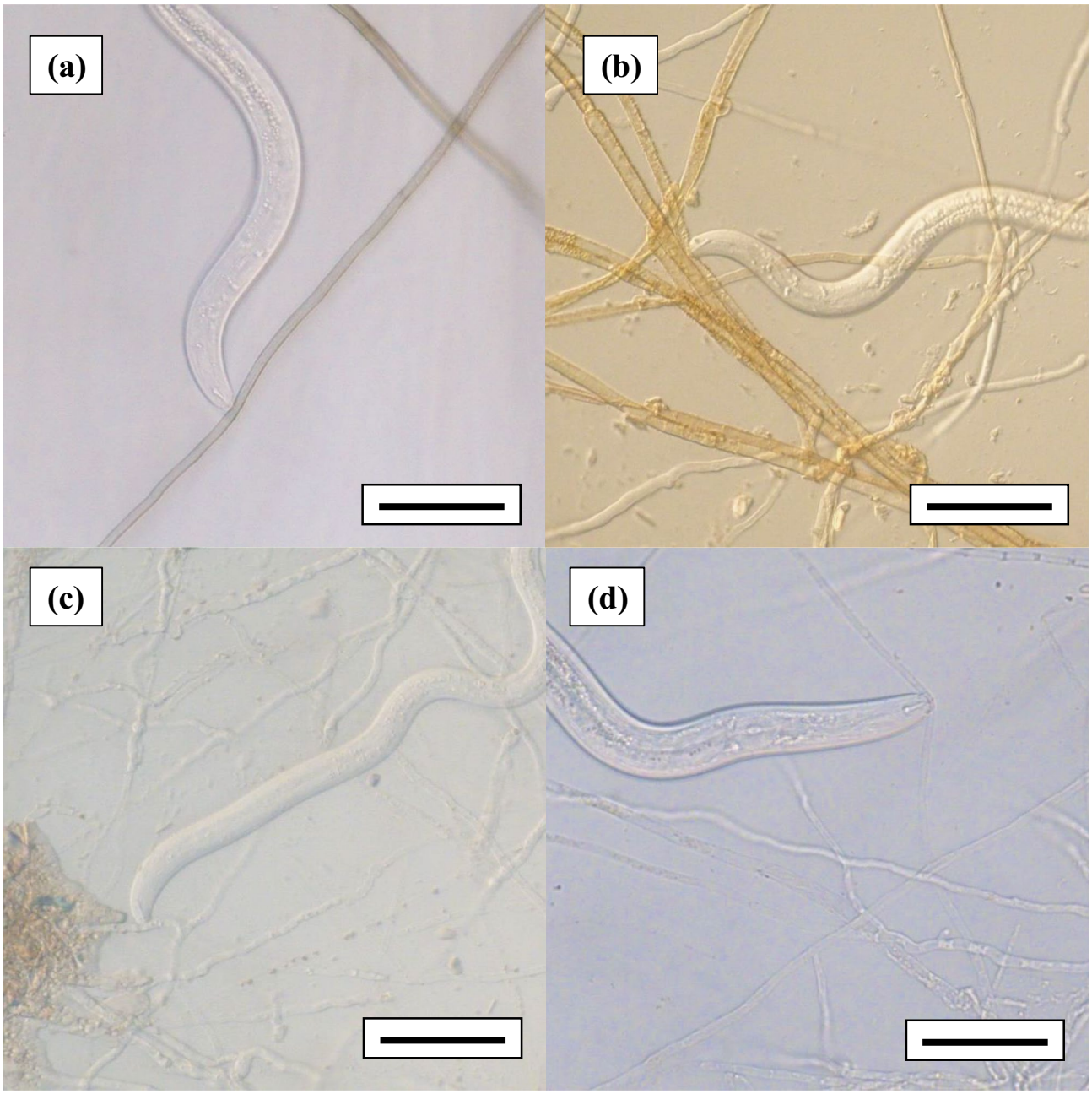

Fig. 1 An *Aphelenchoides* sp. feeding on hyphae of four ectomycorrhizal fungi. (**a**) *Cenococcum geophilum*, (**b**) *Pisolithus tinctorius*, (**c**) *Rhizopogon roseolus* and (**d**) *Suillus granulatus*. Bars indicate 50 µm

their oral spear and consumed the cellular contents. The duration of feeding trials was the shortest at 7 s for *C. geophilum* and the longest at 183 s for *P. tinctorius*.

Population growth of nematodes with four ectomycorrhizal fungi

Nematode populations were sustained under treatment with four ECM fungal species for up to 8 weeks (Fig. 2). In contrast, the control without fungi showed no increase in nematodes and that population crashed at the end of the experimental period (data not shown). The number of nematodes increased significantly with time, except for *P. tinctorius* (Table S1). In general, nematode numbers were significantly lower on *P. tinctorius* when compared to the other three fungal species, which reached similar population growth rates, with *S. granulatus* producing faster the largest number of nematodes at 2, 3 and 4 weeks and *C. geophilum* and *R. roseolus* producing the greatest number of individuals after 8 weeks.

Body size of the nematodes feeding on four ectomycorrhizal fungi

The body sizes of nematodes were 192–700 µm, 196–684 µm, 172–700 µm and 193–820 µm in length and 9–28 µm, 10–27 µm, 8–28 µm and 10–30 µm in width in *C. geophilum*, *P. tinctorius*, *R. roseolus* and *S. granulatus*, respectively. In the histogram of length classes, the smallest number of modes for which the null hypothesis of the Silverman test was fitted was $k=1$ in *P. tinctorius* (Fig. 3b, p = 0.09) and *R. roseolus* (Fig. 3c, p = 0.20). In contrast, the smallest number of modes in which the null hypothesis of the test was fitted was $k=2$ in *C. geophilum* (Fig. 3a, p = 0.20) and *S. granulatus* (Fig. 3d, p = 0.15). In the histogram of width classes, a unimodal pattern ($k=1$) was fitted for *C. geophilum* (Fig. S2a, $p=0.43$), *P. tinctorius* (Fig. S2b, $p=0.15$) and *R. roseolus* (Fig. S2c, $p=0.37$), but a bimodal pattern ($k=2$) was fitted for *S. granulatus* (Fig. S2d, $p=0.93$).

Attraction of nematodes to four ectomycorrhizal fungi

After 24 h of nematode inoculation, nematodes moved around the Petri dishes and some migrated to one of the five discs colonised by ECM fungi or without fungus (i.e. control) (Fig. 4). The mean number of recovered nematodes 1 day later was 86 ranging from 56 to 121 per plate. Thus, the retrieved rate of nematodes was 35.5 ± 2.6%. The attraction of nematodes to *S. granulatus* discs was significantly

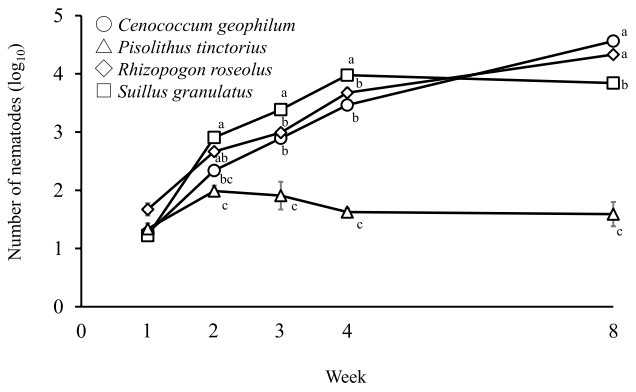

Fig. 2 Population growth of *Aphelenchoides* sp. on four ectomycorrhizal fungi. An initial number of 15 nematode individuals were inoculated per plate. Data are means ($n=5$) with standard errors. Different small letters indicate significant differences in nematode numbers among different fungi at each week (Steel–Dwass test, $p<0.05$)

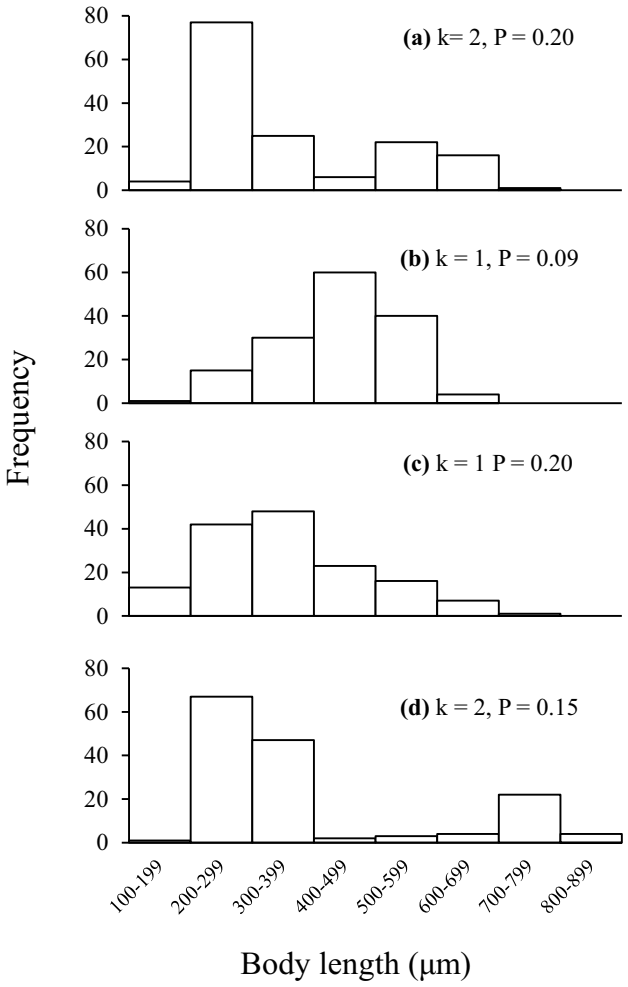

Fig. 3 Distribution of body-length classes of nematodes in four ectomycorrhizal fungi, (**a**) *Cenococcum geophilum*, (**b**) *Pisolithus tinctorius*, (**c**) *Rhizopogon roseolus* and (**d**) *Suillus granulatus*. First 150 individuals including both juveniles and adults encountered in each fungal media were measured. *k* indicates the minimum number of modes when the null hypothesis of the Silverman test is not rejected ($p>0.05$)

higher than that to the other discs (Fig. 4). In addition, the attraction of nematodes on *P. tinctorius* discs was significantly higher than that of the control (Fig. 4). However, the attraction of both *C. geophilum* and *R. roseolus* discs was not significantly different from that of the control (Fig. 4).

Discussion

Changes in population growth of nematodes foraging on four ectomycorrhizal fungi

In the present study, our hypothesis (1) was supported in that fungivorous nematodes can feed on *C. geophilum*, being a constantly accessible food resource as it is a predominant fungus in coastal pine root systems (Matsuda et al. 2009; Obase et al. 2009). The *Aphelenchoides* sp. proliferated more than 1000 times on *C. geophilum* in the eighth week experiment (Fig. 2). This agrees with previous studies in which the population of fungivorous nematodes propagated well in *C. geophilum* media (Ruess et al. 2000). Fungivorous nematodes are well known to dominate in pine forests (De Goede 1996; Háněl 2001; Kitagami et al. 2018), and most root tips of these pines in various forest ecosystems and coastal areas are colonised by *C. geophilum* (Obase et al. 2017). As such, extramatrical hyphae of this and other ECM fungi extending into soils might provide a food resource to fungivorous nematodes, improving the habitat quality for these animals (Cairney 2012; Kitagami and Matsuda 2020; Kudrin et al. 2021). Cytochemically, *C. geophilum* deposits melanin in its cell walls to tolerate water stress or turgor pressure (Fernandes and Koide 2013). Since melanization may strengthen the cell wall (Fernandes and Koide 2013), nematodes may have some physical difficulty to feed on fungal hyphae. However, nematodes feeding on hyphae were visually observed in the *C. geophilum* medium in this study (Fig. 1a, Video 1). In fact, fungivorous nematodes are known to propagate vigorously on some dark-pigmented fungi, such as *Ophiostoma minus* (Maehara and Futai 2000; Ruess et al. 2000). In addition, dark-pigmented fungi contain more carbon or nutrients and are less toxic than hyaline fungi (Maraun et al. 2003). Since fungivorous nematodes secrete cell wall–degrading enzymes, such as cellulase and chitinase, when feeding on hyphae (Karim et al. 2009), nematodes may assimilate carbohydrates by feeding on *C. geophilum*. Thus, our and previous findings suggest that *C. geophilum* supplies food resources widely to belowground fungivorous nematodes.

The nematode population also increased in the *R. roseolus* and *S. granulatus* mycelia. In previous studies, the

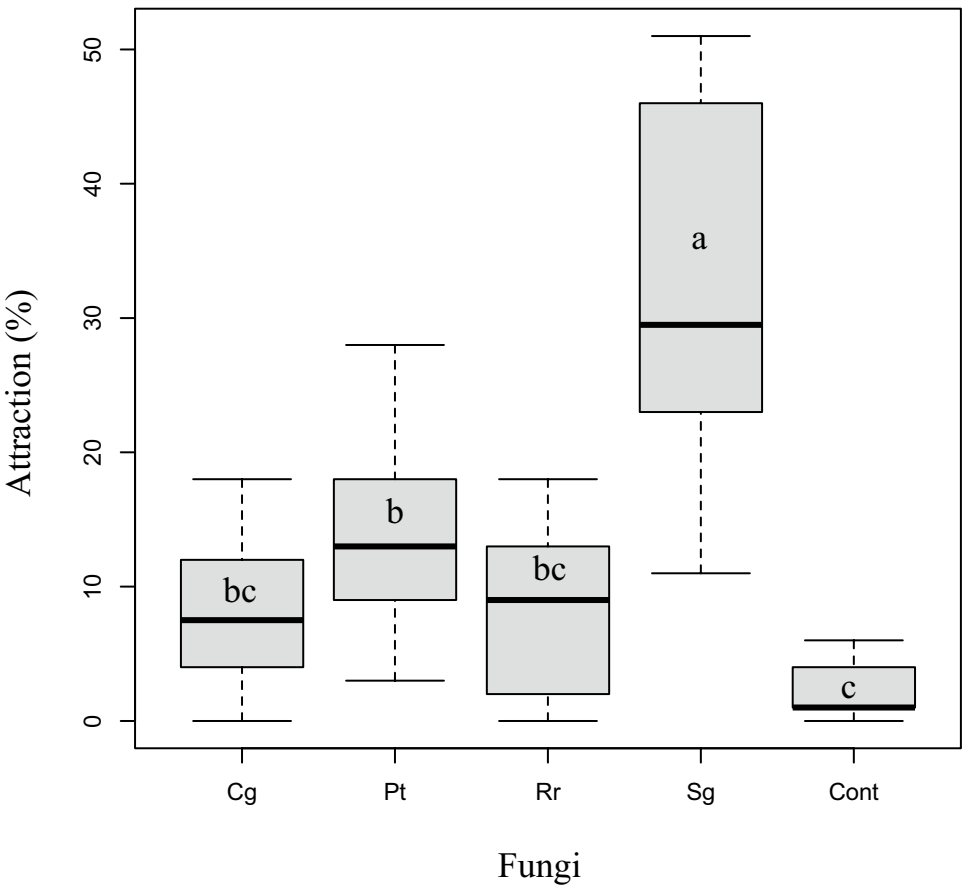

Fig. 4 Food attraction of *Aphelenchoides* sp. to four ectomycorrhizal fungi. Cg, *Cenococcum geophilum*; Pt, *Pisolithus tinctorius*; Rr, *Rhizopogon roseolus*; Sg, *Suillus granulatus*; and Cont, no fungus (i.e., control). Initial number of nematodes including both juveniles and adults was 242 ± 9 ($n = 10$, mean ± *SE*) individuals. Nematodes were extracted 24 h after inoculation. Attraction (%) = (number of nematodes in a certain fungal disc) / (number of nematodes in the medium) × 100. Median (horizontal line), first and third quartiles (bottom and top of the box) and minimum and maximum concentrations (whiskers) are shown. Different small letters indicate significant differences in attraction among different fungi (Steel–Dwass test, $p < 0.05$)

population of fungivorous nematodes also increased in the medium of these ECM fungi (Sutherland and Fortin 1968; Giannakis and Sanders 1990). Thus, they can be used as food sources for nematodes in forest soils. In our study, the number of nematodes increased most on *S. granulatus* plates during the first 4 weeks (Fig. 2). However, population growth of nematodes tended to decrease on *S. granulatus* in the last 4 weeks of the experiment (Table S1). The radial growth of hyphae of *S. granulatus* was greatly reduced (Fig. S3). Moreover, visual observation of fungal cultures showed that its aerial hyphae were almost completely consumed after 4 weeks (Fig. S4). Thus, the hyphae on the medium may be eaten so quickly by nematodes, making it difficult to maintain the mycelium. An asset of fungivorous nematodes could be their ability to switch from one food resource to another depending on availability (Ikonen 2001). In this respect, fungivorous nematode populations can increase quickly owing to their rapid intrinsic population growth when feeding on their preferred food sources (Hofman and S'Jacob 1989). Spatial and temporal variations in nematode population densities may be partly explained by the influence of different food resources on population growth patterns. *Rhizopogon roseolus* and *S. granulatus* have the capacity to remain in soils as spore banks, which are potential fungal inocula for the colonization of fine roots (Glassman et al. 2015). Since the two species are well-known as pioneer ECM fungi (i.e. appearing early in successional series), they are likely to colonise newly recruited host roots earlier than other ECM taxa (Nara 2009). Fungivorous *Aphelenchoides* spp. and *Ditylenchus* spp. were predominant in pine forest soils in the early succession series (De Goede et al. 1993). Grazing of fungal hyphae by fungivorous nematodes has been suggested to enhance nitrogen mineralization in soils (Ingham et al. 1985; Chen and Ferris 1999; Okada and Ferris 2001). Since the early stages in forest ecosystem development are likely to occur in oligotrophic conditions with little organic matter, fungivorous nematodes can accelerate nutrient cycling in this stage by feeding on pioneer ECM fungi.

The nematode population increased slightly in the *P. tinctorius* medium and then decreased after the third week (Fig. 2). Some saprophytic fungi are known to have secretory cells within their hyphae, producing toxins with an anti-feeding function (Tayyrov et al. 2018). For example, hyphae of *Coprinopsis cinerea* produce toxic substances that can kill nematodes upon contact (Schmieder et al. 2019). In this study, yellowish-brown pigmentation around mycelial fronts was confirmed on *P. tinctorius* media. Although the toxicity of the yellowish-brown pigment of the isolate to nematodes is unclear, a yellow pigment called aurovertins produced by the pathogenic fungus *Pochonia chlamydosporia* showed strong toxicity toward the root-knot nematode *Meloidogyne incognita* (Wang et al. 2015). The aurovertins also exerted profound and detrimental effects on the viability of the model nematode *Caenorhabditis elegans* (Wang et al. 2015). *Pisolithus* ECM roots have been suggested to protect host plants against various soil-borne pathogens by producing secondary metabolites (Marx 1972; Kope 1992). In fact, *P. tinctorius* hyphae contain pisosterol and several triterpenoids (Baumert et al. 1997). Moreover, *P. tinctorius* was grazed less than other ECM fungi by collembolans in pot cultures of loblolly pine (Hiol Hiol et al. 1994). Thus, pigment deposition and the production of other secondary metabolites by *P. tinctorius* hyphae may regulate the propagation of nematodes.

Variations of nematode body sizes foraging on four ectomycorrhizal fungi

Our hypothesis (2) was supported in that the distribution of nematode body sizes differs depending on ECM fungal species. The body size distribution of nematodes differed among the four ECM fungal species. For *C. geophilum* and *S. granulatus*, a bimodal pattern with peaks in both small and large nematode individuals was evident (Fig. 3). This pattern might explain the production of next-generation was well. In fact, a large number of nematode eggs were found on *S. granulatus* cultures in the fourth week (Fig. S5). In contrast, *P. tinctorius* and *R. roseolus* showed a unimodal pattern with evident peaks in intermediate-sized individuals (Fig. 3). For *P. tinctorius*, the number of nematodes with newly hatched individuals (200–300 µm) was negligible. According to Monoson (1971), body mass variation can differ when various fungi are used as food source. Yeates (1998) also reported a variation in nematode body length (597–797 µm for *Aphelenchus avenae*) when nematodes were fed with the pathogenic and saprobic fungi *Penicillium oxalicum*, *P. nigricans*, *Sordaria destruens* and *Ustilago zeae*. Nematode body size was suggested to be greater with suitable food resources, leading to an increased nematode population growth rate (Okada and Kadota 2003). Moreover, the feeding on saprophytic and ECM fungal species was shown to affect the fatty acid profile of nematodes (Chen et al. 2001; Ruess et al. 2002); therefore, differences in nutrient content among fungal food sources may influence the developmental level of nematodes. In the case of bacteriovorus nematodes, a 60% difference in the body volume of nematodes was noted when two bacterial strains of *Escherichia coli* were used as food (So et al. 2012). Although the number of nematode cells did not change, the size of the cells and protein content were altered between the two strains (So et al. 2012). Thus, each ECM fungus affects the body size of nematodes differently, suggesting that suitable food sources enhance body size and fecundity.

Food preference of a fungivorous nematode foraging on ectomycorrhizal fungi

Our hypothesis (3) that the fungi that promote population growth most strongly should also attract most nematodes was supported. The present study clearly showed that *Aphelenchoides* sp. was more attracted to *S. granulatus* than to the other fungal species tested (Fig. 4). The population growth of nematodes was highest on *S. granulatus* plates during the first 4 weeks (Fig. 2). Thus, the results from our attraction and population growth tests indicate that the attraction intensity of a fungus as a food source can be related to its preference as a host of fungivorous nematodes.

Nematodes are attracted to root exudates (Ali et al. 2010) or to volatile compounds released by insect vectors (Zhao et al. 2007). Moreover, volatile substances of the nematophagous fungus *Esteya vermicola* have been shown to attract neighbouring nematodes (Wang et al. 2010). *Suillus variegatus* isolated from Scots pine roots showed a high exudation rate of acetic acid (Bäck et al. 2010). Moreover, root-knot nematodes have been shown to be attracted to acetic acid (Wang et al. 2009). Thus, nematodes may reach food resources in response to volatile organic compounds derived from fungal mycelia. Moreover, since nematode attractants are also produced by themselves (Perry 2005), such attractants can facilitate the aggregation of nematodes.

Soil nematodes are estimated to be as many as 1.84×10^{19} individuals in temperate coniferous forests (van den Hoogen et al. 2019). Among them, fungivorous nematodes account for approximately 20% (van den Hoogen et al. 2019), and ECM plants are a dominant mycorrhizal type in temperate regions (Soudzilovskaia et al. 2019). Thus, ECM fungi may contribute to maintaining fungivorous nematode populations in temperate forest ecosystems. Our present study showed the predator–prey relationships of *Aphelenchoides* sp. and four ECM fungi and that such relationships could be more prevalent than we are aware. However, all of our findings were not performed under field conditions, where hyphae grow three dimensionally in soils and nutrient distribution is more heterogeneous compared with controlled medium conditions. Thus, to unveil the feeding preferences of fungivorous nematodes on various ECM fungi in forest ecosystems, these effects should also be investigated on ECM-inoculated plants and at a field scale.

Conclusion

Our study revealed that four ECM fungi associated with *P. thunbergii* affect the population growth of the fungivorous nematode *Aphelenchoides* sp. The nematode was found to propagate well by feeding on three mycorrhizal fungi, *C. geophilum*, *R. roseolus* and *S. granulatus* and to show an attraction towards *S. granulatus*. Our study suggests that nematodes possibly utilise ECM fungi as preferable food resources in *P. thunbergii* coastal forests. Differential ECM fungal grazing by nematodes can alter ECM fungal community structures and further influence the stability and maintenance of ecosystem functioning in various forests.

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Author contribution YK and YM conceived the study, YK carried out the laboratory work and analysed data, YK wrote the first draft and all authors contributed to the manuscript.

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Declarations

Competing interests The authors declare no competing interests.

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