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The effect of soil on the efficacy of a nematode-based biopesticide of slugs

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HIGHLIGHTS

- Soil type may affect the field efficacy of slug parasitic nematodes.
- Nematode survival and the ability to kills slugs was best in compost.
- Surprisingly, nematodes reproduced in many soils potentially due to bacterial colonisation.
- Soil type should be classified before nematode application.
- Use of compost should be encouraged to enhance nematode survival.

GRAPHICAL ABSTRACT

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ABSTRACT

Several slug species are serious pests of agriculture and are difficult to control. One popular control method is the nematode *Phasmarhabditis hermaphrodita,* which has been used in slug control for > 25 years. However, there are reports of it failing to reduce slug numbers and damage in the field for unknown reasons. This may be due to lack of knowledge about how *P. hermaphrodita* performs when applied to different soils. We therefore assessed the survival, movement and pathogenicity of *P. hermaphrodita* infective juveniles (IJs) when added to six different soils (compost with and without peat, clay loam, loam, sandy loam and sandy soil). The soils were either frozen or autoclaved before use to eradicate resident nematodes prior to the experiment. *P. hermaphrodita* survived best in autoclaved compost without peat and in experiments with frozen soils, compost with and without peat was best. Survival of *P. hermaphrodita* was similar in other soils. Interestingly, in peat-free compost *P. hermaphrodita* reproduced prolifically, which may affect the long-term success of the nematode in the field as other life stages, apart from the IJ stage, cannot infect slugs. In infection experiments we found *P. hermaphrodita* added to compost with peat killed slugs faster than nematodes added to a sandy clay loam or sandy soil. In movement experiments, the nematodes remained within 3 cm of the application point in each soil. In summary, soil type severely affects *P. hermaphrodita* survival, and the ability to kill slugs; therefore it should be assessed by farmers and gardeners before use.

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1. Introduction

Several slug species cause significant losses to agricultural and horticultural crops (Barua et al., 2021), mainly by feeding on leaves (South, 1992), contaminating plants with faeces and mucus, which not only reduces crop value but also can impair machinery (Wilson and Thomas, 2017). In the United Kingdom alone it has been estimated that slugs would cause approximately £43.5 million worth of damage annually if not controlled (Nicholls, 2014). The main control method in the U.K. was the molluscicide metaldehyde (Garthwaite et al., 1996), which has been shown to be harmful to non-target organisms such as dogs, cats and cattle (Teichmann-Knorrn et al., 2017; Castle et al., 2017) and has subsequently been banned. Alternative slug control methods include iron phosphate pellets, baits (Barua et al., 2021) and the parasitic nematodes *Phasmarhabditis hermaphrodita* and *P. californica*, which kill several pestiferous species and have been developed as biological control agents (Nemaslug® and Nemaslug 2.0® from BASF Agricultural Specialities) (Wilson et al., 1993; Rae et al., 2023) for use across northern Europe. *Phasmarhabditis hermaphrodita* was released in 1994 and can provide equivalent levels of protection as metaldehyde (Glen et al., 1996; Grewal et al., 2001), and is not harmful to non-target organisms like earthworms (Cutler and Rae, 2022; Grewal and Grewal, 2003). Nematodes are mixed with water and applied to soil at the recommended rate of 300,000 nematodes per m^2 (Grewal et al., 2005). The nematodes seek out slugs in the soil and, on discovery, penetrate through the back of the mantle, move into the shell cavity, and kill the slug in 4–21 days (Wilson et al., 1993); (Tan and Grewal, 2001) . The nematodes then feed on the bacteria proliferating on the decomposing cadaver and reproduce until the resources are depleted and they will then develop into infective juveniles (IJ) and search for more slugs in the soil (Wilson et al., 1993). As a biological control, *P. hermaphrodita* is able to provide protection of plants against slugs in two ways. First, susceptible slug species e.g. *Deroceras reticulatum* are killed by *P. hermaphrodita* and second, other species such as *Arion hortensis*, *A. subfuscus* and *Limax maximus,* are not killed by the nematode but their feeding is severely inhibited (Grewal et al., 2003). In field studies using various crops, *P. hermaphrodita* has been shown to provide protection from slug damage in asparagus (Ester et al., 2003), lettuce (Wilson et al., 1995) and winter wheat (Wilson et al., 1994). However, there are some studies that have recorded failure of *P. hermaphrodita* in providing slug protection or reducing slug numbers (Iglesias et al., 2001; Iglesias et al., 2003; Rae et al., 2009). One of the reasons for the lack of efficacy of *P. hermaphrodita* in the field is due to the presence of adult *Arion lusitanicus*, which are not killed by the nematode (Grimm, 2002). Furthermore, the effect of abiotic and biotic factors (e.g. soil type, temperature, moisture and predators such as mites and collembola) that can severely affect the success of nematode biological control agents in controlling pests (Campos-Herrera, 2015); but is understudied for *P. hermaphrodita.*

There is very little information about how cropping systems, cover crops and practices such as mulching may affect nematode efficacy or even how soil type can affect survival, pathogenicity or movement of *P. hermaphrodita.* Therefore, we assessed the effect of six different diverse soils (compost with and without peat, clay loam, loam, sandy loam and sandy soil) on the survival, pathogenicity and movement of *P. hermaphrodita*, as possible reasons for variable reports in field efficacy of *P. hermaphrodita*.

2. Materials and methods

2.1. Source of soils and nematodes

Six different soil types were used, which were collected from two Royal Horticultural Society (RHS) gardens (Harlow Carr in Harrogate and Wisley in Woking). At each RHS garden there were two sample areas, one from an established garden bed and one collected from under an area of turf. The soils used were: 1. Compost with peat 2. Compost without peat 3. Sandy clay loam from a garden bed from Harlow Carr 4. Sandy clay loam from under turf from Harlow Carr 5. Sandy loam from garden bed from Wisley 6. Sandy soil from under turf from Wisley. Compost (with peat and without) was purchased from local garden centres. To prevent seasonal variance of soil structure and composition, the samples were collected mid-November 2018 and again in mid-November 2019. Fresh samples of soil were used for each repetition of the experiment.

P. hermaphrodita (Nemaslug®, strain DMG0001) was provided by BASF Agricultural Specialities and stored at 10 °C until use. Nematodes were used within one month of arrival. *P. hermaphrodita* strain M2 was grown on rotting slug in White traps to the infective juvenile stage and stored at 10 °C until use (Andrus and Rae, 2019).

2.2. Assessing the effect of different soils and temperatures on the survival of P. hermaphrodita

Soils were either autoclaved at 121°C for 15 mins with a cooling rate of 40 min at 80 °C or frozen at −20 °C for 5 days to eliminate any resident nematodes (unpublished observation). Both approaches were used as autoclaving can affect the physical structure of soil (Berns et al., 2008; Tanaka et al., 2003) and freezing soils represented more realistic natural conditions. After autoclaving and freezing, the soils were rewetted to 10–15 % water content.

Fifteen 5 cm Petri dishes were filled to the lip with each soil. To each Petri dish 2,000*P. hermaphrodita* IJs were applied and incubated at 5, 10 or 15 °C. After 3, 6, 12, 24 and 48 days the nematodes were extracted from 3 separate Petri dishes and the numbers of live infective stage and non-infective stage nematodes were quantified. The whole experiment was repeated twice. As soil moisture affects nematode survival (Grewal and Grewal, 2003), the moisture content of each soil was checked twice a week over 48 days using a Xiaomi flower care monitoring system. If moisture was < 15 %, the soil was misted until it reached 15 % moisture content. Each Petri dish was sealed with Parafilm® to water loss and kept in airtight sealed containers.

To quantify live nematodes, soil from individual Petri dishes was added to 50 ml Falcon tubes and then half filled with tap water. The Falcon tubes were then shaken vigorously for 2 mins and three 1 ml subsamples were pipetted into a 5 cm Petri dish with a grid on the bottom and total population in the Falcon tube calculated. This technique uses a similar method as Circular Estimate Method developed as a simple method to estimate *Caenorhabditis elegans* culture densities in liquid medium (Josende et al., 2019). This process was repeated for each of the three Petri dishes used on each time point.

2.3. Infection assay to test the effects of soils on the pathogenicity of nematodes

Deroceras invadens was chosen as a suitable slug host as it is highly pestiferous, with a worldwide distribution and commonly found in the Merseyside area (Cutler and Rae, 2020). *D. invadens* (>0.10 g and > 2.5 cm) were collected from a garden in Maghull, Liverpool (OS grid reference SD373027), and stored in non-airtight containers and fed lettuce or carrot *ad libitum*. Before the experiment began slugs were examined for any signs of nematode infection e.g. swollen mantle, lesions on the cuticle, and if they displayed these symptoms they were discarded.

To test the pathogenicity of *P. hermaphrodita* a standard protocol was followed (Cutler and Rae, 2020). Briefly, 30 ml universal tubes were filled to a level of 3.5 cm with each soil type. The soil types varied in composition and weight therefore the level of 3.5 cm was used to enable controlled comparisons. Eighteen universal bottles were used for each soil and were split into 9 used for studying the survival of slugs exposed to nematodes and the other 9 were used as untreated controls, with slugs added but with no nematodes, just water. To half of the universal bottles 1000 *P. hermaphrodita* MG2 were added to the soil. This wild strain of

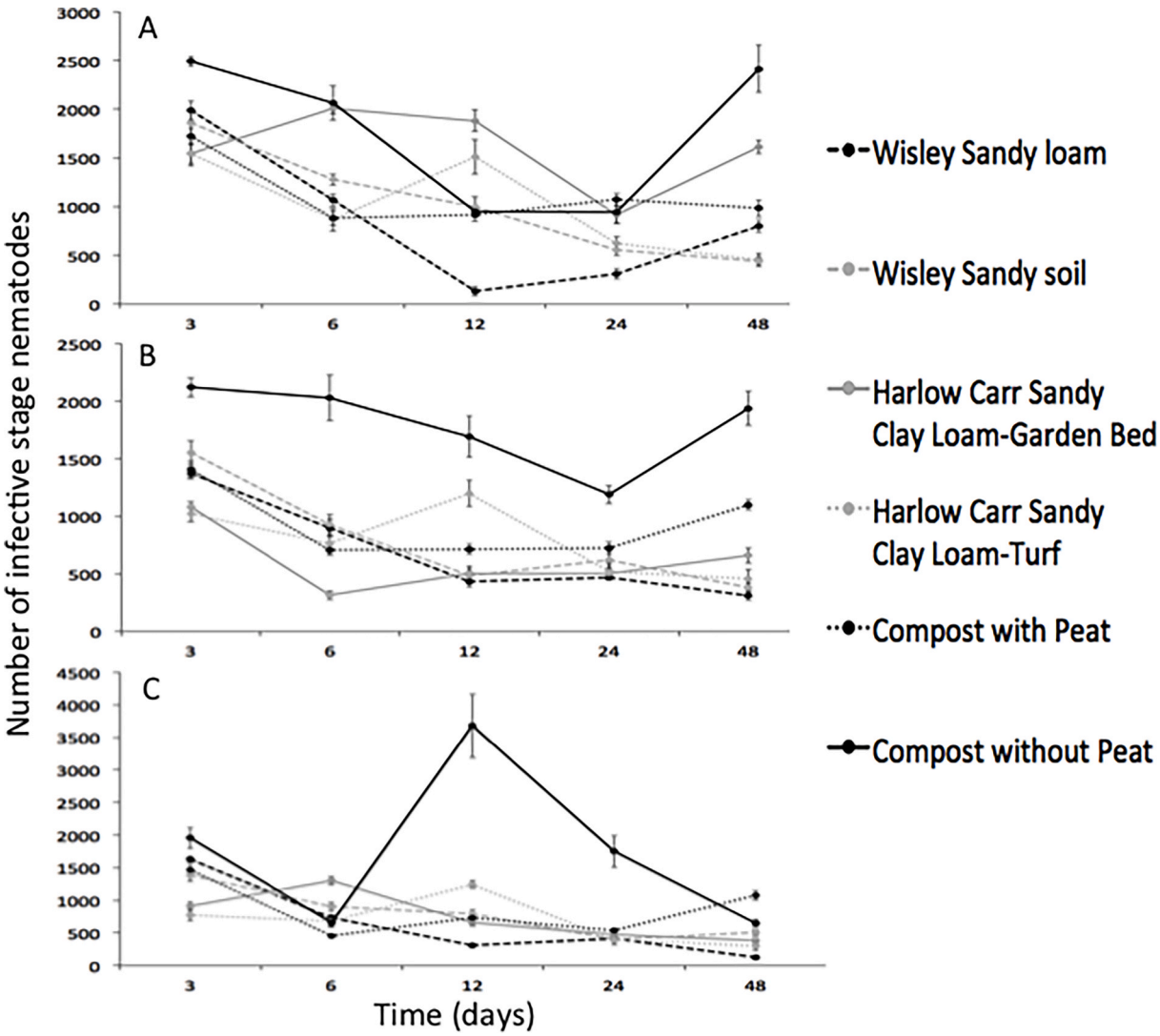

Fig. 1. The mean number of *P. hermaphrodita* IJs added to six different previously autoclaved soils including Wisley sandy loam from a garden bed (long dash black line), Wisley sandy soil from under turf (long dash grey line), Harlow Carr sandy clay loam from a garden bed (solid grey line), Harlow Carr sandy clay loam from under turf (short dash grey line), compost with peat (short dash black line) and compost without peat (solid black line) at 5 °C (A), 10 °C (B) and 15 °C (C) over 48 days (mean ± SE).

P. hermaphrodita was used as in our previous experiments it was more pathogenic than the commercial strain (*P. hermaphrodita* DMG0001) [see ref. 28]. Two *D. invadens* were added (mean weight 0.20 g ± 0.031) to each universal bottle and a piece of moist cotton wool was added on top and the lid loosely placed on top and stored at 10 °C for 5 days. After this, slugs were removed and individually placed on 5 cm Petri dishes with pre-moistened filter paper and a disc of lettuce (3.5 cm in diameter). The survival of the slugs was monitored and after 10 days the amount the slugs had eaten was quantified by tracing the remnants of the lettuce onto 1 x 1 mm^2 graph paper (Rae et al., 2009).

2.4. Movement of *P. hermaphrodita* through six different soils with *D. reticulatum* as an attractant

Plastic 50 ml Falcon tubes were cut into three sections (0 to 3.5 cm, 3.5 to 7 cm and 7 to 9.5 cm), placed on their side and half filled with one of six soils used in the previous experiment to a height of 1.5 cm. To the first section 2,000 *P. hermaphrodita* (DMG0001) IJs were added in 1 ml of water to the top of the soil. Two slugs (*D. reticulatum*) were added to the third section as an attractant for the nematodes (MacMillan et al., 2009) and a disc of lettuce and carrot was also added. A layer of fine netting was added to prevent the slugs from moving into the other sections. The controls for the experiment included the same set-up with lettuce and carrot discs added but no slugs were placed in the tube. All sections were securely fitted back together using Parafilm®. The tubes were stored in an incubator set at 15 °C for 7 days.

Soil moisture was monitored using a Xiaomi Flower Care monitoring system. After 7 days, the sections were separated and the soil from each section was placed in individual 50 ml Falcon tubes. Fifty mls of tap water added, the mixture was homogenised using a vortexer and three 1 ml samples were removed and the numbers of nematodes was quantified using a dissecting microscope. Counts of nematodes were calculated as the total number of nematodes per 50 ml (by multiplying the average in 3 mls by 50). There were 6 tubes for each of the 6 soils (3 with nematodes, 3 without) and the whole experiment was repeated 3 times.

2.5. Data analysis

A Generalised Linear (Poisson loglinear) Model (GLM) was used to compare the survival of either infective stage or non-infective stage nematodes. Predictors were: soil type, soil treatment (frozen vs. autoclaved), nematode type (infective vs non-infective), time (3, 6, 12, 24

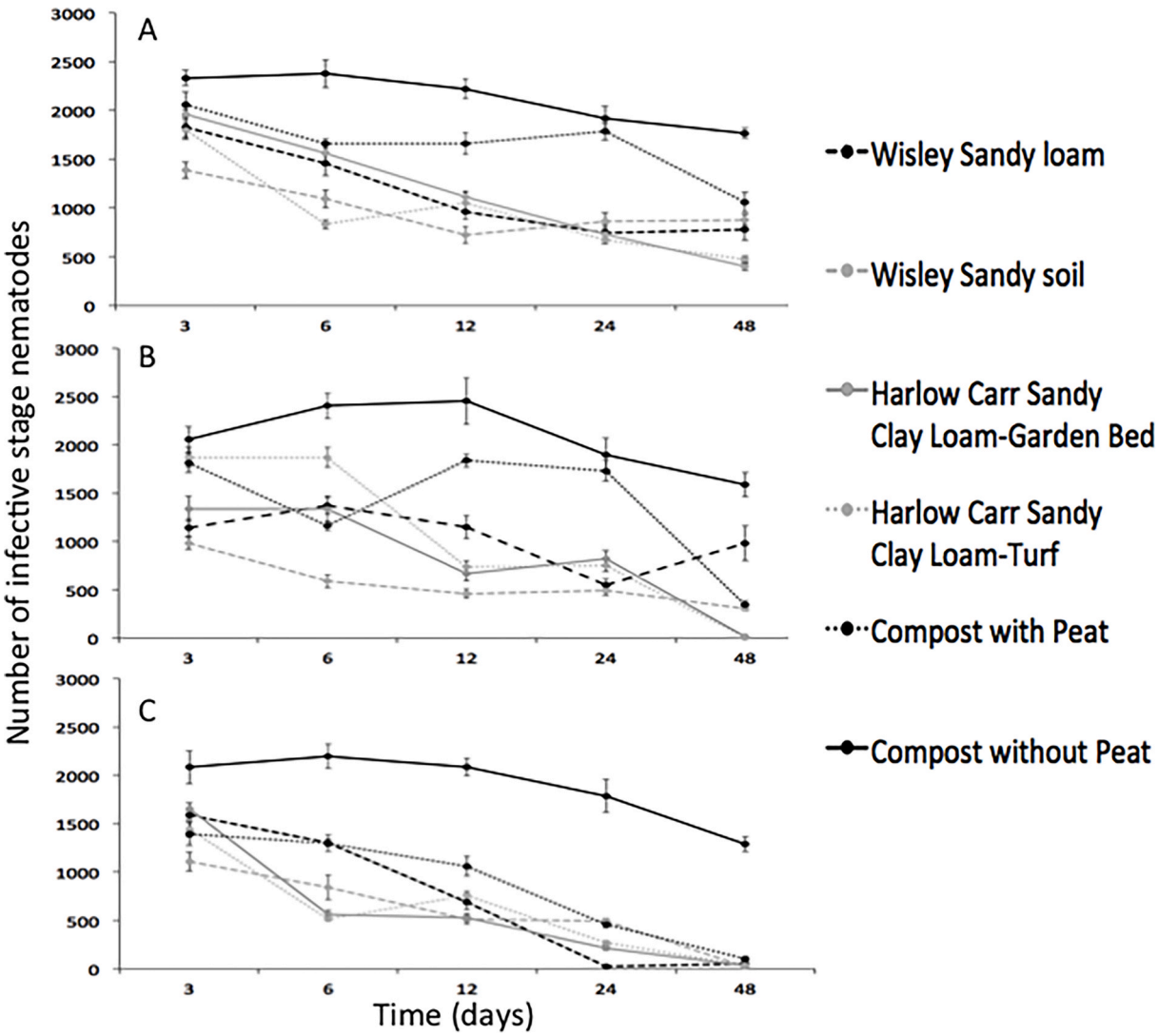

Fig. 2. The mean number of infective stage *P. hermaphrodita* added to six different previously frozen soils including Wisley sandy loam from a garden bed (long dash black line), Wisley sandy soil from under turf (long dash grey line), Harlow Carr sandy clay loam from a garden bed (solid grey line), Harlow Carr sandy clay loam from under turf (short dash grey line), compost with peat (short dash black line) and compost without peat (solid black line) at 5 °C (A), 10 °C (B) and 15 °C (C) over 48 days (mean ± SE).

and 48 days), and temperature (5, 10 or 15 °C) with a ful factorial design.

Survival of *D. invadens* exposed to *P. hermaphrodita* added to the six different soils was compared using a Log Rank test in OASIS (Yang et al., 2011). The number of 1 x 1 mm^2 squares of lettuce the slugs ate was compared using a One Way ANOVA and Tukey's post hoc test.

A Kruskal-Wallis test was used to compare the numbers of *P. hermaphrodita* found in sections 0 to 3.5 cm, 3.5 to 7 cm and 7 to 9.5 cm in each of the six soils with and without a slug added.

3. Results

3.1. Survival of P. hermaphrodita IJs in six different soils (previously autoclaved or frozen) incubated at 5, 10 and 15 °C over 48 days

A Generalised Linear Model (GLM) with a Poisson distribution and log link function was used to model the survival of infective juvenile *P. hermaphrodita* over 48 days based on soil, time, temperature and whether soils had been autoclaved or frozen. The model fit the data well (Goodness-of-fit statistics: Deviance/df = 67.241, Person Chi-Square/df = 65.892, AIC = 110766.047) and the Omnibus test was significant ($\chi^2(179) = 668533.193$, $p < 0.001$) (Supplementary Table 1). The individual predictors were significant predictors of nematode survival, including soil ($P < 0.001$), time ($P < 0.001$), temperature ($P < 0.001$) and whether the soil was autoclaved or frozen ($P < 0.001$) (Figs. 1 and 2). The intercept of the model was significant ($B = 7.162$, $P < 0.001$). Specifically, soil type significantly affected nematode survival with compost (without peat) providing the best substrate for nematode survival compared to the other 5 soils (Figs. 1 and 2). The poorer soils for nematode survival were the sandy loam and sandy soil from Wisley in both autoclaved and frozen soils.

3.2. Numbers of non-infective stage P. hermaphrodita in six different soils (previously autoclaved or frozen) incubated at 5, 10 and 15 °C over 48 days

Surprisingly, when counting the number of nematodes at each time point in the soils at 5, 10 and 15 °C it was clear the nematodes had moulted, exited the IJ stage and had begun to reproduce, as numerous other life stages were present in the soils (Figs. 3, 4). To understand this further a GLM was used with the same parameters as above. The model fit the data well (Goodness-of-fit statistics: deviance/df = 41.948,

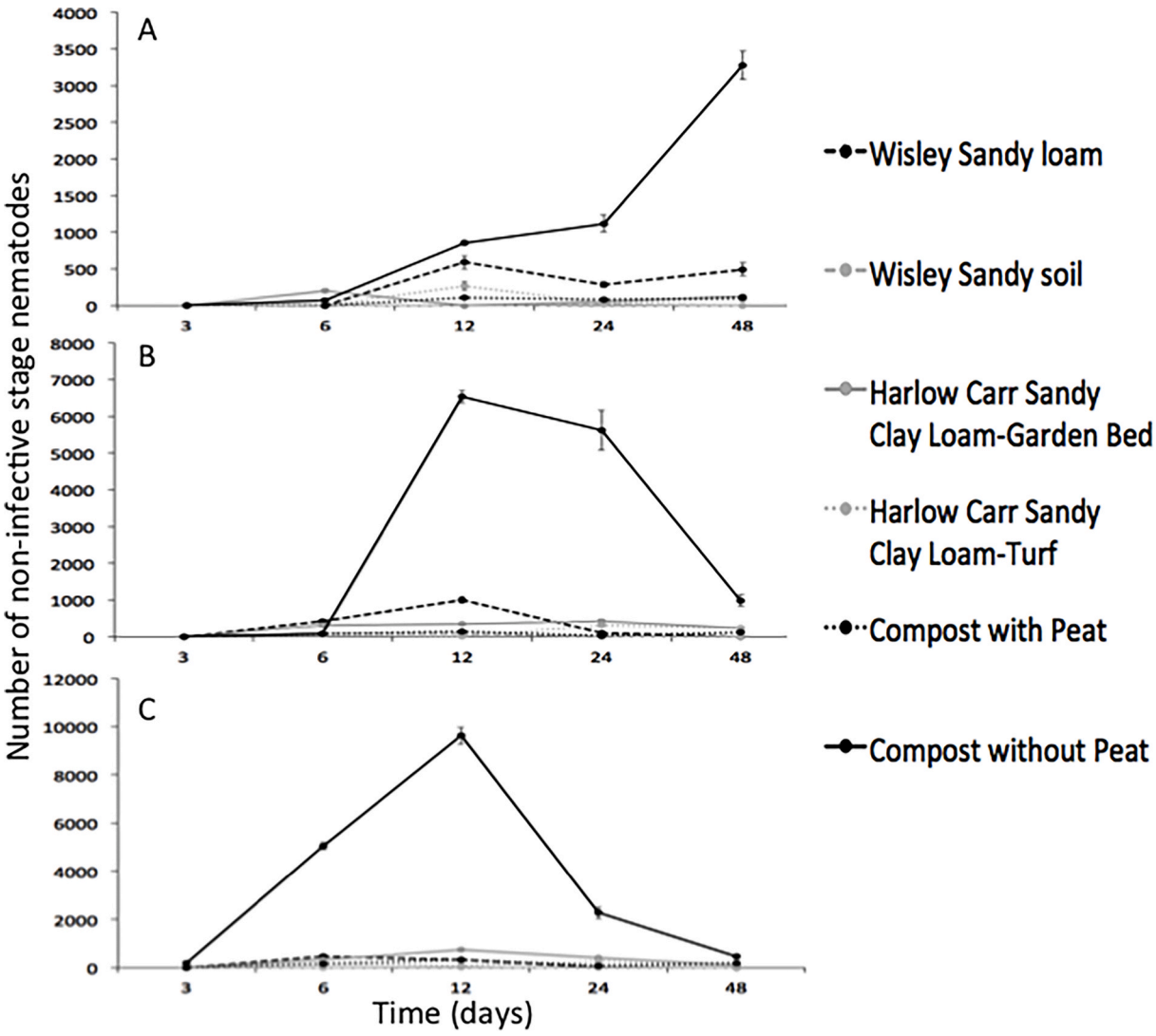

Fig. 3. The mean number of non-infective stage *P. hermaphrodita* added to six different previously autoclaved soils including Wisley sandy loam from a garden bed (long dash black line), Wisley sandy soil from under turf (long dash grey line), Harlow Carr sandy clay loam from a garden bed (solid grey line), Harlow Carr sandy clay loam from under turf (short dash grey line), compost with peat (short dash black line) and compost without peat (solid black line) at 5 °C (A), 10 °C (B) and 15 °C (C) over 48 days (mean ± SE).

Person Chi-Square/df = 41.316, AIC = 66414.666) and the Omnibus test was significant ($\chi^2(179) = 1809840.126$, $p < 0.001$) (Supplementary Table 2). All individual predictors were significant predictors of non-infective stage nematode survival, including soil ($P < 0.001$), time ($P < 0.001$), temperature ($P < 0.001$) and whether the soil was autoclaved or frozen ($P < 0.001$) (Figs. 3, 4; Supplementary Table 2). The intercept of the model was significant ($B = 2.813$, $P < 0.001$). Specifically, the nematodes reproduced prolifically in peat free compost (previously autoclaved) (Fig. 3) where nematode numbers increased rapidly over time ($P < 0.001$) (Fig. 3A-C). However, in frozen soils the numbers of non-infective stage nematodes fluctuated dramatically and differed significantly with soil type at 5 °C ($P < 0.001$) (Fig. 4). For example, the numbers of non-infective nematodes was highest in sandy clay loam soil from a garden bed in Harlow Carr (compared to all other soils). Unlike in autoclaved soils, the numbers of non-infective stage nematodes in peat free compost was negligible and produced the lowest number of nematodes. Other soils that were particularly poor for *P. hermaphrodita* to exit the IJ stage include the autoclaved sandy soil and sandy loam from Wisley.

3.3. Survival of slugs exposed to P. hermaphrodita MG2 in six different soils

The addition of *P. hermaphrodita* MG2 to each of the 6 soils resulted in significantly more slugs dying (Fig. 5A) compared to the untreated control (Fig. 5B) over 14 days ($P < 0.05$). *Phasmarhabditis hermaphrodita* MG2 added to compost with peat resulted in *D. invadens* dying faster than slugs added to sandy clay loam from under turf (from Harlow Carr) and sandy soil from under turf (from Wisley) ($P < 0.05$; Fig. 5A). There was no significant difference in the survival of *D. invadens* exposed to water (untreated control) in the different soils over 15 days (Fig. 5B).

Exposure of *P. hermaphrodita* MG2 to *D. invadens* resulted in severe feeding inhibition with the number of 1 x 1 mm^2 squares of lettuce eaten being significantly different from the untreated control for each soil ($P < 0.05$; Fig. 6). There was no difference in the number of 1 x 1 mm^2 squares eaten by slugs in the six different soils with nematodes ($P > 0.05$; Fig. 6).

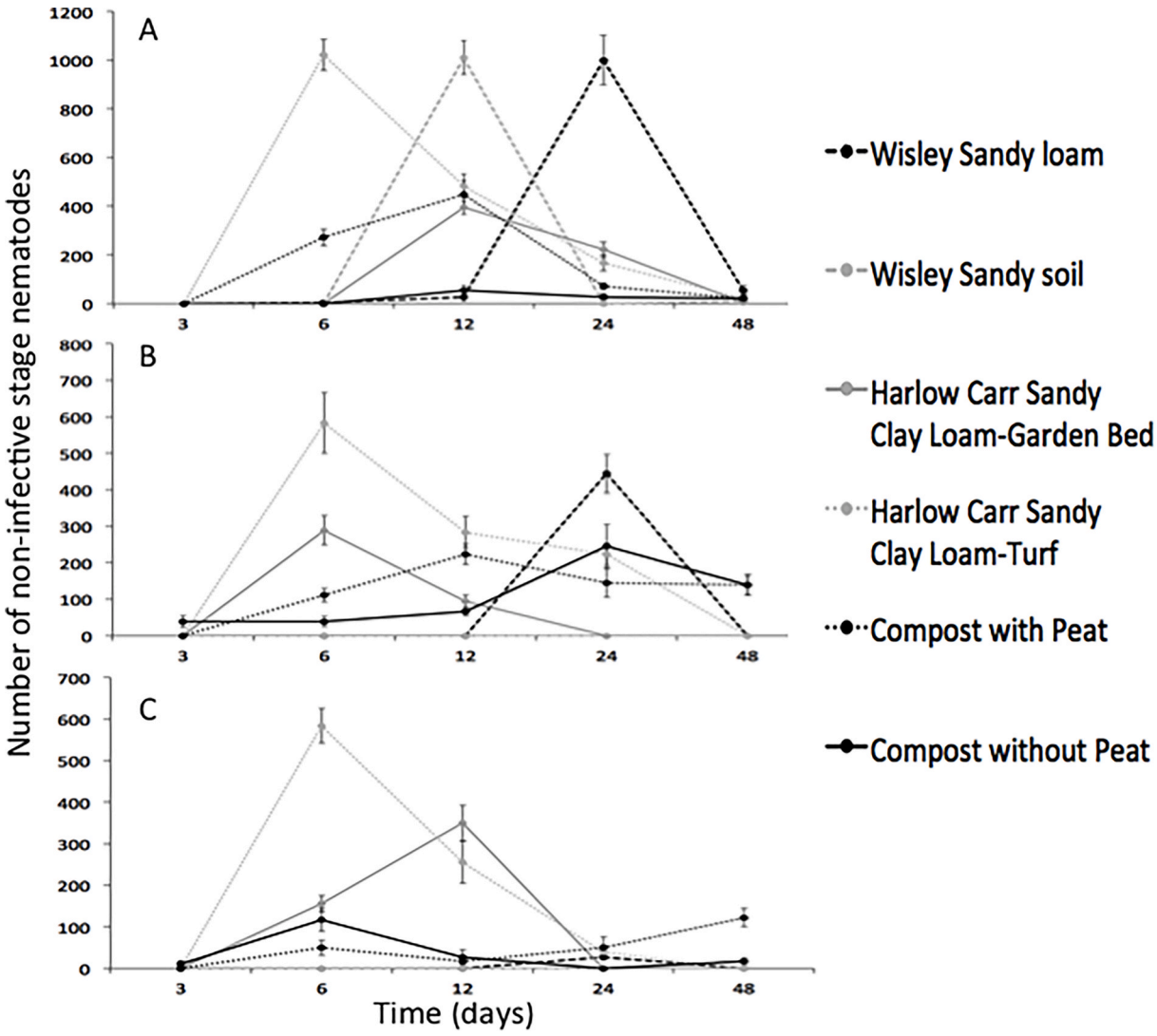

Fig. 4. The mean number of non-infective stage *P. hermaphrodita* added to six different previously frozen soils including Wisley sandy loam from a garden bed (long dash black line), Wisley sandy soil from under turf (long dash grey line), Harlow Carr sandy clay loam from a garden bed (solid grey line), Harlow Carr sandy clay loam from under turf (short dash grey line), compost with peat (short dash black line) and compost without peat (solid black line) at 5 °C (A), 10 °C (B) and 15 °C (C) over 48 days (mean ± SE).

3.4. Movement of P. hermaphrodita through six different soils with D. reticulatum as an attractant

There was a highly significant difference between the numbers of *P. hermaphrodita* (DMG0001) found in section 0 to 3.5 cm, 3.5 to 7 cm and 7 to 9.5 cm when applied to each of the six soils but all nematodes remained at the point of application ($P < 0.001$; Fig. 7). The presence of a slug in the 7 to 9.5 cm section did not encourage *P. hermaphrodita* to migrate through any soil as there was no difference between the numbers of *P. hermaphrodita* moving in soil with and without the slug (Fig. 7).

4. Discussion

We found *P. hermaphrodita* could survive for 48 days in a selection of soils but survived best in compost (without peat) compared to the other soils. It could therefore be recommended to apply *P. hermaphrodita* to compost before the addition to garden soil for better slug control. Indeed, it has been suggested (Herren et al., 2018) that compost could be used as a medium to apply entomopathogenic nematodes (EPNs). The authours found the more mature the compost, the better the survival of EPNs and that EPNs could be applied in infected cadavers in compost as an environmentally friendly method, which could be more beneficial than applying nematodes via water. Also, the addition of organic soil amendments e.g. mulch, compost or potting mix was beneficial for EPN survival (*Heterorhabditis bacteriophora*) as it prevented moisture loss (Khumalo et al., 2021). Conversely, another study (Kapranas et al., 2017) found increasing peat content negatively affected the ability of EPNs (*S. carpocapsae*; *Heterorhabditis downesi* and *S. feltiae*) to find hosts (*Galleria mellonella*). The use of compost as a medium to apply *P. hermaphrodita* certainly warrants further research. There are only a handful of studies that have looked at the effect soils have on *P. hermaphrodita* survival. Persistence of *P. hermaphrodita* has been monitored using real time qPCR techniques (MacMillan et al., 2006) and populations of *P. hermaphrodita* declined sharply after two weeks (Hatteland et al., 2013). However, it was found *P. hermaphrodita* could survive up to 5 months in wet sand, and even 8 months in garden soil and organic horticultural substrate (Nermut', 2012). In field trials *P. hermaphrodita* can survive up to 6 weeks in soil (Kozlowski et al., 1774) and even up to 99 days (Vernavá et al., 2004). These results are similar to studies using EPNs. Upon application Smit (Smits, 1996) proposed a model whereby EPNs experience quick decline (40 to 90 %

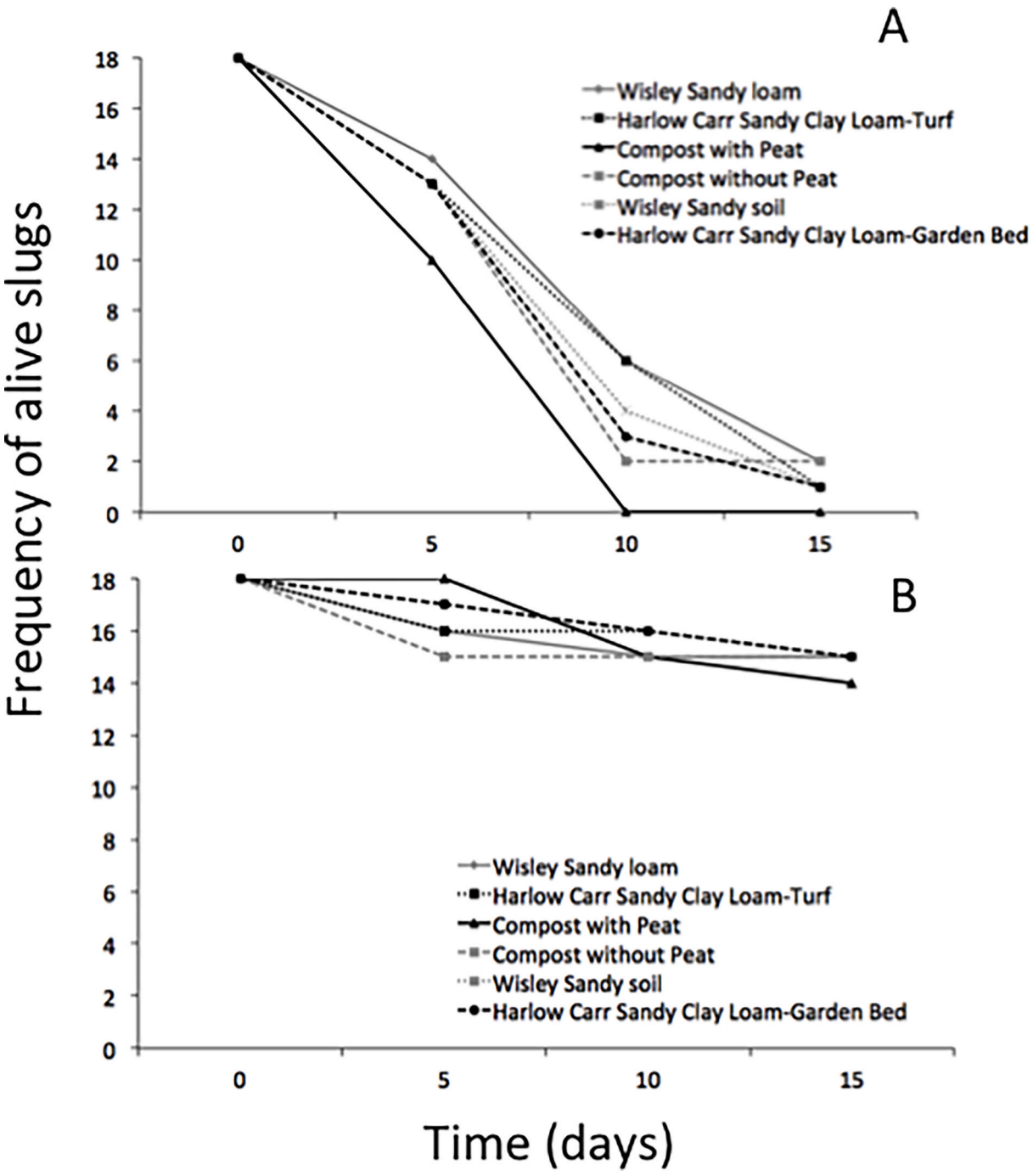

Fig. 5. Frequency of live slugs exposed to *P. hermaphrodita* MG2 applied to the six different soils over 15 days (A) or exposed to water (untreated control) (B).

die within hours or days of application), after which there is a steady decrease and the population is then maintained at low levels due to successful infection and reproduction in hosts. The reasons for the rapid decrease in population are due to exposure to UV light, desiccation, parasites and pathogens (Wilson and Gaugler, 2004). The physical properties of soil e.g. temperature, oxygen, moisture retention and texture (Smits, 1996) (Griffin, 2015) are also important factors for nematode survival, particularly for entomopathogenic nematodes (EPNs). For example, *Steinernema riobrave* and *Heterorhabditis bacteriophora* persisted longer in high slit and clay soil compared to sand soils (Shapiro and McCoy, 2000). Also, survival of *Steinernema glaseri* and *Steinernema carpocapsae* was lowest in clay than silty clay, sand or sandy silt (Kung et al., 1990). In a field experiment, it was found the efficacy of *H. bacteriophora*, *H. megidis* and *Steinernema feltiae* to kill western corn rootworm (*Diabrotica virgifera virgifera*) was best in heavy clay or silty clay soil rather than sandy soils (Toepfer et al., 2004). Finally, the survival of *H. bacteriophora*, *S. carpocapsae* and *S. glaseri* was severely affected by increasing bulk densities of sandy loam soil (Portillo-Aguilar et al., 1999).

We found *P. hermaphrodita* exited the IJ stage and reproduced prolifically in soils e.g. compost without peat. Presumably bacteria transferred with the nematodes are able to proliferate in this substrate compared to the other soils. It may seem surprising *P. hermaphrodita* can reproduce without a host but it is a facultative parasite able to reproduce in leaf litter (MacMillan et al., 2009), on dead earthworms (Rae et al., 2009), and slug faeces (Tan and Grewal, 2001). This is an important

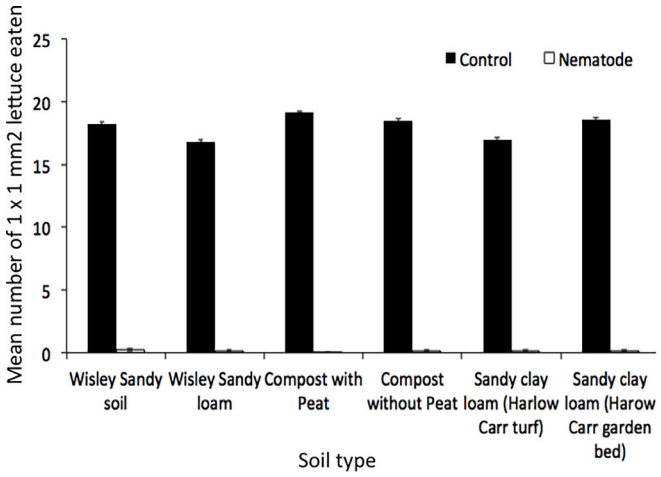

Fig. 6. Mean number of 1 x 1 mm^2 squares of lettuce eaten by *D. invadens* exposed to *P. hermaphrodita* MG2 (white bars) or water (untreated control) (black bars) (mean ± SE).

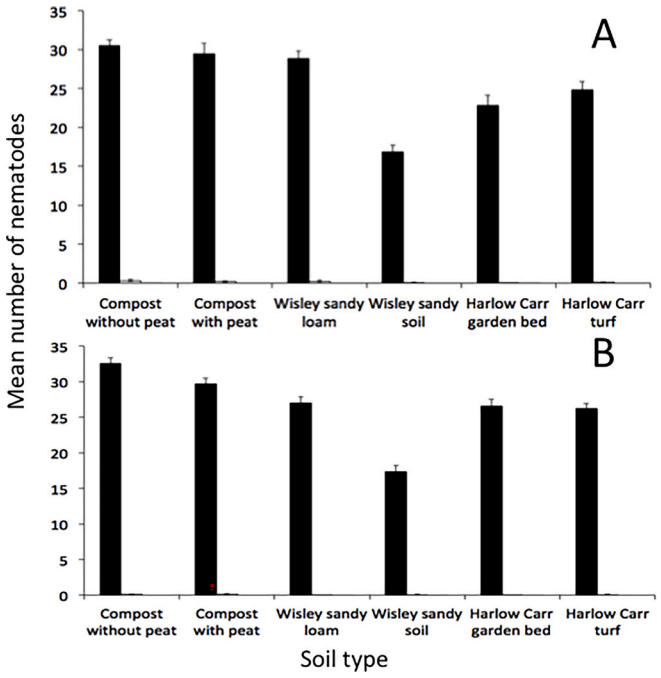

Fig. 7. Mean number of *P. hermaphrodita* (DMG0001) found at application point 0 to 3.5 cm (black bars), 3.5 to 7 cm (white bars) and 7 to 9.5 cm (grey bars) with slug present (A) or absent (B) in the six different soils (mean ± SE).

difference between EPNs and *P. hermaphrodita* in terms of lifestyle that needs to be addressed. Principally, when added to soil, EPNs will never exit the IJ stage as they are obligate parasites that can only reproduce when feeding on their symbiotic bacteria harboured in their intestine (*Xenorhabdus* spp. for the Steinernematidae and *Photorhabdus* spp. for the Heterorhabditidae). However, *P. hermaphrodita* is able to reproduce on an array of bacterial species (Wilson et al., 1995; Wilson et al., 1995; Andrus and Rae, 2019) and substrates, therefore if these nematodes are applied to bacteria rich soil they will not infect slugs but will reproduce in the soil. The ability of theses animals to exit the IJ stage could be problematic for controlling slug damage. The other life stages e.g. L1-L4 and adults do not infect slugs (Tan and Grewal, 2001), therefore may be unable to reduce slug populations. However, it is promising to see the nematodes managed to reproduce so effectively that the subsequent generations developed into high numbers of IJs, and that this may lead to better slug control. However, this is an important point that farmers and gardeners should be aware of and could potentially affect the success of *P. hermaphrodita* in controlling slugs in the field.

Temperature can also severely affect the survival of nematodes in soil (Campos-Herrera, 2015) and *P. hermaphrodita* is no different. It was previously known the survival of *P. hermaphrodita* dramatically decreased at > 25 °C but there is no difference at 5, 10 and 15 °C (Andrus and Rae, 2019; Grewal and Grewal, 2003) with the optimum growth temperature for *P. hermaphrodita* at 17 °C (Wilson et al., 1993). However, we found regardless of temperature (5, 10 and 15 °C) or whether the soils had been autoclaved or frozen, the substrate that was best for nematode survival was compost without the addition of peat.

P. hermaphrodita MG2 was lethal to *D. invadens* when placed in all six soils, though death of the slugs was faster in slugs exposed to the nematodes added to compost with peat, compared to sandy clay loam from under turf from Harlow Carr and sandy soil from under turf from Wisley. The reasons for this are unknown, but soil type has been shown to affect the efficacy of nematodes to control other pests, such as insects. For example, increasing clay content had a dramatic effect on the virulence of 17 strains of *S. feltiae* towards several insects (Campos-Herrera and Gutiérrez, 2009). Also infectivity of insects *Anomala orientalis* and *Popillia japonica* by *H. bacteriophora* was highest in highly organic potting mix and lowest in acidic sand (Koppenhöfer and Fuzy, 2006). Presumably, the different soil structures and contents affect factors such as dispersal of host cues through the soil matrix in sandy loam and sandy soil compared to compost. As compost is a granular matrix with bigger pore spaces compared to turf, which is tightly bound causing smaller pores, this may inhibit host cues permeating the soil. *Phasmarhabditis hermaphrodita* relies on detecting soluble host cues such as mucus and faeces (Rae et al., 2006) to find slugs. If there are difficulties in these cues dispersing through soil pores then it could be problematic for the nematodes to find slugs (though it must be noted in all soils where nematodes were applied, they did manage to rapidly kill the slugs).

When *P. hermaphrodita* is applied to soil, it largely remains within 2 cm of the point of application (Wilson et al., 2000). Similarly, in our experiments *P. hermaphrodita* (DMG0001) largely stayed at the point of application when added to the six different soils. In terms of strategies for EPNs to infect hosts they are broadly split into 'cruisers' or 'ambushers (Lewis et al., 1992). Hunters actively roam through the soil looking for hosts, but ambushers wait for their hosts to pass then latch on. A crucial point about ambushers is they nictate (stand on tail) (Campbell and Gaugler, 1993), but *Phasmarhabditis* nematodes do not, therefore, these nematodes do not seem to fit with the behavioural ecology paradigm for EPNs. In similar research the effect of soil type on *P. hermaphrodita* (DMG0001 – the commercial strain and a wild isolate of *P. hermaphrodita* from Norway) dispersal was investigated (MacMillan et al., 2009). They found, in general, the Norwegian strain moved better through all soil types more than the commercial strain (but they did not look at infectivity or pathogenicity). Furthermore, they found nematode movement was reduced in sandy loam soils compared to clay loam, and both strains moved readily through leaf litter compared to peat (and they recorded *P. hermaphrodita* also reproduced in leaf litter).

In summary, we have shown the survival of *P. hermaphrodita* and the ability to kill slugs is dependant on soil type, with peat-free compost being the best soil for both traits. We found these nematodes readily exit the IJ stage in many soils, which could prove problematic for slug control (though also maybe be beneficial as more nematodes are produced). Therefore, we encourage farmers and gardeners that use *P. hermaphrodita* to check soil type before application.

CRediT authorship contribution statement

Kerry McDonald-Howard: Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Christopher D. Williams:** Writing – review & editing,

Supervision, Formal analysis. **Hayley Jones:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Robbie Rae:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2025.105751.

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