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

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Keywords: *Phasmarhabditis hermaphrodita*, slugs, soil, snails, biological control, nematodes

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.

Keywords: biological control, gastropods, nematodes, parasites, pest management, *Phasmarhabditis hermaphrodita*

41 **1 Introduction**

42 Several slug species cause significant losses to agricultural and horticultural 43 crops [1], mainly by feeding on leaves [2], contaminating plants with faeces and 44 mucus, which not only reduces crop value but also can impair machinery [3]. In the 45 United Kingdom alone it has been estimated that slugs would cause approximately 46 £43.5 million worth of damage annually if not controlled [4]. The main control 47 method in the U.K. was the molluscicide metaldehyde [5], which has been shown to 48 be harmful to non-target organisms such as dogs, cats and cattle [6][7] and has 49 subsequently been banned. Alternative slug control methods include iron phosphate 50 pellets, baits [1] and the parasitic nematodes *Phasmarhabditis hermaphrodita* and *P.* 51 *californica*, which kill several pestiferous species and have been developed as 52 biological control agents (Nemaslug® and Nemaslug 2.0® from BASF Agricultural 53 Specialities) [8][9] for use across northern Europe. *Phasmarhabditis hermaphrodita* 54 was released in 1994 and can provide equivalent levels of protection as metaldehyde 55 [10][11], and is not harmful to non-target organisms like earthworms [12][13]. 56 Nematodes are mixed with water and applied to soil at the recommended rate of 57 300,000 nematodes per m^2 [14]. The nematodes seek out slugs in the soil and, on 58 discovery, penetrate through the back of the mantle, move into the shell cavity, and 59 kill the slug in 4-21 days [8][15]. The nematodes then feed on the bacteria 60 proliferating on the decomposing cadaver and reproduce until the resources are 61 depleted and they will then develop into infective juveniles (IJ) and search for more 62 slugs in the soil [8]. As a biological control, *P. hermaphrodita* is able to provide 63 protection of plants against slugs in two ways. First, susceptible slug species e.g. 64 *Deroceras reticulatum* are killed by *P. hermaphrodita* and second, other species such 65 as *Arion hortensis*, *A. subfuscus* and *Limax maximus,* are not killed by the nematode

66 but their feeding is severely inhibited [16]. In field studies using various crops, *P.*
67 *hermaphrodita* has been shown to provide protection from slug damage in asparagus
68 [17], lettuce [18] and winter wheat [19]. However, there are some studies that have
69 recorded failure of *P. hermaphrodit*a in providing slug protection or reducing slug
70 numbers [20][21][22]. One of the reasons for the lack of efficacy of *P. hermaphrodita*
71 in the field is due to the presence of adult *Arion lusitanicus*, which are not killed by
72 the nematode [23]. Furthermore, the effect of abiotic and biotic factors (e.g. soil type,
73 temperature, moisture and predators such as mites and collembola) that can severely
74 affect the success of nematode biological control agents in controlling pests [24]; but
75 is understudied for *P. hermaphrodita.*
76 There is very little information about how cropping systems, cover crops and
77 practices such as mulching may affect nematode efficacy or even how soil type can
78 affect survival, pathogenicity or movement of *P. hermaphrodita.* Therefore, we
79 assessed the effect of six different diverse soils (compost with and without peat, clay
80 loam, loam, sandy loam and sandy soil) on the survival, pathogenicity and movement
81 of *P. hermaphrodita*, as possible reasons for variable reports in field efficacy of *P.*
82 *hermaphrodita*.
83

84 **2.1 Materials and methods**

85 **2.1.1 Source of soils and nematodes**

86 Six different soil types were used, which were collected from two Royal
87 Horticultural Society (RHS) gardens (Harlow Carr in Harrogate and Wisley in
88 Woking). At each RHS garden there were two sample areas, one from an established
89 garden bed and one collected from under an area of turf. The soils used were: 1.
90 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 [49].

2.1.2 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 minutes 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 [25][26] 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 [50], 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 %,

117 the soil was misted until it reached 15% moisture content. Each Petri dish was sealed
118 with Parafilm® to water loss and kept in airtight sealed containers.
119 To quantify live nematodes, soil from individual Petri dishes was added to 50
120 ml Falcon tubes and then half filled with tap water. The Falcon tubes were then
121 shaken vigorously for 2 mins and three 1 ml subsamples were pipetted into a 5 cm
122 Petri dish with a grid on the bottom and total population in the Falcon tube calculated.
123 This technique uses a similar method as Circular Estimate Method developed as a
124 simple method to estimate *Caenorhabditis elegans* culture densities in liquid medium
125 [27]. This process was repeated for each of the three Petri dishes used on each time
126 point.
127
128 **2.1.3 Infection assay to test the effects of soils on the pathogenicity of nematodes**
129
130 *Deroceras invadens* was chosen as a suitable slug host as it is highly
131 pestiferous, with a worldwide distribution and commonly found in the Merseyside
132 area [28]. *D. invadens* (>0.10 g and >2.5 cm) were collected from a garden in
133 Maghull, Liverpool (OS grid reference SD373027), and stored in non-airtight
134 containers and fed lettuce or carrot *ad libitum*. Before the experiment began slugs
135 were examined for any signs of nematode infection e.g. swollen mantle, lesions on the
136 cuticle, and if they displayed these symptoms they were discarded.
137 To test the pathogenicity of *P. hermaphrodita* a standard protocol was
138 followed [28]. Briefly, 30 ml universal tubes were filled to a level of 3.5 cm with
139 each soil type. The soil types varied in composition and weight therefore the level of
140 3.5 cm was used to enable controlled comparisons. Eighteen universal bottles were
141 used for each soil and were split into 9 used for studying the survival of slugs exposed

142 to nematodes and the other 9 were used as untreated controls, with slugs added but
143 with no nematodes, just water. To half of the universal bottles 1000 *P. hermaphrodita*
144 MG2 were added to the soil. This wild strain of *P. hermaphrodita* was used as in our
145 previous experiments it was more pathogenic than the commercial strain (*P.*
146 *hermaphrodita* DMG0001) [see ref. 28]. Two *D. invadens* were added (mean weight
147 0.20 g ± 0.031) to each universal bottle and a piece of moist cotton wool was added
148 on top and the lid loosely placed on top and stored at 10°C for 5 days. After this, slugs
149 were removed and individually placed on 5 cm Petri dishes with pre-moistened filter
150 paper and a disc of lettuce (3.5 cm in diameter). The survival of the slugs was
151 monitored and after 10 days the amount the slugs had eaten was quantified by tracing
152 the remnants of the lettuce onto 1 x 1 mm^2 graph paper [29].

153 **2.1.4 Movement of *P. hermaphrodita* through six different soils with *D.***
154 ***reticulatum* as an attractant**

155 Plastic 50 ml Falcon tubes were cut into three sections (0 to 3.5 cm, 3.5 to 7
156 cm and 7 to 9.5 cm), placed on their side and half filled with one of six soils used in
157 the previous experiment to a height of 1.5 cm. To the first section 2,000 *P.*
158 *hermaphrodita* (DMG0001) IJs were added in 1 ml of water to the top of the soil.
159 Two slugs (*D. reticulatum*) were added to the third section as an attractant for the
160 nematodes [46] and a disc of lettuce and carrot was also added. A layer of fine netting
161 was added to prevent the slugs from moving into the other sections. The controls for
162 the experiment included the same set-up with lettuce and carrot discs added but no
163 slugs were placed in the tube. All sections were securely fitted back together using
164 Parafilm®. The tubes were stored in an incubator set at 15°C for 7 days.

165 Soil moisture was monitored using a Xiaomi Flower Care monitoring system.
166 After 7 days, the sections were separated and the soil from each section was placed in

167 individual 50 ml Falcon tubes. Fifty mls of tap water added, the mixture was
168 homogenised using a vortexer and three 1 ml samples were removed and the numbers
169 of nematodes was quantified using a dissecting microscope. Counts of nematodes
170 were calculated as the total number of nematodes per 50 ml (by multiplying the
171 average in 3 mls by 50). There were 6 tubes for each of the 6 soils (3 with nematodes,
172 3 without) and the whole experiment was repeated 3 times.

173

174 **2.1.5 Data analysis**

175 A Generalised Linear (Poisson loglinear) Model (GLM) was used to compare
176 the survival of either infective stage or non-infective stage nematodes. Predictors
177 were: soil type, soil treatment (frozen vs. autoclaved), nematode type (infective vs
178 non-infective), time (3, 6, 12, 24 and 48 days), and temperature (5, 10 or 15°C) with a
179 ful factorial design.

180 Survival of *D. invadens* exposed to *P. hermaphrodita* added to the six
181 different soils was compared using a Log Rank test in OASIS [30]. The number of 1 x
182 1 mm^2 squares of lettuce the slugs ate was compared using a One Way ANOVA and
183 Tukey's post hoc test.

184 A Kruskal-Wallis test was used to compare the numbers of *P. hermaphrodita*
185 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
186 and without a slug added.

187

188 **3.1 Results**

189 **3.1.1 Survival of *P. hermaphrodita* IJs in six different soils (previously autoclaved**
190 **or frozen) incubated at 5, 10 and 15°C over 48 days**

191 A Generalised Linear Model (GLM) with a Poisson distribution and log link
192 function was used to model the survival of infective juvenile *P. hermaphrodita* over
193 48 days based on soil, time, temperature and whether soils had been autoclaved or
194 frozen. The model fit the data well (Goodness-of-fit statistics: Deviance/df = 67.241,
195 Person Chi-Square/df= 65.892, AIC= 110766.047) and the Omnibus test was
196 significant ($\chi^2(179) = 668533.193$, $p<0.001$) (Supplementary Table 1). The individual
197 predictors were significant predictors of nematode survival, including soil ($P<0.001$),
198 time ($P<0.001$), temperature ($P<0.001$) and whether the soil was autoclaved or frozen
199 ($P<0.001$) (Fig 1 and 2). The intercept of the model was significant ($B = 7.162$,
200 $P<0.001$). Specifically, soil type significantly affected nematode survival with
201 compost (without peat) providing the best substrate for nematode survival compared
202 to the other 5 soils (Fig 1 and 2). The poorer soils for nematode survival were the
203 sandy loam and sandy soil from Wisley in both autoclaved and frozen soils.
204

205 **3.1.2 Numbers of non-infective stage *P. hermaphrodita* in six different soils**
206 **(previously autoclaved or frozen) incubated at 5, 10 and 15°C over 48 days**

207 Surprisingly, when counting the number of nematodes at each time point in the
208 soils at 5, 10 and 15°C it was clear the nematodes had moulted, exited the IJ stage and
209 had begun to reproduce, as numerous other life stages were present in the soils (Fig 3,
210 4). To understand this further a GLM was used with the same parameters as above.
211 The model fit the data well (Goodness-of-fit statistics: deviance/df = 41.948, Person
212 Chi-Square/df= 41.316, AIC= 66414.666) and the Omnibus test was significant
213 ($\chi^2(179) =1809840.126$, $p<0.001$) (Supplementary Table 2). All individual predictors
214 were significant predictors of non-infective stage nematode survival, including soil
215 ($P<0.001$), time ($P<0.001$), temperature ($P<0.001$) and whether the soil was

autoclaved or frozen ($P<0.001$) (Fig 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.

3.1.3 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).

241

242 **3.1.4 Movement of *P. hermaphrodita* through six different soils with *D.*
243 *reticulatum* as an attractant**

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

251

252 **4.1 Discussion**

253 We found *P. hermaphrodita* could survive for 48 days in a selection of soils
254 but survived best in compost (without peat) compared to the other soils. It could
255 therefore be recommended to apply *P. hermaphrodita* to compost before the addition
256 to garden soil for better slug control. Indeed, it has been suggested [43] that compost
257 could be used as a medium to apply entomopathogenic nematodes (EPNs). The
258 authours found the more mature the compost, the better the survival of EPNs and that
259 EPNs could be applied in infected cadavers in compost as an environmentally friendly
260 method, which could be more beneficial than applying nematodes via water. Also, the
261 addition of organic soil amendments e.g. mulch, compost or potting mix was
262 beneficial for EPN survival (*Heterorhabditis bacteriophora*) as it prevented moisture
263 loss [44]. Conversely, another study [45] found increasing peat content negatively
264 affected the ability of EPNs (*S. carpocapsae*, *Heterorhabditis downesi* and *S. feltiae*)
265 to find hosts (*Galleria mellonella*). The use of compost as a medium to apply *P.*

266 *hermaphrodita* certainly warrants further research. There are only a handful of studies
267 that have looked at the effect soils have on *P. hermaphrodita* survival. Persistence of
268 *P. hermaphrodita* has been monitored using real time qPCR techniques [38] and
269 populations of *P. hermaphrodita* declined sharply after two weeks [39]. However, it
270 was found *P. hermaphrodita* could survive up to 5 months in wet sand, and even 8
271 months in garden soil and organic horticultural substrate [40]. In field trials *P.*
272 *hermaphrodita* can survive up to 6 weeks in soil [41] and even up to 99 days [42].
273 These results are similar to studies using EPNs. Upon application Smit [31] proposed
274 a model whereby EPNs experience quick decline (40 to 90% die within hours or days
275 of application), after which there is a steady decrease and the population is then
276 maintained at low levels due to successful infection and reproduction in hosts. The
277 reasons for the rapid decrease in population are due to exposure to UV light,
278 desiccation, parasites and pathogens [32]. The physical properties of soil e.g.
279 temperature, oxygen, moisture retention and texture [31][33] are also important
280 factors for nematode survival, particularly for entomopathogenic nematodes (EPNs).
281 For example, *Steinernema riobrave* and *Heterorhabditis bacteriophora* persisted
282 longer in high slit and clay soil compared to sand soils [34]. Also, survival of
283 *Steinernema glaseri* and *Steinernema carpocapsae* was lowest in clay than silty clay,
284 sand or sandy silt [35]. In a field experiment, it was found the efficacy of *H.*
285 *bacteriophora*, *H. megidis* and *Steinernema feltiae* to kill western corn rootworm
286 (*Diabrotica virgifera virgifera*) was best in heavy clay or silty clay soil rather than
287 sandy soils [36]. Finally, the survival of *H. bacteriophora*, *S. carpocapsae* and *S.*
288 *glaseri* was severely affected by increasing bulk densities of sandy loam soil [37].

289 We found *P. hermaphrodita* exited the IJ stage and reproduced prolifically in
290 soils e.g. compost without peat. Presumably bacteria transferred with the nematodes

291 are able to proliferate in this substrate compared to the other soils. It may seem
292 surprising *P. hermaphrodita* can reproduce without a host but it is a facultative
293 parasite able to reproduce in leaf litter [46], on dead earthworms [29], and slug faeces
294 [15]. This is an important difference between EPNs and *P. hermaphrodita* in terms of
295 lifestyle that needs to be addressed. Principally, when added to soil, EPNs will never
296 exit the IJ stage as they are obligate parasites that can only reproduce when feeding on
297 their symbiotic bacteria harboured in their intestine (*Xenorhabdus* spp. for the
298 Steinernematidae and *Photorhabdus* spp. for the Heterorhabditidae). However, *P.*
299 *hermaphrodita* is able to reproduce on an array of bacterial species [47][48][49] and
300 substrates, therefore if these nematodes are applied to bacteria rich soil they will not
301 infect slugs but will reproduce in the soil. The ability of theses animals to exit the IJ
302 stage could be problematic for controlling slug damage. The other life stages e.g. L1-
303 L4 and adults do not infect slugs [15], therefore may be unable to reduce slug
304 populations. However, it is promising to see the nematodes managed to reproduce so
305 effectively that the subsequent generations developed into high numbers of IJs, and
306 that this may lead to better slug control. However, this is an important point that
307 farmers and gardeners should be aware of and could potentially affect the success of
308 *P. hermaphrodita* in controlling slugs in the field.

309 Temperature can also severely affect the survival of nematodes in soil [24] and
310 *P. hermaphrodita* is no different. It was previously known the survival of *P.*
311 *hermaphrodita* dramatically decreased at >25ºC but there is no difference at 5, 10 and
312 15ºC [49][50] with the optimum growth temperature for *P. hermaphrodita* at 17°C
313 [8]. However, we found regardless of temperature (5, 10 and 15ºC) or whether the
314 soils had been autoclaved or frozen, the substrate that was best for nematode survival
315 was compost without the addition of peat.

316 *P. hermaphrodita* MG2 was lethal to *D. invadens* when placed in all six soils,
317 though death of the slugs was faster in slugs exposed to the nematodes added to
318 compost with peat, compared to sandy clay loam from under turf from Harlow Carr
319 and sandy soil from under turf from Wisley. The reasons for this are unknown, but
320 soil type has been shown to affect the efficacy of nematodes to control other pests,
321 such as insects. For example, increasing clay content had a dramatic effect on the
322 virulence of 17 strains of *S. feltiae* towards several insects [51]. Also infectivity of
323 insects *Anomala orientalis* and *Popillia japonica* by *H. bacteriophora* was highest in
324 highly organic potting mix and lowest in acidic sand [52]. Presumably, the different
325 soil structures and contents affect factors such as dispersal of host cues through the
326 soil matrix in sandy loam and sandy soil compared to compost. As compost is a
327 granular matrix with bigger pore spaces compared to turf, which is tightly bound
328 causing smaller pores, this may inhibit host cues permeating the soil.
329 *Phasmarhabditis hermaphrodita* relies on detecting soluble host cues such as mucus
330 and faeces [53] to find slugs. If there are difficulties in these cues dispersing through
331 soil pores then it could be problematic for the nematodes to find slugs (though it must
332 be noted in all soils where nematodes were applied, they did manage to rapidly kill
333 the slugs).

334 When *P. hermaphrodita* is applied to soil, it largely remains within 2 cm of
335 the point of application [54]. Similarly, in our experiments *P. hermaphrodita*
336 (DMG0001) largely stayed at the point of application when added to the six different
337 soils. In terms of strategies for EPNs to infect hosts they are broadly split into
338 'cruisers' or 'ambushers [55]. Hunters actively roam through the soil looking for
339 hosts, but ambushers wait for their hosts to pass then latch on. A crucial point about
340 ambushers is they nictate (stand on tail) [56], 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 [46]. 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.

Acknowledgments

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Figure legends

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

365 turf (short dash grey line), compost with peat (short dash black line) and compost
366 without peat (solid black line) at 5°C (A), 10°C (B) and 15°C (C) over 48 days (mean
367 ± SE).

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

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

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

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).

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).

Supplementary Table 1: Table of model effects from GLM comparing the survival of infective stage *P. hermaphrodita* exposed to the six soils (either previously frozen or autoclaved) at three temperatures over 48 days.

Supplementary Table 2: Table of model effects from GLM comparing the survival of non-infective stage *P. hermaphrodita* exposed to the six soils (either previously frozen or autoclaved) at three temperatures over 48 days.

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