Behavioural avoidance by slugs and snails of the parasitic nematode Phasmarhabditis hermaphrodita

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Behavioural avoidance by slugs and snails of the parasitic nematode *Phasmarhabditis hermaphrodita*

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Abstract

The nematode *Phasmarhabditis hermaphrodita* has been developed as a biological control agent for slugs and snails. Slugs avoid areas where *P. hermaphrodita* is present. We investigated whether behavioural avoidance of *P. hermaphrodita* is a common feature of slugs and snails by exposing eight species to *P. hermaphrodita*. We showed that slugs generally avoided *P. hermaphrodita* whereas snails did not. We also showed that slugs specifically avoided the commercial strain and a natural isolate of *P. hermaphrodita* and were not deterred by other nematodes such as *Steinernema kraussei* or *Turbatrix aceti*. We also showed that slugs avoided the dauer stage of *P. hermaphrodita* and not mixed stage cultures. Furthermore, slugs do not avoid dead *P. hermaphrodita* or exudates from live nematodes. Taken together, we have unravelled further factors that are essential for slugs to avoid *P. hermaphrodita* in soil, which could have important implications for the biological control of slugs and snails.

Keywords

Phasmarhabditis hermaphrodita, nematodes, slugs, snails, behaviour, avoidance

1. Introduction

Slugs and snails cause damage to arable, vegetable and horticultural crops by reducing leaf area by eating stems and leaves (Glen and Moens, 2002; Port and Ester, 2002; Port and Port, 1986; South, 1992). They are usually controlled by applications of chemical bait pellets containing metaldehyde, methiocarb and iron phosphate (Purves and Bannon, 1992; Speiser and Kistler, 2002). Other methods of slug control have been developed including the parasitic nematode *Phasmarhabditis hermaphrodita* DMG0001 which is a lethal parasite of numerous slug and snail species such as *Deroceras reticulatum*, *Arion ater*, and *Helix aspersa* (Wilson et al., 1993; Glen et al., 1996). Formulations of *P. hermaphrodita* DMG0001 are sold as Nemaslug® by Becker Underwood-BASF Agricultural Specialties and are routinely used by farmers and gardeners (Rae et al., 2007). Nematodes are mixed with water and applied using spraying equipment to soil where they search for hosts. They are attracted to mucus and faeces from slugs (Rae et al., 2006, 2009) and upon discovery they enter the shell cavity beneath the dorsal surface of the mantle and kill the slug between 4 and 21 days later (Wilson et al., 1993; Tan and Grewal, 2001a). *P. hermaphrodita* DMG0001 has been used successfully to protect against slug damage in oilseed rape (Wilson et al., 1995), winter wheat (Wilson et al., 1994a), strawberries (Glen et al., 2000a), asparagus (Ester et al., 2003a), orchids (Ester et al., 2003b) and hostas (Grewal et al., 2001).

Slugs such as *Deroceras reticulatum* and *Arion ater* avoid *P. hermaphrodita* DMG0001 in soil and spend less time feeding and resting where these nematodes have been applied (Wilson et., 1999). Although this avoidance behaviour is an interesting phenomenon it is uncharacterised and there remain many questions to be answered. For example, what other slug and snail species avoid *P. hermaphrodita* DMG0001? Are susceptible species more likely to avoid *P. hermaphrodita* DMG0001 than resistant gastropods? Do other

nematodes provoke such an extreme behavioural avoidance? Also does *P. hermaphrodita* DMG0001 secrete a chemical or exudate that the slugs detect?

2. Materials and methods

2.1 Source and maintenance of invertebrates

Slugs species used in this study were: *Arion subfuscus*, *A. ater*, *Deroceras reticulatum*, *D. panormitanum*, *Limax flavus* and *Lehmannia valentiana*, which were collected from Festival gardens in Liverpool and in greenhouses at Liverpool John Moores University (LJMU). Snails used in the study consisted of *Cepaea nemoralis* and *H. aspersa*. Slugs and snails were kept in non-airtight containers with moist tissue paper and were fed lettuce and cabbage *ad libitum*. Nematodes (*Steinernema kraussei* and *P. hermaphrodita* DMG0001 were supplied by Becker Underwood BASF Agricultural Specialities. *Turbatrix aceti* was supplied by Sciento, U.K. All nematodes were kept at 15ºC until use. A natural strain of *P. hermaphrodita* was isolated from a separate study looking at the distribution of *Phasmarhabditis* species from around the U.K. This strain, designated "AB38", was dissected from a collected *L. flavus* from Aberdeen and was identified using DNA sequencing of the 18SrRNA gene. It was grown on rotting *L. flavus* in White traps for 28 days at 20ºC.

2.2 Behavioural avoidance assay exposing slugs and snails to *P. hermaphrodita* DMG0001

We used a similar assay to Wilson et al. (1999). Briefly, three non-airtight plastic boxes (9 x 24 x 6 cm) were half filled with moist peat soil (approx. 50 g) and fitted with copper tape to stop slugs escaping *P. hermaphrodita* DMG0001. Nematodes were evenly applied to one side (4.5 x 12 cm) at a rate of 120 nematodes per cm^2 (Wilson et al., 1999). To the other side an equal volume of water was added. Five slugs or snails were added to the

middle of each of the three boxes and stored at 18ºC. Three discs of fresh cabbage (diameter 3.5 cm) were added to each side of each box and replaced every two days. The number of slugs or snails resting on each side was monitored every 12 hours for 4 days. Once recorded, slugs and snails were placed back to the midline of each assay box. Each experiment was repeated twice. The same soil bioassay was used to investigate whether slugs would avoid other nematodes such as *S. kraussei*, *P. hermaphrodita* AB38 and *T. aceti*.

2.3 Assessing the effect of heat killed nematodes, different life stages and nematode suspension on slug behaviour

For further experiments we concentrated on using just one species of slug (*A. subfuscus*) as it was readily available around LJMU gardens. In order to investigate whether nematodes have to be alive to induce behavioural avoidance in *A. subfuscus, P. hermaphrodita* DMG0001 were heat killed by placing nematodes at 80°C for 20 mins and the same soil bioassay was used as above. This temperature and time was chosen as in preliminary studies all nematodes were deceased after 20 mins even with high numbers of *P. hermaphrodita* DMG0001 present. To test whether *P. hermaphrodita* DMG0001 released a compound into the environment which slugs and snails avoided approximately 15,000 ~~a~~live *P. hermaphrodita* DMG0001 were placed in PBS buffer, mixed and stored in non-airtight plastic boxes (9 x 24 x 6 cm) at 18°C for 7 days. We incubated the nematodes for 7 days as this should be sufficient time to release any potential exudates that slugs may avoid. We then harvested the supernatant of the suspension by centrifugation at 5,000 rpm for 10 mins and applied at the treated side of the soil assay compared with PBS, which was applied to the other side. In order to discover whether slugs were deterred by other life stages of *P. hermaphrodita* DMG0001 we grew nematodes on rotting *L. flavus* which had previously

been killed by placing it at -80°C for 20 mins. We grew the nematodes for 5 days after which cultures consisted of mixed life stages of J1-J4 and adult stages. The nematodes were washed several times in PBS, quantified and applied to the soil bioassay at the same rate as used in previous experiments. In each of these experiments, three replicate boxes were used and the experiment was repeated twice.

2.4 Quantification of movement of *P. hermaphrodita* DMG0001 in soil bioassay

We also determined how far *P. hermaphrodita* DMG0001 could move throughout the soil during the 4-day experiment as this may affect the avoidance behaviour of the slugs as the nematodes possibly were not confined to one location. *P. hermaphrodita* DMG0001 were evenly applied to one side of the soil bioassay (4.5 x 12 cm) at a rate of 30 nematodes per cm^2. Over 1, 2, 3 and 4 days, 3 soil samples (approx. 1-2 g) were removed from 3 different boxes from 0-2, 4-6 and 10-12 cm from the midline of the box and the numbers of *P. hermaphrodita* DMG0001 were extracted via sugar solution centrifugation and quantified. The experiment was repeated 3 times. Overall, this revealed how far *P. hermaphrodita* DMG0001 moved throughout the soil in the bioassay.

2.5 Data analysis

Numbers of slugs or snails on each side of the assay box were recorded every 12 hours for 4 days. The mean number of slugs on each side over 4 days was compared using a Two Way Repeated Measures ANOVA. Movement of *P. hermaphrodita* DMG0001 was analysed using a One-way Analysis of Variance (ANOVA).

3. Results

3.1 Slugs but not snails avoided *P. hermaphrodita* DMG0001

D. reticulatum and *D. panormitanum* are rapidly killed by *P. hermaphrodita* DMG0001 (Wilson et al., 1993) and avoided the nematode as significantly more slugs were recorded in the untreated than nematode-treated half of the box ($P<0.001$, Fig 1). The slug species *A. ater* and *A. subfuscus* are only killed by *P. hermaphrodita* DMG0001 when juveniles but adult slugs (which were used in our assays) are resistant but still avoided the nematode ($P<0.001$, Fig 1). Similarly, *L. valentiana* is not killed by *P. hermaphrodita* DMG0001 (Dankowska, 2006; Ester et al., 2003b) but also is deterred by it ($P<0.001$). In contrast to these species, *L. flavus* is resistant to *P. hermaphrodita* DMG0001 (Rae et al., 2008) but does not avoid the nematode (Fig 1) as similar numbers of slugs were recorded in the untreated and nematode-treated halves of the boxes ($P>0.05$). The snail species used in this study (*C. nemoralis* and *H. aspersa*) are resistant to *P. hermaphrodita* DMG0001 and remain alive even when exposed to high doses of this nematode (Rae et al., 2009, Wilson et al., 2000). Equal numbers of *H. aspersa* and *C. nemoralis* were recorded in the untreated and nematode-treated halves of the boxes ($P>0.05$, Fig 1). In general, slugs avoided *P. hermaphrodita* DMG0001 but snails were not deterred by the nematode.

3.2 *A. subfuscus* avoided *P. hermaphrodita* DMG0001 but not other nematodes

A. subfuscus also avoided the recently isolated natural stain of *P. hermaphrodita* AB38 as significantly more slugs were recorded in the untreated and nematode-treated halves of the boxes (Fig 2) ($P<0.001$). In contrast, *A. subfuscus* did not avoid either *T. aceti* or *S. kraussei* and an equal number of slugs were recorded in the untreated and nematode-treated halves of the boxes ($P>0.05$) (Fig 2). Therefore, *A. subfuscus* can differentiate between

nematode species and is deterred specifically by *P. hermaphrodita* (strains AB38 and DMG0001).

3.3 Factors affecting behavioural avoidance of *A. subfuscus* exposed to treated *P. hermaphrodita* DMG0001

A. subfuscus did not avoid heat killed *P. hermaphrodita* DMG0001 (P>0.05) (Fig 3) or the supernatant of a suspension of *P. hermaphrodita* DMG0001 (P>0.05) (Fig 3). Therefore, in order to avoid these nematodes they must be alive and in contact with *A. subfuscus*. Also when *P. hermaphrodita* DMG0001 was applied as a mixture of J1-J4 and adult stages *A. subfuscus* did not exhibit avoidance behaviour and equal numbers of slugs were recorded in the untreated and nematode-treated halves of the boxes P>0.05) (Fig 3).

3.4. Movement of *P. hermaphrodita* DMG0001 in the soil bioassay

Over the four days of the assay the numbers of *P. hermaphrodita* DMG0001 that moved from the nematode side to the water treated side was negligible (Table 1). There was no significant difference between the numbers of *P. hermaphrodita* DMG0001 moving from the nematode treated side to 0-2, 4-6 and 10-12 cm on the untreated side of the boxes over 4 days (P>0.05). Only 0.78 ± 0.46 nematodes per gram of soil had moved onto the water treated side after 4 days. These results are similar results to Wilson et al. (1999), who found *P. hermaphrodita* DMG0001 remained within 2 cm of the point of application. Therefore, natural nematode movement did not affect the results of the bioassay.

4. Discussion

Avoidance behaviour is the first line of defence used by free-living animals in their struggle to maintain fitness in the face of parasite threat, and is likely the most cost-effective strategy as compared to resistance and tolerance (Curtis, 2014). Avoidance behaviour can be defined as the actions taken by an animal (or group of animals) to reduce its (or their) chances of becoming infected with pathogens or parasites (Curtis, 2014) and is widespread in the animal kingdom. For example, pine weevils (*Hylobius abietus*) avoid areas where *Steinernema carpocapsae* are present (Ennis et al., 2010). The model organism *Caenorhabditis elegans* avoids pathogenic bacteria such as *Bacillus thuringiensis* (Schulenburg and Muller, 2004). Bumblebees (*Bombus terrestris*) avoid flowers contaminated with *Escherichia coli* (Fouks and Lattorff, 2011). Rainbow trout (*Oncorhynchus mykiss*) avoid flukes (*Diplostonum spathaceum*) that cause eye infections (Karvonen et al., 2004). Here we have shown that slugs avoided *P. hermaphrodita* (both strains DMG0001 and AB38) but snails did not. We were interested to discover if gastropods that are susceptible to *P. hermaphrodita* DMG0001were more likely to avoid the nematode. Slugs such as *D. reticulatum* and *D. panormitanum* and juveniles of *A. ater* and *A. subfuscus* are susceptible to *P. hermaphrodita* DMG0001 (Wilson et al., 1993; Tan and Grewal, 2001a; Rae et al., 2009) and avoided the nematode. In comparison to susceptible slug species the behavioural response of resistant slug species differed. For example, *L. valentiana* is resistant to *P. hermaphrodita* DMG0001 (Dankowska, 2006; Ester et al., 2003b;) but is deterred by the nematode. Also *L. flavus* is resistant to *P. hermaphrodita* DMG0001 (Rae et al., 2008) and did not show avoidance behaviour. The snails tested (*H. aspersa* and *C. nemoralis*) did not avoid *P. hermaphrodita* DMG0001 but as both snails used in this study are resistant to *P. hermaphrodita* DMG0001 it is perhaps not surprising that these species would not avoid it (Rae et al., 2009; Wilson et al., 2000).

198 *A. subfuscus* only avoided dauer stage *P. hermaphrodita* DMG0001 and was not
199 deterred by mixed stage nematodes~~;~~. As the dauer stage is the life cycle stage responsible for
200 killing slugs and juvenile or adult stages cannot infect slugs (Tan and Grewal, 2001a) it
201 seems reasonable to presume slugs would avoid this stage exclusively. We also showed that
202 slugs were not repelled by the supernatant of a liquid suspension of *P. hermaphrodita*
203 DMG0001. This suggests that nematodes were probing the slug's body and trying to
204 penetrate inside and the slugs were avoiding the mechanical stimulus rather than a chemical
205 cue.

206 We could also show that *A. subfuscus* specifically avoided both the commercial and
207 recently isolated strain of *P. hermaphrodita* (strains DMG0001 and AB38) and did not avoid
208 other nematodes such as *S. kraussei* or *T. aceti*. Entomopathogenic nematodes (EPNs) such as
209 *S. kraussei*, harbour symbiotic bacteria in their intestines that are transported into insect hosts
210 and released which kills the insect in 24-48 hours (Forst et al., 1997). EPNs cannot kill slugs
211 (Wilson et al., 1994b) therefore there is no need for them to avoid these parasites in soil.
212 Similarly, there are no reports of *T. aceti* parasitizing slugs or snails; hence there is no need
213 for gastropods to avoid them either.

214 Other factors have to be taken into account when trying to understand why slugs
215 avoid *P. hermaphrodita* DMG0001. For example, the commercial strain of *P. hermaphrodita*
216 DMG0001 is grown on the bacterium *Moraxella osloensis* and is thought to be responsible
217 for slug death (Tan and Grewal, 2001b). The slugs could possibly be avoiding *M. osloensis*
218 present in the nematodes and future research will investigate this possibility. Also another
219 caveat is that slugs follow slime trails of similar species to find mates, aggregate and avoid
220 desiccation (Ng et al., 2013). As we only looked at groups of slugs together and did not carry
221 out experiments with individual slugs perhaps when slugs began moving towards one side
222 others would follow.

Our results may have important implications for biocontrol and the use of *P. hermaphrodita* DMG0001 in controlling slugs in the field. For example, two outdoor plot trials have shown that a reduced application of *P. hermaphrodita* DMG0001 has the potential to reduce slug damage in Chinese cabbage and winter wheat due to slugs being deterred by areas applied with nematodes (Hass et al., 1999a,b). These studies concentrated on just one slug species (*D. reticulatum*) but our data shows that other pestiferous slug species such as *A. subfucus*, *D. panormitanum* and *L. valentiana* (as well as *A. ater* and *D. reticulatum*) would also be deterred by *P. hermaphrodita* DMG0001.

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References

Curtis, V.A. (2014). Infection-avoidance behaviour in humans and other animals. *Trends in Immunology, 35,* 457-464.

Dankowska, E., (2006). Laboratory studies on the use of a nematode *Phasmarhabditis hermaphrodita* (Schneider) in slug control. *Folia Malacologica, 14,* 61-62.

Ennis, D.E., Dillon, A.B. & Griffin, C.T. (2010). Pine weevils modulate defensive behaviour in response to parasites of differing virulence. *Animal Behaviour, 80,* 283-288.

Ester, A., Van Rozen, K., & Molendijk, L.P.G. (2003a). Field experiments using the rhabditid nematode *Phasmarhabditis hermaphrodita* or salt as control measures against slugs in green asparagus. *Crop Protection, 22,* 689-695.

Ester, A., Van Rozen, K., & Hazendonk, A. (2003b). Efficacy of pesticides to control *Lehmannia valentiana* (Ferussac) in orchids (Cymbidium) in greenhouse experiments, in: Slugs and snails: Agricultural, Veterinary and Environmental perspectives. British Crop protection Council (BCPC) Symposium proceedings, Alton, Hants, U.K.

Forst, S., Dowds, B., Boemare, N. & Stackebrandt, E. (1997). *Xenorhabdus* spp. and *Photorhabdus* spp.: bugs that kill bugs. *Annual Review of Microbiology. 51,* 47-72.

Fouks, B., & Lattorff, H.M. (2011). Recognition and avoidance of contaminated flowers by foraging bumblebees (*Bombus terrestris*). *PLoS One. 6,* e26328. DOI:10.1371/journal.pone.0026328

Glen, D.M., Wilson, M.J., Hughes, L., Cargeeg, P. & Hajjar, A. (1996). Exploring and exploiting the potential of the rhabditid nematode *Phasmarhabditis hermaphrodita* as a biocontrol agent for slugs, in Slugs and Snails: Agricultural, Veterinary and Environmental Perspectives, BCPC Symposium Proceedings Alton, Hants, U.K.

Glen, D.M. & Moens, R. (2002). Agriolimacidae, Arionidae and Milacidae as pests in West European cereals. In G.M. Barker (Ed.), *Molluscs as Crop Pests* (pp. 271-300). CABI Publishing, U.K.

Glen, D.M., Wiltshire, C.W., Hughes, L., Ester, A., Van Rozen, K., Castillejo, J., Iglesias, J., Speiser, B., Coupland, J. & Gwynn, R. (2000~~a~~). The use of slug-parasitic nematodes and

other techniques for control of slug and snail damage in horticultural crops. Brighton Crop Protection Conference (BCPC) Pests and Diseases.

Grewal, P.S., Grewal, S.K., Taylor, R.A.J., & Hammond, R.B. (2001). Application of molluscicidal nematodes to slug shelters: a novel approach to economic biological control of slugs. *Biological Control, 22,* 72-80.

Hass, B., Glen, D.M., Brain, P. & Hughes, L.A. (1999a). Targeting biocontrol with the slug parasitic nematode *Phasmarhabditis hermaphrodita* in slug feeding areas: a model study. *Biocontrol Science and Technology, 9,* 587-598.

Hass, B., Hughes, L.A. and Glen, D.M. (1999b). Overall versus band application of the nematode *Phasmarhabditis hermaphrodita* with and without incorporation into soil, for biological control of slugs in winter wheat. *Biocontrol Science and Technology,* 9, 579-586.

Karvonen, A., Seppala, O., & Valtonen, E.T. (2004). Parasite resistance and avoidance behaviour in preventing eye fluke infections in fish. *Parasitology*, *129,* 159-164.

Ng, T.P.T., Saltin, S.H., Davies, M.S., Johannesson, K., Stafford, R., & Williams, G.A. (2013) Snails and their trails: the multiple functions of trail following in gastropods. *Biological Reviews*, *88*, 683-700.

Port, G., & Ester, A. (2002). Gastropods as pests in vegetables and ornamental crops in Western Europe. In G.M. Barker (Ed.), Molluscs as Crop Pests (pp. 271-300). CABI Publishing, U.K.

Port, C.M., & Port, G. (1986). The biology and behaviour of slugs in relation to crop damage and control. *Agricultural Zoology Reviews, 1,* 255-299.

Purves, G., & Bannon, J.W. (1992). Non-target effects of repeated methiocarb slug pellet application on carabid beetle (Coleoptera: Carabidae) activity in winter-sown cereals. *Annals of Applied Biology, 121,* 215-223.

Rae, R.G., Robertson, J.F., & Wilson, M.J. (2006). The chemotactic response of *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditida) to cues of *Deroceras reticulatum* (Mollusca: Gastropoda). *Nematology, 8,* 197-200.

Rae, R., Verdun, C., Grewal, P.S., Robertson, J.F. & Wilson, M.J. (2007). Biological control of terrestrial molluscs using *Phasmarhabditis hermaphrodita*-progress and prospects. *Pest Management Science, 63,* 1153-1164.

Rae, R.G., Robertson, J.F. & Wilson, M.J. (2009). Chemoattraction and host preference of the gastropod parasitic nematode *Phasmarhabditis hermaphrodita*. *Journal of Parasitology, 95,* 517-526.

Rae, R.G, Robertson, J. & Wilson, M.J. (2008). Susceptibility and immune response of *Deroceras reticulatum*, *Milax gagates* and *Limax pseudoflavus* exposed to the slug parasitic nematode *Phasmarhabditis hermaphrodita*. *Journal of Invertebrate Pathology*, *97,* 61-69.

Schulenburg, H., & Muller, S. (2004). Natural variation in the response of *Caenorhabditis elegans* towards *Bacillus thuringiensis*. *Parasitology, 128,* 433-443.

South, A. (1992). *Terrestrial slugs: Biology, ecology and control*. Chapman and Hall: U.K.

Speiser, B, & Kistler, C. (2002). Field tests with a molluscicide containing iron phosphate. *Crop Protection*, *21*, 389-394.

306 Tan, L., & Grewal, P.S. (2001a). Infection behaviour of the rhabditid nematode
307 *Phasmarhabditis hermaphrodita* to the grey garden slug *Deroceras reticulatum*. *Journal of*
308 *Parasitology, 87,* 1349-1354.

309 Tan, L. & Grewal, P.S. (2001b). Pathogenicity of *Moraxella osloensis*, a bacterium
310 associated with the nematode *Phasmarhabditis hermaphrodita*, to the slug *Deroceras*
311 *reticulatum*. *Applied Environmental Microbiology, 67,* 5010-5016.

312 Wilson, M.J., Glen, D.M. & George, S.K. (1993). The rhabditid nematode *Phasmarhabditis*
313 *hermaphrodita* as a potential biological control agent for slugs. *Biocontrol Science and*
314 *Technology, 3,* 503-511.

315 Wilson, M.J., Glen, D.M., George, S.K., Pearce, J.D. & Wiltshire, C.W. (1994a). Biological
316 control of slugs in winter wheat using the rhabditid nematode *Phasmarhabditis*
317 *hermaphrodita*. *Annals of Applied Biology, 125,* 377-390.

318 Wilson, M.J., Glen, D.M., Hughes, L.A., Pearce, J.D. & Rodgers, P.B. (1994b). Laboratory
319 tests of the potential of entomopathogenic nematodes for the control of field slugs (*Deroceras*
320 *reticulatum*). *Journal of Invertebrate Pathology, 64,* 182-187.

321

322 Wilson, M.J., Hughes, L.A. & Glen, D.M. (1995). Developing strategies for the nematode,
323 *Phasmarhabditis hermaphrodita*, as a biological control agent for slugs in Integrated Crop
324 Management Systems. Integrated Crop protection; Towards Sustainability? British Crop
325 Protection Council (BCPC).

326 Wilson, M.J., Hughes, L.A., Jefferies, D. & Glen, D.M. (1999). Slugs (*Deroceras reticulatum*
327 and *Arion ater* agg.) avoid soil treated with the rhabditid nematode *Phasmarhabditis*
328 *hermaphrodita*. *Biological Control, 16,* 170-176.

Wilson, M.J., Hughes, L.A., Hamacher, G.M. & Glen, D.M. (2000). Effect of *Phasmarhabditis hermaphrodita* on non-target molluscs. *Pest Management Science, 56,* 711-716.

Figure legends

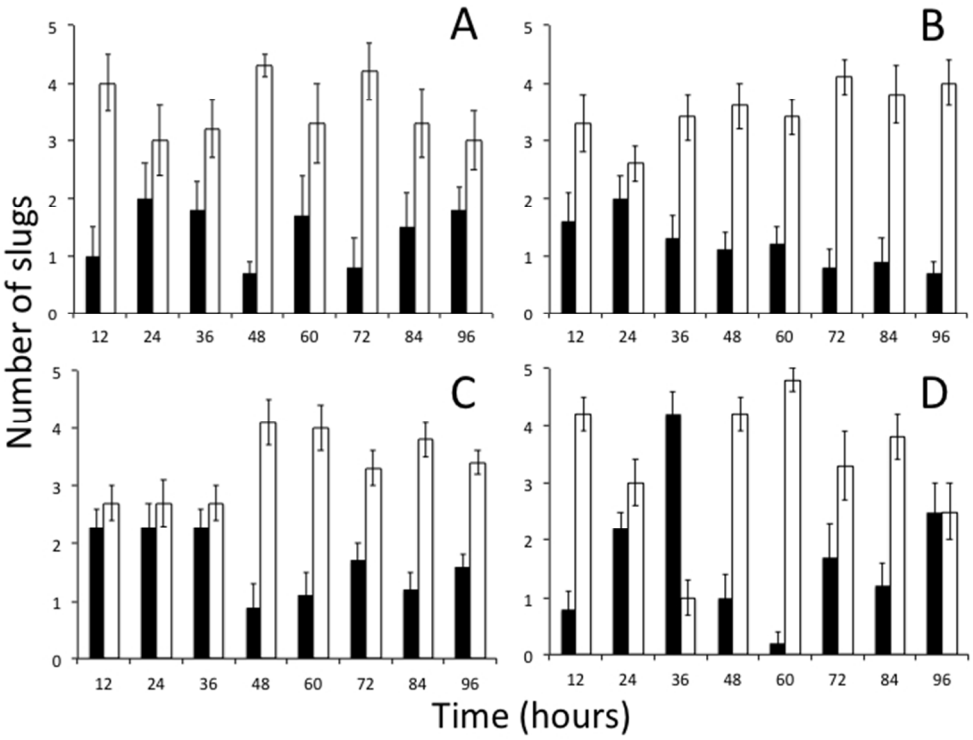
Fig 1. Numbers of slugs or snails recorded on each side of soil bioassay treated with either *P. hermaphrodita* DMG0001 (black) or no nematodes (white) every 12 hours for 4 days. Slug and snail species tested include *D. reticulatum* (A), *D. panormitanum* (B), *A. ater* (C), *A. subfuscus* (D), *L. valentiana* (E), *L. flavus* (F), *C. nemoralis* (G) and *H. aspersa* (H). Bars represent ± one standard error.

Fig 2. Numbers of *A. subfuscus* recorded on each side of soil bioassay treated with either nematodes (black) or on the side with no nematodes (white) every 12 hours for 4 days. Different nematode species tested were: a natural isolate of *P. hermaphrodita* AB38 (A), *T. aceti* (B) and *S. kraussei* (C). Bars represent ± one standard error.

Fig 3. Numbers of *A. subfuscus* recorded on each side of soil bioassay treated with either nematodes (black) or on the side with no nematodes (white) every 12 hours for 4 days. *A. subfuscus* were exposed to heat killed *P. hermaphrodita* DMG0001 (A), the supernatant of a suspension of *P. hermaphrodita* DMG0001 (B) and mixed stage *P. hermaphrodita* DMG0001 (C). Bars represent ± one standard error.

Table legend

Table 1. Mean numbers of *P. hermaphrodita* DMG0001 per 1-2 g of soil in behavioural bioassay monitored daily for 1, 2, 3 and 4 days. Nematodes were extracted -from 0-2, 4-6 and 10-12 cm from midline in water treated and nematode treated sides.



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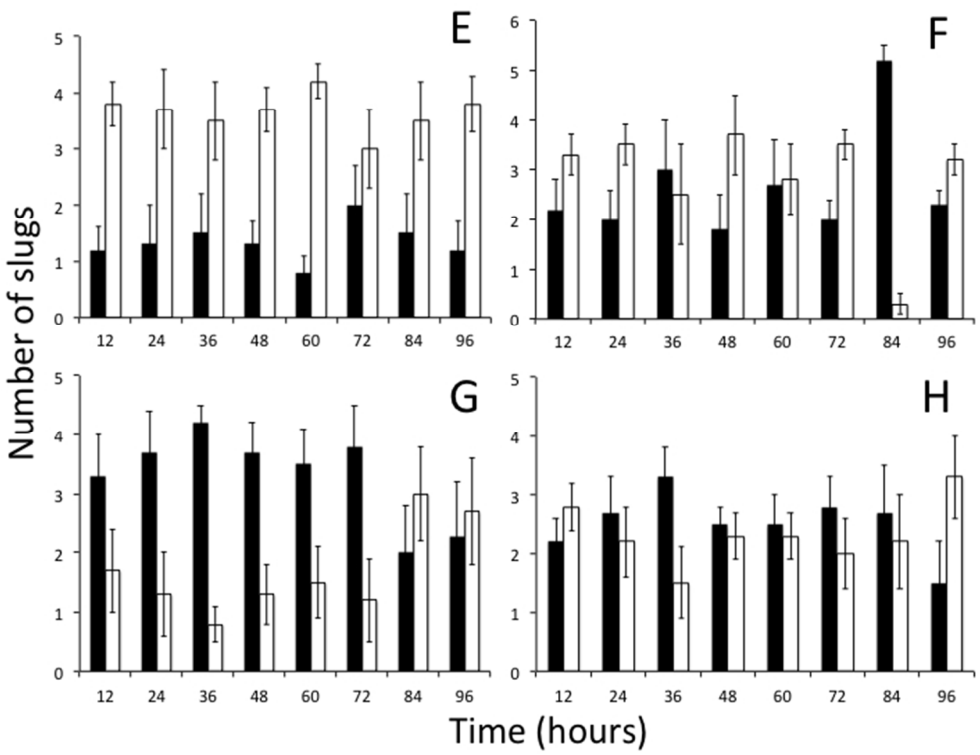
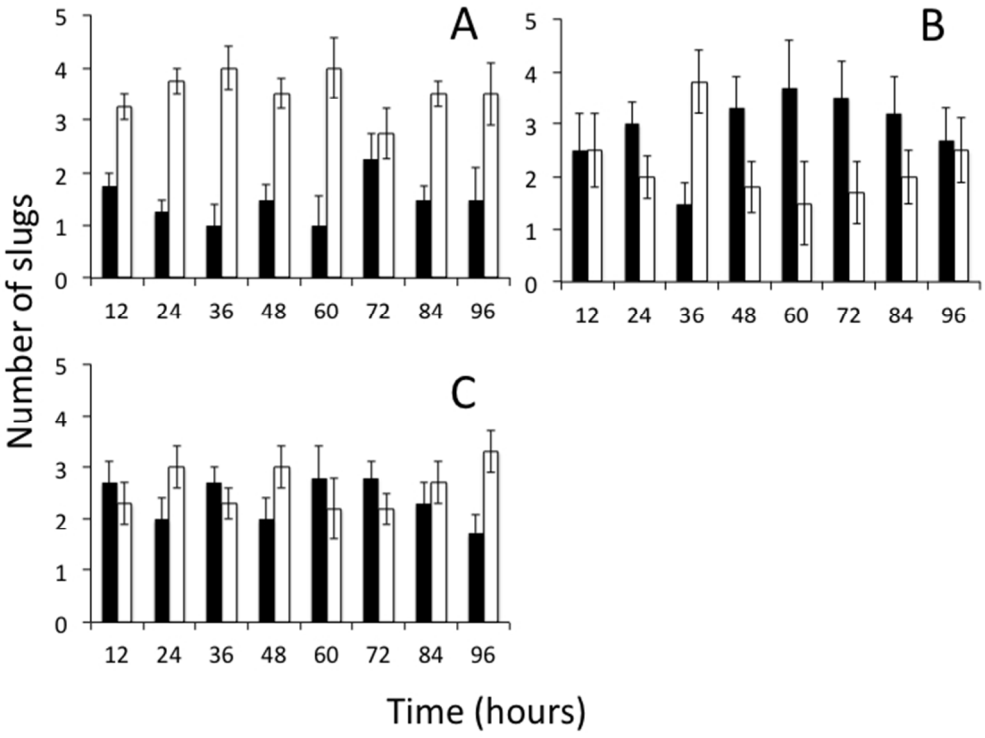
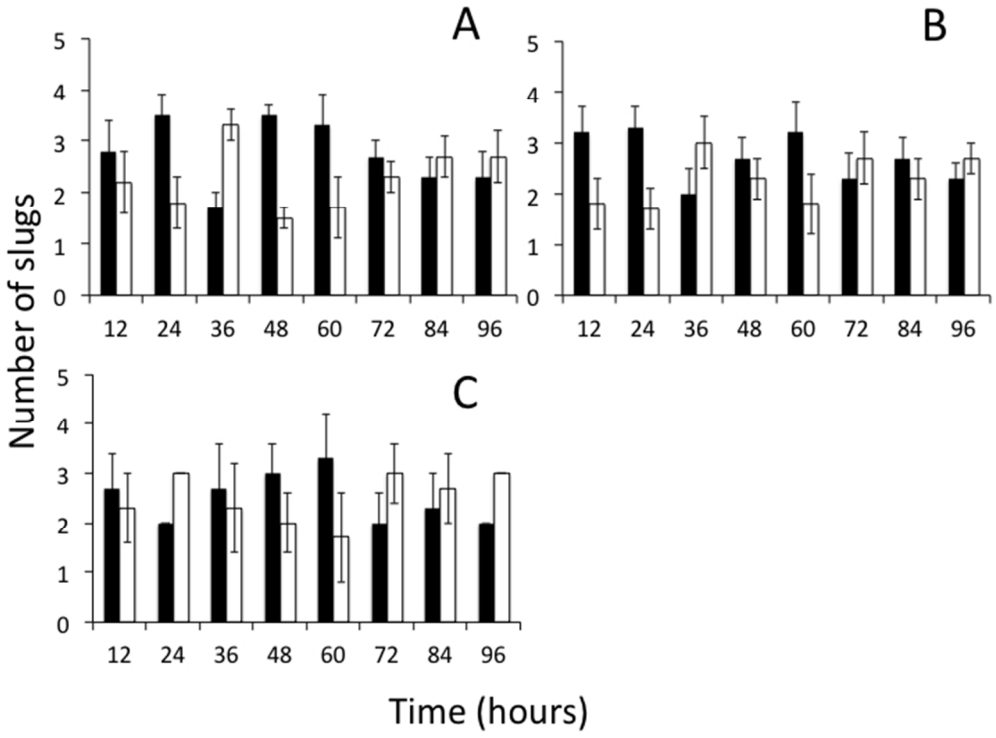


254x190mm (72 x 72 DPI)



254x190mm (72 x 72 DPI)



254x190mm (72 x 72 DPI)

Treatment	Day	Distance* from midline	Numbers of *P. hermaphrodita* (± st. err)
Water treated side	1	0-2	0 ± 0
		4-6	0 ± 0
		10-12	0 ± 0
	2	0-2	0.11 ± 0.11
		4-6	0 ± 0
		10-12	0 ± 0
	3	0-2	0.33 ± 0.24
		4-6	0 ± 0
		10-12	0 ± 0
	4	0-2	0.8 ± 0.46
		4-6	0 ± 0
		10-12	0 ± 0
Nematode treated side	1	0-2	12.1 ± 3.34
		4-6	6.6 ± 1.73
		10-12	2.4 ± 0.73
	2	0-2	17.3 ± 2.36
		4-6	7.7 ± 1.12
		10-12	3.7 ± 0.62
	3	0-2	24.2± 2.85
		4-6	11.2 ± 1.26
		10-12	9.8 ± 3.57
	4	0-2	5.4 ± 1.12
		4-6	5.9 ± 0.98
		10-12	3 ± 0.76

*Distance in centimeters

254x190mm (96 x 96 DPI)