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The gastropod parasitic nematode *Phasmarhabditis hermaphrodita* does not affect non-target freshwater snails *Lymnaea stagnalis*, *Bithynia tentaculata* and *Planorbarius corneus*.

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Abstract

Phasmarhabditis hermaphrodita is a lethal parasite of several slug and snail species that has been formulated into a biological control agent. However, the complete host range of this nematode is poorly understood, in particular its potential to affect non-target aquatic snail species. Here we exposed three species of juvenile and adult freshwater snail (*Lymnaea stagnalis, Planorbarius corneus* and *Bithynia tentaculata*) to 30 and 150 *P. hermaphrodita* per cm^2 and assessed survival, as well as differences in weight for 66 days. We show that *P. hermaphrodita* has no effect on the survival of *L. stagnalis, P. corneus* and *B. tentaculata* after 66 days of exposure. In summary, we found little evidence of *P. hermaphrodita* causing mortality to three freshwater snail species at two different life stages and believe that *P. hermaphrodita* would have little effect on non-target snail species in the wild.

Keywords

Slugs, aquatic snails, parasites, non-target organisms.

The gastropod parasitic nematode *Phasmarhabditis hermaphrodita* is a lethal parasite of several pest slugs and snails including *Deroceras reticulatum* and *Arion ater* (Wilson et al., 1993; 2000) and has been formulated into a biological control agent (Nemaslug®) for farmers and gardeners in Northern Europe available from BASF-Becker Underwood (Rae et al., 2007). Once applied nematodes seek out slugs and snails, responding to mucus and faeces, then penetrate through the mantle and kill the host in between 4 and 21 days (Rae et al., 2006; 2009a; Wilson et al., 1993; Tan and Grewal, 2001). *P. hermaphrodita* has been used to protect many crops from slug damage including Chinese cabbage (Rae et al., 2009b), winter wheat (Wilson et al., 1994) and oilseed rape (Wilson et al., 1995).

The complete host range of *P. hermaphrodita* is poorly understood and many slug and snail species have never been tested for their susceptibility towards this nematode. One group of molluscs that have been neglected are freshwater snails. There are only two studies that have focused on investigating the effects of *P. hermaphrodita* on aquatic snails, which showed that under lab conditions *P. hermaphrodita* can kill the non-target snail *Lymnaea stagnalis* but not *Physa fontalis* (Wilson et al., 1993; Morley and Morritt, 2006). Here we decided to investigate whether *P. hermaphrodita* could kill three common non-target species of freshwater snail including the Great Pond snail (*L. stagnalis*), the Great Ram's-horn snail (*Planorbarius corneus*) and *Bithynia tentaculata*, which are common, widely distributed snails which live in slow moving and large ponds (Beedham, 1972). We also decided to examine whether the susceptibility of snails to *P. hermaphrodita* could be due to differences in size as previously it has been shown that *P. hermaphrodita* can kill juveniles of the snail *Helix aspersa*, and the slugs *A. ater* and *A. lusitanicus* but adults remain resistant (Glen et al., 1996; Grimm, 2002).

Materials and Methods

Source of invertebrates

P. hermaphrodita was purchased from BASF-Becker Underwood and was stored at 10°C prior to use. Freshwater snails (*L. stagnalis, B. tentaculata* and *P. corneus*) were supplied by Sciento, U.K. and collected from ponds at Calderstones Park, Liverpool. Snails were kept in fresh water at 10°C prior to use.

Infection assay with freshwater snails exposed to *P. hermaphrodita*

P. hermaphrodita were mixed with tap water and numbers of nematodes per 100 µl were quantified. Non-airtight plastic boxes (10 x 9 x 6 cm) were filled with 120 ml of fresh water. Evaporation of water was monitored by weighing boxes every 5 days and adding fresh pond water if necessary to maintain approximately the same volume throughout the experiment. To three boxes the recommended rate of *P. hermaphrodita* was applied (30 nematodes per cm^2) and to another three boxes five times the recommended rate was applied (150 per cm^2). Three boxes received no nematodes and acted as the controls. For the first experiment, ten juvenile *L. stagnalis* (mean weight = 0.48 ± 0.03 g, n = 90) were added to each box. To investigate the difference in weight of snails when infected with *P. hermaphrodita* we also exposed adult *L. stagnalis* to a high dose of 150 *P. hermaphrodita* per cm^2 (mean weight = 4.03 ± 0.13 g, n = 60). This experimental set up was also repeated for both sizes of *P. corneus* (juvenile mean weight = 0.118 ± 0.004, n = 90; adult mean weight = 2.14 ± 0.12, n = 60) and only one size of *B. tentaculata* (mean weight = 0.302 ± 0.006, n = 90) was exposed to 0, 30 and 150 *P. hermaphrodita* per cm^2. All species of snails were weighed before and after the experiment to determine if the nematode caused any effect on weight gain and food consumption which has been documented in other molluscan species (Glen et al., 2000). Snails were provided with food including pond weed and cabbage *ad libitum*.

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Survival was monitored every 3-4 days for 66 days. Any dead snails were dissected to examine nematode penetrance.

Data analysis

Survival of snails was analysed using the log rank test carried out in OASIS (Yang et al., 2011) and the weight of snails before and after nematode treatment was compared using a Student t-test.

Results

***The effect of P. hermaphrodita* on the survival of juvenile and adult *L. stagnalis*, *P. corneus* and *B. tentaculata*.**

P. hermaphrodita applied at both 30 and 150 nematodes per cm^2 had no significant effect on the survival of juvenile or adult *L. stagnalis* after 66 days exposure (P>0.05) (Fig 1a, b). Similarly, *P. hermaphrodita* had no effect on the survival of both juvenile and adult *P. corneus* at both doses (30 and 150 nematodes per cm^2) ($P>0.05$) (Fig 2a, b). Also adult *B. tentaculata* were resistant to both doses of *P. hermaphrodita* as there were no significant differences in survival over 66 days ($P>0.05$) (Fig 3). Therefore, *P. hermaphrodita* had no effect on the survival of three species of aquatic snails when applied at two different doses for 66 days.

The effect of *P. hermaphrodita* on the weight of juvenile and adult *P. corneus* and adult *B. tentaculata*

There was no significant difference between the weight of juvenile or adult *P. corneus* on day 0 and day 66 when exposed to no nematodes, 30 and 150 *P. hermaphrodita* per cm^2 ($P>0.05$) (Fig 4a, b). However, there was a significant difference between the weight of *B. tentaculata* on day 0 and day 66 ($P<0.001$) (Fig 4c), but this was the case for the untreated and both doses of *P. hermaphrodita,* hence these snails lost weight in general throughout the experiment regardless of treatment. Therefore, *P. hermaphrodita* has no effect on the weight gain of aquatic snails.

Discussion

Previous studies have shown that *P. hermaphrodita* may affect non-target aquatic molluscs including *L. stagnalis* (Wilson et al., 1993; Morley and Morritt, 2006). However, in our studies we have shown that *P. hermaphrodita* is unable to kill a selection of non-target freshwater snails including *L. stagnalis, B.*

tentaculata and *P. corneus* at two different doses of *P. hermaphrodita* (30 and 150 nematodes per cm^2) after 66 days exposure. Ultimately, this study shows that *P. hermaphrodita* poses little risk to non-target fresh water snails.

The host range of *P. hermaphrodita* is best characterized in terrestrial slugs and snails. Pestiferous slugs such as *D. reticulatum* and *D. panormitanum* are highly susceptible to *P. hermaphrodita* (Wilson et al., 1993) but other species such as *Limax maximus* and *L. pseudoflavus* (Rae et al., 2008; Grewal et al., 2003) are resistant. Resistance in other species is dependent on size as adult *A. lusitanicus* and *A. ater* are resistant to *P. hermaphrodita* but juveniles are susceptible (Glen et al., 1996; Grimm, 2002). Similarly, in terrestrial snails, some species of terrestrial snails are resistant to *P. hermaphrodita* including *Cepaea nermoralis*, *Oxychilus helveticus*, *Pnentina ponentina*, *Discus rotundatus* and *Clausilia bidentat*a (Wilson et al., 2000; Coupland, 1995; Iglesias et al., 2003). It is unknown why there are these differences in susceptibility to *P. hermaphrodita* but some terrestrial snails, such as the Giant African snail (*Achatina fulica*) have the ability to encapsulate and kill invading nematodes in their shell (Williams and Rae, 2015), which has also been shown in slugs (Rae et al., 2008). However, upon dissection of dead snails no encapsulated nematodes were observed so perhaps this defensive ability is only in terrestrial molluscs. Similarly, we rarely found *P. hermaphrodita* inside the snails, but this is not uncommon when *P. hermaphrodita* is exposed to other snails e.g. *H. aspersa* (Rae et al., 2009a). Either it is harder for *P. hermaphrodita* to penetrate into snails than slugs or our experimental assay is suppressive to nematode infection. However, this seems unlikely as two studies (Morley and Morritt, 2006 and Wilson et al., 1993) showed that *P. hermaphrodita* can kill *L. stagnalis* under similar conditions. One

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important factor maybe the way snails were reared. Morley and Morritt (2006) showed that laboratory reared *L. stagnalis* were susceptible to *P. hermaphrodita* In our study we collected snails from the wild, which have been exposed to an array of naturally occurring parasites and may potential have a stronger immune system and are able to cope with *P. hermaphrodita*. Perhaps laboratory reared *L. stagnalis* used in Morley and Morritt (2006) may potentially have unchallenged and impaired immune systems, which made them more susceptible to *P. hermaphrodita*?

In conclusion we have shown that *P. hermaphrodita* has little pathogenicity towards wild caught freshwater snails and therefore poses little threat to non-target aquatic snails.

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