The parasitic nematode *Phasmarhabditis hermaphrodita* defends its slug host from being predated or scavenged by manipulating host spatial behaviour

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Received 11 December 2007; received in revised form 8 February 2008; accepted 14 February 2008

**Abstract**

Infective stages of commercially used molluscicidal rhabditid nematodes *Phasmarhabditis hermaphrodita* contain bacterial symbionts which kill their host by septicaemia. The nematodes feed on the multiplying bacteria and entire host tissue, develop and repeatedly reproduce. Invertebrate cadavers are rapidly (from minutes to hours) removed by scavengers. However nematodes need days to complete their life cycle inside the host.

The post mortem locations of slugs killed by six different treatments (three types of molluscicides, a simulation of unsuccessful predation and two *P. hermaphrodita* nematode treatments) were compared.

In comparison to other pathogenic states, significantly more slugs killed by the nematodes died within the soil, where the scavenging pressure is weaker than on the soil surface (where most of the slugs died regardless treatment). We suggest that this is an outcome of behavioural manipulation, which prevent the parasites from being predated or scavenged together with their host until the nematodes complete development inside the host cadaver.

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**Keywords:** Manipulation; Slug; Nematode; *Phasmarhabditis*; Deroceras; UV-fluorescent

**1. Introduction**

The rhabditid nematode *Phasmarhabditis hermaphrodita* (Schneider, 1859) has been shown to be a successful biocontrol agent of different pest slugs (i.e. Wilson et al., 1993; Wilson et al., 1995; Glen and Wilson, 1997; Glen et al., 2000; Iglesias et al., 2003). The infective third-stage juveniles of *P. hermaphrodita* invade the slug host (Tan and Grewal, 2001) and release the associated bacterial symbiont, which then multiplies in the slug body and kills the host by septicaemia (Tan and Grewal, 2002). After host death, typically within a week from infection (Grewal et al., 2001), the nematodes colonize the entire cadaver and feed on the bacterial symbiont and the host tissue, developing into adult self-fertilizing hermaphrodites and repeatedly reproducing. As food resources in the cadaver are depleted, new infective juveniles carrying symbiotic bacteria leave the cadaver in search for a new host (Wilson et al., 1993).

While inhabiting the cadaver, the nematodes are under serious threat from predatory and scavenging invertebrates, which can destroy them while feeding on their host. This threat is particularly high because the susceptibility of dying hosts to predation increases as their defence efficiency declines (Pakarinen, 1993; Winder et al., 1994; Symondson, 1997; Foltan, 2004). Invertebrate cadavers are rapidly, usually in minutes or hours (Seastedt et al., 1981; Fellers and Fellers, 1982; Young, 1984; Retana et al., 1991; Bestelmeyer and Wiens, 2003; Foltan et al., 2005) and preferentially (Sunderland and Sutton, 1980; Mair and Port, 2001; Langan et al., 2001; Foltan et al., 2005) removed by predators and scavengers, while nematodes require several days to complete their reproduction in the cadaver.

Different parasitic groups have been shown to manipulate feeding, social, spatial or dispersal behaviour or physiological functions of their vectors, which results in an increased probability of their transmission to other hosts (Barnard and Behnke, 1990; Combes, 1991, 1998; Poulin, 1995, 1998; Lafferty et
al., 2000; Moore, 2002; Lion et al., 2006). Such manipulations expose the hosts to increased predation risks, facilitating transmission of the parasites across the food chains. Several studies have shown that manipulation can sometimes evolve as a strategy to protect the infected host from environmental dangers (Brodeur and McNeil, 1989; Eberhard, 2000; Médoc et al., 2006). However, not all cases of reported host behavioural change are necessarily a result of the parasite actively manipulating host behaviour; they may rather be the ‘by-products’ of pathology caused by the parasites (Thomas et al., 2005; Leung and Poulin, 2006).

In this study, we report that *P. hermaphroditus* manipulates the spatial behaviour of its slug host, *Deroceras reticulatum* (Müller, 1774), preventing the host from being predated or scavenged until the nematodes complete their development. We exposed the slugs to the nematodes and four other pathogenic agents. Although all treatments ultimately killed the slugs, nematode treatment resulted in spatial distribution of cadavers differing from the spatial distribution of slugs that have died due to action of mollusccides or mechanical damage. As far as we are aware, this is a rare case where host manipulation protects the infected host (and parasite) from predation and scavenging, rather than manipulating the host to increase the probability of consumption by a consequent host species.

2. Methods

2.1. Slugs

*D. reticulatum* slugs (100–250 mg/individual) were collected from arable fields around Ceske Budejovice, Czech Republic (49°0’0”, 14°30’, alt. 400 m). The slugs were housed individually in plastic containers containing moist soil substrate (AROS Ltd.) and fresh lettuce leaves, and maintained in a controlled environment (L:D 16:8; 20 ± 1°C) for at least 7 days prior to the experiment to become established and equally hydrated. Ninety slugs were labelled by a UV-fluorescent dye (10 μL/individual) 2 days before the experiment (Foltan and Konvicka, in press). The labelling enabled later identification of slug cadavers on the soil surface or in the soil, which otherwise would not be possible.

2.2. UV-fluorescent dye

10% liquid solution of biologically inert powder Radglo JST UV-fluorescent pigment (Radiant colour N.V.) was made up by dilution in Polyethylene glycol, Av. mol. wt.: 400 (Sigma–Aldrich) The solution was subsequently diluted in distilled water 1:1 (Foltan and Konvicka, in press).

2.3. Mini-plots

The experiment was conducted under controlled conditions in 90 mini-plots consisting of translucent plastic cylinders (7.5 cm dia. and 27 cm deep) half filled with 15 cm of moist soil substrate and covered with mosquito-curtain to prevent slugs from escaping. Each container was coated in black paper tape from outside, up to the soil surface, to prevent side illumination through the container wall. To simulate field conditions, barley seeds were sown at a depth of 1 cm (approx. 25 seeds per container) in the soil and allowed to grow up to 10 cm before the experiment. Equal soil moisture of approx. 30% was maintained in all the containers used.

2.4. Experimental design

The 90 slugs were divided into six groups, each consisting of 15 individuals (kept individually in 15 containers) and subjected to six different treatments. Three treatments were different types of mollusccicides, Ferramol (iron phosphate-based, W. Neuedorff GmbH KG), Mesurol (methiocarb-based, Bayer AG) and Vanish slug pellets (metaldehyde-based, TransChem Professional BV). The slugs to be treated with mollusccicides were introduced into the containers 5 days before treatment to become established and find natural refugia. Two treatments were by the nematodes (commercially available *P. hermaphroditus* nematodes (Nemaslug®): one treatment simulated exposure to infected soil, consisted of treating the 5-day established slugs with commercially recommended dose of the parasite. In the second treatment, the slugs were infested by placing them into water containing a high concentration of the nematode for 25 min, washed and placed individually into the containers. Finally, one treatment simulated deaths from unsuccessful predation: the slugs were cut by scissors into the hepatopancreas region (slugs were able to move for several hours before the death) and placed individually into the experimental containers.

After the slugs had died, the post mortem position (vegetation/walls, surface, and soil) of each individual was identified, using a portable UV-lamp (according to Foltan and Konvicka, in press). All slugs that died under the two nematode treatments were checked for the presence of nematodes using a stereomicroscope.

Contingency table (with six treatments and three response levels) was used to compare distribution of post mortem locations.

3. Results

Slugs exposed to all six treatments were able to move for some period of time before they all died regardless of treatment. There were significant differences between the distributions of the slug post mortem locations between all treatments (M–L $\chi^2 f = 24.31$, df = 10, $p < 0.01$). Treatments without nematodes (simulation of predation and the three types of mollusccicides) did not differin their distribution of the slug post mortem positions (M–L $\chi^2 f = 6.30$, df = 6.0, $p = 0.39$). Thus, for further analyses, this group of treatments was chosen as the control group.

Significantly more slugs (M–L $\chi^2 f = 9.0$, df = 2, $p < 0.05$) died on the vegetation or container walls above ground level in the treatment, where slugs were infected inside the experimental containers than in the control group. In the treatment where slugs were infected outside the experimental container, significantly more cadavers were found within the soil compared to the control (M–L $\chi^2 f = 6.39$, df = 2, $p < 0.05$). Slug cadavers from the two “nematode treatments” differed in distribution from each other.
Fig. 1. Counts and position (vegetation/walls, soil surface, soil) of slug cadavers found in the experimental containers \( (N = 90) \) where six different treatments were applied to slugs placed individually into the containers: three different molluscicides, simulation of predation and two nematode treatments were used. Nematodes I: slugs were infected by nematodes inside the experimental containers. Nematodes II: slugs were infected outside the containers and introduced into nematode-free containers.

(M–L \( \chi^2 f = 12.52, df = 2, p < 0.05 \)), see Fig. 1. All slugs that died under the two nematode treatments contained high numbers of reproducing nematodes.

4. Discussion

In the first nematode treatment, a considerable proportion of slugs added to containers containing infective nematodes died on plants or walls of experimental containers, probably trying to escape from the reach of the nematodes present in the soil. Under natural conditions, the nematode spatial distribution varies (Stuart and Gaugler, 1994). The escaping infected slugs thus can reach some nematode-free areas before their death. Slugs have been shown to survive infection to some level (Wilson et al., 2000; Wilson and Gaugler, 2000), thus escaping from the soil inhabited by infective nematodes remains meaningful even for already infected hosts. This observation is supported by findings of Wilson et al. (1999), who placed \textit{D. reticulatum} and \textit{Arion ater} slugs into boxes, where half the soil surface area was treated by \textit{P. hermaphroditus} and the second half remained untreated. Both slug species were more likely to rest and feed on the nematode untreated than nematode treated soil, but showed no preference between the treatments if low \(<38/cm^2\) nematode densities were used. However in a mini-plot experiment using \textit{P. hermaphroditus} to control slugs in Chinese cabbage, Hass et al. (1999) found no evidence of slugs being repelled. In the second nematode treatment, slugs infected by the nematodes were transported into nematode-free containers, where they died from the infection. The slugs would be expected to die on the surface as they were not habituated in the containers and had less time, compared to other treatments excluding the simulation of predation, to create shelters in the soil (Hommay et al., 1998; Grewal et al., 2001). However, the slugs exhibited significant tendency to die in the soil compared to other pathogenic states.

The prevalent post mortem location on the soil surface in molluscicide treatments and consequent significant difference from the two nematode treatments can be potentially caused by extremely rapid death of slugs after direct contact with the molluscicide. However, in all such treatments, at least some slugs were found elsewhere than on the soil surface, which indicates that they moved before they died. Furthermore, in the “predation” treatment, where injured slugs were able to move for a sufficient period of time before their death, the distribution of their post mortem positions did not differ from the molluscicide treatments.

Invertebrate cadavers present on the soil surface are open to extreme scavenging pressure of generalist predators/scavengers (Seastedt et al., 1981; Fellers and Fellers, 1982; Young, 1984; Retana et al., 1991; Bestelmeyer and Wiens, 2003; Foltan et al., 2005), whereas the removal of invertebrate cadavers is much lower under the soil surface (Seastedt et al., 1981). Apart from scavenging, the cadavers present on the soil surface are exposed to high temperatures (Ruth et al., 1975) and quickly desiccate.

It would be beneficial, to the nematode/bacteria complex manipulating slugs, for the slugs to die in soil. The manipulation enables the nematodes to resist scavenging pressure (at least at a certain level) and also to avoid desiccation on the soil surface. Foltan and Puza, submitted for publication revealed significant deterrent effect of slugs infected by \textit{P. hermaphroditus} to a
carabid predator *Pterostichus melanarius*, a prevalent predator of slugs in the majority of crops capable of affecting their temporal and spatial dynamics (Symondson et al., 1996; Bohan et al., 2000; McKemey et al., 2003; Paill, 2004). Combination of the two effects potentially facilitates successful development of the nematodes inside their host cadavers. However, it would be essential to experimentally compare the success of nematode development both on and under the soil surface with and without predator/scavenger presence in order to be able to claim that the manipulation has evolved as a strategy against predators and scavengers. Our experimental design unfortunately excludes to decide whether it is the nematodes, the associated bacterial symbionts or the interaction of both who is responsible for the suggested behavioural manipulation. Further experiments with bacterial and nematode isolates are necessary to test this.

Extrapolation of laboratory mini-plot data to the field situation is also problematic and only further field experiments can reveal whether the manipulation hypothesis can be adopted in the context presented above.

Previously, many different gut content analysis techniques have been developed as a valuable tool for the study of predation on pest slug species (Sunderland et al., 1987; Sunderland, 1996; Symondson and Liddell, 1996a,b; Symondson, 2002a,b).

Foltan et al. (2005) and Calder et al. (2005) used a slug-carabid model to show that scavening represents a significant biasing factor when molecular (PCR-based) or biochemical (monoclonal antibody-based) gut content analysis is used to assess impact of predation on prey populations. Neither analytical approach was able to distinguish between material that was predated or scavenge. To assess the size of error coming from scavening, estimation of carrion availability for scavengers is necessary. In our experiment, regardless of the treatment, a majority of the slug cadavers remained on the surface, easily accessible to scavengers. As many predators regularly scavenge (Sunderland, 1996) or prefer dead to live prey (Sunderland and Sutton, 1980; Mair and Port, 2001; Langan et al., 2001), we assume that many false positive results of predation can be obtained when gut content analyses are used for its assessment.

In terms of biological control, the effects of parasites and predators are often negatively correlated. The predators are likely to destroy parasites within their prey. On the other hand, a parasitic infection can quickly reduce pest abundance, which can be critical for maintaining predators in the field. Success of entomopathogenic and molluscicidal nematodes in reducing pest populations is significantly influenced by persistence of infective juveniles in the soil and the ability of a parasitic stage to develop within the cadaver (Baur et al., 1998). In the case of *P. hermaphrodita*, the suggested manipulation of slug behaviour so that they die in the soil, together with the repellent effect of infected carrion (Foltan and Puza, submitted for publication), reduces the vulnerability of the parasites to predation however potentially beneficial predators are restricted in their access to a valuable source of energy which originates from scavening. Further research on predator–parasite–host and scavenger–parasite–cadaver interactions is needed to improve application success and commercial use of pesticidal nematodes as well as to maintain pest predators in the field as an essential part of the integrated pest management.

Acknowledgements

We would like to thank Oleg Dittrich, Martin Konvicka and Pavel Pech, and for many useful advices and constructive criticism. We thank Cyrille Verdun and Becker-Underwood for providing the nematodes and Frantisek Sedlacek for providing UV-fluorescent dye. Thanks are also due to Graham Small and Peter McEwen for essential language correction, the Institute of Entomology for loaning of a controlled environment room and to the anonymous referees for many helpful improvements to the manuscript.

The study was supported by the Czech Ministry of Education (MSM6007665801).

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