Review

Received: 17 April 2023 Revised: 23 June 2023 Accepted article published: 27 June 2023 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.7636

Thirty years of slug control using the parasitic nematode *Phasmarhabditis hermaphrodita* and beyond

Robbie Rae,* Laura Sheehy and Kerry McDonald-Howard

Abstract

Several slug species are highly pestiferous and threaten global sustainable agriculture. Current control methods rely heavily on metaldehyde pellets, which are often ineffective, harm nontarget organisms and have been banned in some countries. A viable alternative is the parasitic nematode *Phasmarhabditis hermaphrodita* (and recently *P. californica*), which has been formulated into a biological control agent (Nemaslug®) to control slugs across northern Europe. Nematodes are mixed with water and applied to soil where they seek out slugs, penetrate behind the mantle and kill them in 4–21 days. *Phasmarhabditis hermaphrodita* has been on the market since 1994 and since then there has been ample research on its use. Here we review the research carried out on *P. hermaphrodita* over the last 30 years since its development and release as a commercial product. We provide information on life cycle, worldwide distribution, history of commercialisation, gastropod immunity, host range, ecological and environmental factors that affect its success in the field, bacterial relationships, and summarise results of field trials. Finally, we suggest future directions for *P. hermaphrodita* research (and other *Phasmarhabditis* species) to enhance its use as a biological control agent to control slugs for the next 30 years.

Keywords: nematodes; slugs; snails; biocontrol; gastropods

1 INTRODUCTION

Several slug species are highly pestiferous and pose a significant global threat to agriculture, horticulture and floriculture.[1] Slugs cause crop damage by eating seeds, stems, growing points and leaves, leading to a reduction in growth.[2,3] They can be a major pest throughout the lifecycle of field vegetables and in extreme cases, whole fields have to be re-sown resulting in economic losses.[4] Contamination of the harvested crop also occurs from slug mucus and faeces, resulting in poor product quality.[5] It is estimated that a lack of slug control for crops such as oilseed rape and wheat would lead to £43.5 million a year in loss of product in the UK alone.[6] In Europe, wheat and oilseed rape suffer greatly from slug damage;[7] for example in 2010 it was reported that 22% of winter wheat crops suffered damage from slugs, and if left untreated by chemical molluscicides a 5% decrease in yield would be expected.[8] As well as causing damage in agriculture, slug-feeding can affect plant community diversity and richness[9] with preferential feeding on native species aiding in exotic plant growth.[10] Furthermore, slug-feeding reduces conservation efforts such as forest regeneration[11] and threatens endangered species such as lichens.[12] Slugs also can transmit plant pathogens such as *Phytophthora*[13] and parasites,[3,14] including the rat lungworm, *Angiostrongylus cantonensis*, the causal agent of eosinophilic meningitis, which is recognised as an emerging tropical and subtropical zoonotic disease.[15]

Slugs are commonly controlled by chemical bait pellets containing metaldehyde. In the past methiocarb was used, yet it is toxic to beneficial invertebrates and other nontarget organisms[16,17] and was banned in the UK in 2014.[18] Metaldehyde pellets are used globally.[19] For example, from 2008 to 2014 an estimated 1640 t metaldehyde was used in the UK alone.[19] Slugs feed on the pellets and exhibit symptoms such as increased levels of mucus secretion and paralysis, and die within several days from water loss.[20,21] Although effective, these bait pellets also cause harm to nontarget organisms including canines and other vertebrates.[22] Additionally, metaldehyde also is now considered an important emerging pollutant of concern as a consequence of leaching into watercourses[23] caused by its high mobility in soil.[24] Furthermore, in parts of the UK metaldehyde concentrations in water bodies have exceeded the European Union's regulatory drinking water standard for pesticides.[24] An alternative slug pellet (Ferramol®) is composed of iron III phosphate or ferric phosphate and is registered for use in many European countries.[25] Although it has been used to control slugs such as *Arion ater*, studies have

* *Correspondence to: Robbie Rae, Liverpool John Moores University, School of Biological and Environmental Sciences, Byrom St., Liverpool, UK, L33AF. E-mail: r.g.rae@ljmu.ac.uk*

School of Biological and Environmental Sciences, Liverpool John Moores University, Liverpool, UK

shown that high doses can lead to mortality and reduced activity in earthworms.[26]

In agriculture, trapping, drilling at a greater depth, ploughing, crop rotation, increasing crop diversity and firm seedbed preparation also can help to limit slug damage, although some practices such as direct drilling and minimal tillage can result in an increase in pest slug populations.[27] Drilling to depths of 25–45 mm has been shown to provide the most effective protection against slug damage[28] and ploughing, and firm seedbed preparation reduces slug numbers by disrupting their normal surface activity patterns.[29]

In gardens and glasshouses, damage by gastropods can be limited by cultural control methods such as the use of copper (Cu) tape, garlic and mulch, although they are inefficient for larger scale agricultural use.[30] The use of Cu tape or Cu-impregnated matting has been shown to act as a barrier and reduce the velocity of pest slugs, possibly as a result of irritation.[31] In choice experiments, Cu was seen to repel slugs and they nearly always avoided mulch as it dries out quickly.[31] However, these methods are time-consuming, expensive and not always effective. An effective alternative for slug control is the gastropod parasitic nematode *Phasmarhabditis hermaphrodita* (Fig. 1) (for key diagnostic features see Stock and Hunt[32]), which has been formulated into a biological control agent (Nemaslug®) produced and sold by BASF Agricultural Specialities (Littlehampton, UK).[33] *Phasmarhabditis hermaphrodita* (strain DMG0001) is sold in 15 different European countries[34] and has been on the market since 1994; it also is available as a product called SlugTech® sold by Dudutech (Naivasha, Kenya; www.dudutech.com/products/slugtech-sp/).

Over the last 30 years, *P. hermaphrodita* has been successfully used to reduce slug damage in agriculture, floriculture and horticulture to levels comparable to those in crops treated with metaldehyde.[35] Here we describe the research that has been carried out on *P. hermaphrodita* since the first publication outlining its potential as a biocontrol agent of slugs in 1993,[33] and provide information on the *Phasmarhabditis* genus, host range and interactions, bacterial associations, nematode and gastropod behaviour, results of field trials, and suggest future research to enhance the use of *P. hermaphrodita* (and other *Phasmarhabditis* species) in the field.

1.1 Slug parasitic nematodes and the genus *Phasmarhabditis*

There are 108 nematodes associated with slugs and snails[14] used as definitive, intermediate or necromenic hosts.[34] Forty-seven species of nematode, belonging to eight families, use molluscs as a definitive host,[14,34] yet the only nematodes that can kill slugs and snails are those from the genus *Phasmarhabditis*.[33] There are some reports of mortality being caused by *Alloionema appendiculatum* towards *Arion vulgaris* but not at levels considered suitable for a biocontrol agent.[36]

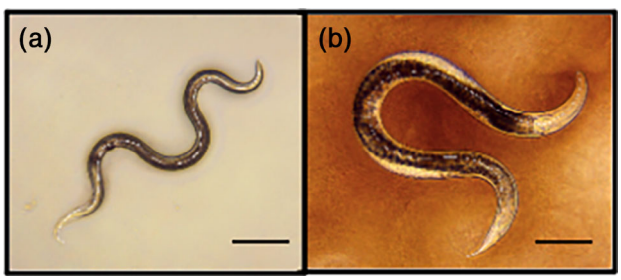

Figure 1. Dauer stage *P. hermaphrodita* (a) seek out slugs in soil and then penetrate inside. Once the slug dies the nematodes exit the dauer stage and grow to young adult nematodes (b) and reproduce on the cadaver. Bars, 100 µm.

Phasmarhabditis hermaphrodita is in clade V of the Nematoda,[37] and along with other *Phasmarhabditis* species, are easy to isolate from slugs and snails,[33,38–40] with many different species isolated from around the world. Identification can be accomplished using standard genotyping methods using 18SrRNA primers,[41] species-specific primers and quantitative polymerase chain reaction (qPCR) methodologies for nematodes isolated from soil or hosts.[42,43] *Phasmarhabditis he hermaphrodita* was first described from Germany by Schneider in 1859,[44] then in 1900, Maupas isolated *P. hermaphrodita* in Normandy, France,[45] and 50 years later in 1953 it was re-isolated by Mengert in Germany.[46] The species was found in the UK in the early 1990's from diseased grey field slugs (*Deroceras reticulatum*) at Long Ashton Research Station, University of Bristol[33] as part of a project to identify potential biocontrol agents of slugs.[47] Further research focused on finding a suitable bacterium for mass production[48,49] and proof that the nematode could be used to control slugs under field conditions.[50,51] This research carried out by Mike Wilson and David Glen was used as a blueprint to commercially produce *P. hermaphrodita* first by MicroBio, then Becker Underwood and now BASF Agricultural Specialities. Subsequently, interest in *P. hermaphrodita* grew, and it was subsequently found in France,[52] Chile,[53] Iran,[54] Czech Republic,[55] Egypt,[56] New Zealand,[38,57] Norway[58] and Belgium.[59] One of the biggest markets for slug control is the USA, but for years *P. hermaphrodita* was never isolated despite several surveys.[60–62] However, recently numerous strains of *P. hermaphrodita* and other *Phasmarhabditis* species have been found in North America, specifically California, Oregon[63–66] and Canada.[67,68] The US strains of *P. hermaphrodita* have been shown to kill neonate giant African snails (*Lissachatina fulica*),[69] and several other *Phasmarhabditis* species can kill *D. reticulatum*,[70,71] the snails *Succinea* spp.[72] and *Theba pisana*,[73,74] as well as the subterranean slug *Testacella haliotidea*.[75] As well as *P. hermaphrodita* it has recently been shown another three species in the genus (*P. bohemica*, *P. bonaquanense* and *P. apuliae*) can infect and kill slugs (*D. reticulatum*).[76] Interestingly, full mitochondrial analysis of European and US strains of *P. hermaphrodita*, *P. californica* and *P. papillosa*, (as well as the Nemaslug® product) implies that the commercial strain *P hermaphrodita* DMG0001 was introduced to the US.[77]

Nematodes from the genus *Phasmarhabditis* are problematic to classify as there are some poorly described species, but currently 18 species have been isolated from terrestrial gastropods, including *P. apuliae*, *P. bohemica*, *P. bonaquaense*, *P. californica*, *P. circassica*, *P. clausilliae*, *P. hermaphrodita*, *P. meridionalis*, *P. neopapillosa*, *P. papillosa*, *P. safricana*, *P. akhaldaba*, *P. kenyaensis*, *P. thesamica*, *P. quinamensis*, *P. zhejiangensis* and *P. tawfiki*, and one species (*P. huizhouensis*) from rotting leaf litter.[78–91] There were another two *Phasmarhabditis* species including *P. nidrosienses* (isolated from a marine habitat) and *P. valida* (isolated from littoral detritus),[92] but after revision they were moved to the genus *Buetschlinema*.[93]

It is clear from the numerous surveys carried out over the last 30 years that *Phasmarhabditis* nematodes are commonly found in many countries from diverse terrestrial gastropod hosts. Whether or not there is any specific host preference the nematode has to a particular slug or snail species is unknown, but from survey results it would seem that there is a looser association with numerous terrestrial gastropod species: *P. tawfiki* was isolated from the snail *Eobania vermiculata* and the slug *Limacus flavus* in

Egypt;[78] *P. bonaquaense* was found in the slug *Malacolimax tenellus* in the Czech Republic; *P. apuliae* was isolated from slugs *Milax sowerbyi* and *Milax gagates* from Italy;[80,81] *P. bohemica* from the Czech Republic was isolated from *D. reticulatum*;[82] *P. papillosa* was isolated from *D. invadens* (previously called *D. panormitanum*) and *Tandonia sowerbyi* from the UK and *D. reticulatum* in the USA.[62,79] and South Africa;[94] *P. neopapillosa* was isolated from *D. reticulatum*, *D. panormitanum*, *L. flavus*, *Arion ater* and *Arion distinctus* in Scotland and England.[40,62] A new species (*P. safricana*) was collected from *D. reticulatum* in South Africa.[90,95] *Phasmarhabditis californica* was isolated from the USA from numerous species including *D. reticulatum*, *D. laeve*, *Arion hortensis* and *Ambigolimax valentianus*,[79] as well as being found in *Geomalacus maculosus* in Ireland[96] and from the snail *Oxychilus draparnaudi* in Wales[40] and Germany;[97] it also was isolated from *Arion rufus* from Edmonton, Canada,[67,68] and along with *P. hermaphrodita* has been infecting *D. reticulatum* in New Zealand.[57,77] *Phasmarhabditis meridionalis* was described from snails (*Quantula striata*) in Vietnam[85] and in 2019, *P. circassica* and *P. clausiliiae* were found in snails *Oxychilus* sp. and Clausiliidae sp., respectively, in Russia.[86] Therefore, *Phasmarhabditis* nematodes have a cosmopolitan distribution across the globe and can be easily isolated from a diverse range of slugs and snails.

There are several *Phasmarhabditis* species still awaiting description, including two *Phasmarhabditis* species in Japan,[98] and two species (called '*Phasmarhabditis* sp. SA3' and '*Phasmarhabditis* sp. SA4') isolated from slugs in nurseries in South Africa.[99] A possible *Phasmarhabditis* species was found reproducing on the earthworm *Lumbricus terrestris*,[100] and was described as being virulent towards earthworms, which is highly unusual for a *Phasmarhabditis* species. Finally, *Phasmarhabditis* sp. EM434 was discovered in North America[101] but there is only limited information on this species, which amounts to only a few DNA sequences in the National Centre for Biotechnology Information (NCBI) database.

Out of all the currently described species, *P. hermaphrodita*,[33] *P. neopapillosa*,[102,103] *P. tawfiki*,[104] *P. papillosa*,[94] *P. safricana*,[90,95] *P. bohemica*, *P. bonaquaense* and *P. apuliae*,[76,105] and *P. californica*[73] have been shown to kill slugs and snails. Taken together, these results demonstrate that pathogenicity towards terrestrial gastropods is not confined to one *Phasmarhabditis* species and appears to be a common trait across the genus.

1.1.1 *Life cycle of* P. hermaphrodita

Phasmarhabditis hermaphrodita is a facultative parasite, able to kill several species of terrestrial gastropods and grow and reproduce on a variety of organic matter[45,106,107] (Fig. 2). It also is able to infect larger host species such as *A. ater* where it will remain until the host dies and reproduce on the cadaver, termed 'necromeny'[108] (Fig. 2). *Phasmarhabditis hermaphrodita* is a hermaphroditic nematode and the occurrence of males is extremely rare,[92] with one study finding just one male in 14 888 hermaphrodites.[45]

1.1.2 *Chemoattraction of* P. hermaphrodita *to slug and snail host cues*

In order to locate hosts, *P. hermaphrodita* dauer stage nematodes seek out slugs in soil by following mucus, faecal and volatile cues.[109–115] Nictation (where entomopathogenic nematodes stand on their tail hoping to latch on to hosts passing by) and body waving has not been observed in *P. hermaphrodita*, potentially as a consequence of their long length.[116,117] Alternatively, these nematodes employ a 'cruiser'-based foraging strategy where they actively search for hosts following cues. *Phasmarhabditis hermaphrodita* is attracted to faeces, foot and mantle mucus of *D. reticulatum*.[109] As many slugs and snails display homing behaviour and return to the same location each night,[118] faecal attraction of *P. hermaphrodita* may be beneficial for infecting new hosts. Volatile host cues such as CO_2 were found to be the least attractive cues to *P. hermaphrodita*,[109] potentially owing to the vast quantities of CO_2 released by microorganisms in soil,[119] but also as a result of *P. hermaphrodita* entering the slug host through the back of the mantle and not the respiratory pore.[33] When *P. hermaphrodita* is exposed to *D. reticulatum* mucus, speed, movement, distribution of turning angles and the fractal dimension of nematode foraging trails significantly increase.[111,112] *Phasmarhabditis hermaphrodita* not only responds to mucus from *D. reticulatum*, but also is positively attracted mucus from a wide range of diverse slug and snail species.[110,120] Of the species tested, *P. hermaphrodita* showed a preference for slugs such as *Arion subfuscus*, *D. invadens* and the snail *Cornu aspersum* (even though the nematode finds it difficult to infect and kill this species). These hosts represent a range of parasitic and necromenic life cycles. *Phasmarhabditis hermaphrodita* was more attracted to slugs than earthworms (*L. terrestris* and *Eisenia hortensis*). Reproductive success of *P. hermaphrodita* was not greater on attractive slug species (compared to nonattractive species), and the reason for this preference to certain slug species is still unknown.[110] In a similar experiment recently[121] the chemotactic response of *P. papillosa* was recorded when exposed to mucus from a selection of species, of which *L. maximus* and *C. aspersum* were particularly attractive to compared to *A. vulgaris* and *D. reticulatum* (for reasons unknown). The pathogenicity of *P. papillosa* to these slug and snail species is unknown; therefore conclusions about the reasons for their attraction cannot be made.

All these studies have focused on using the commercial strain of *P. hermaphrodita* (strain DMG0001) that has been in culture since 1994. To gain more insight into how wild strains of *P. hermaphrodita* would behave, several wild isolated strains of *P. hermaphrodita*, *P. neopapillosa* and *P. californica* were exposed to mucus from seven different slug species.[122] The wild strains differed in their preference to the slug species tested with *P. neopapillosa* preferring *Arion* spp. In a similar study[123] the response of *P. hermaphrodita*, *P. neopapillosa* and *P. californica* to snail mucus was recorded. Surprisingly, the commercial strain of *P. hermaphrodita* DMG0001 showed little chemotactic response and remained at the point of application, whereas wild isolates of *P. hermaphrodita* and *P. californica* were attracted to mucus of *Cepaea nemoralis*, *Cepaea hortensis* and *Arianta arbustorum*[123] (even though they are all resistant to the nematode). There is little information about what the exact compounds in slug and snail mucus *Phasmarhabditis* nematodes are attracted to, but metal ions (e.g. $MgCl_2$, $FeSO_4$) and hyaluronic acid (an abundant component of slug mucus) play a role.[123] Furthermore, there is natural variation in the chemotactic response of wild strains of *P. hermaphrodita*, *P. californica* and *P. neopapillosa* to hyaluronic acid, suggesting that it must be an important component for host finding.[124]

The majority of chemotaxis experiments investigating the behaviour of *P hermaphrodita* have been carried out on agar plates and therefore may not be applicable to their natural soil environment. A more realistic experimental design, where sand grains were placed on agar plates, found that the speed, turning angle distribution, fractal dimension and mean square displacement of *P. hermaphrodita* was reduced when in contact with mucus.[112] Furthermore, in soil olfactometers *P. hermaphrodita* was averted from dead slugs (which are usually attractive) leading

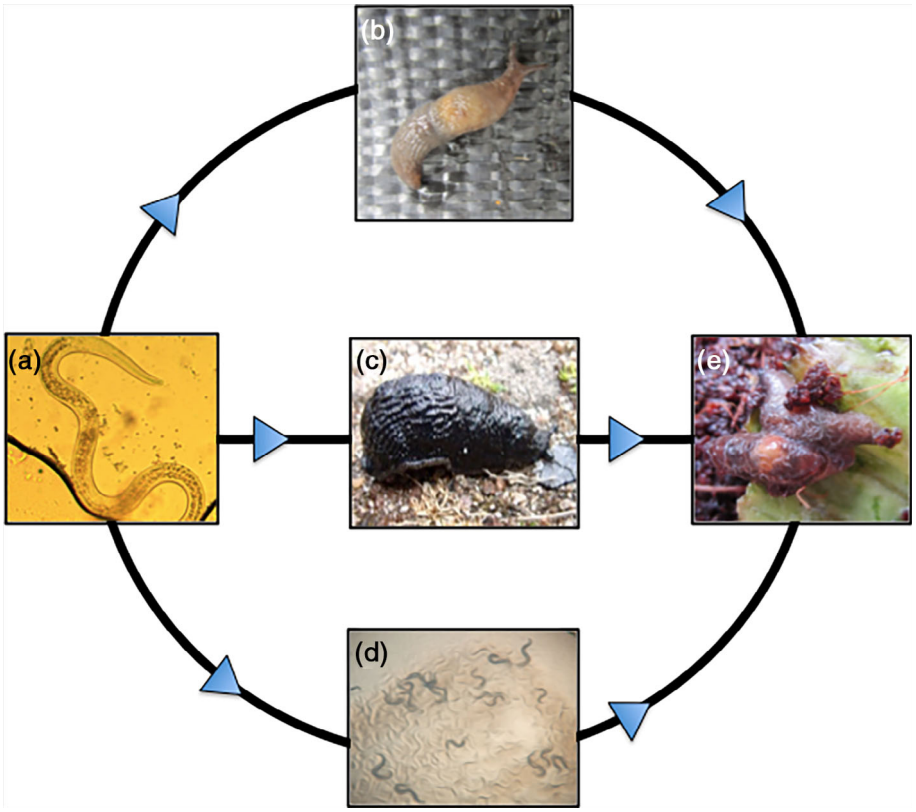

Figure 2. *Phasmarhabditis hermaphrodita* (a) can complete its life cycle in three ways. It can parasitise and kill susceptible hosts such as *D. invadens* (b), infect resistant slug species such as *A. ater* and wait for it to die (a 'necromenic' relationship) (c), or feed and reproduce on the bacteria that proliferate on decomposing organic matter (a 'saprobic' relationship) or can be kept under laboratory conditions on an agar plate with *E. coli* as a food source (d). In each case once the food supply has been depleted the nematode will develop to the dauer stage and move through soil to find more hosts to infect and kill (e).

the authors to hypothesise that the large variety of decay gases caused *P. hermaphrodita* to suffer from a lack of oxygen and move away.[115] In columns packed with different substrates *P. hermaphrodita* moved best through organic matter, uncompacted soil and soil containing large aggregates.[107] Dispersal of *P. hermaphrodita* was increased when placed in mineral soils with the earthworm *L. terrestris*. They also showed that the commercial strain of *P. hermaphrodita* was unable to move through the soil column, but a wild isolated strain from Norway dispersed significantly more.[107]

1.1.3 How P. hermaphrodita *kills slugs—the questionable role of bacteria*

When *P. hermaphrodita* locates a slug host it enters through the back of the mantle through a pore and migrates to the shell cavity.[33,106] Larvae then develop into self-fertilising hermaphrodites and start to reproduce. This produces characteristic signs of infection such as a swollen mantle and shell ejection (Fig. 3). Host death occurs 4–21 days after initial infection,[33] and nematodes feed and reproduce on bacteria proliferating on the cadaver. When the food source is depleted, dauer juveniles enter the soil to locate a new host. It is currently unknown how *P. hermaphrodita* kills slugs. Early research focused on a paradigm similar to entomopathogenic nematodes (EPNs) and their symbiotic relationship with bacteria. EPNs of the families Steinernematidae and Heterorhabditidae associate with *Xenorhabdus* spp. and *Photorhabdus* spp., respectively, that are responsible for killing host insects.[125] It was previously thought that *P. hermaphrodita* functioned in a similar way to EPNs and acted as a vector for the bacterium *Moraxella osloensis*, and the host died due to septicaemia.[126] When the first strain of *P. hermaphrodita* (DMG0001) was isolated an attempt was made to identify a bacterium that could be used for industrial production of these nematodes. Indeed, it is clear that bacterial diet, substrate and inoculation density can have dramatic effects on growth, lipid content and length of nematodes.[48,49,105,127,128] Initial studies focused on understanding the best bacterium that could be used to produce high numbers of consistently virulent nematodes. In these experiments *P. hermaphrodita* were fed a selection of bacteria that had been isolated from *P. hermaphrodita* infected slugs and from *P. hermaphrodita* dauer juveniles emerging from dead slugs.[48,49] Many different bacterial species were isolated and tested including: *Acinetobacter calcoaceticus*, *Aeromonas hydrophila*, *Aeromonas* sp., *Bacillus cereus*, *Flavobacterium breve*, *Flavobacterium odoratum*, *Moraxella osloensis*, *Providencia rettgeri*, *Pseudomonas fluorescens* (isolate no. 1a), *Pseudomonas fluorescens* (isolate no. 140), *Pseudomonas fluorescens* [isolate no. 141, *P. fluorescens* (pSG)], *Pseudomonas paucimobilis*, *Serratia proteamaculans*, *Sphingobacterium spiritocorum* and *Xenorhabdus bovienii*.[48,49] Successful feeding and growth of *P. hermaphrodita* also has been recorded on *Pseudomonas* sp. 1, *Bacillus* sp. 1, *Escherichia coli* OP50 and *E. coli* BR.[40] *Moraxella osloensis* was chosen as it produced consistently high yields of pathogenic nematodes.[48,49] It should be stressed that this bacterium was chosen for commercial production and does not reflect the natural tritrophic interactions that may be occurring between slugs, *P. hermaphrodita* and bacteria in the wild. Indeed, when *P. hermaphrodita* was grown on rotting slugs or emerging after parasitising slugs (*D. reticulatum*), there was no evidence of *M. osloensis* being present inside the

Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

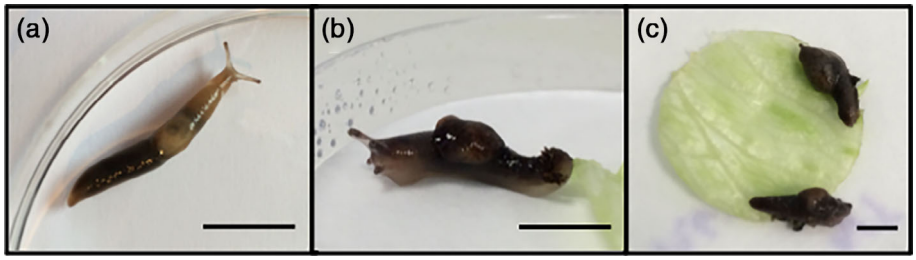

Figure 3. *Phasmarhabditis hermaphrodita* produces characteristic signs of infection when parasitising pestiferous hosts such as *D. invadens* (a). Nematodes infect the slug through a pore in the back of the mantle and reproduce, causing a swelling of the mantle area (b), this eventually leads to death in 4 to 21 days (c). Bars, 1 cm.

nematodes,[129] and, therefore, these nematodes do not vertically transmit this bacterium. Likewise, *M. osloensis* was lost after repeated culturing of *P. hermaphrodita* strain (DMG0001) over several generations on homogenised pig kidney.[128] However, research has shown that injection of 40- and 60-h cultures of *M. osloensis* into the haemocoel of *D. reticulatum* will kill slugs, with the 60-h cultures being more pathogenic than the 40-h cultures.[126] This is thought to be due to a lipopolysaccharide (LPS) which acts as an endotoxin,[130,131] and *ubiS* and *dsbC* genes that are upregulated by *M. osloensis* when infecting *D. reticulatum*.[132] *Moraxella osloensis* is only toxic to *D. reticulatum* when injected and showed no contact or oral toxicity to slugs.[131] The relationship between *M. osloensis* and *P. hermaphrodita* has been categorised as 'symbiotic' yet there are compelling reasons why this may not be the case. This is out with the scope of this review but see Wilson and Rae[133] for further details. What is clear is that *P. hermaphrodita* is a facultative parasite, able to grow on a multitude of different bacterial species which can dramatically affect the numbers of offspring produced and the nematode's pathogenicity. Whether or not the nematode relies on a strict symbiotic relationship with one bacterium is a matter of debate, but profiling the bacterial species wild *P. hermaphrodita* associate with in nature will provide insight. For example, a plethora of different bacterial species including *Acinetobacter* sp., *Alcaligenes faecalis*, *Bacillus cereus* and *Stenotrophomonas* sp. were identified from dauer juveniles of *P. hermaphrodita* DMG0001 and wild strains of *P. hermaphrodita*.[128] Likewise, by using 16SrRNA metagenomics the microbiome of wild *Phasmarhabditis* from California was profiled and the most predominant bacteria identified were *Shewanella*, *Clostridium perfringens*, Aeromonadaceae, Pseudomonadaceae and Actinetobacter;[134] however, the authors did not carry out any other experiments so it is difficult to come to any major conclusions about the role of bacteria in US strains of *Phasmarhabditis*. By contrast, a recent study[135] showed that *P. hermaphrodita* (wild and commercial strains), *P. californica* or *P. neopapillosa* dauer juveniles which had killed a slug harboured a plethora of bacterial species, including *M. osloensis* but in minute amounts. Furthermore, genotyping of the *M. osloensis* strains used by BASF Agricultural Specialities used to grow *P. hermaphrodita* revealed that the species was actually more closely related to *Psychrobacter faecaelis*, and thus there seems to be limited use of *M. osloensis* in the pathogencity process.[135]

2 REPRODUCTION

Upon host death, nematodes proliferate on the slug cadaver, and multiple factors can influence progeny dynamics. *Phasmarhabditis hermaphrodita* grown on tissue from different species of slugs and snails yielded different numbers of offspring with *D. invadens* producing the highest number of progeny followed by *Limax marginata*, *M. gagates*, *C. hortensis* and *D. reticulatum*.[110] Development and quality of *P. hermaphrodita* can be severely affected by growing substrate:[105,128] it was able to successfully grow on multiple substrates including a mixture of homogenised pig kidney with different homogenised slug species (*Arion lusitanicus* and *D. reticulatum*) and homogenised moth (*Galleria mellonella*), faeces from *D. reticulatum* and *A. lusitanicus*, and leaf compost. The authors found the yield of *P. hermaphrodita* to be greater on invertebrate-based substrates, although the quality of *P. hermaphrodita* produced remained stable based on body size and lipid content.[105,128] Similar findings of dauer juveniles of *P. hermaphrodita* recovering and multiplying in slug faeces but not soil samples have been reported.[106] These results indicate that reproducing on an invertebrate can produce similar numbers of progeny as when the nematode kills a slug host and reproduces on it.[128] As well as *P. hermaphrodita*, other *Phasmarhabditis* species such as *P. bohemica*, *P. bonaquaense* and *P. apuliae*[136] can all be grown under laboratory conditions on dead slugs and have different generation times.

Intraspecific competition for resources can influence *P. hermaphrodita* development; lipid content, yield and body length,[127,128] and nematodes may leave areas of dense populations to find other resources.[127] Also, the time it takes for new dauer juveniles to develop can differ with species. For example, *P. bohemica* had the shortest development cycle compared to *P. hermaphrodita*, *P. papillosa* and *P. kenyaensis* when grown on rotting slug (*D. invadens*), but it should be noted for industrial production that *P. hermaphrodita* is best as it is a hermaphrodite and not gonochoristic like the other species.[136] As well as differences between species, temperature also can severely affect the survival and growth of *P. hermaphrodita*. Survival dramatically decreases at 25 °C and 35 °C but there is no difference at 5, 10 and 15 °C,[137] with the optimum growth temperature for *P. hermaphrodita* at 17 °C.[33]

2.1 Susceptibility of terrestrial gastropods to *P. hermaphrodita*

There are currently 22 species of slug and 21 species of snail that have been tested for susceptibility to *P. hermaphrodita* under laboratory conditions (Fig. 4; Table 1). To date, 12 slug species and eight snail species can clearly be killed by *P. hermaphrodita*. There is little research into understanding how *P. hermaphrodita* is able to kill terrestrial gastropods and very little information about why there is this difference in susceptibility of different species. Some studies have shown that younger stages of certain slug species are susceptible to *P. hermaphrodita* whereas adults are not

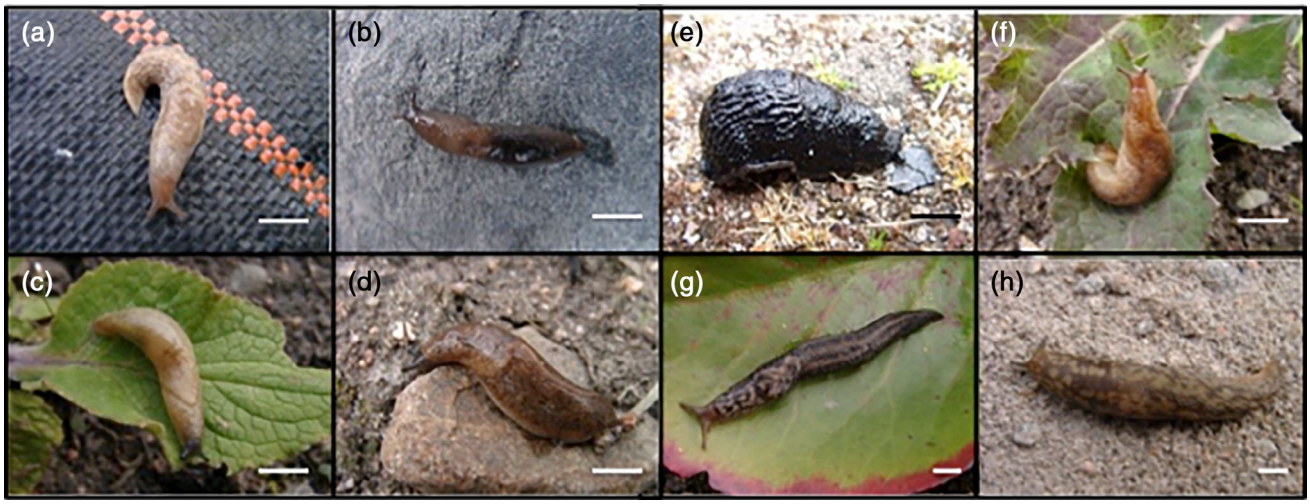

Figure 4. *Phasmarhabditis hermaphrodita* can cause rapid mortality to the susceptible slugs *D. reticulatum* (a), *D. invadens* (b), *M. gagates* (c) and *T. sowerbyi* (d), but *A. ater* (e), *A. subfuscus* (f), *L. maximus* (g) and *L. flavus* (h) are resistant, for reasons unknown. Bars, 0.5 cm.

including *A. vulgaris* (previously known as *A. lusitanicus*)[142–145] and *A. ater*[33,110] (although it should be noted that *P. papillosa* can supposedly kill adult *A. vulgaris*[155]). It also has been recorded that *P. californica* can kill neonate *C. aspersum* but not adults,[156] similar to *P. hermaphrodita*.[103] Confusingly, studies that have carried out the same experiment have reported different results. For example, neonate stages of the giant African snail (*L. fulica*) can be killed by a wild strain of *P. hermaphrodita* from the US,[69] whereas the commercial strain *P. hermaphrodita* DMG0001 had no negative effect on juvenile stages of these snails.[149] Also the freshwater snail *Lymnaea stagnalis* was killed by *P. hermaphrodita*[151] but another study observed no mortality when the same experiment was repeated.[150] These differences could be to the result of using laboratory-reared or wild-collected nematodes or hosts. For example, in the former study[151] a laboratory strain of *L. stagnalis* was used whilst wild-collected *L. stagnalis* were used in the latter study.[150] Likewise, the commercial strain of *P. hermaphrodita* was exposed to *L. fulica* in the UK study[149] but a wild strain of *P. hermaphrodita* was used in the US study.[69] It is interesting to speculate why there are such differences; perhaps it could be a consequence of continuous laboratory culturing, which can have severe effects on the health of laboratory animals.[157] and possibly nematodes. For example, traits such as heat, UV light and desiccation tolerance, and reproductive potential have been shown to be reduced in *H. bacteriophora* through continuous culturing in *Galleria mellonella*.[158] The effect of continuous laboratory culturing in nematodes and hosts could therefore play a role in the differences found in these experiments

One common symptom of *P. hermaphrodita* infection is host feeding inhibition, which is strongly observed in slugs such as *D. reticulatum* and *D. invadens* but also has been observed in slug species that it cannot kill.[33,110] It has been suggested that slug control in field trials is probably from host-feeding inhibition as opposed to slug mortality.[27,50,139] Feeding inhibition may be a defensive behaviour of slugs to contract and reduce the numbers of nematodes penetrating inside.[139] Some species, however, are not killed by *P. hermaphrodita* and their feeding is not inhibited, such as *L. maculatus*.[140] Interestingly, it has been recently shown that as well as affecting feeding behavior, infection by *P. hermaphrodita* can alter the microbiome of the susceptible slug *D. invadens*, but has no effect on the bacterial communities of the resistant slug *A. valentianus*.[159]

In contrast to slugs, the effect that *P. hermaphrodita* has on snails has not been investigated in detail (although these nematodes have been isolated regularly from snails[160]). *Phasmarhabditis hermaphrodita* has been shown to cause high levels of mortality to snails (*T. pisana*, *Trochoidea elegans* and *Monacha cantiana*).[52,73,161] There are many snail species resistant to infection by *P. hermaphrodita* and one reason for this may be the snail shell. An observation during an infection experiment using *P. hermaphrodita* and *L. fulica* found nematodes trapped and encased in the inner layer of the shell.[149] Evidence of this process also has been shown in live *C. nemoralis*[148] (Fig. 5), *A. arbustorum*,[146] and in museum collections of *C. aspersum* and *H. pomatia*.[162] This process is remarkably well-conserved across the Stylommatophora and has been thought to be present when the two major clades diverged 80–130 million years ago (Ma);[163] nematodes have even been observed in the vestigial shell of the slug *L. pseudoflavus*.[140] Nematodes have been infecting gastropods since the late Cambrian[14] and this evolutionary arms race has resulted in slugs and snails co-opting their shell to encapsulate and encase parasitic nematodes instead of just using the shell for shelter.[163] Interestingly, dark morphs of the snail *Cernuella virgata* were found to be more resistant to *P. hermaphrodita* than light morphs and this was not due to phenoloxidase levels;[164] those authors did not dissect the snails or examine the shells for nematodes, but perhaps this difference in susceptibility was due to the effectiveness of the shell morphs in encasing invading nematodes?

As well as the shell, the immune system of slugs and snails must play a role in combating infection, but this has been poorly researched. There have only been a couple of studies looking at the immune system of snails when infected by *P. hermaphrodita*.[154,165] Oxidative stress and cell metabolism were affected in the nematode-infected freshwater golden apple snails (*Pomacea canaliculata*)[165] and specifically *Pc-bpi*, a mammalian bactericidal/permeability increasing protein orthologue, was highly upregulated in the kidney and gills of the snail.[154] How abundantly upregulated this protein is and its role in combatting nematode infection in terrestrial gastropods is unknown.

Table 1. Susceptibility of slugs and snails exposed to *Phasmarhabditis hermaphrodita*

Gastropod	Family	Species	Susceptible to *P. hermaphrodita*?	Relationship with host	References
Slugs	Agriolimacidae	*Deroceras reticulatum*	Yes	Parasitic	33,70,71,106,110
		Deroceras invadens	Yes	Parasitic	33,110,135,138
		Deroceras laeve	Yes	Parasitic	139
	Limacidae	*Limax maximus*	No	Necromenic	139
		Limax maculatus	No	Necromenic	140
		Limax marginata	No	Necromenic	110
		Ambigolimax valentianus	No	Necromenic	141
	Arionidae	*Arion ater*	Only juveniles	Parasitic/Necromenic?	33,110
		Arion silvaticus	Yes	Parasitic	33
		Arion intermedius	Yes	Parasitic	33
		Arion distinctus	Yes	Parasitic	33,142
		Arion lusitanicus	Only juveniles	Parasitic/Necromenic?	143,144
		Arion subfuscus	No	Necromenic	110,139
		Arion hortensis	No	Necromenic	139,142
		Arion fasciatus	Yes	Parasitic	145
		Arion vulgaris	No	Necromenic	145
		Geomalacus maculosus	No	Necromenic	96
	Milacidae	*Tandonia sowerbyi*	Yes	Parasitic	33,110
		Tandonia budapestensis	Yes	Parasitic	33
		Milax gagates	Yes	Parasitic	110,140
	Testacellidae	*Testacella haliotidea*	Yes	Parasitic	75
	Veronicelloidae	*Leidyula floridana*	Yes	Parasitic	139
Snails	Helicidae	*Cornu aspersum*	Only juveniles	Parasitic/Necromenic?	103,110
		Arianta arbustorum	No	Necromenic	145,146
		Cepaea hortensis	Yes/No	Parasitic/Necromenic?	110,147
		Cepaea nemoralis	No	Necromenic	147,148
		Theba pisana	Yes	Parasitic	52,73,74
	Geomitridae	*Cochlicella acuta*	Yes	Parasitic	52
		Cernuella virgata	Yes	Parasitic	52
	Hygromiidae	*Monacha cantiana*	Yes	Parasitic	147
	Succineidae	*Succinea* spp.	Yes	Parasitic	72
	Pomatiasidae	*Pomatias elegans*	No	Necromenic	147
	Oxychilidae	*Oxychilus helveticus*	No	Necromenic	147
	Clausiliidae	*Clausilia bidentata*	No	Necromenic	147
	Discidae	*Discus rotundatus*	No	Necromenic	147
	Achatinidae	*Lissachatina fulica*	No/Yes	Parasitic/Necromenic?	69,149
	Bithyniidae	*Bithynia tentaculata*	No	Necromenic	150
	Lymnaeidae	*Lymnaea stagnalis*	Yes/No	Parasitic/Necromenic?	150,151
	Physidae	*Physa fontinalis*	No	Necromenic	150
	Planorbidae	*Planorbarius corneus*	No	Necromenic	150
		Biomphalaria pfeifferi	Yes	Parasitic	152
		Biomphalaria alexandrina	Yes	Parasitic	153
	Ampullariidae	*Pomacea canaliculata*	Yes	Parasitic/Necromenic?	154

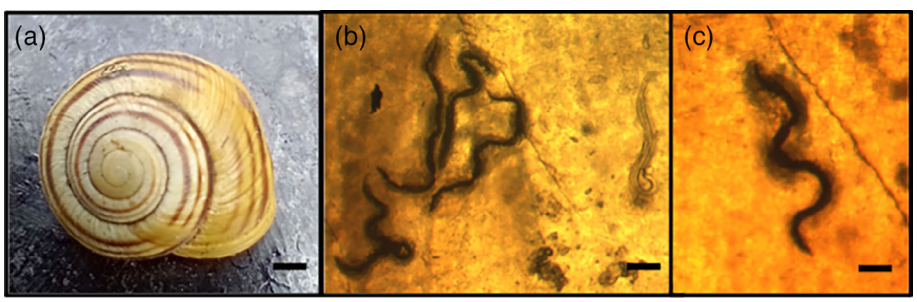

Figure 5. Snails such as *C. nemoralis* (a) can be infected with *P. hermaphrodita* under laboratory and field conditions and nematodes are trapped, encased and killed in the shell (b and c). Bars, 2 mm (a) and 100 µm (b and c).

Owing to its ability to kill snails *P. hermaphrodita* could be used to reduce snail populations that vector medically important parasites. Specifically, application of the nematode has been shown to negatively affect the freshwater snails *Biomphalaria alexandrina* and *B. pfeifferi* (under laboratory conditions), which could potentially result in a diminished transmission of schistosomiasis.[152,153] The potential of these nematodes to control *Biomphalaria* snails warrants significant attention and could be highly promising.

2.2 Host avoidance and behavioural manipulation

Avoidance behaviour is the first strategy an organism can employ to reduce the threat of parasitism.[166] In order to reduce parasitism by *P. hermaphrodita*, slugs avoid areas where nematodes are present. Slugs such as *D. invadens* and *A. ater* are able to detect and avoid areas where *P. hermaphrodita* is present, and spend less time feeding and resting in such areas.[167] It could be presumed that slugs would avoid all parasitic *Phasmarhabditis* species, but this is not the case: *D. invadens* avoids *P. hermaphrodita* and *P. californica* but curiously is attracted to areas were *P. neopapillosa* has been applied.[168] The reasons for this are unknown but it has important ramifications for the use of other *Phasmarhabditis* species in the field.

Avoidance behaviour in slugs when exposed to *P. hermaphrodita* has been observed in several diverse slug species from three different families, yet snails (e.g. *C. aspersum*) do not avoid the nematodes.[169] Slugs specifically avoid *P. hermaphrodita* and not other nematodes such as the EPN *Steinernema kraussei* or the vinegar eelworm (*Turbatrix aceti*)—both of which are not parasites of terrestrial gastropods. Resistant slug species *A. subfuscus*, *A. hortensis* and *A. valentianus* avoid *P. hermaphrodita*, although *L. flavus* also is resistant to *P. hermaphrodita* infection but does not avoid the nematode.[169,170] Slugs do not avoid areas treated with the supernatant of a liquid suspension of *P. hermaphrodita* suggesting that the slugs are avoiding the mechanical stimulus of the nematodes probing the slug's body, rather than a chemical cue.[169] However, when a slug is infected with *P. hermaphrodita* the usual avoidance behaviour is abrogated and slugs are oddly more likely to be found on soil where *P. hermaphrodita* is present.[170] The exact reason why the nematodes are influencing slug behaviour is unclear, but it could increase chances for more successful infection and therefore reproduction.[170] It is unclear how *P. hermaphrodita* is able to manipulate slug behaviour, but it could be linked to neurotransmitter signaling. Uninfected slugs (*D. invadens*) fed fluoxetine or sertraline, which increase serotonin levels, were driven towards the nematodes, whereas infected slugs treated with cyproheptadine, which suppresses serotonin levels, were no longer attracted to the nematodes.[170] Uninfected slugs treated with apomorphine, which stimulates dopamine receptors, failed to avoid *P. hermaphrodita*, and infected slugs treated with a dopamine antagonist (haloperidol) no longer moved towards *P. hermaphrodita*.[171] This suggests that *P. hermaphrodita* is somehow able to influence levels of biogenic amines to alter slug behaviour.[170,171]

As well as the ability to alter attraction or avoidance behaviour in slugs, *P. hermaphrodita* has been reported to have caused other extreme effects on slug behaviour. For example, infected slugs eat less,[27] are slower,[172] are more likely to be found under refuge traps[50] and move underground to die,[173] and infected freshwater snails are more likely to be found outside the water.[151] Not only does *P. hermaphrodita* influence host behaviour, but also it has been suggested they exhibit an anti-feeding effect on scavenging beetles (*Carabus nemoralis* and *Pterostichus melanarius*) by deterring them from feeding on dead, infected slugs where the nematodes are reproducing.[174] Whether the nematode is actively manipulating the behaviour of the slugs or this is a by-product of infection of sick slugs warrants further investigation.

2.2.1 *The effect of* P. hermaphrodita *on nontarget organisms*

Concern has been raised about the use of *Phasmarhabditits* species on nontarget organisms,[175] particularly native snail populations, yet there has not been one observation of these nematodes significantly affecting the health or populations of nontarget slugs or snails in 30 years of use across northern Europe. Also, there has been unease about the potential spread of *M. osloensis* (an opportunistic human pathogen) used to grow *P. hermaphrodita*, yet the bacterium these nematodes are reared on is not *M. osloensis* but a species closely related to *P. faecalis*,[135] which poses no threat to humans, so the level of risk to nontarget organisms associated with the use of the these nematodes remains low. Nevertheless, the commercial strain of *P. hermaphrodita* has been tested against nontarget beneficial invertebrates. As expected for a parasite of gastropods, *P. hermaphrodita* has been shown not to harm several insect species including *Tenebrio molitor*,[176] *G. mellonella* or *Pterostichus melanarius*.[33] The earthworms *L. terrestris*, *Eisenia fetida*, *E. hortensis*, *E. fetida*, *E. andrei* and *Dendrodrilus rubidus* also are unaffected by the nematode as well as the platyhelminth *Arthurdendyus triangulatus*.[177–179] A *Phasmarhabditis*-like nematode that potentially killed earthworms (e.g. *L. terrestris*) has been reported[100] but there has been no subsequent research. This nematode was only identified morphologically and causing earthworm mortality would be highly unusual for a gastropod parasitic nematode. Another *Phasmarhabditis* species (*P. californica*) also has been exposed to earthworms (*L. terrestris* and *E. fetida*), as well as the insect larvae *T. molitor* and *G. mellonella*, with no mortality of any species tested observed.[180]

The effect of *P. hermaphrodita* on nontarget gastropods also has been investigated in the field. From seven snail species commonly found in hedgerows, *P. hermaphrodita* caused mortality to just two (*M. cantiana* and *C. hortensis*).[147] Also, over a 2-year field trial[181] there was no effect of *P. hermaphrodita* on the snail species *Ponentina ponentina* and *Oxychilus helveticus*, or on acarids, collembolans or earthworm populations. Therefore, the effect of *P. hermaphrodita* on nontarget organisms is limited in Europe but there are no data on nontarget effects in other parts of the world where *Phasmarhabditis* species have been isolated for example, South Africa, New Zealand, USA and Canada.

2.3 Production of *P. hermaphrodita*

Consistent and efficacious pest control as well as low cost, storage, delivery, handling and marketing are required for any biocontrol product (including nematodes) to become commercial.[182] *Phasmarhabditis hermaphrodita* has successfully been in production since 1994 by MicroBio, which was bought by Becker Underwood and then by BASF Agricultural Specialities. *Phasmarhabditis hermaphrodita* is grown in *in vitro* liquid culture with a bacterium closely related to *P. faecalis*[48,49] with upwards of 100 000 dauers mL^{-1} being produced.[183] Monoxenic liquid culture of nematodes for mass production allows for more predictable and high virulent yields.[48,49,184] After monoxenic fermentation, dauers are harvested and the most effective dauer recovery methodology is using a combination of continuous phase density and flotation by adjustment.[185] The same authors also found that the introduction of an air supply to break apart and clear insoluble spent media was recommended. To separate

dauers and other life stages, the product can be sieved at an aperture size of 75–106 μL[186] or by using vibrating membrane filtration.[187] Centrifugation and repeated washing also can be used.[35] After extraction, dauer juveniles are mixed with an inert gel polymer and packaged.[34]

2.3.1 *Field use and application of* P. hermaphrodita

Phasmarhabditis hermaphrodita is formulated into a water-dispersible formulation that can be suspended in water and applied to soil at a rate of 3×10^9 dauer juveniles ha^{-1},[35] via spraying equipment[183] and irrigation lines.[188] As well as being applied to the soil surface, *P. hermaphrodita* can be incorporated into soil through cultivation to kill subterranean slugs though this has mixed results in terms of efficacy at reducing slug damage and slug numbers.[189] *Phasmarhabditis hermaphrodita* has been used to successfully control slug damage in an array of plants including lettuce,[51,190] winter wheat,[50,189] oilseed rape,[191,192] cabbage,[192,193] asparagus,[194] Brussels sprouts,[195] glasshouse orchids[141] and sugar beet.[196]

In general, there have been few field trials using *P. hermaphrodita* since 2009 but many before (see the complete list of field trials and results[35]). It has been largely successful at controlling slugs, yet there are reports of failures. For example, *P. hermaphrodita* was unable to reduce slug damage[51] or slug numbers[181,197] in some field trials. The most likely reasons for the lack of slug control are exposure to abiotic (e.g. UV light, desiccation, temperature) and biotic (e.g. predators) factors that the nematodes face once they have been applied or the presence of nonsusceptible slug species.

Other factors which may influence the efficacy of *P. hermaphrodita*, such as watering regime and earthworm activity, were investigated in comparison to chemical controls.[198] No effect on slug feeding or mortality was observed, but this could be a result of the presence of the slug *A. vulgaris*, which is known to be resistant to *P. hermaphrodita*.[143,145] It has, however, been suggested that failures could be avoided by following recommended protocols.[39]

The effect of treatments of crops before nematode application also has been investigated. When manure was applied before *P. hermaphrodita* dauer juveniles, the nematodes were rendered ineffective, possibly as a result of poor dauer survival, manure interfering with chemoreception or the manure attracting more slugs.[181] By contrast, there was no effect of cover crops or lupin on the ability of nematodes to control slugs in the next crop planted.[199,200]

Novel application strategies that improve efficiency and economic use of nematode biological control products will improve their attractiveness,[201] which have been investigated with *P. hermaphrodita*. For example, the most efficient control method of slugs in sugar beet utilised nematode application and methiocarb pellets in furrow treatment;[202] however, it has been found that methiocarb can reduce nematode survival but not infectivity.[203] In spite of this, there is limited scope for this combination as methiocarb affects nontarget organisms such as birds and has been banned in the UK and Europe.[6]

Multiple lower rate applications of *P. hermaphrodita* can sometimes offer better control,[196] or the same level of control as standard recommended broadcast rates,[194,195,197,204] but they require more time to achieve a reduction in slug damage.[205–207] Lower application rates and concentration could be beneficial for larger areas of crop, as *P. hermaphrodita* can be applied via irrigation lines,[208] instead of broadcast application. Nematodes also have been applied in bands but offered no economic advantage over recommended broadcast application at the standard rate, possibly as a result of too few nematodes being applied.[205,206] Other application strategies such as dipping root plugs in a nematode/carboxymethyl cellulose solution also have been found to be successful, thereby providing protection against slugs using a lower number of nematodes and reducing the cost.[197,209] More targeted application methods have been proposed[208] including nematode application machinery (Wroot water Nemaslug xtra applicator) which injects nematodes onto irrigation water and aerates and agitates the nematode solution, allowing nematodes to be applied over a longer timescale. In plots of hostas, targeted application of *P. hermaphrodita* to slug shelters at a reduced application rate provided similar protection to that of uniform broadcast application.[204] Likewise, damage to oilseed rape by *A. lusitanicus* was reduced for 25 days by spraying *P. hermaphrodita* on the plants at a rate of 2×10 nematodes cm^{-2},[210] rather than a broadcast spray. In order to optimise the numbers of *P. hermaphrodita* used for slug control several models have been developed.[206,211–213]

2.3.2 *Persistence and environmental factors affecting the success of* P. hermaphrodita *in the field*

In order for *P. hermaphrodita* to be successfully used as a biological control agent, it must persist in soil after application, but there is little research on this. Soil type can affect the movement of *P. hermaphrodita*[42,107] and its persistence has been monitored using real time qPCR techniques[42] showing that the *P. hermaphrodita* population declines sharply after 2 weeks.[214] However, in other studies survival of *P. hermaphrodita* has been recorded up to 5 months in wet sand, and even 8 months in garden soil and organic horticultural substrate.[215] In field trials *P. hermaphrodita* can survive up to 6 weeks in soil[209] and even up to 99 days.[199] Under laboratory conditions, the survival of *P. hermaphrodita* was best at 5, 10 and 15 °C, and osmotic desiccation in 10% glycerol could increase survival of the nematodes at temperature extremes.[137]

Unfavourable abiotic and biotic conditions including UV light, temperature and desiccation affect nematode survival and persistence.[216] This can be reduced by cultivating the land immediately after nematode application.[189,205] As well as abiotic factors, nematodes are killed by mites, collembolans and fungi.[216,217] DNA analysis has shown that mites and collembola including *Heteromurus nitidus* devour *P. hermaphrodita* under laboratory conditions and in the field,[218–220] and fungi have been speculated to affect the survival of these nematodes.[215]

With temperatures increasing in parts of the world due to climate change, the efficacy of *P. hermaphrodita* in controlling slugs may be affected; in particular slug-feeding was not reduced in infected slugs as temperatures increased from 14 °C to 24 °C.[221] It is thought that *P. hermaphrodita* is well adjusted to the cooler climate of northern Europe,[222] yet *P. hermaphrodita* could be used to reduce slug damage in warmer conditions in Spain, where the mean air temperature was 19.8 ± 2.6 °C.[207] The impact of temperature on the efficacy of *P. hermaphrodita* also was investigated through field trials using predicted winter warming conditions.[223] They found that damage to plants and slug survival was much lower in the predicted wintering conditions than under normal wintering conditions. Therefore, *P. hermaphrodita* may perform better at controlling slug damage under winter warming conditions.[223]

Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

2.3.3 Combining chemical and biological control methods with P. hermaphrodita

There is evidence to show that *P. hermaphrodita* combined with other methods could enhance slug control. In 2007 the efficacy of combining *P. hermaphrodita* infection with cadmium and *Bacillus thuringiensis* (BT) in the snail *C. aspersum* was investigated.[224] The growth rate of *C. aspersum* was reduced by both BT and cadmium and increasing doses of *P. hermaphrodita*.[224]

The repellent effect of Birch tar oil (BTO) has been examined and suggested for possible complementary use with *P. hermaphrodita* to control *A. arbustorum* and *A. vulgaris*.[225] The authors found that BTO repels *A. arbustorum* and *A. vulgaris* in confined heavily nematode-infested areas, and that repeated application of BTO over several weeks was required to deter *A. lusitanicus* with weekly treatments offering the best slug control.

Other more novel strategies have been investigated. *Phasmarhabditis hermaphrodita* has been used in combination with wasp venom from *Pimpla hypochondriaca* to kill and inhibit feeding of *D. reticulatum*.[226] The authors concluded that together with *P. hermaphrodita* the venom can be more effective than *P. hermaphrodita* on its own and is more successful at causing slug fatality and significantly reducing slug-feeding. One of the suggested strategies for future studies is to genetically engineer *P. hermaphrodita* to express individual venom factors[226] for slug control.

More recently the behaviour and feeding of *Tetanocera elata* fly larval, (a parasitoid and predator of slugs) and its potential for use with *P. hermaphrodita* have been explored.[227] The results demonstrate that *T. elata* larvae suffer in development and pupariation if feeding from an infected slug with only 20% pupating. Oddly, however, the larvae did show a preference for slugs previously infected with *P. hermaphrodita*. Ultimately further work is needed to examine if they can provide a consistently efficient synergistic level of slug control.

3 FUTURE DIRECTIONS AND CONCLUSIONS

Over the 30 years since *P. hermaphrodita* was first developed as a biological control agent, interest in this nematode has slowly increased as chemical usage is being reduced. However, compared to other nematodes used in biological control such as EPNs, the number of researchers investigating *P. hermaphrodita* is low[133] and subsequently, there are still many unanswered questions about the use and basic biology of *P. hermaphrodita*. Here we outline several research avenues which we think could improve the use of *P. hermaphrodita*: an appreciation of co-evolution between host and parasite; genetic improvement and genomic understanding of *P. hermaphrodita* and other *Phasmarhabditis* species; and investigating new application strategies of *P. hermaphrodita* in the field.

3.1 The importance of understanding the co-evolution between host and parasite

Nematodes and slugs have been co-evolving in an arms race for 540 Ma.[14] The geographical mosaic theory of co-evolution predicts genetic variation in the ability of hosts to combat parasites as well as pathogenicity of parasites.[228] There is little information on natural variation in pathogenicity of *P. hermaphrodita* strains, with only one study[138] recently demonstrating several wild strains of *P. hermaphrodita* that were more virulent than the commercial strain DMG0001 to *D. invadens* and other strains poor at killing slugs. Also there is no information on whether local and global populations of specific slug species differ in their susceptibility to the nematode. It seems highly likely that there would be genetic variation in both host defence and pathogenic potential of the parasite, which has been observed in other animals. For example, there is considerable variation in the resistance of the fruit fly *Drosophila melangaster* to the fungal pathogen *Entomophthora muscae*[229] and in wild populations of *Daphnia magma* exposed to the bacterial pathogen *Pasteuria ramosa*.[230] For *Phasmarhabditis* nematodes this has only been investigated at the interspecies level (see the 'Susceptibility of terrestrial gastropods to *P. hermaphrodita*' section and Table 1), where species such as *A. ater* are resistant, and *D. invadens* and *D. reticulatum* are highly susceptible.[33,106,110] There are limited data on host susceptibility to *P. hermaphrodita* at the intraspecies level. The only evidence comes from two studies focused on the snail *C. hortensis* where a population from Bristol, UK was found to be resistant to *P. hermaphrodita*,[147] yet *C. hortensis* from Aberdeen, UK were susceptible to the nematode.[110] This has important ramifications for gastropod control. If different populations have evolved resistance to *P. hermaphrodita* then application of the current strain (DMG0001) for control of resistant populations will be futile. Therefore, we propose that mechanistic understanding of how different populations of slugs and snails overcome parasitism and infection by *P. hermaphrodita* would be beneficial. Furthermore, as well as examining the pathogenic potential of wild *P. hermaphrodita* strains, variation in beneficial traits also should be examined. This approach is commonly used in EPN research; for example, wild strains of *Steinernema* and *Heterorhabditis* have been isolated and screened for superior virulence,[231] host finding and stress tolerance for example, heat, desiccation[232] and longevity[233] (to name but a few traits). This approach has never been utilised for *P. hermaphrodita*, however, as researchers tend not to keep their wild isolated strains in culture. Therefore, natural variation of different traits has not been investigated in great detail for *P. hermaphrodita* apart from tolerance to extreme pHs and temperature,[40] as well as chemotactic response to slug and snail mucus and hyaluronic acid.[122–124]

3.2 Genetic tools and genomic sequencing of parasitic nematodes

Coupled with the isolation of wild strains, the development of genetic techniques could enhance the efficacy of *P. hermaphrodita* in the field. This also is inspired by approaches used in EPN research. There have been numerous successful examples of selection of different advantageous traits using EPNs for example, high responsiveness to foraging cues,[234] heat tolerance and low-temperature activity,[235] which could potentially increase their viability as biological control agents. Other techniques such as inbreeding, hybridisation and mutagenesis have been employed to improve oxidative stress tolerance and longevity in *H. bacteriophora*,[236,237]–methods that also could be employed for *P. hermaphrodita*. More sophisticated genetic techniques have been shown to work in EPNs, such as RNAi in *S. carpocapsae*[238] and *H. bacteriophora*,[239] and even transgenic techniques in *H. bacteriophora*.[240] Although *P. hermaphrodita* has been proposed as a model nematode to understand the genetic mechanisms of parasitism,[241-244] development of techniques for genetic manipulation are in their infancy.[40] With the subsequent sequencing of the genome ongoing (Sheehy, Rae, unpublished data), the unravelling of the genetic blueprint of *P. hermaphrodita* may aid in the development of molecular tools. As seen with *C. elegans* and parasitic helminths, genomic investigations can lead to valuable

insights regarding the evolution of these organisms[245–247] as well as the development of beneficial online resources such as WormBase and WormBase ParaSite. The availability of genomic data would enable the identification of key genes such as those for pathogenicity, dauer formation, longevity and chemoattraction as well as their manipulation, which could lead to improvements in the use of *P. hermaphrodita* as a biological control agent. In terms of genomics, research on EPNs is well ahead of *P. hermaphrodita* with the genomes and transcriptomes of several *Steinernema* species including *S. carpocapsae*, *S. scapterisci*, *S. monticolum*, *S. feltiae* and *S. glaseri* already sequenced[248] as well as *Heterorhabditis bacteriophora*[249] and their bacterial symbionts *Xenorhabdus* and *Photorhabdus*.[250]

3.2.1 *Novel application strategies of* P. hermaphrodita

Novel application strategies can reduce the cost of using nematodes and increase attractiveness to the consumer.[201] Instead of standard broadcast spraying, these techniques include dipping roots of plants into adhesive mixtures containing nematodes, using lower, more frequent applications of nematodes as well applying infected cadavers or applying nematodes to slow-release bags. Some of the techniques have been shown to work well in field trials for example, mixing *P. hermaphrodita* with carboxymethylcellulose to adhere to root plugs and smaller more frequent doses of nematodes to control slug damage in Chinese cabbage.[197] However, methods such as using already infected hosts, gels and slow-release tea bags have not received commercial or research attention using *P. hermaphrodita*. Another promising method is encapsulating nematodes in alginate beads providing a more targeted approach, which has been shown to work with EPNs to control *Diabrotica balteata* larvae.[251] These methods also could be combined with others to allow synergistic slug control for example, using essential oils, such as clove bud oil that kills snail eggs,[252] and spearmint and thyme oil that kill slugs[253] (*P. hermaphrodita* is unaffected by several essential oils that kill gastropods[254]), or combining with other biocontrol agents such as the fly *T. elata*.[227]

4 CONCLUSION

With the discovery of *Phasmarhabditis* nematodes from slugs and snails in many countries across the world,[35] including North America[65] there is ample opportunity for expansion of the Nemaslug® product across the globe. Ultimately, we hope by focussing on the approaches that we have suggested previously, *P. hermaphrodita* (and other *Phasmarhabditis* species) could be developed and used as successful biological control agents of slugs for the next 30 years. In fact, at the time of writing BASF have announced that a new *Phasmarhabditis* product (Nemaslug 2.0®) will be launched for use in gardens in spring 2023 containing not *P. hermaphrodita* but *P. californica*, owing to its pathogenicity towards slugs,[135] snails[156] and its lack of effect on nontarget organisms.[180]

ACKNOWLEDGEMENTS

We are grateful to BASF Agricultural Specialities and the Royal Horticultural Society who fund research on *P. hermaphrodita* at LJMU, as well as Tom Goddard, Jack Shepherd and Jeremy Pearce for discussions. We dedicate this work to our friend and fellow *Phasmarhabditis* researcher James Cutler who sadly passed away in 2022 and is sorely missed.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

REFERENCES

1 Barua A, Williams CD and Ross JL, A literature review of biological and bio-rational control strategies for slugs: current research and future prospects. *Insects* **12**:541 (2021). https://doi.org/10.3389/insects12060541.
2 Port CM, The biology and behaviour of slugs in relation to crop damage and control. *Agric Zoo Rev* **1**:243–287 (1986).
3 South A, *Terrestrial Slugs: Biology, Ecology and Control*. Springer, Netherlands (1992).
4 Willis JC, Bohan DA, Choi YH, Conrad KF and Semenov MA, Use of an individual based model to forecast the effect of climate change on the dynamics, abundance and geographical range of the pest slug *Deroceras reticulatum* in the UK. *Glob Change Biol* **12**:1643–1657 (2006).
5 Port G and Ester A, Gastropods as pests in vegetable and ornamental crops in western Europe, in *Molluscs as Crop Pests*, ed. by Barker GM. Wallingford, CABI Publishing, pp. 337–351 (2002).
6 Nicholls CJ, Implications of Not Controlling Slugs in Oilseed Rape and Wheat in the UK (2014). https://cereals.ahdb.org.uk/publications/2014/march/10/implications-of-not-controlling-slugs-in-oilseed-rape-and-wheat-in-the-uk.aspx.
7 Simms LC, Mullins CE and Wilson MJ, Seed dressings to control slug damage in oilseed rape. *Pest Manag Sci* **58**:687–694 (2002).
8 ADAS, A review of slug control in winter cereal and oilseed rape, *Research Project Final Report PS2803*. Department for Environment, Food and Rural Affairs (DEFRA), London (2010) https://sciencesearch.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=17215&FromSearch=Y&Publisher=1&SearchText=slug&GridPage=1&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description.
9 Wilby A and Brown VK, Herbivory, litter and soil disturbance as determinants of vegetation dynamics during early old-field succession under set-aside. *Oecologia* **127**:259–265 (2001).
10 Joe SM and Daehler CC, Invasive slugs as under-appreciated obstacles to rare plant restoration: evidence from the Hawaiian islands. *Biol Invasions* **10**:245–255 (2008).
11 Côté M, Ferron J and Gagnon R, Invertebrate predation of post dispersal seeds and juvenile seedlings of black spruce (*Picea mariana*) in boreal forest of eastern Canada. *Can J For Res* **35**:674–681 (2005).
12 Cameron R, Are non-native gastropods a threat to endangered lichens? *Can Field Nat* **123**:169–171 (2009).
13 Telfer KH, Brurberg MB, Haukeland S, Stensvand A and Talgø V, *Phytophthora* survives the digestive system of the invasive slug *Arion vulgaris*. *Eur J Plant Pathol* **142**:125–132 (2015).
14 Grewal PS, Grewal SK, Tan L and Adams BJ, Parasitism of molluscs by nematodes: types of associations and evolutionary trends. *J Nematol* **35**:146–156 (2003a).
15 Yong HS, Eamsobhana P, Lim PE, Razali R, Aziz FA, Rosli NS *et al*., Draft genome of neurotropic nematode parasite *Angiostrongylus cantonensis*, causative agent of human eosinophilic meningitis. *Acta Trop* **148**:51–57 (2015).
16 Purvis G and Bannon JW, Non-target effects of repeated methiocarb slug pellet application on carabid beetle (Coleoptera: Carabidae) activity in winter-sown cereals. *Ann Appl Biol* **121**:401–422 (1992).
17 Jones D, EU votes to ban methiocarb slug pellets, Farmers Weekly http://www.fwico.uk/arable/eu-votes-to-ban-methiocarb-slug-pellets.html (2014).
18 Nicholls CJ, Implications of not controlling slugs in oilseed rape and wheat in the UK. *Res Rev* **79**, Agriculture and Horticulture Board, pp. 1–9 (2014).
19 Castle GD, Mills GA, Gravell A, Jones L, Townsend I, Cameron DG *et al*., Review of the molluscicide metaldehyde in the environment. *Environ Sci Water Res* **3**:415–428 (2017).
20 Campbell A, Audsley N and Port G, The fate of *Deroceras reticulatum* following metaldehyde poisoning. *Insects* **12**:344 (2021). https://doi.org/10.3390/insects12040344.
21 Bailey SER, Molluscicidal baits for control of terrestrial gastropods, in *Molluscs as Crop Pests*, ed. by Barker GM. CABI Publishing, Wallingford, U.K., pp. 33–54 (2002).

22 Cope RB, White KS, More E, Holmes E, Nair A, Chauvin P *et al*., Exposure to treatment interval and clinical severity in canine poisoning: a retrospective analysis at a Portland veterinary emergency center. *J Vet Pharmacol Ther* **29**:233–236 (2006).
23 Stuart M, Lapworth D, Crane E and Hart A, Review of risk from potential emerging contaminants in UK groundwater. *Sci Total Environ* **416**:1–21 (2012).
24 Kay P and Grayson R, Using water industry data to assess the metaldehyde pollution problem. *Water and Environ J* **28**:410–417 (2014).
25 Speiser B and Kistler C, Field tests with a molluscicide containing iron phosphate. *Crop Prot* **21**:389–394 (2001).
26 Langan AM and Shaw EM, Responses of earthworm *Lumbricus terrestris* (L.) to iron phosphate and metaldehyde slug pellet formulations. *Appl Soil Ecol* **34**:184–189 (2006).
27 Glen DM, Wiltshire CW, Hughes L, Ester A, Van Rozen K, Castillejo J *et al*., *The Use of Slug-Parasitic Nematodes and Other Techniques for Control of Slug and Snail Damage in Horticultural Crops, in Proceedings of the Brighton Crop Protection Conference–Pests and Diseases*, pp. 345–350 (2002).
28 Glen DM, Milsom NF and Wiltshire CW, Effect of seed depth on slug damage to winter wheat. *Ann Appl Biol* **117**:693–701 (1990).
29 Glen DM, Bamber G, Batchelor C, Bohan DA, Fisher J, Foster V *et al*., Integrated Slug Control in Arable Crops: Risk Assessment, Trapping, Agronomy and Chemical Control HGCA Project Report (2006). https://ahdb.org.uk/integrated-slug-control-in-arable-crops-risk-assessment-trapping-agronomy-and-chemical-control.
30 Schüder I, Port GR and Bennison J, Barriers, repellents and antifeedants for slug and snail control. *Crop Prot* **22**:1033–1038 (2003).
31 Schüder I, Port GR and Bennison J, The behavioural response of slugs and snails to novel molluscicides, irritants and repellents. *Pest Manag Sci* **60**:1171–1177 (2005).
32 Stock SP and Hunt DJ, Morphology and systematics of nematodes used in biocontrol, in *Nematodes as Biological Control Agents*, ed. by Grewal PS, Ehlers R-U and Shapiro-Ilan D. CABI Publishing, Wallingford, U.K., pp. 3–43 (2005).
33 Wilson MJ, Glen DM and George SK, The rhabditid nematode *Phasmarhabditis hermaphrodita* as a potential biological control agent for slugs. *Biocontrol Sci Technol* **3**:503–511 (1993).
34 Pieterse A, Malan AP and Ross JL, Nematodes that associate with terrestrial molluscs as definitive hosts, including *Phasmarhabditis hermaphrodita* (Rhabditida: Rhabditidae) and its development as a biological molluscicide. *J Helminthol* **91**:517–527 (2017a).
35 Rae RG, Verdun C, Grewal PS, Robertson JF and Wilson MJ, Biological control of terrestrial molluscs using *Phasmarhabditis hermaphrodita*–progress and prospects. *Pest Manag Sci* **63**:1153–1164 (2007).
36 Nermut J, Půža V and Mráček Z, Bionomics of the slug-parasitic nematode *Alloionema appendiculatum* and its effect on the invasive pest slug *Arion vulgaris*. *BioControl* **64**:697–707 (2019a).
37 Ross JL, Ivanova ES, Spiridonov SE, Waeyenberge L, Moens M, Nicol GW *et al*., Molecular phylogeny of slug-parasitic nematodes inferred from 18S rRNA gene sequences. *Mol Phylogenet Evol* **55**: 738–743 (2010a).
38 Wilson MJ, Wilson DJ, Aalders LT and Tourna M, Testing a new low-labour method for detecting the presence of *Phasmarhabditis* spp. in slugs in New Zealand. *Nematology* **18**:925–931 (2016).
39 Wilson MJ, Pathogens and parasites of terrestrial molluscs, in *Manual of Techniques in Invertebrate Pathology Lacey*, ed. by Lacey LA. San Diego Academic Press, San Diego, California, U.S., pp. 429–441 (2012).
40 Andrus P and Rae R, Development of *Phasmarhabditis hermaphrodita* (and members of the *Phasmarhabditis* genus) as new genetic model nematodes to study the genetic basis of parasitism. *J Helminthol* **93**: 319–331 (2019a).
41 Blaxter ML, de Ley P, Garey JR, Liu LX, Scheldeman P, Vierstraete A *et al*., A molecular evolutionary framework for the phylum Nematoda. *Nature* **392**:71–75 (1998).
42 MacMillan K, Blok V, Young I, Crawford J and Wilson MJ, Quantification of the slug parasitic nematode *Phasmarhabditis hermaphrodita* from soil samples using real time qPCR. *Int J Parasitol* **36**:1453–1461 (2006).
43 Jaffuel G, Půža V, Hug AS, Meuli RG, Nermut J, Turlings TCJ *et al*., Molecular detection and quantification of slug parasitic nematodes from the soil and their hosts. *J Invertebr Pathol* **160**:18–25 (2019).
44 Schneider A, Uber eine Nematoden larvae und gewisse verschiedenheiten in den geschlechtsorganen der nematoden. *Zeitschrift fur wissenschaftliche Zoologie* **10**:176–178 (1859).
45 Maupas E, Modes et forms de reproduction des nematodes. *Archives de Zoologie* **8**:464–642 (1900).
46 Mengert H, Nematoden and Schneken. *Zeitschrift für Morphologie und Öekologie Tiere* **41**:311–349 (1953).
47 Glen DM and Coupland J, The discovery and commercialization of a slug parasitic nematode, in *Biocontrol Agents: Entomopathogenic and Slug Parasitic Nematodes*, ed. by Abd-Elgawad MMM, Askary TH and Coupland J. CABI Publishing, Wallingford, U.K., pp. 548–559 (2017).
48 Wilson MJ, Glen DM, George SK and Pearce JD, Selection of a bacterium for the mass production of *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae) as a biocontrol agent for slugs. *Fundam Appl Nematol* **18**:419–425 (1995a).
49 Wilson MJ, Glen DM, George SK and Pearce JD, Monoxenic culture of the slug parasite *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae) with different bacteria in liquid and solid phase. *Fundam Appl Nematol* **18**:159–166 (1995b).
50 Wilson MJ, Glen DM, George SK, Pearce JD and Wiltshire CW, Biological control of slugs in winter wheat using the rhabditid nematode *Phasmarhabditis hermaphrodita*. *Ann Appl Biol* **125**:377–390 (1994a).
51 Wilson MJ, Glen DM, George SK and Hughes LA, Biocontrol of slugs in protected lettuce using the rhabditid nematode *Phasmarhabditis hermaphrodita*. *Biocontrol Sci Technol* **5**:233–242 (1995c).
52 Coupland JB, Susceptibility of helicid snails to isolates of the nematode *Phasmarhabitis hermaphrodita* from southern France. *J Invertebr Pathol* **66**:207–208 (1995).
53 France A and Gerding M, Discovery of *Phasmarhabditis hermaphrodita* in Chile and its pathological differences with the UK isolate in slug control. *J Nematol* **32**:430 (2000).
54 Karimi J, Kharazi-Pakadel A and Robert SJ, Report of pathogenic nematodes from slugs, *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditida) in Iran. *Journal of Entomological Society of Iran* **22**:77–78 (2003).
55 Nermut J, Půža V and Mráček Z, The first report on the slug parasitic nematode *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae) in The Czech Republic, in *30th International Symposium of the European Society of Nematologists*, Vol. **56**. Vienna (2010).
56 Genena MAM, Mostafa FAM, Fouly AH and Yousef AA, First record for the slug parasitic nematode, *Phasmarhabditis hermaphrodita* (Schneider) in Egypt. *Arch Phytopathol* **44**:340–345 (2011).
57 Wilson MJ, Burch G, Tourna M, Aalders LT and Barker GM, The potential of a New Zealand strain of *Phasmarhabditis hermaphrodita* for biological control of slug. *N Z Plant Prot* **65**:161–165 (2012).
58 Ross JL, Ivanova ES, Hatteland BA, Brurberg MB and Haukeland S, Survey of nematodes associated with terrestrial slugs in Norway. *J Helminthol* **90**:583–587 (2016).
59 Singh PR, Couvreur M, Decraemer W and Bert W, Survey of slug-parasitic nematodes in East and West Flanders, Belgium and description of *Angiostoma gandavensis* n. sp. (Nematoda: Angiostomidae) from arionid slugs. *J Helminthol* **94**:e35 (2019). https://doi.org/10.1017/S0022149X19000105.
60 Grewal SK, Grewal PS, Brown I, Tan L, Hammond RB and Gaugler R, First north American survey for the recovery of nematodes associated with molluscs. *J Nematol* **32**:432 (2000).
61 Kaya HK and Mitani DR, Molluscicidal nematodes for the biological control of pest slugs. *Slosson Report* **14**:1–4 (2000).
62 Ross JL, Ivanova ES, Severns PM and Wilson MJ, The role of parasite release in invasion of the USA by European slugs. *Biol Invasions* **12**: 603–610 (2010b).
63 Tandingan De Ley I, McDonnell RD, Paine TD and De Ley P, *Phasmarhabditis*: the slug and snail parasitic nematodes in North America, in *Biocontrol Agents: Entomopathogenic and Slug Parasitic Nematodes*, ed. by Abd-Elgawad MMM, Askary TH and Coupland J. CABI Publishing, Wallingford, pp. 560–578 (2017).
64 Mc Donnell RJ, Lutz MS, Howe DK and Denver DR, First report of the gastropod-killing nematode, *Phasmarhabditis hermaphrodita*, in Oregon, USA. *J Nematol* **50**:77–78 (2018a).
65 Tandingan De Ley I, McDonnell RD, Lopez S, Paine TD and De Ley P, *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae), a potential biocontrol agent isolated for the first time from invasive slugs in North America. *Nematology* **16**:1129–1138 (2014).
66 Schurkman J, Tandingan De Ley I, Anesko K, Paine T, Mc Donnell R and Dillman AR, distribution of *Phasmarhabditis* (nematode: Rhabditidae) and their gastropod hosts in California plant nurseries and

garden centers. *Front Plant Sci* **13**:856863 (2022a). https://doi.org/10.3389/fpls.2022.856863.
67 Brophy T, Mc Donnell RJ, Howe DK, Denver DR, Ross JL and Luong LT, Nematodes associated with terrestrial slugs in the Edmonton region of Alberta, Canada. *J Helminthol* **94**:e200 (2020a). https://doi.org/10.1017/S0022149X20000838.
68 Brophy T, Howe DK, Denver DR and Luong LT, First report of a gastropod parasitic nematode *Phasmarhabditis californica* (Nematoda: Rhabditidae) in Alberta, Canada. *J Nematol* **52**:e2020-92 (2020b). https://doi.org/10.21307/jofnem-2020-092.
69 Mc Donnell R, Tandingan De Ley I and Paine TD, susceptibility of neonate *Lissachatina fulica* (Achatinidae: Mollusca) to a US strain of the nematode *Phasmarhabditis hermaphrodita* (Rhabditidae: Nematoda). *Biocontrol Sci Technol* **28**:1091–1095 (2018b).
70 Mc Donnell RJ, Colton AJ, Howe DK and Denver DR, Lethality of four species of *Phasmarhabditis* (Nematoda: Rhabditidae) to the invasive slug, *Deroceras reticulatum* (Gastropoda: Agriolimacidae) in laboratory infectivity trials. *Biol Control* **150**:104349 (2018c).
71 Schurkman J, Dodge C, McDonnell R, De Ley IT and Dillman AR, Lethality of *Phasmarhabditis* spp. (*P. hermaphrodita*, *P. californica*, and *P. papillosa*) nematodes to the grey field slug *Deroceras reticulatum* on *canna* lilies in a lath house. *Agronomy* **12**:20 (2022b). https://doi.org/10.3390/agronomy12010020.
72 Schurkman J, De Ley IT and Dillman AR, Lethality of three *Phasmarhabditis* spp. (*P. hermaphrodita*, *P. californica*, and *P. papillosa*) to *Succinea* snails. *Agri* **12**:837 (2022c). https://doi.org/10.3390/agriculture12060837.
73 Tandingan De Ley I, Schurkman J, Wilen C and Dillman AR, Mortality of the invasive white garden snail *Theba pisana* exposed to three US isolates of *Phasmarhabditis* spp (*P. hermaphrodita*, *P. californica*, and *P. papillosa*). *PloS One* **15**:e0228244 (2020). https://doi.org/10.1371/journal.pone.0228244.
74 Schurkman J, De Ley IT and Dillman AR, Dose dependence of *Phasmarhabditis* isolates (*P. hermaphrodita*, *P. californica*, *P. papillosa*) on the mortality of adult invasive white garden snails (*Theba pisana*). *PloS One* **17**:e0270185 (2022d). https://doi.org/10.1371/journal.pone.0270185.
75 Mc Donnell RJ, Colton AJ, Howe DK and Denver DR, Susceptibility of *Testacella haliotidea* (Testacellidae: Mollusca) to a U.S. strain of *Phasmarhabditis hermaphrodita* (Rhabditidae: Nematoda). *Biocontrol. Sci Technol* **32**:262–266 (2022).
76 Nermut J, Holley M, Ortmayer LF and Půža V, New players in the field: ecology and biocontrol potential of three species of mollusc-parasitic nematodes of the genus *Phasmarhabditis*. *BioControl* **67**:543–5552 (2022a).
77 Howe DK, Ha AD, Colton A, De Ley IT, Rae RG, Ross J *et al*., Phylogenetic evidence for the invasion of a commercialized European *Phasmarhabditis hermaphrodita* lineage into North America and New Zealand. *PloS One* **15**:e0237249 (2020). https://doi.org/10.1371/journal.pone.0237249.
78 Azzam KM, Description of the nematode *Phasmarhabditis tawfiki* n. sp. isolated from Egyptian terrestrial snails and slugs. *journal of Egyptian German society of Fortschr Zool* **42**:79–88 (2003).
79 Tandingan De Ley I, Holovachov O, McDonnell RJ, Bert W, Paine TD and De Ley P, Description of *Phasmarhabditis californica* n. sp and first report of *P. papillosa* (Nematoda: Rhabditidae) from invasive slugs in the USA. *Nematology* **18**:175–193 (2016).
80 Nermut J, Půža V, Mekete T and Mráček Z, *Phasmarhabditis bonaquaense* n. sp (Nematoda: Rhabditidae), a new slug-parasitic nematode from The Czech Republic. *Zootaxa* **4179**:530–546 (2016a).
81 Nermut J, Půža V and Mráček Z, *Phasmarhabditis apuliae* n. sp (Nematoda: Rhabditidae), a new rhabditid nematode from milacid slugs. *Nematology* **18**:1095–1112 (2016b).
82 Nermut J, Půža V, Mekete T and Mráček Z, Phasmarhabditis bohemica n. sp. (Nematoda: Rhabditidae), a slug-parasitic nematode from The Czech Republic. *Nematology* **19**:93–107 (2017).
83 Gorgadze O, Troccoli A, Fanelli E, Tarasco E and De Luca F, Phasmarhabditis thesamica n. sp. (Nematoda: Rhabditidae), a new slug nematode from southern slope of Caucasus, Georgia. *Nematology* **24**:617–629 (2022).
84 Huang RE, Ye W, Ren X and Zhao Z, Morphological and molecular characterization of *Phasmarhabditis huizhouensis* sp. nov. (Nematoda: Rhabditidae), a new rhabditid nematode from South China. *PloS One* **10**:e0144386 (2015). https://doi.org/10.1371/journal.pone.0144386.
85 Ivanova ES and Spiridonov SE, *Phasmarhabditis meridionalis* sp n. (Nematoda: Rhabditidae) from a land snail *Quantula striata* (Gastropoda: Dyakiidae) from southern Vietnam. *Russ J Nematol* **25**:129–140 (2017).
86 Ivanova ES, Geraskina AP and Spiridonov SE, Two new species of *Phasmarhabditis* Andrássy, 1976 (Nematoda: Rhabditidae) associated with land snails in Northwest Caucasus, Russian Federation: description and molecular affiliation. *Nematology* **22**:179–197 (2020).
87 Ivanova ES and Spiridonov SE, Phasmarhabditis quinamensis sp. n. (Nematoda: Rhabditidae) from tropical terrestrial gastropods in southern Vietnam. *Nematology* **24**:225–239 (2021).
88 Ivanova ES, Gorgadze OA, Lortkhipanidze MA and Spiridonov SE, *Phasmarhabditis akhaldaba* n. sp. associated with a slug *Deroceras reticulatum* in lesser Caucasus mountains in republic of Georgia. *Russ J Nematol* **29**:75–88 (2021).
89 Pieterse A, Rowson B, Tiedt L, Malan AP, Haukeland S and Ross JL, *Phasmarhabditis kenyaensis* n. sp. (Nematoda: Rhabditidae) from the slug, *Polytoxon robustum*, in Kenya. *Nematology* **23**:229–245 (2020).
90 Ross JL, Pieterse A, Malan AP and Ivanova E, *Phasmarhabditis safricana* n. sp (Nematoda: Rhabditidae), a parasite of the slug *Deroceras reticulatum* from South Africa. *Zootaxa* **4420**:391–404 (2018).
91 Zhang C-N and Liu Q-A, *Phasmarhabditis zhejiangensis* sp. nov. (Nematoda: Rhabditidae), a new rhabditid nematode from Zhejiang, China. *PloS One* **15**:e0241413 (2020). https://doi.org/10.1371/journal.pone.0241413.
92 Andrassy I, *A Taxonomic Review of the Sub-Order Rhabditina (Nematoda: Secernentae)*. Office de la Recherche Scientifique et Technique, Paris (1983).
93 Sudhaus W, Phylogenetic systematisation and catalogue of paraphyletic "Rhabditidae" (Secernentea, Nematoda). *Journal of Nematode Morphology and Systematics* **14**:113–178 (2011).
94 Pieterse A, Tiedt LR, Malan AP and Ross JL, First record of *Phasmarhabditis papillosa* (Nematoda: Rhabditidae) in South Africa, and its virulence against the invasive slug. *Deroceras panormitanum Nematology* **19**:1035–1050 (2017b).
95 Ross JL, Ivanova ES, Sirgel WF, Malan AP and Wilson MJ, Diversity and distribution of nematodes associated with terrestrial slugs in the Western Cape Province of South Africa. *J Helminthol* **86**:215–221 (2012).
96 Carnaghi M, Rae R, de Ley IT, Johnston E, Kindermann G, Mc Donnell R *et al*., Nematode associates and susceptibility of a protected slug (*Geomalacus maculosus*) to four biocontrol nematodes. *Biocontrol Sci Technol* **27**:294–299 (2017).
97 Keyte M, Grannell A, Sheehy L, Shepherd J and Rae R, *Phasmarhabditis californica* in Germany. *Nematology* **24**:475–480 (2022).
98 Waki T, Diversity of terrestrial mollusks and their helminths in artificial environments in Yoyogi Park, Tokyo, Japan. *Journal of Asia-Pacific Biodiversity* **10**:254–256 (2017).
99 Pieterse A, Malan AP, Kruitbos LM, Sirgel W and Ross JL, Nematodes associated with terrestrial slugs from canola fields and ornamental nurseries in South Africa. *Zootaxa* **4312**:194–200 (2017c).
100 Zaborski ER, Gittenger LAS and Roberts SJ, A possible *Phasmarhabditis* sp(Nematoda: Rhabditidae) isolated from Lumbricus terrestris (Oligochaeta: Lumbricidae). *J Invertebr Pathol* **77**:284–287 (2001).
101 Kiontke K, Barrière A, Kolotuev I, Pobilewicz B, Sommer R, Fitch DHA *et al*., Trends, stasis, and drift in the evolution of nematode vulva development. *Curr Biol* **17**:1925–1937 (2007).
102 Hooper DJ, Wilson MJ, Rowe JA and Glen DM, Some observations on the morphology and protein profiles of the slug parasitic nematodes *Phasmarhabditis hermaphrodita* and *P. neopapillosa* (Nematoda: Rhabditidae). *Nematology* **1**:173–182 (1999).
103 Glen DM, Wilson MJ, Hughes L, Cargeeg P and Hajjar A, 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. *British Crop Protection Council (BCPC) Symposium Proceedings* **66**:271–280 (1996).
104 Azzam KM and Tawfiki MFS, Capability of the nematode *Phasmarhabditis tawfiki* Azzam to infect some snails, slugs and insects larvae in the laboratory. *Egyptian German Society Journal of Zoology* **42**:27–32 (2003).
105 Nermut J, Holley M and Půža V, Growth and reproduction of monoxenic and polyxenic cultures of the slug-parasitic nematodes

Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Phasmarhabditis spp. in naturally occurring organic substrates. *Bio-Control* **67**:533–542 (2022b).
106 Tan L and Grewal PS, Infection behaviour of the rhabditid nematode *Phasmarhabditis hermaphrodita* to the grey garden slug *Deroceras reticulatum*. *J Parasitol* **87**:1349–1354 (2001a).
107 MacMillan K, Haukeland S, Rae RG, Young IM, Crawford JW, Hapca S *et al*., Dispersal patterns and behaviour of the nematode *Phasmarhabditis hermaphrodita* in mineral soils and organic media. *Soil Biol Biochem* **41**:1483–1490 (2009).
108 Schulte F, The association between *Rhabditis necromena* Sudhaus and Schulte, 1989 (Nematoda: Rhabditidae) and native and introduced millipedes in South Australia. *Nematologica* **35**:82–89 (1989).
109 Rae RG, Robertson JF and Wilson MJ, The chemotactic response of *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditida) to cues of *Deroceras reticulatum* (Mollusca: Gastropoda). *Nematology* **8**:197–200 (2006).
110 Rae RG, Robertson JF and Wilson MJ, Chemoattraction and host preference of the gastropod parasitic nematode *Phasmarhabditis hermaphrodita*. *J Parasitol* **95**:517–526 (2009a).
111 Hapca S, Crawford J, Rae R, Wilson M and Young I, Movement of the parasitic nematode *Phasmarhabditis hermaphrodita* in the presence of mucus from the host slug *Deroceras reticulatum*. *Biol Control* **41**:223–229 (2007a).
112 Hapca SM, Budha PB, Crawford J and Young I, Movement of the nematode, *Phasmarhabditis hermaphrodita*, in a structurally heterogeneous environment. *Nematology* **9**:731–738 (2007b).
113 Hapca S, Crawford JW, MacMillan K, Wilson MJ and Young IM, Modelling nematode movement using time-fractional dynamics. *J Theor Biol* **248**:212–224 (2007c).
114 Hapca S, Crawford JW and Young IM, Anomalous diffusion of heterogeneous populations characterized by normal diffusion at the individual level. *J R Soc Interface* **6**:111–122 (2009).
115 Nermut J, Půža V and Mráček Z, The response of *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae) and *Steinernema feltiae* (Nematoda: Steinernematidae) to different host-associated cues. *Biol Control* **61**:201–206 (2012a).
116 Kruitbos LM, Heritage S, Hapca SM and Wilson M, Influence of substrate on the body-waving behaviour of nematodes. *Nematology* **11**:917–925 (2009).
117 Brown FD, D'Anna I and Sommer RJ, Host-finding behaviour in the nematode *Pristionchus pacificus*. *Proc R Soc B* **278**:3260–3269 (2011a).
118 Dunstan DJ and Hodgson DJ, Snails home. *Phys Scr* **89**:068002 (2014). https://doi.org/10.1088/0031-8949/89/06/068002.
119 Bradley NC and Wiel RR, *The Nature and Properties of Soil*. Prentice Hall, New Jersey (1999).
120 Small RW and Bradford C, Behavioural responses of *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditida) to mucus from potential hosts. *Nematology* **10**:591–598 (2008).
121 Laznik Ž, Franin K, Trdan S, Vidrih M and Majić I, Chemotactic response and motility of the mollusc parasitic nematode *Phasmarhabditis papillosa* towards mucus from different mollusc species. *Bio-Control* **67**:345–356 (2022).
122 Andrus P and Rae R, Natural variation in chemoattraction in the gastropod parasitic nematodes *Phasmarhabditis hermaphrodita*. *P neopapillosa and P californica Nematol* **21**:479–488 (2019b).
123 Andrus P, Ingle O, Coleman T and Rae R, Gastropod parasitic nematodes (*Phasmarhabditis* sp.) are attracted to hyaluronic acid in snail mucus by cGMP signalling. *J Helminthol* **94**:e9 (2018). https://doi.org/10.1017/S0022149X18000986.
124 Cutler J and Rae R, Natural variation in host-finding behaviour of gastropod parasitic nematodes (*Phasmarhabditis* spp.) exposed to host-associated cues. *J Helminthol* **95**:e10 (2021).
125 Campos-Herrera R, *Nematode Pathogenesis of Insects and Other Pests: Ecology and Applied Technologies for Sustainable Plant and Crop Protection*. Springer International Publishing, Switzerland (2015).
126 Tan L and Grewal PS, Pathogenicity of *Moraxella osloensis*, a bacterium associated with the nematode *Phasmarhabditis hermaphrodita*, to the slug *Deroceras reticulatum*. *Appl Environ Microbiol* **67**:5010–5016 (2001b).
127 Nermut J, Půža V and Mráček Z, The effect of intraspecific competition on the development and quality of *Phasmarhabditis hermaphrodita* (Rhabditida: Rhabditidae). *Biocontrol. Sci Technol* **22**:1389–1397 (2012b).
128 Nermut J, Půža V and Mráček Z, The effect of different growing substrates on the development and quality of *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae). *Biocontrol. Sci Technol* **24**:1026–1038 (2014).
129 Rae RG, Tourna M and Wilson MJ, The slug parasitic nematode *Phasmarhabditis hermaphrodita* associates with complex and variable bacterial assemblages that do not affect its virulence. *J Invertebr Pathol* **104**:222–226 (2010).
130 Tan L and Grewal PS, Endotoxin activity of *Moraxella osloensis* against the grey garden slug. *Deroceras reticulatum Appl Environ Microbiol* **68**:3943–3947 (2002).
131 Tan L and Grewal PS, Characterization of the first molluscicidal lipopolysaccharide from *Moraxella osloensis*. *Appl Environ Microbiol* **69**:3646–3649 (2003).
132 An R, Sreevatsan S and Grewal PS, *Moraxella osloensis* gene expression in the slug host *Deroceras reticulatum*. *BMC Microbiol* **8** (2008). https://doi.org/10.1186/1471-2180-8-19.
133 Wilson M and Rae R, *Phasmarhabditis hermaphrodita* as a control agent for slugs, in *Nematode Pathogenesis of Insects and Other Pests: Ecology and Applied Technologies for Sustainable Plant and Crop Protection*, ed. by Campos-Herrera R. Switzerland, Springer International Publishing, pp. 509–521 (2015).
134 Schurkman J, Liu R and Alavi S, Tandingan De Ley I, Hsiao a and Dillman AR, the native microbial community of gastropod-associated *Phasmarhabditis* species across central and southern Californica. *Front Microbiol* **13**:903136 (2022e). https://doi.org/10.3389/fmicb.2022.903136.
135 Sheehy L, Cutler J, Weedall GD and Rae R, Microbiome analysis of malacopathogenic nematodes suggests no evidence of a single bacterial symbiont responsible for gastropod mortality. *Front Immunol* **20**:878783 (2022).
136 Pieterse A, Haukeland S, Půža V, Ross JL and Malan AP, *Ex vivo* development of *Phasmarhabditis* spp. associated with terrestrial molluscs. *J Helminthol* **96**:E6 (2022). https://doi.org/10.1017/S0022149X21000663.
137 Grewal SK and Grewal PS, Effect of osmotic desiccation on longevity and temperature tolerance of *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae). *J Parasitol* **89**:434–438 (2003a).
138 Cutler J and Rae R, Pathogenicity of wild and commercial *Phasmarhabditis hermaphrodita* exposed to the pestiferous slug *Deroceras invadens*. *J Invertebr Pathol* **174**:107435 (2020).
139 Grewal SK, Grewal PS and Hammond RB, Susceptibility of north American native and non-native slugs (Mollusca: Gastropoda) to *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae). *Biocontrol Sci Technol* **13**:119–125 (2003b).
140 Rae RG, Robertson JF and Wilson MJ, Susceptibility and immune response of *Deroceras reticulatum*, *Milax gagates* and *Limax pseudoflavus* exposed to the slug parasitic nematode *Phasmarhabditis hermaphrodita*. *J Invertebr Pathol* **97**:61–69 (2008).
141 Ester A, Rozen Van K and Hazendonk A, Efficacy of pesticides to control *Lehmannia valentiana* (Férussac) in orchids (*cymbidium*) in greenhouse experiments, in slugs and snails: agricultural, veterinary and environmental perspectives. *British Crop Protection Council (BCPC) Symposium Proceedings* **80**:89–94 (2003c).
142 Iglesias J and Speiser B, Consumption rate and susceptibility to parasitic nematodes and chemical molluscicides of the pest slugs *Arion hortensis* and *A. distinctus*. *Pestic Sci* **74**:159–166 (2001).
143 Speiser B, Zaller JG and Newdecker A, Size susceptibility of the pest slugs *Deroceras reticulatum* and *Arion lusitanicus* to the nematode biocontrol agent *Phasmarhabditis hermaphrodita*. *Biocontrol* **46**:311–320 (2001).
144 Grimm B, Effect of the nematode *Phasmarhabditis hermaphrodita* on young stages of the pest slug *Arion lusitanicus*. *J Moll Stud* **68**:25–28 (2002).
145 Antzée-Hyliseth H, Trandem N, Torp T and Haukeland S, Prevalence and parasite load of nematodes and trematodes in an invasive slug and it susceptibility to a slug parasitic nematode compared to native gastropods. *J Invertebr Pathol* **173**:107372 (2020). https://doi.org/10.1016/j.jip.2020.107372.
146 Rae R, Shell encapsulation of parasitic nematodes by *Arianta arbustorum* (Linnaeus, 1758) in the laboratory and in field collections. *J Moll Stud* **84**:92–95 (2018).
147 Wilson MJ, Hughes LA, Hamacher GM and Glen DM, Effects of *Phasmarhabditis hermaphrodita* on non-target molluscs. *Pest Manag Sci* **56**:711–716 (2000a).

148 Williams A and Rae R, *Cepaea nemoralis* (Linnaeus, 1758) uses its shell as a defence mechanism to trap and kill parasitic nematodes. *J Moll Stud* **82**:349–350 (2016).
149 Williams A and Rae R, Susceptibility of the Giant African snail (*Achatina fulica*) exposed to the *gastropod parasitic nematode Phasmarhabditis hermaphrodita*. *J Invertebr Pathol* **127**:122–126 (2015).
150 Whitaker G and Rae R, The gastropod parasitic nematode *Phasmarhabditis hermaphrodita* does not affect non-target freshwater snails *Lymnaea stagnalis*. *Bithynia tentaculata and Planorbarius corneus Nematology* **17**:679–683 (2015).
151 Morley NJ and Morritt D, The effects of the slug biological control agent, *Phasmarhabditis hermaphrodita* (Nematoda), on non-target aquatic molluscs. *J Invertebr Pathol* **92**:112–114 (2006).
152 Okonjo E, Achieng G, Adundo J, Ojowi D and Bayo J, Evaluation of the beneficial nematode *Phasmarhabditis hermaphrodita* in the control of *Biomphalaria pfeifferi*. *Afr J Health Sci* **28**:168–170 (2015).
153 Abou-Elnour BM, El-Wahab El-Emam MA, Mahmoud MB, Ibrahim WL and Youssef AA, Alternations in parasitological, biochemical and molecular parameters of *Biomphalaria* snails intermediate host of *Schistosoma mansoni*, induced post exposure to the proposed snail biocontrol agent *Phasmarhabditis hermaphrodita* nematode. *Asian Pac J Trop Dis* **5**:957–963 (2015).
154 Montanari A, Bergamini G, Ferrari A, Ferri A, Nasi M, Simonini R *et al*., The immune response of the invasive golden apple snail to a nematode-based molluscicide involves different organs. *Biology* **9**: 371 (2020). https://doi.org/10.3390/biology9110371.
155 Laznik Ž, Majić I, Trdan S, Malan AP, Pieterse A and Ross JL, Is *Phasmarhabditis papillosa* (Nematoda: Rhabditidae) a possible biological control agent against the Spanish slug, *Arion vulgaris* (Gastropoda: Arionidae)? *Nematology* **23**:577–585 (2020).
156 Grannell A, Cutler J and Rae R, Size-susceptibility of *Cornu aspersum* exposed to the malacopathogenic nematodes *Phasmarhabditis hermaphrodita* and *P. californica*. *Biocontrol. Sci Technol* **31**:1149–1160 (2021).
157 Huey RB and Rosenzweig F, Laboratory evolution meets Catch-22: balancing simplicity and realism, in *Experimental Evolution: Concepts, Methods and Applications of Selection Experiments*, ed. by Garland T Jr and Rose MR. University of California Press, Berkeley, California, U.S., pp. 671–702 (2009).
158 Wang X and Grewal PS, Rapid genetic deterioration of environmental tolerance and reproductive potential of an entomopathogenic nematode during laboratory maintenance. *Biol Cont* **23**:71–78 (2002).
159 Sheehy L, MacDonald-Howard K, Williams CD, Weedall GD, Jones H and Rae R, A parasitic nematode induces dysbiosis in susceptible but not resistant gastropod hosts. *Microbiology* **12**:e1346 (2023). https://doi.org/10.1002/mbo3.1346.
160 Morand S, Wilson MJ and Glen DM, Nematodes (Nematoda) parasitic in terrestrial gastropods, in *Nematodes (Nematoda) Parasitic in Terrestrial Gastropods, in Natural Enemies of Terrestrial Molluscs Ed by Barker GM*. CABI Publishing, Wallingford, pp. 525–557 (2004).
161 Genena MAM and Mostafa FAM, Biological control of the clover land snail, *Monacha cantiana* (Montagu) using the rhabditid nematode, *Phasmarhabditis hermaphrodita* (Schneider) under mini-plot field conditions. *Egypt Journal of Agronematology* **9**:149–156 (2010).
162 Cowlishaw R, Andrus P and Rae R, An investigation into nematodes encapsulated in shells of wild, farmed and museum specimens of *Cornu aspersum* and *Helix pomatia*. *J Conchol* **43**:1–8 (2019).
163 Rae R, The gastropod shell has been co-opted to kill parasitic nematodes. *Sci Rep* **7**:4745 (2017a). https://doi.org/10.1038/s41598-017-04695-5.
164 Scheil AE, Hilsmann S, Triebskorn R and Köhler K-R, Shell colouration and parasite tolerance in two helicoid snail species. *J Invertebr Pathol* **117**:1–8 (2014).
165 Boraldi F, Lofaro FD, Bergamini G, Ferrari A and Malagoli D, *Pomacea canaliculata* ampullar proteome: a nematode based biopesticide induces changes in metabolic and stress-related pathways. *Biology* **10**:1049 (2021). https://doi.org/10.3390/biology10101049.
166 Curtis VA, Infection-avoidance behaviour in humans and other animals. *Trends Immunol* **35**:457–464 (2014).
167 Wilson MJ, Hughes LA, Jefferies D and Glen D, Slugs (*Deroceras reticulatum* and *Arion ater* agg.) avoid soil treated with the rhabditid nematode *Phasmarhabditis hermaphrodita*. *Biol Cont* **16**:170–176 (1999).
168 Rae R, Avoidance and attraction behaviour of slugs exposed to parasitic nematodes. *J Invertebr Pathol* **197**:107896 (2023). https://doi.org/10.1016/j.jip.2023.107896.
169 Wynne R, Morris A and Rae R, Behavioural avoidance by slugs and snails of the parasitic nematode *Phasmarhabditis hermaphrodita*. *Biocontrol Sci Technol* **26**:1129–1138 (2016).
170 Morris A, Green M, Martin H, Crossland K, Swaney WT, Williamson SM *et al*., A nematode that can manipulate the behaviour of slugs. *Behav Processes* **151**:73–80 (2018).
171 Cutler J, Williamson SM and Rae R, The effect of sertraline, haloperidol and apomorphine on the behavioural manipulation of slugs (*Deroceras invadens*) by the nematode *Phasmarhabditis hermaphrodita*. *Behav Processes* **165**:1–3 (2019).
172 Bailey SER, Cairns A, Latham R, Abdel Kasi M and Manning P, Onset of immobilization on the slug *Deroceras reticulatum* Müller parasitized by the nematode *Phasmarhabditis hermaphrodita* Schneider, in slugs and snails: agricultural, veterinary and environmental perspectives. *British Crop Protection Council (BCPC) Symposium Proceedings* **80**:215–220 (2003).
173 Pechova H and Foltan P, The parasitic nematode *Phasmarhabditis hermaphrodita* defends its slug host from being predated or scavenged by manipulating host spatial behaviour. *Behav Processes* **78**:416–420 (2008).
174 Foltan P and Puza V, To complete their life cycle, pathogenic nematode-bacteria complexes deter scavengers from feeding on their host cadaver. *Behav Processes* **80**:76–79 (2009).
175 Christensen CC, Cowie RH, Yeung NW and Hayes KA, Biological control of pest non-marine molluscs: a Pacific perspective on risks to non-target organisms. *Insects* **12**:583 (2021). https://doi.org/10.3390/insects12070583.
176 Wilson MJ, Glen DM, Hughes LA, Pearce JD and Rodgers PB, Laboratory tests of the potential of entomopathogenic nematodes for the control of field slugs (*Deroceras reticulatum*). *J Invertebr Pathol* **64**:182–187 (1994b).
177 Grewal SK and Grewal PS, Survival of earthworms exposed to the slug-parasitic nematode *Phasmarhabditis hermaphrodita*. *J Invertebr Pathol* **82**:72–74 (2003b).
178 DeNardo EAB, Sindermann AB, Grewal SK and Grewal PS, Non-susceptibility of earthworm *Eisenia fetida* to the rhabditid nematode *Phasmarhabditis hermaphrodita*, a biological agent of slugs. *Biocontrol Sci Technol* **14**:93–98 (2004).
179 Rae RG, Robertson JF and Wilson MJ, Susceptibility of indigenous U.K. earthworms and an invasive pest flatworm to the slug parasitic nematode *Phasmarhabditis hermaphrodita*. *Biocontrol. Sci Technol* **15**:623–626 (2005).
180 Cutler J and Rae R, The malacopathogenic nematode *Phasmarhabditis californica* does not affect the survival of earthworms (*Lumbricus terrestris* and *Eisenia fetida*) or insects (*galleria mellonella* and *Tenebrio molitor*). *Biocontrol Sci Technol* **32**:765–770 (2022).
181 Iglesias J, Castillejo J and Castro R, The effect of repeated applications of the molluscicides metaldehyde and the biocontrol nematode *Phasmarhabditis hermaphrodita* on molluscs, earthworms, nematodes, acarids and collembolans: a two-year study in north West Spain. *Pest Manag Sci* **59**:1217–1224 (2003).
182 Georgis R, Koppenhöfer AM, Lacey LA, Bélair G, Duncan LW, Grewal PS *et al*., Successes and failures in the use of parasitic nematodes for pest control. *Biol Control* **38**:103–123 (2006).
183 Glen DM, Wilson MJ, Pearce JD and Rodgers PB, *Discovery and Investigation of a Novel Nematode Parasite for Biological Control of Slugs, in Proceedings of the Brighton Crop Protection Conference–Pests and Diseases*, pp. 617–624 (1994).
184 Ehlers R-U and Shapiro-Ilan DI, Mass production, in *Mass Production of Entomopathogenic Nematodes, in Nematodes as Biological Control Agents*, ed. by Grewal PS, Ehlers R-U and Shapiro-Ilan D. CABI Publishing, Wallingford, pp. 65–79 (2005).
185 Young JM, Dunhill P and Pearce JD, Separation characteristics of liquid nematode cultures and the design of recovery operations. *Biotechnol Prog* **18**:29–35 (2002).
186 Wilson JA, Pearce JD and Ayazi Shamlou P, Scaleable downstream recovery of nematodes used as biopesticides. *Biotechnol Bioeng* **75**:733–740 (2001).
187 Wilson JA, Postlethwaite J, Pearce JD, Leach JD, Lye GJ and Ayazi Shamlou P, Vibrating membrane filtration for recovery and concentration of insect killing nematodes. *Biotechnol Bioeng* **83**:235–240 (2003).
188 Wilson MJ and Gaugler R, Terrestrial Mollusc Pests, in *Terrestrial Mollusc Pests, in Field Manual of Techniques in Invertebrate Pathology: Application and Evaluation of Pathogens for Control of Insects and*

Other Invertebrate Pests, Ed by Lacey LA and Kaya HK. Kluwer Academic Publishers, Netherlands, pp. 787–804 (2000).
189 Wilson MJ, Hughes LA, Hamacher GM, Carahona LD and Glen DM, Effects of soil incorporation on the efficacy of the rhabditid nematode, *Phasmarhabditis hermaphrodita*, as a biological control agent for slugs. *Ann Appl Biol* **128**:117–126 (1996).
190 Grubišić D, Čuljak G, Mešić T, Juran I, Loparić A, Starčević D *et al*., Slug control in leafy vegetable using nematode *Phasmarhabditis hermaphrodita* (Schneider). *Appl Ecol Environ Res* **16**:1739–1747 (2018).
191 Speiser B and Andermatt M, Field trials with *Phasmarhabditis hermaphrodita* in Switzerland. *Slug and Snail Pests in Agriculture, British Crop Protection Proceedings* **66**:419–424 (1996).
192 Kozlowski J, Jaskulska M, Kozlowska M and Gawlor A, Effectiveness of nemaslug in reducing damage plants caused by grey field slug *Deroceras reticulatum* (O.F. Müller, 1774). *Prog Plant Prot* **52**:721–724 (2012).
193 Grubišić D, Ostrec LJ and Dusak I, Biological control of slugs in vegetable crops in Croatia, in Slugs and Snails: Agricultural, Veterinary and Environmental. *Perspectives* **80**:115–120 (2003).
194 Ester A, Rozen Van K and Molendijk LPG, Field experiments using the rhabditid nematode *Phasmarhabditis hermaphrodita* or salt as control measures against slugs in green asparagus. *Crop Prot* **22**:689–695 (2003a).
195 Ester A, Huiting HF, Molendijk LPG and Vlaswinkel MET, The rhabditid nematode *Phasmarhabditis hermaphrodita* Schneider as a potential biological agent to control field slugs *Deroceras reticulatum* (Müller) in Brussels sprouts, in slugs and snails: agricultural, veterinary and environmental perspectives. *British Crop Protection Council (BCPC) Symposium Proceedings* **80**:313–318 (2003b).
196 Ester A and Wilson MJ, Application of slug-parasitic nematodes, in *Application of Slug Parasitic Nematodes, in Nematodes as Biocontrol Agents*, ed. by Ehlers RU, Shapiro-Ilan DI and Grewal PS. CABI Publishing, Wallingford, pp. 431–444 (2005).
197 Rae RG, Robertson JF and Wilson MJ, Optimization of biological (*Phasmarhabditis hermaphrodita*) and chemical (iron phosphate and metaldehyde) slug control. *Crop Prot* **28**:765–773 (2009b).
198 Dörler D, Scheucher A and Zaller JG, Efficacy of chemical and biological slug control measures in response to watering and earthworms. *Sci Rep* **9**:2954 (2019). https://doi.org/10.1038/s41598-019-39585-5.
199 Vernavá MN, Phillps-Aalten PM, Hughes LA, Rowcliffe H, Wiltshire CW and Glen DM, Influences of preceding cover crops on slug damage and biological control using *Phasmarhabditis hermaphrodita*. *Ann Appl Biol* **145**:279–284 (2004).
200 France A, Gerding M, Céspedes C and Cortez C, Slug control (*Deroceras reticulatum* Müller) with *Phasmarhabditis hermaphrodita* Schneider (Nematoda: Rhabditidae) in non-tillage crop systems. *Agri Téc* **62**:181–190 (2002).
201 Grewal PS, *Ehlers R-U and Shapiro-Ilan DI (2005) Nematodes as Biocontrol Agents*. CABI Publishing, Wallingford (2005).
202 Ester A and Geelen PMTM, Integrated control of slugs in a sugar beet crop growing in a rye cover crop, in slug and snail pests in agriculture. *British Crop Protection Council Monograph* **66**:445–450 (1996).
203 Wilson MJ, Major B, Hughes LA and Glen DM, integrating biological and chemical control of slugs, in crop protection conference–pests and diseases. *British Crop Protection Council* **1**:1007–1012 (2000b).
204 Grewal SK, Grewal PS, Taylor RAJ and Hammond RB, Application of molluscicidal nematodes to slug shelters: a novel approach to economic biological control of slugs. *Biol Control* **22**:72–80 (2001).
205 Hass B, Hughes LA and Glen DM, Overall versus band application of the nematode *Phasmarhabditis hermaphrodita* with and without incorporation into soil, for biological control of slugs in winter wheat. *Biocontrol Sci Technol* **9**:579–586 (1999a).
206 Hass B, Glen DM, Brain P and Hughes LA, Targeting biocontrol with the slug-parasitic nematode *Phasmarhabditis hermaphrodita* in slug feeding areas: a model study. *Biocontrol Sci Technol* **9**:571–578 (1999b).
207 Iglesias J, Castillejo J and Castro R, Field test using the nematode *Phasmarhabditis hermaphrodita* for biocontrol of slugs in Spain. *Biocontrol Sci Technol* **11**:93–98 (2001).
208 Brown AP, Barker A, Hopkins A and Nelson D, Application of *Phasmarhabditis hermaphrodita* (Nemaslug) to broad acre crops, in international organisation for biological and integrated control (IOBC/WPRS). *Bulletin* **64**:99–104 (2011b).
209 Kozlowska J, Jaskulska M and Kozlowska M, Evaluation of the effectiveness of iron phosphate and the parasitic nematode *Phasmarhabditis hermaphrodita* in reducing plant damage caused by the slug *Arion vulgaris* Moquin-Tandon, 1885. *Folia Malacol* **22**:293–300 (2014).
210 Jaskulska M and Kozlowski J, Possible use of the nematode *Phasmarhabditis hermaphrodita* to limit plant damage by gastropods. *Folia Malacol* **20**:43–71 (2012).
211 Glen DM, Wilson MJ, Brain P and Stroud G, Feeding activity and survival of slugs, *Deroceras reticulatum*, exposed to the rhabditid nematode, *Phasmarhabditis hermaphrodita*: a model of dose response. *Biol Cont* **17**:73–81 (2000).
212 Wilson MJ, Glen D, Hamacher GM and Smith JU, A model to optimise biological control of slugs using nematode parasites. *Appl Soil Ecol* **26**:179–191 (2004).
213 Schley D and Bees MA, The role of time delays in a non-autonomous host-parasitoid model of slug biocontrol with nematodes. *Ecol Model* **193**:543–559 (2006).
214 Hatteland BA, Haukeland S, Roth S, Brurberg MB, Vaughan IP and Symondson WOC, Spatiotemporal analysis of predation by carabid beetles (Carabidae) on nematode infected and uninfected slugs in the field. *PloS One* **8**:e82142 (2013). https://doi.org/10.1371/journal.pone.0082142.
215 Nermut' J, The persistence of *Phasmarhabditis hermaphrodita* (Rhabditida: Rhabditidae) in different substrates. *Russ J Nematol* **20**:61–64 (2012).
216 Wilson MJ and Gaugler R, Factors limiting short-term persistence of entomopathogenic nematodes. *J Appl Entomol* **128**:250–253 (2004).
217 Strong DR, Populations of entomopathogenic nematodes in foodwebs, in *Entomopathogenic Nematology*, ed. by Gaugler R. CABI Publishing, Wallingford, pp. 225–240 (2002).
218 Read DS, Sheppard SK, Bruford MW, Glen D and Symondson WOC, Molecular detection of predation by soil micro-arthropods on nematodes. *Mol Ecol* **15**:1963–1972 (2006).
219 Heidemann K, Scheu S, Ruess L and Maraun M, Molecular detection of nematode predation and scavenging in oribatid mites: laboratory and field experiments. *Soil Biol Biochem* **43**:2229–2236 (2011).
220 Fiera C, Detection of food in the gut content of *Heteromurus nitidus* (Hexapoda: Collembola) by DNA/PCR-based molecular analysis. *North-West J Zool* **10**:67–73 (2014).
221 Wilson MJ, Digweed AJ, Brown J, Ivanova ES and Hapca SH, Invasive slug pests and their parasites-temperature responses and potential implications of climate change. *Biol Fertil Soils* **51**:739–748 (2015a).
222 Glen DM and Wilson MJ, Slug parasitic nematodes as biocontrol agents for slugs. *Agro Food Ind Hi-Tech* **8**:23–27 (1997).
223 El-Danasoury H and Iglesias-Pineiro J, Performance of the slug parasitic nematode *Phasmarhabditis hermaphrodita* under predicted conditions of winter warming. *Pestic Sci* **42**:62–66 (2017).
224 Kramarz PE, de Vaufleury A, Zygmunt PM and Verdun C, Increased response to cadmium and *bacillus thuringiensis* maize toxicity in the snail *Helix aspersa* infected by the nematode *Phasmarhabditis hermaphrodita*. *Environ Toxicol Chem* **26**:73–79 (2007).
225 Lindqvist I, Lindqvist B and Tiilikkala K, Birch tar oil is an effective mollusc repellent: field and laboratory experiments using *Arianta arbustorum* (Gastropoda: Helicidae) and *Arion lusitanicus* (Gastropoda: Arionidae). *Agric Food Sci* **19**:1–12 (2010).
226 Richards EH, DeMarzo D, Port GR, Dani MP and Walters KF, Effects of the nematode *Phasmarhabditis hermaphrodita* and of venom from the endoparasitic wasp *Pimpla hypochondriaca* on survival and food consumption of the pest slug *Deroceras reticulatum*; implications for novel biocontrol strategies. *Pest Manag Sci* **64**:711–719 (2008).
227 Ahmed KSD, Stephens C, Bistline-East A, Williams CD, Mc Donnell RJ, Carnaghi M *et al*., Biological control of pestiferous slugs using *Tetanocera elata* (Fabricius) (Diptera: Sciomyzidae): larval behaviour and feeding on slugs exposed to *Phasmarhabditis hermaphrodita* (Schneider, 1859). *Biol Control* **135**:1–8 (2019).
228 Thompson JN, *The Geographic Mosaic of Coevolution*. The University of Chicago Press, U.S. (2005).
229 Wang JB, Elya C and St Leger RJ, Genetic variation for resistance to the specific fly pathogen *Entomophthora muscae*. *Sci Rep* **10**:14284 (2020). https://doi.org/10.1038/s41598-020-71262-w.
230 Bento G, Fields PD, Duneau D and Ebert D, An alternative route of bacterial infection associated with a novel resistance locus in the *daphnia-Pasteuria* host-parasite system. *Heredity* **125**:173–183 (2020).
231 Batalla-Carrera L, Morton a and Garcia-del-Pino, virulence of entomopathogenic nematodes and their symbiotic bacteria against the hazelnut weevil *curculio nucum*. *J Appl Entomol* **140**:115–123 (2016).

232 Levy N, Faigenboim A, Salame L, Molina C, Ehlers R-U, Glazer I *et al*., Characterization of the phenotypic and genotypic tolerance to abiotic stresses of natural populations of *Heterorhabditis bacteriophora*. *Sci Rep* **10**:10500 (2020). https://doi.org/10.1038/s41598-020-67097-0.
233 Sumaya NH, Aryal S, Vandenbossche B, Barg M, Doerfler V, Strauch O *et al*., Phenotyping dauer juvenile oxidative stress tolerance, longevity and persistence within wild type and inbred lines of the entomopathogenic nematode *Heterorhabditis bacteriophora*. *Nematology* **19**:971–986 (2017).
234 Hiltpold I, Baroni M, Toepfer S, Kuhlmann U and Turlings TCJ, Selection of entomopathogenic nematodes for enhanced responsiveness to a volatile root signal helps to control a major root pest. *J Exp Biol* **213**:2417–2423 (2010).
235 Ehlers R-U, Oestergaard J, Hollmer S, Wingen M and Strauch O, Genetic selection for heat tolerance and low temperature of the entomopathogenic nematode-bacterium complex *Heterorhabditis bacteriophora-Photorhabdus luminescens*. *BioControl* **50**:699–716 (2005).
236 Sumaya NH, Gohli R, Okolo C, Addis T, Doerfler V, Ehler R-U *et al*., Applying inbreeding; hybridization and mutagenesis to improve oxidative stress tolerance and longevity of the entomopathogenic nematode *Heterorhabditis bacteriophora*. *J Invertebr Pathol* **151**:50–58 (2018).
237 Okolo CT, Molina C, Bulus J, Chindo PS and Ehlers RU, Molecular screening of *Heterorhabditis bacteriophora* inbred lines for polymorphism and genetic crosses for the development of recombinant inbred lines. *Niger J Biotechnol* **36**:27–33 (2019).
238 Morris R, Wilson L, Sturrock M, Warnock ND, Carrizo D, Cox D *et al*., A neuropeptide modulates sensory perception in the entomopathogenic nematode *Steinernema carpocapsae*. *PLoS Pathog* **13**:e1006185 (2017). https://doi.org/10.1371/journal.ppat.1006185.
239 Ratnappan R, Vadnal J, Keaney M, Eleftherianos I, O'Halloran D and Hawdon JM, RNAi-mediated gene knockdown by microinjection in the model entomopathogenic nematode *Heterorhabditis bacteriophora*. *Parasit Vectors* **9**:160 (2016). https://doi.org/10.1186/s13071-016-1442-4.
240 Hashmi S, Hashmi G and Gaugler R, Genetic transformation of an entomopathogenic nematode by microinjection. *J Invertebr Pathol* **66**:293–296 (1995).
241 Wilson MJ, Ivanova ES and Spiridonov SE, Born to be wild–don't forget the invertebrates. *Trends Parasitol* **31**:530–532 (2015b).
242 Rae R, *Phasmarhabditis hermaphrodita*-a new model to study the genetic evolution of parasitism. *Nematology* **19**:375–387 (2017b).
243 Rae R, My favourite nematode-*Phasmarhabditis hermaphrodita*. *Nematology* **21**:1–4 (2019).
244 Luong LT and Mathot KJ, Facultative parasites as evolutionary stepping-stones towards parasitic lifestyles. *Biol Lett* **15**:20190058 (2019).
245 Hunt VL, Tsai IJ, Coghlan A, Reid AJ, Holroyd N, Foth BJ *et al*., The genomic basis of parasitism in the *Strongyloides* clade of nematodes. *Nat Genet* **48**:299–307 (2016).
246 International Helminth Genomes Consortium, Comparative genomics of the major parasitic worms. *Nat Genet* **51**:163–174 (2019).
247 Stevens L, Felix MA, Beltran T, Braendle C, Caurcel C, Fausett S *et al*., Comparative genomics of 10 new *Caenorhabditis* species. *Evol Lett* **3**:217–236 (2019).
248 Dillman AR, Macchietto M, Porter CF, Rogers A, Williams B, Antoshechkin I *et al*., Comparative genomics of *Steinernema* reveals deeply conserved gene regulatory networks. *Genome Biol* **16**:200 (2015). https://doi.org/10.1186/s13059-015-0746-6.
249 Bai X, Adams BJ, Ciche TA, Clifton S, Gaugler R, Kim K-S *et al*., A lover and a fighter: the genome sequence of an entomopathogenic nematode *Heterorhabditis bacteriophora*. *PloS One* **8**:e69618 (2013).
250 Chaston JM, Suen G, Tucker SL, Andersen AW, Bhasin A, Bode E *et al*., The entomopathogenic bacterial endosymbionts *Xenorhabdus* and *Photorhabdus*: convergent lifestyles from divergent genomes. *PloS One* **6**:e27909 (2011). https://doi.org/10.1371/journal.pone.0027909.
251 Jaffuel G, Sbaiti I and Turlings TCJ, Encapsulated entomopathogenic nematodes can protect maize plants from *Diabrotica balteata* larvae. *Insects* **11**:27 (2020). https://doi.org/10.3390/insects11010027.
252 Mc Donnell RJ, Yoo J, Patel K, Rios L, Hollingsworth R, Millar J *et al*., Can essential oils be used as novel drench treatments for the eggs and juveniles of the pest snail *Cornu aspersum* in potted plants? *J Pest Sci* **89**:549–555 (2016).
253 Klein ML, Chastain TG, Garbacik CJ, Qian YPL and McDonnell RJ, Acute toxicity of essential oils to the pest slug *Deroceras reticulatum* in laboratory and greenhouse bioassays. *J Pest Sci* **93**:415–425 (2019).
254 Barua A, McDonald-Howard K, McDonnell RJ, Rae R and Williams CD, Toxicity of essential oils to slug parasitic and entomopathogenic nematodes. *J Pest Sci* **93**:1411–1419 (2020).

