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**Pathogenicity of wild and commercial *Phasmarhabditis hermaphrodita*  
exposed to the pestiferous slug *Deroceras invadens***

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## 1 **Abstract**

2 Many terrestrial gastropods are pestiferous and pose a significant threat to agriculture,  
3 horticulture and floriculture. They are usually controlled by metaldehyde based pellets but an  
4 alternative control method is the slug parasitic nematode *Phasmarhabditis hermaphrodita*  
5 which has been formulated into a biological control agent (Nemaslug®) for use by farmers  
6 and gardeners to kill certain pestiferous slug species in 4-21 days. The current strain of *P.*  
7 *hermaphrodita* (called DMG0001) has been used in commercial production since 1994, but  
8 there is little information about the pathogenicity of wild strains of *P. hermaphrodita* towards  
9 slugs. Here, we exposed the pestiferous slug *Deroceras invadens* to nine wild isolated strains  
10 of *P. hermaphrodita* (DMG0002, DMG0003, DMG0005, DMG0006, DMG0007, DMG0008,  
11 DMG0009, DMG0010 and DMG0011) and the commercial strain (DMG0001) to three doses  
12 (0, 500 and 1000 nematodes per ml). Survival and feeding were recorded over 14 days. All  
13 wild *P. hermaphrodita* strains (other than DMG0010) and *P. hermaphrodita* (DMG0001),  
14 applied at 500 nematodes per ml caused significantly mortality to *D. invadens* compared to  
15 an uninfected control. Similarly, all *P. hermaphrodita* strains (apart from DMG0003) caused  
16 significant mortality to *D. invadens* when compared to an uninfected control at 1000  
17 nematodes per ml. Overall, all wild *P. hermaphrodita* strains (other than DMG0011) caused  
18 significantly more mortality than *P. hermaphrodita* DMG0001 at one or both dose rates. In  
19 summary, we have found some wild *P. hermaphrodita* strains were more virulent than *P.*  
20 *hermaphrodita* (DMG0001). Ultimately, these strains could potentially be developed as  
21 alternative, efficient biological control agents for use against slugs.

22

23 **Keywords:** Biological control; Molluscicide; Pests; Slugs

24

## 1. Introduction

On average, 32% of crop production is lost on a global scale due to pests (Dhawan *et al.*, 2010). Terrestrial gastropods (slugs and snails) inhabit all continents and are important agricultural pests in Europe, America and Australia (South, 1992; Barker, 2002). Uncontrolled slug damage could result in £43 million loss of oilseed rape and winter wheat product in the UK (Nicholls, 2014). In some cases, whole fields have to be re-sown with economic repercussions (Willis *et al.*, 2006). Slugs can also act as vectors for pathogens and parasites such as Metastrongyloidea nematodes (Grewal *et al.*, 2003). The main method to control slugs is by using metaldehyde based slug pellets (Castle *et al.*, 2017). In the UK, it was estimated that 1640t of metaldehyde was used between 2008 and 2014 (Fera, 2016). Metaldehyde pellets can harm non-target organisms including canines and other vertebrates (Cope, 2006), and due to leaching into watercourses it is now considered an emerging pollutant of concern (Stuart *et al.*, 2012). There are other chemical slug control options available to growers and farmers, including iron phosphate based products (Koch *et al.*, 2000; Iglesias *et al.*, 2001; Kozlowiski *et al.*, 2014). Iron phosphate based products containing chelating agents however, affect earthworm activity, growth and may be toxic (Langan and Shaw, 2006; Edwards *et al.*, 2009).

An alternative to chemical control is the slug parasitic nematode *Phasmarhabditis hermaphrodita*, which is capable of killing several pestiferous slug species (Wilson *et al.*, 1993). In 1994, this nematode was developed into a biological control agent and is sold by BASF Agricultural Specialities under the product name 'Nemaslug®'. Nemaslug® is now sold to gardeners and farmers across Northern Europe and has a market value of £1 million per annum (Pieterse *et al.*, 2017). Nematodes are formulated into a water dispersible formulation and upon soil application dauer larvae (the infective stage of the lifecycle) locate slugs via faecal and mucus cues (Rae *et al.*, 2006), enter host slugs via the dorsal

1 integumental pouch and migrate to the shell cavity (Wilson *et al.*, 1993). They then develop  
2 into self-fertilising hermaphrodites and proliferate, killing the host in 4-21 days (Wilson *et*  
3 *al.*, 1993; Tan and Grewal, 2001a). Nematodes proliferate on the cadaver until the food  
4 source is depleted, then new dauers enter the soil to locate a new host. Commercial *P.*  
5 *hermaphrodita* is produced in monoxenic conditions with the bacterium *Moraxella osloensis*.  
6 It is thought that *P. hermaphrodita* vectors *M. osloensis* into a slug host, causing death via  
7 septicaemia (Tan and Grewal, 2001b; 2002). One common symptom of *P. hermaphrodita*  
8 infection is host-feeding inhibition, which may be a reason why fast efficient control against  
9 slug damage is seen (Glen *et al.*, 2000). *P. hermaphrodita* has been used to control slug  
10 damage to many crops including winter wheat (Wilson *et al.*, 1994), cabbage (Grubišič *et al.*,  
11 2003, 2018; Kozolowski *et al.*, 2012) and sugar beet (Ester & Wilson, 2005).

12 The current strain of *P. hermaphrodita* (called DMG0001) has been used in the  
13 production of Nemaslug® for 25 years and there is little information on the pathogenicity of  
14 wild strains of *P. hermaphrodita*. Therefore, we investigated the pathogenicity and host  
15 feeding inhibition caused by nine recently isolated *P. hermaphrodita* strains (Supplementary  
16 Table 1) towards the common pest slug *Deroceras invadens* and compared them to *P.*  
17 *hermaphrodita* (DMG0001) to help understand if there is natural variation in the  
18 pathogenicity of these nematodes. We chose *D. invadens* as the host in our studies as it has  
19 been reported invading new areas over the last century and matures faster than other common  
20 pest slugs, such as *D. reticulatum* (Hutchinson *et al.*, 2014). It is now an important  
21 pestiferous slug of UK agricultural crops (Williams *et al.*, 2010). Ultimately, by identifying  
22 more pathogenic strains of *P. hermaphrodita* further studies could investigate how these  
23 nematodes kill slugs by first focussing on potential bacterial symbionts.

24

## 1 **2. Materials and methods**

### 2 ***2.1 Source and maintenance of invertebrates***

3 *P. hermaphrodita* commercial strain DMG0001 (Nemaslug®) was supplied by BASF  
4 Agricultural Specialities and stored at 10°C before use. Wild *P. hermaphrodita* strains  
5 (DMG0002, DMG0003, DMG0005, DMG0006, DMG0007, DMG0008, DMG0009,  
6 DMG0010 and DMG0011) were isolated from slugs collected from locations around  
7 Liverpool, UK (Supplementary Table 1). They have been in culture at Liverpool John  
8 Moores University (LJMU) since 2014 on modified White traps (White, 1927). Each strain  
9 began as an isogenic line from a single hermaphrodite mother and was identified to species  
10 via amplification and sequencing of the ITS1, 18SrRNA and the D2-D3 domain of large  
11 subunit (LSU) rDNA genes (Andrus and Rae, 2018). For experimentation the nematodes  
12 were grown up on decaying slug (*Limax flavus*) on modified White traps until they had  
13 reached the dauer stage and were then stored in cell culture flasks at 10°C (see Andrus and  
14 Rae, 2018 for more details). *L. flavus* were frozen at -80°C before use to kill any existing  
15 nematodes. For each experiment, fresh dauers were grown.

16 *D. invadens* (mean weight 0.70 g ± 0.55, n = 900) were collected from parks around  
17 Liverpool and stored at 10°C in the dark. Slugs were fed lettuce *ad libitum* and kept for 1  
18 week before use to screen for any previous nematode infection.

19

### 20 ***2.2 Survival and feeding inhibition of D. invadens exposed to P. hermaphrodita***

21 An adapted method by Wilson *et al.* (1993) was used to test the pathogenicity and  
22 feeding inhibition of *D. invadens* exposed to *P. hermaphrodita*. Two *D. invadens* were placed  
23 in a 20 ml plastic tube with a cotton wool bung pushed to the bottom and 2 ml of water was  
24 added to the tube. Slugs were exposed to 2 ml of 500 or 1000 nematodes per ml (or 2 ml of

1 water as a control). A cotton wool bung was used to stop the slugs escaping and the lid was  
2 loosely screwed on to allow airflow. Slugs were then incubated at 10°C in the dark for 5  
3 days. After 5 days of infection, each slug was placed on a 5 cm Petri dish containing a 3 cm  
4 diameter disk of lettuce (area 700mm<sup>2</sup>). Petri dishes were then incubated at 10°C for 9 days.  
5 Mortality was recorded every 2-3 days and the volume of lettuce disk eaten was recorded 8  
6 and 14 days after initial infection by tracing the remaining lettuce disk on 1 mm<sup>2</sup> graph paper  
7 (Rae *et al.*, 2009). Ten *D. invadens* were used per experiment and it was repeated three times  
8 for each *P. hermaphrodita* strain (DMG0001, DMG0002, DMG0003, DMG0005, DMG0006,  
9 DMG0007, DMG0008, DMG0009, DMG0010 and DMG0011). A no nematode control (dose  
10 rate 0) and *P. hermaphrodita* (DMG0001) (dose rates 500, 1000) were run with each group of  
11 wild *P. hermaphrodita* tested.

12

### 13 **2.3 Statistical analysis**

14 Survival of *D. invadens* exposed to *P. hermaphrodita* (DMG0001, DMG0002,  
15 DMG0003, DMG0005, DMG0006, DMG0007, DMG0008, DMG0009, DMG0010 and  
16 DMG0011) at 0, 500 and 1000 nematodes per ml was analysed using a Log Rank test in  
17 OASIS (Yang *et al.*, 2011). A One-Way ANOVA with Tukey's post hoc test was used to  
18 compare the amount of lettuce eaten by *D. invadens*.

19

20

## 21 **3. Results**

22 **3.1 Survival of *D. invadens* exposed to wild *P. hermaphrodita* strains and commercial *P.***  
23 ***hermaphrodita* strain.**

1           At a dose rate of 500 nematodes per ml the commercial strain of *P. hermaphrodita*  
2 (DMG0001) and all wild strains (DMG0002, DMG0003, DMG0005, DMG0006, DMG0007,  
3 DMG0008, DMG0009, and DMG0011), other than *P. hermaphrodita* DMG0010, caused  
4 significant mortality to *D. invadens* when compared to an uninfected control after 14 days ( $p$   
5  $< 0.05$ ) (Fig. 2A) (Supplementary Table 2A). When compared to the commercial *P.*  
6 *hermaphrodita* strain (DMG0001) the wild *P. hermaphrodita* strains DMG0002, DMG0005,  
7 DMG0007, and DMG0008 were significantly more pathogenic. DMG0002 and DMG0008  
8 caused rapid and consistent mortality, killing 12.2% and 10.6% of slugs per day respectively.  
9 ( $p < 0.05$ ) (Fig. 2A) (Supplementary Table 2A).

10           At the higher dose rate of 1000 nematodes per ml *P. hermaphrodita* commercial strain  
11 (DMG0001) and all wild strains (DMG0002, DMG0005, DMG0006, DMG0007, DMG0008,  
12 DMG0009, DMG0010 and DMG0011), other than *P. hermaphrodita* DMG0003, caused  
13 significant mortality to *D. invadens* when compared to an uninfected control after 14 days ( $p$   
14  $< 0.05$ ) (Fig. 2B) (Supplementary Table 2B). Wild *P. hermaphrodita* strains DMG0002,  
15 DMG0003, DMG0006, DMG0009 and DMG0010 caused significantly more *D. invadens*  
16 mortality than the commercial *P. hermaphrodita* strain (DMG0001) at 1000 nematodes per  
17 ml after 14 days. The fastest mortality rate was seen in *P. hermaphrodita* strains DMG0009  
18 (15% per day) and DMG0010 (13.9% per day) at 1000 nematodes per ml ( $p < 0.05$ ) (Fig. 2B)  
19 (Supplementary Table 2B).

### 20           **3.2 Feeding inhibition of *D. invadens* caused by *P. hermaphrodita* infection**

21

22           There was a significant difference between the amount of lettuce consumed by *D.*  
23 *invadens* exposed to all treatments after 8 days ( $F(10, 272) = 3.716, p < 0.0001$ , and after 14  
24 days ( $F(10, 227) = 5.922, p < 0.0001$  at a dose rate of 500 nematodes per ml. After 8 days

1 only *P. hermaphrodita* DMG0008 ( $43.3 \pm 18.3\text{mm}^2$ ,  $p = 0.007$ ) and DMG0009 ( $114.9 \pm$   
2  $32.4\text{mm}^2$ ,  $p = 0.044$ ) caused significant feeding inhibition to *D. invadens* compared to the  
3 uninfected control ( $317.6 \pm 38.6\text{mm}^2$ ) at a dose rate of 500 nematodes per ml (Fig. 3A). After  
4 14 days *P. hermaphrodita* DMG0003 ( $297.4 \pm 58.5\text{mm}^2$ ,  $p = 0.007$ ), DMG0005 ( $215.4 \pm$   
5  $47.5\text{mm}^2$ ,  $p = 0.001$ ), DMG0006 ( $296.8 \pm 60.9\text{mm}^2$ ,  $p = 0.014$ ), DMG0007 ( $255.4 \pm$   
6  $64.8\text{mm}^2$ ,  $p = 0.009$ ), DMG0008 ( $81.6 \pm 25.1\text{mm}^2$ ,  $p < 0.001$ ), DMG0009 ( $208.9 \pm 60.2\text{mm}^2$ ,  
7  $p < 0.001$ ), DMG0010 ( $244.8 \pm 49.6\text{mm}^2$ ,  $p < 0.001$ ) and DMG0011 ( $301.7 \pm 42.4\text{mm}^2$ ,  $p =$   
8  $0.031$ ) caused significant feeding inhibition to *D. invadens* when compared with the  
9 uninfected control ( $534.5 \pm 33.6\text{mm}^2$ ) at a dose rate of 500 nematodes per ml (Fig. 3B). Only  
10 *P. hermaphrodita* DMG0008 ( $81.6 \pm 25.1\text{mm}^2$ ,  $p = 0.040$ ) significantly inhibited *D. invadens*  
11 feeding more than commercial strain DMG0001 ( $354.9 \pm 45.9\text{mm}^2$ ) after 14 days at 500  
12 nematodes per ml (Fig. 3B).

13 After a dose rate of 1000 nematodes per ml there was a significant difference between  
14 the amount of lettuce consumed by *D. invadens* exposed to all treatments after 8 days ( $F(10,$   
15  $246) = 11.890$ ,  $p = < 0.0001$ ) and after 14 days ( $F(10, 169) = 9.156$ ,  $p < 0.0001$ ). Feeding was  
16 inhibited significantly more than the uninfected control ( $348.9 \pm 36.4\text{mm}^2$ ) after 8 days by *P.*  
17 *hermaphrodita* DMG0001 ( $180.4 \pm 30.5\text{mm}^2$ ,  $p = 0.008$ ) and the wild strains DMG0002  
18 ( $76.8 \pm 22.6\text{mm}^2$ ,  $p = 0.001$ ), DMG0003 ( $57.0 \pm 22.3\text{mm}^2$ ,  $p = 0.007$ ), DMG0005 ( $28.6 \pm$   
19  $9.6\text{mm}^2$ ,  $p = 0.032$ ), DMG0006 ( $139.8 \pm 34.2\text{mm}^2$ ,  $p < 0.001$ ), DMG0007 ( $111.5 \pm 25.5\text{mm}^2$ ,  
20  $p < 0.001$ ) and DMG0009 ( $30.3 \pm 14.5\text{mm}^2$ ,  $p < 0.001$ ) (Fig. 3C). After 14 days *P.*  
21 *hermaphrodita* DMG0001 ( $214.5 \pm 38.7\text{mm}^2$ ,  $p < 0.001$ ) and the wild strains DMG0002  
22 ( $164.1 \pm 46\text{mm}^2$ ,  $p < 0.001$ ), DMG0003 ( $148.1 \pm 41.3\text{mm}^2$ ,  $p < 0.001$ ), DMG0005 ( $138.4 \pm$   
23  $52.8\text{mm}^2$ ,  $p < 0.001$ ), DMG0006 ( $283.4 \pm 68.5\text{mm}^2$ ,  $p < 0.001$ ), DMG0007 ( $185.4 \pm$   
24  $43.8\text{mm}^2$ ,  $p < 0.001$ ), DMG0009 ( $85.0 \pm 59.7\text{mm}^2$ ,  $p = 0.005$ ) and DMG0011 ( $285.7 \pm$   
25  $48.2\text{mm}^2$ ,  $p = 0.002$ ) caused more feeding inhibition compared to the uninfected control

1 (534.6 ± 33.5mm<sup>2</sup>) at 1000 nematodes per ml ( $p < 0.05$ ) (Fig. 3D). None of the wild strains  
2 inhibited feeding significantly more than *P. hermaphrodita* DMG0001 after 8 or 14 days at a  
3 dose rate of 1000 nematodes per ml.

4

#### 5 **4. Discussion**

6 Here we show wild isolated *P. hermaphrodita* from the UK are highly pathogenic  
7 towards *D. invadens*. Little research has investigated the pathogenic potential of wild *P.*  
8 *hermaphrodita* strains, even though *P. hermaphrodita* has been isolated globally (Pieterse *et*  
9 *al.*, 2017). Our findings corroborate those of Wilson *et al.* (2012), Tandingan De Ley *et al.*  
10 (2020) and McDonnell *et al.* (2018), who found wild *P. hermaphrodita* strains that were  
11 pathogenic to the slug *D. reticulatum*, the snail *Theba pisana* and neonate life stages of the  
12 snail *Lissachatina fulica* respectively. Interestingly, commercial *P. hermaphrodita* DMG0001  
13 did not kill 12 week old *L. fulica* (Williams and Rae, 2015), but a wild *P. hermaphrodita*  
14 strain (called ITD290) was highly virulent (McDonnell *et al.*, 2018). The reasons for this  
15 difference could be due to the larger snails used by Williams and Rae (2015) as bigger snails  
16 and slugs tend to be more resistant to nematode infection, which has been observed in  
17 infection studies using *P. hermaphrodita* exposed to *Cornu aspersum*, *Arion lusitanicus* and  
18 *Arion ater* (Glen *et al.*, 1996; Speiser *et al.*, 2001; Grimm, 2002). Only *P. hermaphrodita*  
19 DMG0002 caused significantly more mortality than *P. hermaphrodita* DMG0001 at both  
20 dose rates. *P. hermaphrodita* DMG0005, DMG0007 and DMG0008 only caused significantly  
21 more pathogenicity at the lower dose rate of 500 nematodes per ml. Higher pathogenicity at a  
22 lower dose rate indicates that these wild isolates are highly virulent, and a lower worm  
23 burden is required for infection, inducing mortality and proliferation. Other factors may be  
24 influencing this result as well, such as the ability of each strain to locate a host. For example,

1 wild strains may display better chemoattraction, a trait that has diminished in the commercial  
2 strain (Andrus *et al.*, 2018; Andrus and Rae, 2019). In a study by Andrus and Rae (2019),  
3 using the same wild strains as above, it was found that when exposed to multiple slug mucus  
4 samples the commercial strain responded poorly to all mucus, with the majority of the  
5 nematodes remaining at the application point. Differences in chemoattraction between the  
6 wild isolated *P. hermaphrodita* strains were also seen. Interestingly, DMG0010 caused no  
7 mortality at 500 nematodes per ml, yet was highly virulent at 1000 nematodes per ml.  
8 Presumably a higher worm burden is required for DMG0010 to establish a lethal infection  
9 and when such infection is established this strain kills more rapidly than others. Natural  
10 variation in pathogenicity between nematode strains is well documented in entomopathogenic  
11 nematodes, such as wild isolated strains of *Heterorhabditis bacteriophora* and *Steinernema*  
12 *carpocapsae* , that were found to be more virulent to their targeted hosts (*Cephalcia*  
13 *tannourinensis* and *Agrotis ipsilon*, respectively) than the commercial formulations (Noujeim  
14 *et al.*, 2015; Bélair *et al.*, 2013). Further host range testing of the more virulent wild isolated  
15 *P. hermaphrodita* strains is needed.

16 We found wild *P. hermaphrodita* strains (DMG0002 DMG0005, DMG0007 and  
17 DMG0008) were more pathogenic than the commercial strain (DMG0001) (depending on  
18 dose). The reasons for this pathogenic difference could be due to their associated bacteria.  
19 Infectivity and pathogenicity of *P. hermaphrodita* are strongly influenced by bacterial diet  
20 (Wilson *et al.*, 1995a,b). One bacterium, *Moraxella osloensis*, was chosen for commercial  
21 production from a selection of 16 isolates as it constantly produced high yields of pathogenic  
22 nematodes (Wilson *et al.*, 1995 a,b). *P. hermaphrodita* is still grown on this bacterium. It is  
23 thought that *M. osloensis* is introduced and released into the slug's haemocoel by dauer stage  
24 nematodes and causes septicemia by production of an endotoxin (Tan and Grewal, 2001a, b;  
25 2002). However, there is evidence to show that *M. osloensis* is not vertically transmitted to

1 the next generation of pathogenic nematodes (Rae *et al.*, 2010; Nermut' *et al.*, 2014).  
2 Therefore, the current relationship *P. hermaphrodita*, and especially these wild strains, have  
3 with bacteria warrants further investigation. One such approach could be to use 16S  
4 metagenomics of bacteria present in the nematode gut, which has worked well in profiling the  
5 associated microflora in other nematodes like *Caenorhabditis elegans* (Dirksen *et al.*, 2016).

6 As well as killing slugs, the wild strains of *P. hermaphrodita* were able to inhibit  
7 feeding of *D. invadens*. This is a common symptom of *P. hermaphrodita* infection and  
8 enhances the use of these nematodes as a biological control agent. Host feeding inhibition is  
9 also caused in resistant species (Glen *et al.*, 2000), such as juvenile *A. lusitanicus* (Grimm,  
10 2002). Variation across dose rate, day and strain was also observed. Wild *P. hermaphrodita*  
11 strain DMG0009 had the largest effect, inhibiting slug feeding more than the control at both  
12 doses and inhibiting a greater level of feeding than the commercial strain at 1000 nematodes  
13 per ml.

14 In conclusion, wild isolated strains of *P. hermaphrodita* are capable of killing and  
15 inducing feeding inhibition in the slug *D. invadens*, some more than the commercial strain  
16 (DMG0001). Additional research on the better performing strains will be needed, including  
17 understanding the effects associated bacteria have on yield and virulence, and optimising *in*  
18 *vitro* culturing techniques. This research shows that understanding natural variation between  
19 strains used for biological control purposes could result in a more effective product. Having  
20 multiple wild strains displaying genetic variation in virulence could also help with  
21 understanding which genes are associated with pathogenicity, aid in understanding the  
22 evolution of parasitism and even enhance *P. hermaphrodita* as a biological control agent  
23 (Rae, 2017).

## 24 **Acknowledgments**

1 We are grateful to BASF Agricultural Specialities for funding and to Tom Goddard, Jack  
2 Shepherd, Craig Wilding and Will Swaney for discussions.

3

#### 4 **Figure legends**

5 Fig. 1. *P. hermaphrodita* dauer juveniles (A) are the infective stage and develop into adult  
6 self-fertilising hermaphrodites (B). Infection in *D. invadens* (C) can cause a swollen mantle  
7 and shell ejection in *D. invadens* (D). Scale bar in A represents 100  $\mu\text{m}$ , in B represents  
8 250 $\mu\text{m}$  and in C represents 0.5 cm.

9 Fig. 2. (A) Percentage survival of *D. invadens* exposed to 0 (Black) and 500 nematodes per  
10 ml of *P. hermaphrodita* (DMG0001) (Grey), *P. hermaphrodita* (DMG0002) (Red), *P.*  
11 *hermaphrodita* (DMG0003) (Orange), *P. hermaphrodita* (DMG0005) (Light Green), *P.*  
12 *hermaphrodita* (DMG0006) (Dark Green), *P. hermaphrodita* (DMG0007) (Light Blue), *P.*  
13 *hermaphrodita* (DMG0008) (Dark Blue), *P. hermaphrodita* (DMG0009) (Purple), *P.*  
14 *hermaphrodita* (DMG0010) (Brown) and *P. hermaphrodita* (DMG0011) (Dark Grey).

15 Fig. 2. (B) Percentage survival of *D. invadens* exposed to 0 (Black) and 1000 nematodes per  
16 ml of *P. hermaphrodita* (DMG0001) (Grey), *P. hermaphrodita* (DMG0002) (Red), *P.*  
17 *hermaphrodita* (DMG0003) (Orange), *P. hermaphrodita* (DMG0005) (Light Green), *P.*  
18 *hermaphrodita* (DMG0006) (Dark Green), *P. hermaphrodita* (DMG0007) (Light Blue), *P.*  
19 *hermaphrodita* (DMG0008) (Dark Blue), *P. hermaphrodita* (DMG0009) (Purple), *P.*  
20 *hermaphrodita* (DMG0010) (Brown), *P. hermaphrodita* (DMG0011) (Dark Grey).

21 Fig. 3. Feeding inhibition of *D. invadens* exposed to 0 and 500 nematodes per ml of *P.*  
22 *hermaphrodita* (DMG0001, DMG0002, DMG0003, DMG0005, DMG0006, DMG0007,  
23 DMG0008, DMG0009, DMG0010 and DMG0011) after 8 days (A) and 14 days (B) and 0

1 and 1000 nematodes per ml after 8 days (C) and 14 days (D). Significant differences between  
2 the amount consumed by the control and treatments at  $p < 0.05$  are denoted by \* and at  $p <$   
3  $0.001$  denoted by \*\*. Bars represent  $\pm 1$  standard error.

4 Supplementary Table 1. *Phasmarhabditis hermaphrodita* strains isolated from slugs around  
5 Liverpool.

6 Supplementary Table 2. Mean ( $\pm$  s.e.) number of slugs alive 0,2,5,7,9,12 and 14 days after  
7 exposure to *P. hermaphrodita* (DMG0001, DMG0002, DMG0003, DMG0005, DMG0006,  
8 DMG0007, DMG0008, DMG0009, DMG0010, DMG0011) at 0, 500 (A) or 1000 (B)  
9 nematodes per ml.

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