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Size-specific susceptibility of the pest slugs *Deroceras reticulatum* and *Arion lusitanicus* to the nematode biocontrol agent *Phasmarhabditis hermaphrodita*

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Abstract. The nematode *Phasmarhabditis hermaphrodita* is a commercially available biocontrol agent against slugs. This product is especially interesting for use in organic farming, where products containing metaldehyde or carbamates cannot be used for controlling pest slugs. We investigated the potential of *P. hermaphrodita* for the control of the pest slugs *Deroceras reticulatum* and *Arion lusitanicus*. These two species are the most harmful slug pests in Switzerland. At different times of the year, we collected slug specimens of different weight and assessed their susceptibility to *P. hermaphrodita* in the laboratory. Batches of five slugs were subjected to five different doses of nematodes plus an untreated control and replicated three times. During six weeks, feeding and survival of the slugs were recorded. *D. reticulatum* was strongly affected by increasing nematode doses, irrespective of the slugs' body weight. In small specimens of *A. lusitanicus*, feeding and survival were strongly affected by the nematodes, while larger specimens remained almost unaffected. Because *A. lusitanicus* has an asynchronous development in Switzerland, it seems difficult to control the entire population with a single nematode application. To what extent nematodes will be used in practice for slug control depends on their effectivity against the pest slugs of major importance, on the longevity of the molluscicidal effect and on the price of nematodes.

Key words: biocontrol, nematodes, organic farming, slugs, *Arion lusitanicus*, *Deroceras reticulatum*, *Phasmarhabditis hermaphrodita*

Introduction

In temperate regions all over the world, slugs are important pests in many crops (reviews in Godan, 1979; Port and Port, 1986; South, 1992). *Deroceras reticulatum* Müller (Agriolimacidae), a slug species originally endemic to the palaearctic, was introduced to other continents by human activities. It is now the most widespread slug species worldwide, occurring in temperate regions of Europe, Asia, North and South America, Australia and New Zealand

(Godan, 1979; South, 1992). This species is also responsible for most of the slug damage worldwide in economic terms. *D. reticulatum* occurs in a wide range of habitats, but is particularly pestiferous in arable crops. It frequently attacks winter wheat, winter barley, oilseed rape, maize, sugar beet, soybean, potato and freshly sown leys. Besides, it attacks a range of horticultural crops, such as strawberry, Brussels sprouts and other Brassicaceae, green asparagus and lettuce.

In recent years, the slug species *Arion lusitanicus* Mabille (Arionidae) has gained importance as a serious pest throughout Central Europe (Fechter and Falkner, 1990; Turner et al., 1998). This species was endemic to the Iberian peninsula until the first half of the 20th century, but is now frequent in Central, Northern and Eastern Europe, where it was apparently spread by international trade activities (De Winter, 1989; von Proschwitz, 1997; Briner and Frank, 1998). This slug species is particularly pestiferous in home gardens and in horticulture, but it can also damage arable crops, where they border on unmanaged field margins, riverboards, fallows or meadows (Frank, 1996, 1998b; Friedli and Frank, 1998). *A. lusitanicus* reaches a length of up to 12 cm, and can devour crop plants completely within a few nights. In contrast to *D. reticulatum*, *A. lusitanicus* is active throughout spring and summer, even when moisture is relatively low. In any slug control measure intended for use in Europe, effectivity against *A. lusitanicus* is an important aspect.

At present, slugs are mainly controlled with bait pellets, which usually contain either metaldehyde or a carbamate as active ingredient (Garthwaite and Thomas, 1996). In organic agriculture, the use of slug pellets containing metaldehyde or carbamates is severely restricted or not allowed. In 1994, the nematode *Phasmarhabditis hermaphrodita* has been commercialized as a biocontrol agent against slugs (Glen et al., 1996). The nematode is applied onto the soil in large numbers (10^5–$10^6/m^2$), where it seeks out slugs and infects them. Infection rapidly leads to feeding inhibition, and later kills the slugs. In laboratory trials, *P. hermaphrodita* infected the slugs *D. reticulatum*, *D. caruanae*, *Arion ater*, *A. intermedius*, *A. distinctus*, *A. silvaticus*, *Tandonia sowerbyi* and *T. budapestensis* and the snail *Helix aspersa* (Wilson et al., 1993; Glen et al., 1996). However, these studies have also shown that not all slug species were killed equally fast, and it remains unclear if other slug species not tested so far may be less or not at all susceptible to this biocontrol agent. Additionally, there are some indications that in large Arionid species, susceptibility to *P. hermaphrodita* decreases with body size (unpublished data, see Glen et al., 1996).

In several field trials conducted in England, *P. hermaphrodita* has successfully reduced slug damage to winter wheat and chinese cabbage (Wilson et al., 1993, 1994a, 1994c, 1996; Hass et al., 1999). In some experiments, however,

nematode application did not reduce slug damage (see Wilson et al., 1995a, 1996). In Switzerland, nematode application was successful in some experiments, but not in all (Speiser and Andermatt, 1996). We hypothesize that the nematode treatment might be more successful in controlling *D. reticulatum* (at all ages) and freshly hatched *A. lusitanicus* than larger or adult *A. lusitanicus*. To test this hypothesis, we performed a series of laboratory experiments, in which we determined the susceptibility of differently sized *D. reticulatum* and *A. lusitanicus* to *P. hermaphrodita*.

Materials and methods

Laboratory trials. All trials were conducted with individuals of *D. reticulatum* and *A. lusitanicus* hand-collected a few days before the trials from fields near Frick, Switzerland. To test the size dependence of slug susceptibility to nematodes, six experimental series were carried out at different times of the year (May, June, August and November 1998, March and April 1999). Four size-classes of *D. reticulatum* and seven size-classes of *A. lusitanicus* were tested. In the last series of trials, two different size classes of *A. lusitanicus* were tested separately (see Table 1). Each of these 13 batches of slugs was subjected to five different nematode doses and an untreated control. Each treatment was replicated three times, with each replicate comprising five slugs. Because *D. reticulatum* was rare in spring, there were only two replicates with two slugs in trial 5, and three replicates with two slugs in trial 6.

Table 1. Sampling months and fresh weight (mean & standard error) in mg of slugs (n = 90; [a]n = 24; [b]n = 36) used in the six trials. *D. reticulatum* from trials 2 and 3 and those from trials 5 and 6 were pooled for analysis because they had similar weights

Trial no.	Start of trial	*D. reticulatum*	*A. lusitanicus*
1	May 1998	31 ± 3	1058 ± 25
2	June 1998	95 ± 7	2594 ± 138
3	Aug 1998	89 ± 5	5136 ± 222
4	Nov 1998	801 ± 22	27 ± 1
5	March 1999	589 ± 43[a]	77 ± 2
6	April 1999	565 ± 26[b]	149 ± 5
6	April 1999	—	487 ± 11

Trials were conducted in plastic boxes (8 cm × 8 cm × 6 cm) filled approximately 2 cm high with 80 g of moistened heavy clay soil from our field site in Frick. Dry, fine-grained soil was moistened with 12 ml of tap water per 80 g of soil. The water was either pure, or contained 2,000, 5,000, 12,600, 32,000 or 80,000 *P. hermaphrodita* per box (commercial product: NemaSlug®; MicroBio Ltd, UK). To make sure that slugs remained in contact with the soil and did not crawl up the box walls, these had been painted with Fluon® (Symondson, 1993). Groups of five slugs of the same size class and species were weighed and placed into the boxes. Boxes were kept at 18 °C with 16 h light and 8 h darkness. Because *A. lusitanicus* is known to be cannibalistic, attacking especially unhealthy individuals, this species was kept individually (only the freshly hatched *A. lusitanicus* in trial 4 were kept in groups of five). The lowest dose tested in the laboratory is comparable to the dose recommended for field application.

Each series of trials lasted for six weeks. During the first three weeks, feeding and survival of slugs were recorded twice a week. Later, assessments were made once a week. To measure feeding activity, Chinese cabbage leaf discs of 4 cm diameter were offered to the slugs as food. Depending on the body size of the batch tested, one to three leaf discs were offered. At the next assessment, the leaf discs were replaced and the area eaten by the slugs (in cm^2) was estimated by eye. Slugs were scored healthy when they were alive and when their mantle was not swollen (Wilson et al., 1993). Dead slugs were removed without replacement.

Data analysis. Statistical analyses were made with the programme SYSTAT 5 (SYSTAT Inc., 1990–1992). Slug fresh weights and nematode doses were log-transformed prior to statistical analysis, to obtain a normal distribution of the data. The amounts of Chinese cabbage eaten during each period were integrated over time and divided by the duration of the experiment (Campbell and Madden, 1990; Speiser, 1997). The number of healthy slugs at each assessment was integrated in the same way; this integral is referred to as 'survival' throughout this paper. Because feeding activity and background mortality of slugs naturally vary over the season, feeding activity and survival of the nematode-treated slugs were expressed as a percentage relative to the performance of the untreated slugs of the same batch.

To test whether different nematode treatments in the trials comprised slugs of comparable weight, slug weights were compared with ANCOVA (Analysis of co-variance), treating slug batches as categories, and nematode dose as covariate. The overall impact of the nematode treatment and of slug weight on slug feeding and survival was determined for each slug species separately with multiple regression analysis. In addition, the impact of nematode dose

on slug feeding and survival was determined separately for each size class of each slug species with linear regression analysis. The data for *D. reticulatum* from trials 2 and 3 and from trials 5 and 6 were pooled because these slugs had similar weights.

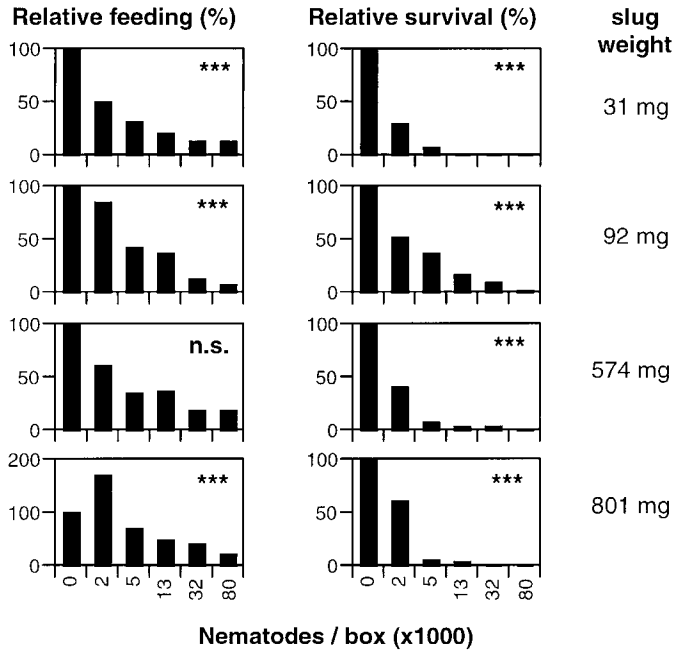

Figure 1. Dose related effect of *Phasmarhabditis hermaphrodita* on feeding and survival (relative to untreated controls) of *D. reticulatum* of different weight classes. Bars represent means from three replicates, asterisks indicate significance of increase or decrease (regression analysis: $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$; n.s. = not significant).

Results

The slugs of different batches had different weights, but within one batch, the slugs subjected to different nematode treatments were of similar weight (ANCOVA for slug weight; effect of slug batch: $p < 0.001$, effect of nematode dose: $p > 0.8$). Mean slug weights are given in Table 1. For both slug species, background mortality (=mortality in the untreated controls) was higher in the batches of presumable adults (*D. reticulatum* >250 mg and *A. lusitanicus* >3 g) than in presumable juveniles (data not shown).

The size-specific response to the nematode treatment was different for the two slug species. In *D. reticulatum*, increasing nematode doses

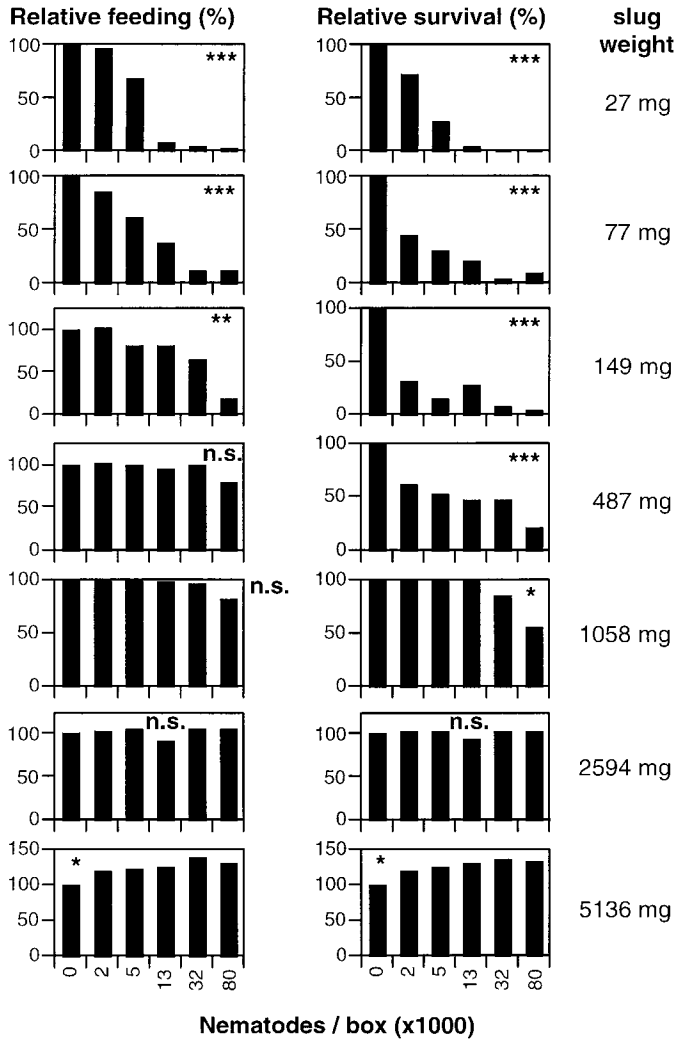

Figure 2. Dose related effect of *Phasmarhabditis hermaphrodita* on feeding and survival (relative to untreated controls) of *A. lusitanicus* of different weight classes. Bars represent means from three replicates, asteriks indicate significance of increase or decrease (regression analysis: $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$; n.s. = not significant).

reduced feeding and survival in all but one size classes (Figure 1). In *A. lusitanicus*, the response to nematodes was strongly size-dependent (Figure 2). Increasing nematode doses significantly reduced feeding and survival of small specimens (up to ca 150 mg). In intermediately sized animals (ca 500 mg to 1,000 mg), only survival, but not feeding was affected by nematode dose and in large specimens (above 1,000 mg), neither feeding

nor survival were affected. For the effects of nematode dose and slug weight on feeding and survival of each slug species, levels of significance are summarized in Table 2.

Table 2. Effects of nematode dose and slug weight on feeding and survival of two slug species in the laboratory trials (determined with multiple regression analysis)

Measurement	Factor	*D. reticulatum*	*A. lusitanicus*
Feeding	nematode dose	$p < 0.001$	$p < 0.001$
	slug weight	$p < 0.05$	$p < 0.001$
Survival	nematode dose	$p < 0.001$	$p < 0.001$
	slug weight	$p > 0.9$	$p < 0.001$

Discussion

In our trials, *D. reticulatum* was highly susceptible to the nematodes. This is in agreement with previous studies and shows that the laboratory conditions were adequate for this nematode species (Wilson et al., 1993, 1994b, 1995b; Glen et al., 2000). The nematodes were effective against this slug species regardless of slug size. By contrast, *A. lusitanicus* was only attacked and killed by nematodes in its early juvenile stages. It is assumed that *P. hermaphrodita* infects *D. reticulatum* dorsally, through a small opening at the posterior end of the mantle (Wilson et al., 1993). We assume that this opening becomes more difficult for nematodes to reach, the larger a slug gets. In our trials, some specimens of nematode treated *A. lusitanicus* (particularly small-sized animals) exhibited the same symptoms after nematode infection as those reported for *D. reticulatum* by Wilson et al. (1993): a swollen mantle and/or a small lesion at the posterior end of the mantle. We therefore assume that *A. lusitanicus* is infected through the same dorsal opening as *D. reticulatum*. Additionally, there is evidence that small *A. lusitanicus* rest within soil crevices, while larger specimens usually stay on the soil surface (Frank, 1998a: average weight of specimens on the soil surface: 970–4490 mg; average weight of specimens within the soil: 60 mg). Thus, for nematodes, which live inside the soil, it could be difficult to infect large *A. lusitanicus* living on the soil surface. It has been shown in laboratory experiments that some *Arion* spp. are killed by *P. hermaphrodita* at a similar rate as *D. reticulatum*. However, *A. ater*, which grows to a similar size as *A. lusitanicus*,

was killed more slowly than the other *Arion* spp. (Wilson et al., 1993). Size-specific susceptibility to *P. hermaphrodita* (with small specimens being most susceptible) has been reported for *A. ater* and for the snail Helix aspersa (unpublished data, see Glen et al., 1996). In a series of laboratory and field trials, *P. hermaphrodita* had contradictory effects on *A. lusitanicus*, but in the majority of cases, neither feeding nor survival were affected (Koch et al., 2000). Unfortunately, the weight of the slugs tested is not reported.

It could be argued that the smallest size class of *A. lusitanicus* was less infected than the other size classes, because the nematodes in one box were shared by five slugs instead of one. However, the results for the second smallest size class were very similar as for the smallest, and these slugs were kept individually. 2,000 to 80,000 nematodes were applied to each box, and we assume that only a very small proportion of these entered the slugs. Thus, the opportunity for competition for nematodes by slugs kept in groups seems very limited.

According to our findings in the laboratory, the most promising way to control *A. lusitanicus* with *P. hermaphrodita* seemed to be an application of nematodes when *A. lusitanicus* is very small in size. In Switzerland the majority of the population of *A. lusitanicus* lay eggs in autumn. Some of the eggs hatch in autumn, the rest hatches in spring. Because the slugs grow little through winter, both cohorts are small in early spring (length ca 1 cm). Thus, early spring seems to be the optimal time for controlling *A. lusitanicus* with nematodes. However, a certain proportion of the population has a different life cycle and is already fairly large in spring. Also, *A. lusitanicus* migrates considerable distances (Grimm et al., 2000), and may therefore quickly reinvade treated areas. In March 1999, we performed a preliminary field test of early nematode application. *A. lusitanicus* was not effectively controlled (data not shown), probably for one or both of the reasons given above.

At the recommended rate of $3 \times 10^5/m^2$, nematode treatment is approximately 100 times more expensive than a single application of chemical molluscicides. The nematode treatment is effective against *D. reticulatum*, but this slug species does much of its damage in arable crops, where expensive molluscicides are economically not competitive. The most promising applications of *P. hermaphrodita* are in high value crops (e.g. Brussels sprouts) and generally in organic horticulture, where chemical molluscicides may not be used. By contrast, *A. lusitanicus* is difficult to control with the present nematode strain. However, there is scope for improvements, as strains of *P. hermaphrodita* have been isolated not only from *D. reticulatum*, but also from other slug hosts including *A. lusitanicus* (Iglesias and Speiser, unpublished). At present, slug control in organic agriculture is a strategy combining crop rotation, soil cultivation, choice of crop variety and various adjustments

in cultural practice. To what extent biocontrol with nematodes will become part of this strategy depends on its effectivity against the pest slugs of major importance, on the longevity of the molluscicidal effect and on the price of nematodes.

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