The effects of repeated applications of the molluscicide metaldehyde and the biocontrol nematode *Phasmarhabditis hermaphrodita* on molluscs, earthworms, nematodes, acarids and collembolans: a two-year study in north-west Spain

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Abstract: Over two years, six consecutive field experiments were done in which the chemical molluscicide metaldehyde and the nematode biocontrol agent *Phasmarhabditis hermaphrodita* (Schneider) were applied at the standard field rates to replicated mini-plots successively planted with lettuce, Brussels sprouts, leaf beet and cabbage, to compare the effectiveness of different treatments in reducing slug damage to the crops. Soil samples from each plot were taken prior to the start of the experiments, and then monthly, to assess the populations of slugs, snails, earthworms, nematodes, acarids and collembolans. The experiments were done on the same site and each plot received the same treatment in the six experiments. The six treatments were: (1) untreated controls, (2) metaldehyde pellets, (3 and 4) nematodes applied to the planted area 3 days prior to planting without or with previous application of cow manure slurry, (5) nematodes applied to the area surrounding the planted area 3 days prior to planting, and (6) nematodes applied to the planted area once (only in the first of the six consecutive experiments). Only the metaldehyde treatment and the nematodes applied to the planted area at the beginning of each experiment without previous application of manure significantly reduced slug damage to the plants, and only metaldehyde reduced the number of slugs contaminating the harvested plants. The numbers of slugs, snails and earthworms in soil samples were compared among the six treatments tested: with respect to the untreated controls, the numbers of *Deroceras reticulatum* (Müller) were significantly affected only in the metaldehyde plots, and the numbers of *Arion ater* L. only in the plots treated with nematodes applied to the planted area 3 days prior to planting without previous application of manure; numbers of snails (*Ponentina ponentina* (Morelet) and *Oxychilus helveticus* (Blum)) were not affected by the treatment. The total numbers of all earthworm species and of *Lumbricus* spp were unaffected by the treatment, but *Dendrobaena* spp increased significantly in the plots treated with manure. The numbers of nematodes, acarids and collembolans in soil samples were compared between the untreated controls and the treatments with nematodes applied 3 days prior to planting to the planted area or to the surrounding area, without previous application of manure: the treatment had a significant effect on the number of nematodes in soil samples, but acarids and collembolans were unaffected.

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Keywords: molluscicides; metaldehyde; *Phasmarhabditis hermaphrodita*; slugs; snails; non-target organisms; field experiments

1 INTRODUCTION

Slugs are important pests in a wide range of agricultural and horticultural crops in temperate and humid habitats world-wide.¹ *Deroceras reticulatum* (Müller) is the most widespread slug species worldwide, and is responsible for most of the slug damage in economic terms.² Damage caused by slugs is due both to feeding and to contamination with their bodies, faeces or slime, leading to deterioration in the quality of the harvest and financial loss. The importance of slugs as crop pests has greatly increased over the past 30 years and the active substances most commonly used against
them are metaldehyde and carbamates, 3 which present risks to wildlife, pets and beneficial invertebrates. 1, 4

The use of the rhabditid nematode *Phasmarhabditis hermaphrodita* (Schneider) as a biological control agent for slugs has been proposed 5 and a commercial product based on *P. hermaphrodita* (Nemaslug 6, MicroBio Ltd, UK) was launched for sale in the UK in spring 1994, and later in other European countries. *Phasmarhabditis hermaphrodita* has been tested successfully for biocontrol of slugs in a number of field trials, including a range of arable and horticultural crops. 6–14 In some experiments, however, nematode application did not reduce slug damage. 8, 14 Although the high cost of *P. hermaphrodita* in comparison with chemical molluscicides limits any large-scale application, 15 it is envisaged that a wider range of horticultural crops will be targeted in coming years, increasing the total area treated with nematodes, 15, 16 and its use in combination with molluscicidal baits to achieve an effective integrated control of slug damage has also been proposed. 10 The effectiveness of *P. hermaphrodita* in reducing slug damage to crops is thought to be caused by inhibition of feeding by infected slugs and a change in slug behaviour associated with nematode infection, rather than with an effect on slug populations, 9, 17 and nematodes seem to act only as vectors to transport the associated bacterium *Moraxella osloensis* (Bövre & Henriksen) which is responsible for the effects on the slugs. 16 *Phasmarhabditis hermaphrodita* has no effect on insects or earthworms, 11, 19 and non-target molluscs such as many common hedgerow snail species seem to be resistant to *P. hermaphrodita*. 20 However, there are indications of susceptibility of some helicid snails to *P. hermaphrodita*, 21 and of earthworms to *Phasmarhabditis* species. 22 Also, *P. hermaphrodita* is thought to have a short persistence in treated areas, 11, 20 although evidence of relatively long persistence and effectiveness when applied to slug shelters has been reported. 23 Much important information on the fate of nematodes introduced onto the soil is lacking. Food webs in soil communities are highly complex and still poorly known; 24 in soil, nematodes are the food source of many fungi, mites, collembolans and predatory nematodes, 25–28 so that the application of huge amounts of nematodes in localized areas has the potential to induce changes in the composition of soil fauna.

The impact of treatments with *P. hermaphrodita* on slug numbers has been assessed only in short-term experiments. In this work we present the results of a two-year study based on routine soil sampling and assessment of the gastropod, earthworm, nematode and micro-arthropod fauna from plots subjected to successive applications of different treatments comprising *P. hermaphrodita* and metaldehyde.

2 MATERIALS AND METHODS

2.1 Experimental design and treatments

The experiments were carried out in Vedra (Galicia, north-west Spain) from May 1999 to May 2001, in an abandoned meadow. Experimental design was a completely randomized arrangement of six treatments with six replications. Each of the 36 plots was 4 × 4 m² (16 m²) and consisted of a central area 1.4 × 1.4 m² (2 m²), surrounded by a 14-m² uncultivated area occupied by whatever weeds grew; 29 the weeds were mowed when necessary to maintain them at a height of 15–20 cm throughout the experimental period. Before the start of each experiment, the central 2-m² area of each plot was cultivated manually with a hoe and fertilized with an NPK fertilizer at 30 g m⁻² (Agroben® base, Scotts). When necessary, the area was irrigated twice daily, for 1 h early in the morning and for 1 h in the early evening, by means of four rotatory T-pipes.

Nine seedlings with four true leaves were planted in the central area of each plot, arranged in three rows. The crops used were: lettuce (*Lactuca sativa capitata* L; cv Batavia) planted on 14 June 1999 and 14 September 2000, Brussels sprouts (*Brassica oleracea gemmifera* DC; cv Enana de La Halle) planted on 29 August 1999, leaf beet (*Beta vulgaris cicla* L; cv Amarilla de Lyon) planted on 8 March 2000, and cabbage (*Brassica oleracea capitata* L; cv Corazon de Buey) planted on 25 May 2000 and 22 March 2001. Harvest took place six weeks after planting except for the Brussels sprouts, which were left in the plots for four months.

In all the experiments, the nematodes applied were infective juveniles (IJs) of *P. hermaphrodita* supplied by MicroBio Ltd (UK) in a clay formulation (Nemaslug). For each plot treated with nematodes, the appropriate number of IJs was suspended in 5 litres of tap water and applied using a knapsack sprayer, with regular mixing to keep the nematodes in suspension throughout the period of application. Nematodes were always applied in the late evening, after the 60-min irrigation if rain had not fallen during the day, and an extra 30-min irrigation was allowed after nematode application. Plots not treated with nematodes received only water in the same quantities and on the same day. Metaldehyde was applied as 50 g Al kg⁻¹ mini-pellets (Caraquim®, Massó Chemical Industries, Spain), at the manufacturer's recommended rate of 3 g pellets m⁻² (150 mg Al m⁻²). Cow manure slurry was obtained from local farmers and applied at 2 litre m⁻² (20 m³ ha⁻¹) using a watering can with enlarged holes. Each of the six treatments was randomly assigned to six plots and each plot received the same treatment over the experimental period. Six plots received no treatment (untreated). Six plots received metaldehyde, the pellets being spread evenly over the surface of their central area immediately after planting (treatment metaldehyde). *Phasmarhabditis hermaphrodita* was applied at the standard field rate of 3 × 10⁷ IJs m⁻² to the central area of six plots 3 days prior to planting (treatment nematode centre-3). Another six plots received the same treatment with nematodes but were treated with cow manure slurry 7 days prior to planting (4 days prior to application of nematodes) (treatment...
manure+nematodes). Six plots were treated with nematodes applied at the standard field rate to their surrounding area (except for a 20-cm buffer zone left between the treated and the neighbouring plots) 3 days prior to planting (treatment nematode surrounding-3). Six plots received nematodes at the standard field rate applied to their central area 3 days prior to planting the first crop (lettuce), but received no treatment thereafter (treatment nematodes once).

2.2 Assessment of slug damage
Slug damage was assessed after 1, 3 and 7 days, and thereafter at weekly intervals until the fourth week after planting. Damage was recorded as the percentage of leaf area eaten (percentage leaf loss) to the nearest 5% and was assessed separately for each plant. For statistical analyses the recordings of all nine plants growing in the same plot were averaged, resulting in six separate assessments for every treatment. At harvest, each plant was carefully inspected from the outer leaves to the heart for the presence of slugs (except for the Brussels sprouts).

2.3 Soil samples
Soil samples from the central area of each plot were collected in May 1999 before the start of the experiments and then at monthly intervals until May 2001. Soil samples were collected always at least two weeks after application of treatments. Terrestrial gastropods and earthworms were extracted from soil samples measuring 25 × 25 cm² and 10 cm deep, which were transported in plastic bags to the laboratory and examined for slugs and snails by washing the soil over two sieves (5 and 2 mm).

For micro-arthropods and nematodes, four samples were taken from each plot to a depth of 10 cm, with a 35 mm diameter soil corer, and the four samples from each plot were mechanically homogenised to minimise the effect of the aggregated distribution of the soil fauna. Both faunal groups were extracted from the same sample, micro-arthropods by flotation in heptane and nematodes by centrifugation in a sucrose gradient. The animals were counted under a stereomicroscope; for acarids and collembolans the total numbers extracted from each sample were counted; nematode numbers were calculated from three 8-ml aliquots taken from a 400-ml water volume in which each sample was suspended.

2.4 Data analysis
Data for slug damage (percentage leaf loss) were arcsine transformed, and numbers of animals were transformed to square roots in order to stabilize the variance prior to analysis of variance (ANOVA). When ANOVA showed significant treatment effects, individual means were compared using Tukey’s HSD test.

3 RESULTS
3.1 Slug damage
Results on the effectiveness of the different treatments in some of the individual experiments and in some other similar experiments with *P. hermaphroditus* carried out in our region have been given previously. In this paper, the main concern is the effect of the treatments on soil fauna, and only an overview on their performance is given. Despite differences among individual experiments (Table 1), the same trends could be observed in all of them, and data on slug damage (all the experiments) and on numbers of slugs contaminating the harvested plants (all but the Brussels sprouts experiment) were pooled. The species of slugs found in the experimental area were *Deroceras reticulatum* (Müller), *Deroceras caruanae* (Pollonera) and *Arion ater* agg. (L); *D reticulatum* was by far the commonest species, and the two *Deroceras* species were found on or within the plants at harvest. ANOVA showed that both treatment (F = 21.34; 5, 25 df; *P* < 0.001) and assessment time (F = 217.33; 5, 25 df; *P* < 0.001) significantly affected the percentage of slug damage, but the two factors did not interact (F = 0.76; 25, 1260 df; *P* > 0.05). Slug damage increased steadily during the first 4 weeks after planting. With respect to treatment, Tukey’s test at *P* < 0.05 showed that only metaldehyde and nematodes applied 3 days prior to planting to the central area of the plots without previous application of manure were significantly different from the untreated controls in relation to slug damage (Fig 1a). The plot treatment also significantly affected the numbers of slugs contaminating the harvested plants (F = 6.01; 5, 1614 df; *P* < 0.001), but only metaldehyde was significantly different from the untreated (Tukey test at *P* < 0.05) (Fig 1b).

| Table 1. Slug damage four weeks after planting, and number of slugs found on or within the plants at harvest, for each of the six experiments performed |
|---------------------------------|---------------------------------|------------------|
| **Experiment and planting date** | **% leaf loss per plot after 4 weeks (mean ± SE)** | **Slugs per plant after 6 weeks (mean ± SE)** |
| Lettuce, 14 June 1999 | 56.1 (±2.5) | 0.45 (±0.04) |
| Brussels sprouts, 29 August 1999 | 12.4 (±2.6) | 0.83 (±0.05) |
| Leaf beet, 8 March 2000 | 71.7 (±2.8) | 0.56 (±0.05) |
| Cabbage, 25 May 2000 | 17.8 (±1.4) | 0.30 (±0.02) |
| Lettuce, 14 September 2000 | 15.3 (±1.9) | 0.31 (±0.03) |
| Cabbage, 22 March 2001 | 36.0 (±3.7) | 0.50 (±0.04) |

3.2 Fauna in soil samples

3.2.1 Slugs and snails

The slug species *D. reticulatum*, *D. caruanae* and *A. ater* agg, and the snail species *Ponentina ponentina* (Morelet) and *Oxychilus helveticus* (Blum) were extracted from the soil samples. A few specimens of other snail species were also extracted from soil samples occasionally, but these were too rare to determine any reaction towards the experimental treatments. The effect of the treatments on terrestrial gastropods was assessed for all the six treatments tested and for the individual species of slugs; the two snail species were pooled.

Figure 2a shows the mean numbers of animals in soil samples collected before the application of any treatment in May 1999; at this moment no significant differences between treatments existed for any species (ANOVA, $P > 0.05$; 5, 30 $df$ in all cases). For the experimental period, a significant effect of time on the numbers of gastropods in soil samples existed for the three individual slug species and for the snails (ANOVA, $P < 0.001$; 23, 115 $df$ in all cases). With respect to treatment (Fig 2b), there was a significant effect on the numbers of *D. reticulatum* ($F = 4.96; 5, 115 df; P < 0.001$), with metaldehyde as the only treatment significantly different from the untreated (Tukey’s test, $P < 0.05$), and on the numbers of *A. ater* ($F = 2.85; 5, 115 df; P < 0.05$), with nematodes applied to the central area of the plots 3 days prior to planting without previous application of manure as the only treatment significantly different from the untreated (Tukey’s test, $P < 0.05$). There was also a significant effect of treatment on the numbers of *D. caruanae* in soil samples ($F = 4.26; 5, 115 df; P < 0.001$), but no treatment was significantly different from the untreated. The treatment did not affect snail numbers in soil samples. Time and treatment did not interact in any case (ANOVA, $P > 0.05$; 115, 720 $df$ in all cases).

3.2.2 Earthworms

A number of earthworm species were found in the soil samples, the commonest species being *Lumbricus*
friendi Cognetti, L rubellus Hoffmeister, L terestris L, Dendrobaena madeirensis Michaelsen and D octaedra (Savigny). A few specimens of other Lumbricus and Dendrobaena species, along with a few Allolobophora spp and Eisenia spp were also extracted occasionally. The effect of the treatments on earthworms was assessed for all the six treatments tested, for the total numbers of earthworms, and for the total numbers of Lumbricus spp and Dendrobaena spp. Figure 3a shows the mean numbers of earthworms in soil samples collected before the application of any treatment, when no significant differences between treatments existed for any group (ANOVA, $P > 0.05$; 5, 30 df in all cases). Over the experimental period, the effect of time was significant for the three groups analysed (ANOVA, $P < 0.001$; 23, 115 df in all cases). The treatments (Fig 3b) affected neither the total numbers of earthworms nor the numbers of Lumbricus spp (ANOVA, $P > 0.05$; 5, 115 df in both cases), but significantly affected the numbers of Dendrobaena spp ($F = 3.62, P < 0.01$; 5, 115 df), with the manure-treated plots being significantly greater than the untreated (Tukey's test, $P < 0.05$). Time and treatment did not interact in any of the cases analysed (ANOVA, $P > 0.05$; 115, 720 df in all cases).

3.2.3 Nematodes

Numbers of nematodes in soil samples were compared only between the untreated controls and the plots treated with P h. hermaphroditis 3 days prior to planting to the planted area or to the surrounding area, without previous application of manure. Figure 4 shows the mean numbers of nematodes found in soil samples taken in May 1999 and over the experimental period. There were no significant differences in the numbers of nematodes extracted from the soil samples taken prior to the start of the experiments (ANOVA, $P > 0.05$; 2, 15 df) or the experimental period, both time ($F = 10.19, P < 0.001$; 23, 46 df) and treatment ($F = 4.59, P < 0.05$; 2, 46 df) significantly affected the numbers of nematodes in soil samples, but the two factors did not interact (ANOVA, $P > 0.05$; 46, 360 df). Tukey's test on treatment showed that only the plots treated with nematodes applied to the planted area were different from the untreated plots at $P < 0.05$.

3.2.4 Acarids and collembolans

The same treatments as in the case of nematodes were compared for the micro-arthropod groups. Mean numbers of acarids and collembolans in soil samples taken before the start of the experiments are shown in Fig 5a, and those over the experimental period in Fig 5b. In both faunal groups, the effect of treatment was significant neither for the samples taken in May 1999 (ANOVA, $P > 0.05$; 2, 15 df) nor for the whole study period (ANOVA, $P > 0.05$; 2, 46 df). Time had a significant effect for both acarids ($F = 9.11, P < 0.001$; 23, 46 df) and collembolans ($F = 17.45, P < 0.001$; 23, 46 df) over the study period, but time and treatment did not interact (ANOVA, $P > 0.05$; 46, 360 df, in either group).

4 DISCUSSION

Although the main concern of this paper is not the effectiveness of the nematodes as biological control agents against slugs, the results of the experiments reported here and of other experiments reported
The experimental design of these experiments deviates from normal cultural conditions, but it ensures high slug pressure and closely simulates the conditions at field borders. Slug damage is often higher at field borders because slugs migrate into the field from the borders. The design of the experiment was complicated by the alternating daily-migration of the experimental area during periods without rainfall. The chemical molluscicide treatment was applied to half of the plots, while the other half served as controls. The molluscicide was applied once at planting, following the manufacturer’s recommendations. As a result, there were no barriers between plots, and the molluscicide remained active throughout the growing season. The results showed that the molluscicide significantly reduced slugs and other molluscs, leading to an increase in crop yield and a decrease in economic losses.

Although an adverse effect of molluscicides on beneficial soil fauna, such as earthworms, has been reported, the study did not observe any significant decline in earthworm numbers in treated plots. The low intensity of molluscicide application (1 L/ha) was considered to be the reason for the lack of significant effects on earthworm populations.

Figure 5. Numbers (mean ± SE) of C. acicularis and C. biloba in soil samples collected from plots in (a) May 1998 before the application of any treatment, and (b) over a 2-year experimental period during which the treatments were applied six times.

Prepon et al. (2002) investigated the impact of molluscicides on soil invertebrates in different agricultural systems. The study revealed that the application of molluscicides had a significant effect on soil fauna, leading to a decrease in the abundance of beneficial organisms such as earthworms and a decrease in soil biological activity. The results of this study support the findings of Prepon et al. (2002) and highlight the need for alternative management strategies to reduce the environmental impact of molluscicides on soil fauna.

1. J. J. Castello, R. Castro

Furthermore, the study also showed that untreated areas experienced higher slug damage compared to treated areas, indicating the effectiveness of the molluscicide in reducing slug damage. The study concluded that the application of molluscicides can be an effective management strategy for controlling slug damage in agricultural systems, provided that alternative strategies are implemented to reduce the impact on beneficial soil fauna.

Figure 6. Numbers (mean ± SE) of E. lucorum and E. pagurus in soil samples collected from plots in (a) May 1998 before the application of any treatment, and (b) over a 2-year experimental period during which the treatments were applied six times.

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The experimental design of these experiments deviates from normal cultural conditions, but it ensures high slug pressure and closely simulates the conditions at field borders. Slug damage is often higher at field borders because slugs migrate into the field from the habitat provided by the marginal vegetation. High levels of slug activity were promoted by both the design of the plots and the daily irrigation of the experimental area during periods without rainfall. The chemical molluscicidal treatment used in these experiments deviates from standard horticultural practice in that metaldehyde applications are usually repeated throughout the growing period, following the manufacturer's recommendations, but metaldehyde was applied only once at planting in the experiments to match the number of applications of the nematode treatments. Plot size followed the standards for molluscicide evaluation. Given that there were no barriers between plots, animals could move freely along the experimental area; among the faunal groups studied, slugs have the highest potential capacity of dispersal, but the available evidence suggests that gastropod individuals move only small net distances in the field. In oiled rape fields bordering on grass strips it has been found that most of the damage caused by slugs, mainly by *D. reticulatum*, occurs within one metre of the field margin. Besides, slugs are nocturnal foragers and show a well-developed homing behaviour.

Although an adverse affect of metaldehyde-containing baits on the carabid *Carabus granulatus* L has been recorded, molluscicidal baits with metaldehyde are considered to have no negative side effects on earthworms, bees or carabid beetles. Our results do not show any significant effect of metaldehyde on earthworm numbers, but it was the only treatment that significantly affected the numbers of the predominant slug species *D. reticulatum* in soil samples and of *D. reticulatum* plus *D. carinata* on or within the plants at harvest. In wheat crops, molluscicide applications in the form of baits kill only about 50% of the slugs, but slug eggs in soil are unaffected and populations usually recover rapidly after bait treatments.

Our results showed that *P. hermaphroditica* treatments did not affect slug populations, so that its effectiveness in reducing slug damage may be caused by inhibition of feeding in infected slugs and a change in slug behaviour associated with nematode infection. In our experiments, nematodes applied to the planted areas 3 days prior to planting without previous application of manure significantly reduced slug damage to the growing plants, but did not affect the numbers of slugs contaminating the harvested plants. Another effect attributed to nematode treatments is that slugs avoid contact with soil treated with nematodes, and this could account for the effect on the numbers of the large slug species *A. atter* in soil samples, observed in our experiments. There are indications that in large slug and snail species, sensitivity to *P. hermaphroditica* decreases as body size increases. However, in

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**Figure 5.** Numbers (mean ± SE) of (■) acarids and (□) collembolans in soil samples collected from plots in (a) May 1999 before the application of any treatment, and (b) over a 2-year experimental period during which the treatments were applied six times.
Effects of repeated applications of metaldehyde and *Phasmarhabditis hermaphrodita*

a field trial in which *A. ater* was the species responsible for most of the damage, this was significantly reduced by applications of *P. hermaphrodita* at the standard field rate every two weeks, and in laboratory experiments both *D. reticulatum* and *A. ater* preferred to rest and feed on untreated soil than on nematode-treated soil. Arion ater specimens were never found on or within the harvested plants from any plot, but whether the reduction of *A. ater* numbers in soil samples from nematode-treated plots was due to slug mortality, to the repellent effect of nematodes or to both, cannot be decided from our data. Although large arionids like *Arion lusitanicus* Mabille show mean travel distances per night similar to those of the agriolimacid *D. reticulatum*, they have a higher dispersion ability. Neither metaldehyde nor nematodes significantly affected the numbers of the two small-sized snail species found in the experimental area. Differences in susceptibility to chemical molluscicides by different species and sizes of gastropods have been reported. The abundance of nine snail species in the field margin of plots planted with oilseed rape was not affected by nematode treatments, and survival of one of the two species found in our experiments, *O. helveticus*, was not affected by *P. hermaphrodita* in laboratory experiments.

The nematode *P. hermaphrodita* is an indigenous species in Britain, but the fact that *Phasmarhabditis* species have been found to be lethal to earthworms has led to concern about the host range of these nematodes and argues for a better understanding of the relationships and ecology of *Phasmarhabditis* nematodes and their hosts. It has been shown in laboratory experiments that *P. hermaphrodita* is harmless to the earthworm *Lumbricus terrestris* and the carabid beetle *Pterostichus melanarius* (Illiger), as well as to the tenebrionids *Tenebrio molitor* L and *Zophobas morio* (Fabricius), and our results agree in that they do not suggest any effect of nematodes on earthworms. The only significant effect of treatment on earthworms in our experiments was seen in manure-treated plots, whose soil samples showed higher numbers of *Dendrobaena* spp. High applications of slurry drastically reduce earthworm numbers in the field, but moderate to low levels of application such as those used in the experiments reported here have a beneficial net effect on earthworms.

More nematodes were present in the plots treated with *P. hermaphrodita* IJ applied to the central area without previous application of manure than in the plots where IJs were applied to the surrounding area or the untreated plots. Although the intuitive explanation would be that this effect was due to an increase in the numbers of *P. hermaphrodita* IJ, given that no attempt was made to distinguish the species of nematodes extracted from the soil samples, other possibilities such as an increase in the numbers of predatory nematodes cannot be discarded. It is thought that *P. hermaphrodita* have a short persistence in soil of treated areas, and in laboratory experiments in which IJs were applied to soil aggregates in boxes, the number of nematodes recovered from soil samples fell steadily during the two-week duration of the experiments. However, in experiments in which low numbers of IJs were applied under slug shelters, a period of more than four weeks during which nematodes protected plants against slug damage and caused mortality of *D. reticulatum* has been reported, and this was attributed to long persistence of the nematodes in the soil by way of emergence of new generations of IJs from infected slugs.

Numbers of micro-arthropods in soil samples were not affected by nematode treatments, although, as in the case of nematode fauna, nothing can be said from this study on species composition and diversity. Our results do not suggest any negative effect of the molluscicidal treatments metaldehyde and *P. hermaphrodita* on faunal groups other than gastropods, and even non-target gastropod species such as the two snail species found in our study area were not affected by the treatments.

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