Field Test Using the Nematode *Phasmarhabditis hermaphrodita* for Biocontrol of Slugs in Spain

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An experiment was carried out between May and July 1999 in Galicia (North-West Spain) to test the capacity of the nematode *Phasmarhabditis hermaphrodita* to protect field grown lettuces from slug damage in our field conditions. The experiment compared a single dose of nematodes (3 x 10⁷ ha⁻¹) with mint-pellets containing 5% metaldehyde, applied at the recommended field rate (3 g pellets m⁻²), and untreated plots. Slug damage for each lettuce head was estimated on six dates during the first 4 weeks after planting. At harvest, each lettuce head was weighed, scored as marketable or not by weight and external aspect, and inspected for slugs. Metaldehyde significantly reduced slug damage to lettuce plants from the first day after planting to the third week. Nematodes significantly reduced slug damage from the second to the third week. At harvest, 6 weeks after planting, the mean weight of the lettuce heads and the number of marketable heads in the nematode plots were as good as in the metaldehyde plots, and both treatments were significantly better than the untreated plots. The number of slugs within the harvested plants was significantly reduced only with the metaldehyde treatment.

Keywords: Phasmarhabditis hermaphrodita, slug, Deroceras Arion, nematodes, lettuce, damage

INTRODUCTION

The battle against pest gastropods relies mainly on chemical control, with metaldehyde and carbamates as the active substances most commonly used (Garthwaite & Thomas, 1996). This chemical control presents risks to wildlife, pets and beneficial invertebrates (South, 1992). The use of the rhhabitid nematode *Phasmarhabditis hermaphrodita* as a biological control agent for slugs has been proposed (Wilson et al., 1993) and a commercial product based on *P. hermaphrodita* (Nemaslug<sup>®</sup>, MicroBio Ltd, UK) was launched for sale in the home garden market in the UK in spring 1994. The nematode is able to infect and kill a variety of pest slugs and snails, provides rapid protection from slug damage through inhibition of slug feeding soon after infection, and has a repellent effect so that slugs avoid feeding or resting in areas of soil treated with nematodes (Glen et al., 1996). The nematode
P. hermaphrodita has been tested for biocontrol of slugs in a number of field trials in northern Europe, including a range of arable and horticultural crops. Most of them concluded that the nematode is an effective biocontrol agent for slugs, as good as, or better than chemical molluscicides (Glen et al., 1996). Glen & Wilson (1997) stressed that the current commercial strain of P. hermaphrodita is well adapted to the relatively cool conditions of northern Europe, but that it is necessary to look for other strains better adapted to warmer conditions in order to extend the use of the nematode further south into Europe. To the best of our knowledge, this is the first time that P. hermaphrodita has been tested in the field in southern Europe.

This article describes an experiment investigating the effects of P. hermaphrodita on slug damage to field grown lettuces. The nematode treatment was compared with the chemical molluscicide metaldehyde and with untreated plots. The trial was done with lettuces, a high-value horticultural crop which is grown both in the field and in polythene tunnels in Galicia and which is always treated with chemical molluscicides.

MATERIALS AND METHODS

The experiment was carried out in an area of grass meadow in Vedra (Galicia, North-West Spain) between May and July 1999. The experimental design followed the recommendations of Speiser (1997) and consisted of 18 plots (three treatments × six replicates) arranged randomly in a block. The experimental area was divided into 4 x 4 m plots, and the central area (1.4 x 1.4 m) of each plot was cleared manually with a hoe. Thus, each plot was 16 m² and consisted of a central 2 m² area surrounded by a 14 m² living grass sward. The plots were prepared between 17-21 May and henceforth the entire experimental area was irrigated twice daily, for 1 h early in the morning and for one hour in the evening, by means of four rotatory T-pipes. Six plots (metaldehyde plots) received the manufacturer’s recommended rate of 3 g m⁻² of mini-pellets (Caraquim®, Massó Chemical Industries, Spain) containing 5% metaldehyde, spread evenly over the surface of the central area of the plots immediately after lettuce planting. Six plots (nematode plots) received 3 x 10⁵ nematodes m⁻² applied to the central area of the plots on 11 June, three days prior to planting the lettuces. Six plots received no treatment (untreated plots). Lettuce seeds (Iceberg lettuce) were germinated and seedlings were grown in a polythene tunnel until they reached the four leaf stage before they were planted in the plots on 14 June. Nine lettuces, arranged in three rows, were planted in the central area of each plot.

The nematodes used were the commercial formulation of P. hermaphrodita known as Nemaslug® (MicroBio Ltd, UK), consisting of infective juveniles of the nematode formulated in a calcium montmorillonite clay. On the day of nematode application, the clay formulation was suspended in tap water and the number of nematodes in the resulting suspension was estimated. The appropriate number of nematodes was suspended in 5 l of tap water and was applied to the plots using a knapsack sprayer. Nematodes were applied after the evening irrigation and an extra 30 min irrigation was allowed after nematode application.

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 lettuce head. For statistical analyses the recordings of all nine lettuces growing in the same plot were averaged, because they reflect the activity of the same slug population (Speiser, 1997), resulting in six independent assessments for every treatment. At harvest, six weeks after planting, each lettuce head was weighed separately and scored as marketable or unmarketable according to its size and external aspect. Finally, each lettuce head was carefully inspected from the outer leaves to the heart for the presence of slugs. Daily air temperatures during the experiment were recorded in a nearby meteorological station.

Data for slug damage (percentage leaf loss) were transformed to angles prior to analysis
of variance. Data for number of marketable lettuce heads and for number of slugs were compared non-parametrically by the Mann-Whitney U-test.

RESULTS

Mean air temperature during the experiment was 19.8 ± 2.6°C (mean ± SD) (Figure 1). Three species of slugs were found in the plots by refuge-trapping and nocturnal observation: Derocerus reticulatum (Müller), Derocerus caruanae (Pollonera) and Arion ater agg. (L.). D. reticulatum was by far the commonest species.

Damage to the lettuce heads increased steadily on all treatments during the first 4 weeks after planting (Figure 2(a)). Plants in the untreated plots suffered more slug damage than lettuces in the metaldehyde or the nematode treated plots over the first four weeks (Figure 2(b)). When analysed date by date the following significant differences were found (Fisher’s LSD test: $P < 0.05$): 1; % leaf loss was significantly different between untreated plots and metaldehyde plots on all dates from day 1 to day 21. 2; % leaf loss was significantly different between untreated plots and nematode plots on day 14 and day 21. There were no significant differences between the metaldehyde and the nematode treatments.

At harvest, lettuces in the treatments metaldehyde and nematode had mean weights of

![Figure 1](image1.png)

**FIGURE 1.** Daily air temperatures recorded in a nearby meteorological station during the experiment. Daily maximum (-----), daily mean (---) and daily minimum (----).

![Figure 2](image2.png)

**FIGURE 2.** Mean percentage leaf loss of lettuce plants over the first four weeks after planting: (a) all treatments and (b) in different treatments. □ untreated, ■ metaldehyde, □ nematode.
93.65 and 88.16 g, respectively, which were both significantly different \((P < 0.05)\) from the mean weight of lettuces grown in the untreated plots (mean weight = 41.51 g) (Figure 3(a)).

The number of marketable heads according to weight and external aspect, number of slugs found within the lettuce heads and number of marketable heads without slugs are shown in Figure 3(b). The treatments metaldehyde and nematode yielded the highest number of marketable plants, with 16 and 15 lettuce heads, respectively, which were both significantly different (Mann-Whitney U-test: \(P < 0.05\)) from the yield of the untreated plots. With respect to the untreated plots, the number of slugs within the lettuce heads was significantly different only in the metaldehyde plots (Mann-Whitney U-test: \(P < 0.05\)), but the metaldehyde and nematode treatments differed significantly (Mann-Whitney U-test: \(P < 0.01\)) in the number of slugs within the plants. When the presence of slugs within the lettuce heads was taken into account in the score of marketable heads, metaldehyde differed significantly (Mann-Whitney U-test: \(P < 0.05\)) from the nematode treatment and from untreated plots.

During the experiment, moribund or dead slugs were seen only in the metaldehyde-treated plots. None of the slugs found within the harvested lettuce plants, which were all \(D.\ reticulatum\), showed symptoms of nematode infection. Also, nematode-infected slugs were never observed on the soil or beneath refuge-traps.

**DISCUSSION**

The experimental design of this experiment deviates from standard horticultural practice and particularly from normal cultural conditions within a lettuce field, but it ensures high slug pressure and closely simulates the conditions at field borders (Speiser, 1997). Slug damage in crops such as lettuce and oilseed rape is often higher at the field borders because slugs migrate into the field from more favourable habitats (Speiser, 1997; Frank, 1998). The metaldehyde treatment was applied only once in order to match the application rate of the nematode treatment, but in lettuce crops in Galicia the farmers usually repeat the application of chemical molluscicides before harvest. Also, the level of irrigation was higher than usual in order to favour slug activity and survival of the nematodes.

In this experiment, with both the application of metaldehyde mini-pellets at the time of planting and the application of nematodes three days prior to planting, slug damage to field grown lettuce was reduced to the same extent three weeks after planting. Previous field experiments comparing \(P.\ hermaphrodita\) with chemical treatments have obtained similar results. In Switzerland, Speiser and Andermatt (1996) found that the effect of the nematodes
was similar to metaldehyde or better in reducing the percentage leaf loss in lettuce, kale and Chinese cabbage during two weeks, and in reducing the number of slug damaged rape plants during a two week period. Glen et al. (1996) reported that nematodes were as effective as metaldehyde in protecting wheat seeds and strawberries from slug damage in the field. Treatment with *P. hermaphrodita* has been reported as better than methiocarb treatment in field experiments with winter wheat (Wilson et al., 1994a), Chinese cabbage (Wilson et al., 1994b) and strawberries (Glen et al., 1996). In The Netherlands, Ester and Geelen (1996) found treatment with *P. hermaphrodita* to be as effective as methiocarb treatment in a sugar beet crop.

In lettuce, the small size of the head is a consequence of early slug feeding, while holed outer leaves indicate late slug attacks (Speiser, 1997). In this experiment, differences in damage between untreated and metaldehyde were significant since the first examination (one day after planting) while significant differences in damage between untreated and nematode-treated plots were noticed two weeks after planting. Consequently, lettuces from the metaldehyde plots were bigger than those from the nematode plots, although the difference between mean weight of lettuces from metaldehyde plots and from nematodetreated plots was not significant.

When scored by weight and aspect, the number of marketable heads was significantly different from the untreated plots in both the metaldehyde and the nematode treatments. However, the number of slugs found within the lettuce plants was significantly reduced only in the metaldehyde-treated plots, and there existed a significant difference between the metaldehyde and the nematode treatment with respect to this criterion. Thus, when the presence of slugs within the lettuce heads was taken into account in the score of marketable heads (Anon, 1984), only metaldehyde significantly increased the yield of the crop. Wilson et al. (1995a) carried out an experiment in which the nematode treatment at the same rate as in our trial (3 x 10⁸ nematodes ha⁻¹) significantly reduced slug damage to the lettuce heads, but the number of slugs within lettuce plants in plots treated with 3 x 10⁸ and 3 x 10⁹ nematodes ha⁻¹ was higher than that in untreated plots. It is difficult to provide an explanation for the high number of slugs within the lettuce heads from the nematode treated plots. The most intuitive explanation is the repellent effect of nematodes, in which slugs avoid resting on soil treated with nematodes (Wilson et al., 1994a, 1995b, 1999), and then slugs climb up the lettuce plants trying to prevent contact with the parasite. Laboratory bioassays have demonstrated that slug feeding activity is strongly inhibited shortly after infection with *P. hermaphrodita* (Glen et al., 2000), and it has been suggested that the rapid activity of the nematode in protecting plants from slug damage is probably due to feeding inhibition after infection rather than death of slugs (Wilson et al., 1994b). In this experiment, the reduced slug damage and the good yield in the nematode plots indicate that slugs from these plots must have been infected and must have been suffered feeding inhibition, but no slug in the lettuce heads showed the characteristic physical symptoms caused by the parasite as described by Wilson et al. (1993).

The results of this experiment show that *P. hermaphrodita* (Nemaslug™) is capable of reducing slug damage to lettuce grown in our field conditions. However, comparing the results of this experiment with those of field experiments with *P. hermaphrodita* carried out in northern Europe, two facts seem to indicate that, in our field conditions, the course of the infection with *P. hermaphrodita* may be in some way altered or slowed down. First, previous field experiments have shown an early significant effect of the nematode in protecting plants from slug damage (Wilson et al., 1994b, 1995a, 1996), while in this experiment a significant reduction in slug damage was noticed in the nematode treated plots only 14 and 21 days after planting (17 and 24 days after treatment). Second, slugs with the characteristic physical symptom (swollen mantle) caused by the parasite were found after nematode application in the field by Wilson et al. (1994a), Speiser and Andrenatti (1996) and Ester and Geelen (1996), but they were never found in this experiment. Further experiments are needed to confirm the effectiveness of this biocontrol agent in our region.
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REFERENCES


