

## Artículo de Revisión

### Biological control of slugs with the rhabditid nematode *Phasmarhabditis hermaphrodita*

### Control biológico de babosas con el nemátodo *rhabditido* *Phasmarhabditis hermaphrodita*

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<https://doi.org/10.26807/remcb.v41i1.836>

Recibido 5-10-2019; Aceptado 10-05-2020

**RESUMEN.-** Esta revisión literaria se basa en el control biológico de babosas usando el nemátodo *Phasmarhabditis hermaphrodita*. Se describe la efectividad y modo de acción del nemátodo según el ambiente. Además de su método de aplicación y dosis, se compara con molusquicidas químicos y su asociación a la bacteria *Moraxella osloensis*.

*P. hermaphrodita* prefiere condiciones húmedas de suelo y actúa en babosas en estado juvenil de la especie *Deroceras*. Las aplicaciones parciales del nemátodo son menos costosas y deberían hacerse en lugares donde las babosas descansan. Existen otras especies de nemátodos controladores, no siendo efectivas para el control de babosas. Se requiere mayor investigación para probar en que momento actúa el nemátodo *P. hermaphrodita* pues no ha sido tan efectivo para el control de otras especies de babosas.

**PALABRAS CLAVES:** control biológico, nemátodo rhabditido *Phasmarhabditis hermaphrodita*, babosa *Deroceras reticulatum*, cultivos hortícolas, bacteria *Moraxella osloensis*.

**ABSTRACT.-** This literature review is based on the biological control of slugs using the nematode *Phasmarhabditis hermaphrodita*. It describes effectiveness and mode of action according to the environment. The method of application and doses compared to chemical molluscicides and the bacteria *Moraxella osloensis* is also described.

*P. hermaphrodita* seems to prefer soil moisture conditions and infect young slugs of the *Deroceras* species. Partial nematode applications are less expensive and should be done in slug resting areas. Other nematode species exist for slug control, but these are ineffective. More research is still needed to prove when the action stage of the nematode *P. hermaphrodita* is, because it has not been proven as effective for the control of many other slug species.

**KEYWORDS:** biological control, rhabditid nematode *Phasmarhabditis hermaphrodita*, slug *Deroceras reticulatum*, horticultural crops, bacterium *Moraxella osloensis*.

## INTRODUCTION

Slugs (Mollusca: Gastropoda) are highly problematic pests of floriculture, horticulture, and agriculture in many parts of the world, with *Deroceras reticulatum* generally being the most destructive species (Grewal et al. 2001). Horticultural crops commonly damaged by slugs

are ripening strawberries and tomatoes. The underground portions of flowers and ornamentals are bored through and the emerging foliage is shredded (South 1992). Damage in green asparagus is observed because of its long growth period in the same field: its spears are deformed by tiny feeding marks on the growing tips (Ester et al. 2003). In

several crops, the presence of slugs and their faeces results in unmarketable products (Glen et al. 2000; Huiting and Ester, 2001). The most serious losses in agricultural crops due to slug damage occur with winter cereals: grains are hollowed out and potatoes have holes bored through them. Damage also occurs in the early growth stages of sugar beet, oilseed rape, field beans and forage crops (alfalfa, clover, lucerne, kale, cabbage and maize) (South 1992).

The rhabditid nematode, *Phasmarhabditis hermaphrodita*, is capable of infecting and killing a wide range of pest slug species from the families Arionidae, Limacidae, and Milacidae. Nonfeeding third stage (dauer) juveniles enter the shell cavity beneath the dorsal surface of the slug mantle, grow into hermaphrodite adults, and reproduce there. The shell cavity of infected slugs gradually becomes swollen with fluid and their ability to feed is inhibited. After the host slug dies, nematodes reproduce on the host cadaver, eventually producing juveniles that move into soil, where they can infect new hosts (Cross et al. 2001; Wilson et al. 1993). At least two generations of nematodes can be found in the swelling (Wilson et al. 1993).

The nematode is mass-reared in monoxenic liquid culture with the gram-negative bacterium *Moraxella osloensis* in fermenters. The product is available commercially in a water-dispersible formulation which is mixed with water and applied to soil. Plant damage control can be sustained for 5-8 weeks (Cross et al. 2001). In agriculture, a recommended dose is 300,000 nematodes per m<sup>2</sup> as a treatment for plant protection, as is used with sugar beet plants in the Netherlands (Ester and Geelen 1996).

Experiments with the nematode to control the small slug *Deroceras reticulatum* have showed some good control results. Wilson and Rae (2015) searched Scopus databases and found there to be fewer studies for *P. hermaphrodita* as compared to other entomopathogenic nematodes, and that there was still little knowledge regarding this nematode's biology and ecology. The aim of our work is to review previous papers regarding the effectiveness against slugs of applying *Phasmarhabditis hermaphrodita* to crops. We will then look at field strategies for more efficient slug control, such as how many slug species are controlled in one treatment, how many nematodes are suitable for slug control, and finally, if there is always an obligate action when rearing *P. hermaphrodita* with *Moraxella osloensis*.

## DESCRIPTION OF RELEVANT *P. hermaphrodita* METHODOLOGIES FOR SLUG CONTROL

Tan and Grewal (2001) found that the dauer juveniles of *P. hermaphrodita* can be recovered and reproduce in slug cadaver or faeces. This has both negative and positive effects. A negative effect is that nematodes may lose their ability to infect living slugs, usually when used as an inundative biological control agent. On the other hand, the recovery and multiplication of dauer juveniles in nonparasitic conditions enhances nematodes, allowing them persist in the environment despite the lack of a host. In addition, Nermut et al. (2014) and McMillan et al. (2009) found that *P. hermaphrodita* can reproduce on many organic substrates, such as dead insects and vegetable residues. As a result, *P. hermaphrodita* may be suitable for long-term inoculative slug control.

*P. hermaphrodita* has been shown to infect and kill a wide variety of slugs, not only *Deroceras reticulatum* but also *Deroceras caruanae*, *Arion ater*, *Arion intermedius*, *Arion distinctus*, *Arion silvaticus*, *Tandonia budapestensis* and *Tandonia sowerbi*. All of this was under laboratory bioassays, however, and not enough field experiments exist that have shown effective slug control with this nematode. Slug body size is important for its susceptibility. One option is to apply nematodes when only juvenile slugs are present in the crops, as adults may be resistant (Wilson et al., 1993; Wilson, M and Gaugler, R. 2000). In a bioassay, Wilson et al. (1993), tested *P. hermaphrodita* with a dose of  $1.9 \times 10^5$  dauer larvae. After 5-day infection period, the differences in mortality between nematode-treated and untreated slugs were highly significant ( $P < 0.001$ ) for *D. reticulatum*, *D. caruanae* and *Arion intermedius*.

Iglesias et al. (2000) carried out a field test in Spain on lettuce using *P. hermaphrodita*, showed in comparison with the use of mini-pellets containing 5% metaldehyde (3 g pellets per m<sup>2</sup>) and a single dose of nematodes ( $3 \times 10^9$  per ha). Leaf damage caused by slugs was reduced sooner (from the first day following planting) with the pesticide than with the nematodes (which were effective starting the second week following planting). The weight of the lettuce heads and the number of marketable heads was also evaluated, and no significant differences were shown. The number of slugs within the harvested plants was significantly lower with metaldehyde treatment.

In order to reduce nematode dose, Hass et al. (1999) carried out a trial in plots of Chinese cabbage. Nematodes were applied in bands, along plant rows, and in spot applications around individual plants. The researchers concluded that focused (partial) treatments of soil around all plants to be protected from slug damage is a valuable method in reducing the overall nematode dose needed for control. It is a good method for controlling slug damage due to the delay in nematode action, and since a certain amount of slug damage can be tolerated. This was demonstrated with the log time to 50% reduction in slug damage (t50) related to the area treated and the dose applied.

Grewal et al. (2001) mentioned that slugs possess a well-developed homing behaviour, with an ability to locate homing sites or shelters during the day. It was hypothesized that the application of nematodes (*P. hermaphrodita*) to the homing sites may be as effective as overall application to the entire area. This was tested in a bioassay arena, using  $0.3 \times 10^6$  or  $0.6 \times 10^6$  infective juveniles (IJs) per m<sup>2</sup>, either to the entire area or only under a 30 by 30-cm roofing shingle that served as an artificial slug shelter. In reducing the number of leaves and leaf area eaten, nematode treatment provided plant protection equal to or better than the metaldehyde treatment. A reduction of 63% in the total number of nematodes required was obtained with the rate  $0.6 \times 10^6$  IJs per m<sup>2</sup> applied only under the shingles. This means an application of  $1.1 \times 10^9$  IJs per ha vs the normally required dose of  $3 \times 10^9$  IJs per ha.

An actual field trial with green asparagus was carried out by Ester et al. (2003). Three kinds of treatments were applied: 1 to 4 times during the spear season; doses of slug-parasitic nematodes (Nemaslug) (from 10000 to 600000 nematodes per m<sup>2</sup>); salt in doses of 500-4000 kg NaCl; and metaldehyde pellets (0.35 kg a.i. per ha). The percentages of affected and unmarketable spears were evaluated. Satisfactory results were achieved with three applications in rows of 50000 nematodes per m<sup>2</sup>. Salt treatments with 2000 kg per ha (applied twice) or 1000 kg per ha (applied four times) provided significant slug control. Nevertheless, with metaldehyde the lowest percentage of damage was seen in the final harvest periods.

Ester et al. (2003), applied nematodes in a 0.6 m-wide band three times, at a rate of 50000 per m<sup>2</sup>. This proved to be the most protective treatment for brussels sprouts (buttons) against slug damage when compared to treatment with metaldehyde

bait pellets (448 g per ha, scattered by hand) and nematodes (50000 per m<sup>2</sup>, band application), both applied six times. Nematodes also provided a longer period of protection against slugs than metaldehyde when sprout damage was recorded.

Slug activity was quite high along the drill furrow in sugar beets that were grown in a rye cover crop. However, a dosage of 300000 nematodes per m<sup>2</sup> as a furrow treatment provided adequate slug control (Ester and Geelen, 1996). Glen et al. (2000) also found that exposing slugs to 300000 dauer juveniles in boxes for 3 or 5 days resulted in them being killed rapidly, within a few days of exposure.

Speiser and Andermatt (1996) did a field trial in Switzerland, demonstrating that nematodes can be effective for slug control. A pilot experiment (where a mixture of several vegetables and ornamental plants were sown) and an experiment with rape showed reduced leaf damage with the use of *P. hermaphrodita*. In the pilot experiment, the effect of the nematodes was similar to that of the metaldehyde treatment, but the effect was better in the experiment with rape.

Grubisic et al. (2003) carried out a trial in Croatia, where cabbage sown in plastic tunnels showed protection against slugs using *P. hermaphrodita*, and in particular against *D. reticulatum*, which showed significantly better results than a methiocarb treatment. The leaf damage observed at picking time was reduced from the 10<sup>th</sup> to the 30<sup>th</sup> day following application. For instance, on the 30<sup>th</sup> day there was 15% leaf damage with the chemical molluscicide, while damage was less than 5% using the nematode treatment. However, this technique is very restricted due to its high costs. Suitable temperatures for *P. hermaphrodita* growth and efficient slug parasitism were found in the 10 to 21° C range.

By using a dish trial, Kaya (2001) studied the effectiveness of entomopathogenic nematodes against *D. reticulatum* or *Limax marginatus*. The nematode *Heterorhabditis marelatus* had potential for controlling slugs (*D. reticulatum*), since a 100% mortality rate was obtained by introducing 1000 infective juveniles per cm<sup>2</sup>. It did not replicate on the host, however, and the high mortality control in the bioassay system masked the nematode's impact.

The storage stability of *P. hermaphrodita* could limit its shelf life. Grewal and Grewal (2003) carried out a series of experiments indicating that temperatures between 5 to 15 °C were an optimal range for

nematode storage, and that osmotic desiccation in 10% glycerol can increase survival at extreme temperatures (as high as 35 °C or as low as -20 °C). The resistance of osmotically desiccated nematodes to extreme temperature can enable their transport under non-refrigerated conditions, contributing to lower shipping costs.

## DISCUSSION

Most of the experiments found were conducted under laboratory conditions or in mini plots. More research in the form of field experiments is needed to show the effectiveness of the nematode *Phasmarhabditis hermaphrodita* against the slug *Deroceras reticulatum*. A common practice in field trials is to cover the plot borders with living grass sward and to plant the sensitive vegetables in a central area, such as in the experiments carried out by Iglesias et al. (2001) and Speiser and Andermatt (1996).

As shown in the reference herein cited, slug-parasitic nematodes have provided positive results for the control of slug damage in a wide range of crops in Europe.

*P. hermaphrodita* is recommended specifically for crops with underground production (green asparagus), high value crops, and for organic horticulture. Good slug control was obtained for brussels sprouts and salad vegetables (e.g. Chinese cabbage, radish, lettuce) (Speiser et al. 2001). *D. reticulatum* control seems to occur at all biological stages, as demonstrated in the plastic box trial carried out by Speiser et al. (2001), where reduced feeding and survival rates were seen, especially with higher nematode doses and a slug weight from 574 to 801 mg.

Under certain field conditions, the typical symptom of a swollen mantle, caused by the nematodes, was not always found, and neither was the effect of rapid death, such as in the experiment of Iglesias et al. (2000). The continued presence of some slugs even after nematode use diminished its preference on the market, especially for controlling slugs in lettuce crops.

According to Speiser and Andermatt (1996) and Iglesias et al. (2000), there were no significant differences between the use of chemical molluscicides and nematodes for slug damage and yields. Applying the biological method for slug control would depend on the development of a low-cost strategy to produce the nematode. Grubisic

et al. (2003) showed that the limiting factor in introducing *P. hermaphrodita* is its high cost. At the nematode treatment rate of  $3 \times 10^5$  per m<sup>2</sup>, the treatment is 100 times more expensive than a single application of chemical molluscicide (Speiser et al. 2001).

Partial nematode treatments seem to be a good alternative to slug control, since there is a reduction in the dose that must be applied. This is particularly true when slug resting areas can be identified. Ester et al. (2003) showed that control of slugs on asparagus spears was possible when small amounts of nematodes were applied during the critical harvest period. This small dose (50,000 nematodes per m<sup>2</sup>) is less than that usually recommended to control slugs in Chinese cabbage (300,000 nematodes per m<sup>2</sup>) mentioned by Wilson et al. (1995). Previously, Speiser and Andermatt (1994) had demonstrated slug damage control with a rate of 1 million nematodes per m<sup>2</sup> in field Chinese cabbage, cabbage, lettuce and tagetes.

The method of applying nematodes could also affect its effectiveness. Nematodes are thought to have a repellent effect, and slugs avoid resting on soil treated with nematodes (Wilson et al. 1994). Slugs climb up the plants avoiding contact with the parasite (Iglesias et al. 2000). However, Hass et al (1999) found that slugs were not repelled from nematode-treated areas. Slug damage may therefore not be impacted by a repellent effect, and more studies such as this are needed. The effectiveness of *P. hermaphrodita* could depend on soil conditions such moisture. Shallow incorporation of nematodes has proven beneficial in dry soil conditions (Glen et al. 1996).

El-Danasoury and Iglesias-Piñeiro (2017) concluded that by emulating winter climates on field lettuce in Spain, a reduction in slug damage could be observed when using *P. hermaphrodita*.

Due to the restriction on molluscicides use, research on environmentally friendly options is needed. An alternative could be the use of salts, while also observing the negative long-term effects of salt.

Port et al. (2003) monitored slugs on lettuce and Brussel sprout crops. By trapping and taking soil samples and applying different control methods (molluscicide pellets applied in the traditional way and where slugs were detected, as well as treatment with *P. hermaphrodita*). They suggest that growers can only be advised to make rational use of



molluscicides by predicting and detecting slug populations. The ELISA test for slug identification could be relevant in guiding how slug populations should be managed before planting, since all of the alternative treatments described above showed no difference with the traditional use of pellets, and failed to improve slug control. According to McKemey et al. (2006), the ELISA test appeared to be advantageous for identifying slugs.

The effectiveness of other nematode species in slug control has been shown to be limited. Kaya (2001) revealed the inefficiency of other nematodes (such as *Heterorhabditis* spp. and *Steinernema* spp.) against slugs. His surveys across the United States found that *P. hermaphrodita* was not naturally occurring. The slug species *Deroceras reticulatum* was tested with the nematode *Heterorhabditis marelatus*, and this led to slug mortality but not replication in the host. More scientific research on other slug species controlled by nematodes is needed.

Under laboratory conditions, Speiser and Andermatt (1996) found that the slug *Arion lusitanicus* was not highly sensitive to *P. hermaphrodita*, suggesting that probably slug control might be age or size-dependent. Dorler et al. (2019) did a pot experiment with lettuce and tested the slug *Arion vulgaris*. Metaldehyde, iron-III-phosphate and *P. hermaphrodita* were applied, and no slug herbivory was seen in the nematodes, probably due to slug size and repellence against nematodes. The different water regimes used did have any influence, either. Speiser et al. (2001) found that *A. lusitanicus* was only attacked and killed by nematodes in its early juvenile stages. *P. hermaphrodita* apparently attacked dorsally, through a small opening at the posterior end of the mantle. the larger a slug gets, the more difficult it becomes for nematodes to reach this opening. Frank (1998) mentioned that small *A. lusitanicus* slugs (weighing 60 mg) rest within soil crevices, while larger specimens (970-4490 mg) remain on the soil surface. It could thus be difficult for soil nematodes to infect large *A. lusitanicus* living on the soil surface. *Arion ater* has been reported to be killed by nematodes, but slowly.

Regarding the action of the bacterium living inside *P. hermaphrodita*, it has been found to be associated with several different bacteria rather than with one particular species. However, the nematode's yield in in vitro cultures and its pathogenicity to slugs differs among different species of bacteria.

## CONCLUSIONS

- The use of *P. hermaphrodita* at the dauer larvae (infective juvenile) stage against slugs is recommended for horticultural crops such as green asparagus, brussels sprouts, cabbage, or lettuce.
- To reduce costs, focus should be given to slug resting areas for partial nematode applications.
- Nematode application should take place with moist soil conditions.
- Nematodes should be applied when slugs are at their juvenile size, before slugs turn into resistant adults.
- Osmotic nematode desiccation to extreme temperatures could improve its storage stability during transport.
- Cost/benefit and pesticide restrictions should be considered before choosing a slug control method.
- P. hermaphrodita* appeared to be more effective in controlling *Deroceras* slug species than *Arion* slug species.
- Other nematode species, such as *Heterorhabditis* or *Steinernema*, have not proven effective for slug control.
- Moraxella osloensis* is the commonly used bacterium for mass-producing *P. hermaphrodita*.

## Acknowledgements

In memory of Dr. Jan Van Benzooijen, for guiding me in organizing certain ideas for this review, and graded it for the course "Fundamental and Applied Biology of Nematodes," during my Master's programme in Plant Sciences in Wageningen University - The Netherlands, in January 2004.

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