Indigenous nematode use in canola

By Annika Pieterse, Jenna L Ross, Willem F Sirgel and Antoinette P Malan, Stellenbosch University

Invasive European molluscs (slugs and snails) have become significant economic pests in South Africa, especially in the Western Cape province, where the climate is favourable. One crop that is particularly targeted is canola (*Brassica napus*), which is a winter-arable crop that is commercially produced for animal feed protein.

Canola is sown between March and May in the Western Cape, with the seedlings being most susceptible to mollusc damage during the first four weeks after planting. The three mollusc species that are particularly pestiferous in canola are *Milax gagates*, *Deroceras panormitanum* and *Deroceras reticulatum*. All three species, which are slugs (as opposed to snails), are European exotic invaders.

Current methods for controlling the invasive European slugs rely on chemical molluscicide pellets containing *metaldehyde* and *carbaryl*, but they are often ineffective and toxic to non-target organisms. Therefore, it is important that a method of biological control be identified.

Control method

The most effective commercial method for the biological control of molluscs in Europe is the mollusc-parasitic nematode, *Phasmarhabditis hermaphrodita*. The product, which is available in Europe from BASF, is sold under the trade name of Nemaslug®. The product provides protection against many terrestrial mollusc families, including *Agriolimacidae*, *Arionidae*, *Limacidae*, *Milacidae*, and *Vagnulidae* (Rae *et al*., 2007).

To date, Nemaslug® cannot be sold in South Africa due to current legislation (amendment of Act 18 of 1989 under the *Agricultural Pest Act* 36 of 1947), thus an indigenous method of biological control must be developed. Consequently, methods of indigenous control require investigation. In 2012, a study was published on nematodes associated with terrestrial slugs in South Africa (Ross *et al*., 2012).



The five slug species collected during the survey: (a) Deroceras reticulatum; (b) Deroceras panormitanum; (c) Milax gagates; (d) Chlamydephorus gibbonsi; and (e) Lehmannia valentiana.

The Western Cape has a unique climate, with warm, damp winters that provide the ideal conditions in which European molluscs can thrive.

The authors identified four true slug-parasitic nematodes, two of which were previously undescribed *Phasmarhabditis* spp, indicating that South Africa is rich in indigenous species (Ross *et al*., 2012). Expansion of the existing understanding with the knowledge to be gained from further surveys, in order to identify, characterise and mass-produce the nematodes, is, therefore, of great importance.

The overall aim of the current study is to develop a nematode as a biological control agent for slugs and snails in South Africa.

Diversity and distribution

The current research was focused on slugs, as opposed to snails, as the former lack a shell, causing them to have greater exposure to nematode parasites in the soil than do snails. Sample sites, including canola crops and commercial nurseries,

were found with the assistance of local farmers and growers.

After identification by means of morphological characterisation (Ross *et al*., 2012), the slugs were then rinsed to remove surface nematodes. The slugs were dissected and examined for the presence of internal nematodes. Nematodes showing similar infection methods as the European isolate, *P. hermaphrodita* (*i.e.* infection of the mantel region), were placed on white traps with organic matter (frozen dead slugs). The nematodes that showed good growth were then used for mass production.

A total of 1 944 slugs were collected from twelve sample sites from around the Western Cape, with the sample sites concerned including those of canola crops and commercial nurseries. A total of five slug species were identified, representing four different families.

Seven of the twelve sample sites examined were found to have slugs infected with nematode parasites, which amounted to 58% of the sample sites. Eight percent of all slugs were found to be infected with nematode parasites. Nematodes were found parasitising all four exotic species, leaving *Chlamydephorus gibbonsi* free from nematode parasites (*Table 1*).

Of the seven sites that had nematodes present, *Caenorhabditis elegans* was found at six sites, *Angiostoma margaretae* at four sites, with the other nematodes, namely *Angiostoma* sp, *Phasmarhabditis* sp SA3, *Phasmarhabditis* sp SA4, and a mermithid species, being found at single sites. The nematodes concerned represented three families: *Angiostomatidae*; *Mermithidae*; and *Rhabditidae*.

Non-harmful molluscicide

The Western Cape has a unique climate, with warm, damp winters that provide the ideal conditions in which European molluscs can thrive. One of the many crops in the Western Cape that is affected by molluscs is canola. The most common mollusc control method that is currently used in canola is the surface broadcasting of molluscicide pellets containing *metaldehyde* and *carbaryl*.

However, the chemicals concerned have been shown to cause adverse effects on non-target organisms. Therefore, a control method is required that can be effective against molluscs, but which is non-harmful both to the environment and to non-target organisms.

The mollusc-parasitic nematode, *P. hermaphrodita*, has been developed into a highly successful, commercially available biological molluscicide, known as Nemaslug®. Although the product is currently sold in fifteen different European countries, the nematode has not yet been isolated in South Africa, and, for the reason mentioned, its importation is prohibited under the amended Act 18 of 1989, which falls under the *Agricultural Pests Act* (No 36 of 1947).

Therefore, the objectives of the study were to conduct a survey of slugs to isolate the local nematode isolates, to determine their mollusc control capabilities, to describe their morphology, biology and phylogeny, and to establish monoxenic cultures, with optimum growth temperatures.

Parasitic nematode survey

The first objective involved a survey of parasitic nematodes associated with slugs in the Western Cape. Slugs were investigated as opposed to snails, as slugs have been identified as being the main pests of canola crops, with their absence of an external shell meaning that they can burrow deep into the soil, thus increasing their exposure to nematode parasites. Sample sites focused on canola crops and commercial nurseries.

A total of six nematode species were identified, including: *Angiostoma margaretae*; *Angiostoma* sp; *Caenorhabditis elegans*; mermithid sp; *Phasmarhabditis* sp SA3; and *Phasmarhabditis* sp SA4. Of these species, four were previously undescribed (*Angiostoma* sp; mermithid sp; *Phasmarhabditis* sp SA3; *Phasmarhabditis* sp SA4).

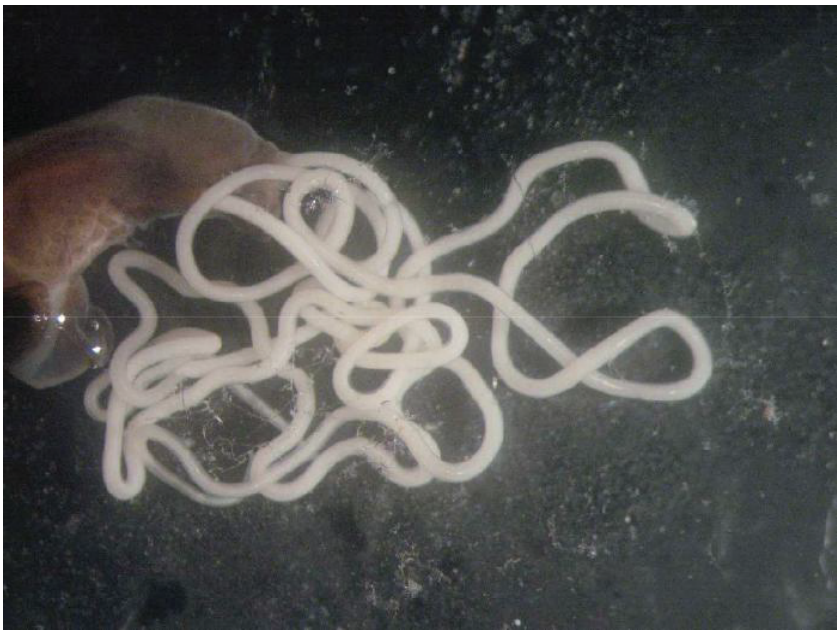
The discovery of these undescribed nematode species indicates that South Africa is rich in biological diversity, and that additional surveys are required to increase the understanding of nematodes that are associated with molluscs in Africa.

Test results

Nematodes isolated in the survey were tested for their ability to reproduce on decaying organic matter (consisting of dead frozen slugs), with the results showing that one of the nematodes, *Phasmarhabditis* sp SA4, could complete its life cycle under such conditions. *Phasmarhabditis* sp SA4 is characterised

Table 1: Prevalence and intensity of nematodes and their associated hosts collected from sites in the Western Cape province of South Africa.

Slug family/species	Native/ introduced	Number collected	Species
Agriolimacidae:			
Deroceras panormitanum	Introduced	1100	Mermitid sp. *Angiostoma margaretae* *Caenorhabditis elegans* *Phasmarhabditis* sp. SA3
D. reticulatum	Introduced	173	*A. margaretae* *C. elegans* *Phasmarhabditis* sp. SA4
Limacidae:			
Lehmannia valentiana	Introduced	387	*A. margaretae* *C. elegans*
Milacidae:			
Milax gagates	Introduced	112	*A. margaretae* *Angiostoma* sp. *C. elegans*
Chlamydephoridae: *Chlamydephorus gibbonsi*	Native	172	-



A mermithid species found after dissecting Deroceras panormitanum, collected from a nursery in George in the Western Cape.

by the shape and length of the female tail, and by the presence of males.

Phylogenetic analysis demonstrated that *Phasmarhabditis* sp SA4 was placed in a monophyletic clade along with *Phasmarhabditis* sp SA2 (previously identified by Dr Jenna Ross), *Phasmarhabditis papillosa*, and the mollusc-parasitic nematode, *Angiostoma dentiferum*.

Monoxenic cultures of *Phasmarhabditis* sp SA4 were established using methods described for the *in vitro* production of *P. hermaphrodita*. Monoxenic cultures are known to offer a relatively predictable result, and to help to create high numbers of infective juveniles with consistent infectivity. Bacterial isolates were obtained by means of swabbing the intestines and internal cavities of *D. reticulatum* specimens, and their pathogenic effects were tested by means of injecting them directly into the haemocoel of *D. reticulatum* hosts, and then monitoring the mortality.

Although five bacterial isolates were identified, *Kluyvera* sp had the highest mortality rate, leading to it being chosen for the monoxenic cultures. The monoxenic cultures were then optimised, using growth temperatures. Three different temperatures were tested, namely 15°C, 20°C, and 25°C. The results showed that the infective juvenile numbers were highest at 15°C.

The pathogenicity of the *in vitro* nematodes was then tested, with the results showing that *Phasmarhabditis* sp SA4 grown in monoxenic cultures with *Kluyvera* sp was found to cause significant mortality in *D. panormitanum*. The above, therefore, demonstrates that the nematode/bacterial combination would make an ideal biocontrol candidate.

Future work should focus on optimising the *in vitro* production of *Phasmarhabditis* sp SA4 and *Kluyvera* sp by means of manipulating the growth conditions concerned, such as extreme temperatures, overcrowding, inoculum density, alteration of the growth medium, and variation of the pH. The pathogenicity of new bacterial isolates should also be tested, both on invasive and native mollusc species, as well as on non-target organisms.

Current project importance

The results of the current project are extremely important, as they indicate that an indigenous nematode could be developed as a biological control agent in South Africa. The commercialisation of this nematode would result in the control of the relevant molluscs in agriculture, horticulture, and viticulture. In order to achieve such control, we must conduct further research into nematode mass production, as well as into formulating a suitable product. Further field trials must also be undertaken to obtain data on application rates and methods.

The results from the present project have been presented at a number of meetings, including at the integrated pest management meetings held monthly at Stellenbosch University, and at canola-related meetings. Details of the project, which has obtained international recognition, were previously presented at the International Congress of Nematology, and at the Nematological Society of Southern Africa.

In addition, outputs from the project have been used as teaching material for both undergraduate and postgraduate research progranunes at Stellenbosch University, with the results having been published in two peer review papers. An additional two papers are currently ready to submit for review. Results from this study have also been included in a book chapter published by Springer in 2017.

An MSc student, Annika Pieterse, who had successfully completed her MSc (*cum laude*) thanks to funding from the project, has since acquired funding from the National Research Foundation (NRF) to continue the project for her doctoral studies. In addition, a fully detailed business plan is being compiled, thanks to the contribution made by Dr Jenna Ross, with the aim of seeking funds from the Technology Innovation Agency, in order to move the project forward into a commercialisation phase.

Conclusion

The extreme importance of the results of the current project is to indicate that an indigenous nematode isolate could be developed as a biological control agent in South Africa, which should have a significant impact on the agricultural, horticultural, and viticulture industries. The aim is to continue the project to the commercialisation phase.

References are available from the authors. This article has been shortened for publication in *Oilseeds Focus*.