Investigating the potential of indigenous nematode isolates to control invasive molluscs in canola

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Executive summary

European molluscs (slugs and snails) have become significant pests in South Africa, especially in terms of the targeting of canola crops. The local production of canola is an important objective for the Protein Research Foundation, as it is the only method that has, so far, been successfully employed for the large-scale production of animal feed protein in the Western Cape province. Therefore, it is important to protect the crop. Current methods for controlling molluscs rely on the use of chemical molluscicide pellets, which are often overused, ineffective, and toxic to non-target organisms and the environment. Therefore, the identification of a biological remedy is important. The use of mollusc-parasitic nematodes is a possible environment-friendly alternative.

Current knowledge indicates eight nematode families associate with molluscs, including Agfidae, Alaninematidae, Alloionematidae, Angiostomatidae, Cosmocercidae, Diplogastridae, Mermithidae, and Rhabditidae. To date, *Phasmarhabditis hermaphrodita* is the only nematode that has been developed as a biological molluscicide. The nematode, which was commercially released in 1994 by MicroBio Ltd, Littlehampton, UK (formally Becker Underwood, now BASF), under the trade name Nemaslug®, is now sold in fifteen different European countries. Due to current legislation, Nemaslug® can neither be sold, nor used in South Africa. A survey was, therefore, conducted in the Western Cape province of South Africa to isolate a local slug-parasitic nematode, capable of causing mortality in invasive mollusc pests.

A total of 1944 slugs were collected from 12 different study sites. On identifying the slugs, they were dissected alive, and examined for internal nematodes. Nematodes were identified using morphological and molecular techniques (18S rRNA). Seven of the 12 sites had nematodes present, with 8% of the slugs examined being found to be infected with nematodes. Six nematode species were identified, including *Angiostoma margaretae*, *Angiostoma* sp., *Caenorhabditis elegans*, a mermitid sp., and *Phasmarhabditis* spp. (SA3 and SA4). Of the six species mentioned, four were previously undescribed. The isolation of new *Phasmarhabditis* spp. indicates the importance of conducting further surveys of mollusc-parasitic nematodes in South Africa.

Nematodes isolated in the survey were tested for their ability to reproduce on decaying organic matter (dead slugs), with results demonstrating that one of the nematodes,

Phasmarhabditis sp. SA4, could complete its life cycle under such conditions. *Phasmarhabditis* sp. SA4 was then fully described and characterised 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, *Phasmarhabditis papillosa*, and the mollusc-parasitic nematode, *Angiostoma dentiferum*.

Phasmarhabditis sp. SA4 was then established in monoxenic cultures. Five bacterial isolates were isolated from the intestine of slug hosts, and identified using 16S rRNA gene sequences. Their pathogenicity was then tested by means of direct injection into the haemocoel of *D. reticulatum*, and by then monitoring the mortality over time. *Kluyvera* sp., which was found to cause the highest mortality rate among the slugs concerned, was chosen for monoxenic culturing. Cultures containing *Phasmarhabditis* sp. SA4 and *Kluyvera* sp. were optimised, using temperatures ranging from 15°C to 25°C, with results showing that 15°C was the optimum growth temperature.

The results of this project have been presented at various national seminars (Integrated Pest Management meetings), as well as at international meetings (International Congress of Nematology and the Nematological Society of Southern Africa). In addition, two papers have emanated from this project, with another two papers being ready for submission to peer review.