Entomopathogenic nematodes for possible control of false codling moth in subtropical tree crops in South Africa

Willem Steyn and Mieke Daneel
AGRICULTURAL RESEARCH COUNCIL – INSTITUTE FOR TROPICAL AND SUBTROPICAL CROPS

The false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) is endemic and indigenous to Africa (Schwartz, 1981). It is a serious pest of citrus in South Africa and can cause major economic losses (Moore *et al.*, 2004). Several other subtropical fruit crops have also been identified as hosts of false codling moth, including macadamia, avocado and litchi (Schwartz, 1981). False codling moth have become an increasing problem on subtropical fruit crops in all producing areas in the last few years, causing concern especially for the export market because false codling moth is a pest of phytosanitary concern with restrictive import regulations having been imposed by importing countries (Bloem *et al.*, 2003).

Entomopathogenic nematodes

Entomopathogenic nematodes (EPNs) are soil-inhabiting, lethal insect parasitoids. EPNs of the families Steinernematidae and Heterorhabditidae, with their associated symbiotic bacteria, *Xenorhabdus* for Steinernematidae and *Photorhabdus* for Heterorhabditidae, are widely distributed in soils throughout the world (Hominick *et al.*, 1996; Hominick, 2002; Adams *et al.*, 2006). These nematodes are parasites of insects, killing them within 48 hours with the aid of their associated bacterial symbiont. Since the late 1970s these nematodes have gained status as one of the best non-chemical alternatives for the control of insect pests, mainly due to their ability to reach insects in cryptic habitats, their reproductive ability, the ease of mass producing them and their safety to humans and other vertebrates (Gaugler, 2007). The infective juvenile EPN is microscopic, 0.5 mm to 1.5 mm long and has a closed mouth and anus and cannot feed until it finds an insect. It enters into the body of the insect through the insect's natural openings, the mouth, anus or respiratory inlets (Poinar, 1990). Once in the insect's blood, the EPN infective juvenile releases a highly specialised symbiotic bacterium found only in EPNs. These bacteria multiply and rapidly kill the insect. No special methods are required for the application of these nematodes, as they can be applied as an aqueous suspension, using ordinary agrochemical spray equipment.

Various tests against rats, rabbits and monkeys (Gaugler, 1979; Wang *et al.*, 1983, 1984; Wang & Lu, 1983; Boemare *et al.*, 1996) have shown that the EPNs tested are harmless when ingested, injected or inhaled. They are also harmless to earthworms (Capinera *et al.*, 1982) and non-target organisms such as plants. They have now been used on a large scale in various countries over the past years and large numbers of production workers have been exposed to thousands of billions of them without any adverse effects been recorded. These biological control agents have proved to be effective against several soil insects and pests that occur in cryptic habitats (Georgis & Manweiler, 1994; Koppenhöfer, 2000).

Entomopathogenic nematodes in South Africa

In South Africa, the first occurrence of a *Steinernema* species, retrieved from the maize beetle in a maize field

in Grahamstown, was documented in 1953 (Harrington, 1953). Several years later, in a survey to obtain effective nematodes for the possible control of the African sugarcane stalk-borer, many isolates of both *Heterorhabditis* and *Steinernema* were found (Spaull, 1988, 1990, 1991). De Waal (2008) did a study on the potential of using EPNs on the codling moth, *Cydia pomonella*, under South African conditions and concluded that EPNs can provide effective control of the codling moth. In a survey done by Malan *et al.* (2011) in citrus orchards in the Western Cape, Eastern Cape and Mpumalanga provinces, they found six species of EPNs as potential bio-control agents for the false codling moth on citrus. Laboratory bioassays have shown that isolates of all six species found during this survey to be highly virulent against the final instar larvae of false codling moth. It also showed that emerging adult moths were infected with nematodes which may aid in control and dispersal. Few other surveys have been conducted in South Africa (Malan *et al.*, 2006; Hatting *et al.*, 2009) and throughout the rest of the African continent, which remains relatively unexplored, offering a fertile field for bio-prospecting.

EPNs are commercially available in numerous countries in several formulations (Grewal & Peters, 2005). Concerns with using exotic EPNs include the possible displacement of native nematodes, effects on non-target organisms (Ehlers, 2005) and strict South African regulations regarding the importation of exotic organisms (amendment of Act 18 of 1989 under the Agricultural Pest Act 36 of 1947). Furthermore exotic nematodes are not adapted to local environmental conditions. Surveys are currently being conducted in many countries, other than South Africa, to find endemic nematode isolates with good efficacy against a specific target insect.

Study on EPNs in subtropical tree crop orchards

The main objectives of this study will be:

- To obtain EPNs especially from South African macadamia, avocado and litchi orchards since species can differ from those found in citrus orchards in the study done by Malan *et al.* in 2011.
- To mass rear EPNs found in samples for use in laboratory bioassays to determine the most virulent species against the target insect.
- To determine the potential of the EPNs found in these soils for control of the soil stages of the target insect and identifying the most promising isolate by means of bioassays and lastly to evaluate the most promising isolate(s) in field trials where the effects of concentration, temperature, humidity and other environmental conditions will be determined on the efficacy of the EPN isolate(s) on the target insect.

Design of the study

A survey in macadamia, avocado and litchi orchards in the different production areas to determine the diversity and frequency of endemic EPNs in these orchards by taking soil samples and rearing EPNs from the samples. Then, bioassays in the laboratory will be done to determine the potential of the EPNs found and also to identify the most virulent isolate(s). Lastly, the most virulent isolate(s) found will be used in field trials to determine the efficacy of the EPN on false codling moth. These trials will be conducted in all three mentioned fruit tree crops.

Willem Steyn and Dr Mieke Daneel can be contacted at the Institute for Tropical and Subtropical Crops in Nelspruit on 013 753 7000.

References

ADAMS, B.J., FODOR, A., KOPPENHÔFER, H.S., STACKEBRANDT, E., STOCK, S.P. & KLEIN, M.G. 2006. Biodiversity and systematics of nematode-bacterium entomopathogens. *Biological Control* 37: 32-49.

BLOEM, S., CARPENTER, J.E. & HOFMEYER, J.H. 2003. Radiation biology and inherited sterility in false codling moth (Lepidoptera: Tortricidae). *Journal of Economical Entomology* 96: 1724-1731.

BOEMARE, N., LAUMOND, C. & MAULEON, H. 1996. The Entomopathogenic nematode-bacterium complex: biology, life cycle and vertebrate safety. *Biocontrol Science and Technology* 6(3): 333-345.

CAPINERA, J.L., BLUE, S.L. & WHEELER, G.S. 1982. Survival of earthworms exposed to *Neoaplectana carpocapsae* nematodes. *Journal of Invertebrate Pathology* 39(3): 419-421.

DE WAAL, J.Y. 2008. Entomopathogenic nematodes (Rhabditidae: Steinernematidae and Heterorhabditidae) for the control of Codling moth, *Cydia pomonella* (L.) under South African conditions. M.Sc. Dissertation, Stellenbosch University, Stellenbosch.

EHLERS, R.-U. 2005. Forum on safety and regulation. In: Grewal, P.S., Ehlers, R. -U., Shapiro-Ilan, D.I. (eds.), Nematodes as biocontrol agents. CABI Publishing, Wallingford, UK, 107-114.

GAUGLER, R. 1979. The entomogenous nematode Neoaplectana carpocapsae: radiation and mammalian safety

TO PAGE 14

Entomopathogenic nematodes

FROM PAGE 13

studies. *Dissertation Abstracts International* 40B (3): 1050-1051.

GAUGLER, R. 2007. Entomopathogenic Nematology. CABI Publishing, Wallingford, UK.

GEORGIS, R. & MANWEILER, S.A. 1994. Entomopathogenic nematodes: a developing biological control technology. *Agricultural Zoological Reviews* 6: 63-94.

GREWAL, P.S. & PETERS, A. 2005. Formulation and quality. In: Grewal, P.S., Ehlers, R.-U., Shapiro-Ilan, D.I. (eds.), Nematodes as biocontrol agents. CABI Publishing, Wallingford, UK, 79-90.

HARRINGTON, J.S. 1953. Observation on the biology, the parasites and the taxonomic position of the maize-beetle – *Heteronychus sanctae-helenae* Blanch. *South African Journal of Science* 50: 11-14.

HATTING, J., STOCK, P.S. & HAZIR, S. 2009. Diversity and distribution of Entomopathogenic nematodes (Steinernematidae, Heterorhabditidae) in South Africa. *Journal of Invertebrate Pathology* 102: 120-128.

HOMICK, W.M., REID, A.P., BOHAN, D.A. & BRISCOE, B.R. 1996. Entomopathogenic nematodes: biodiversity, geographical distribution and the Convention on Biological Diversity. *Biocontrol Science and Technology* 6: 331.

HOMICK, W.M. 2002. Biogeography. In: Gaugler, R. (ed.), Entomopathogenic Nematology, CABI Publishing, Wallingford, UK, 115-143.

KOPPENHöFER, A.M. 2000. Nematodes. In: Lacey, L.A., Kaya, H.K. (eds.), Field manual of techniques in invertebrate pathology. Kluwer Academic Publishers, The Netherlands, 283-301.

MALAN, A.P., NGUYEN, K.B. & ADDISON, M.F. 2006. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from the south-west parts of South Africa. *African Plant Protection* 12: 65-69.

MALAN, A.P., KNOETZE, R. & MOORE, S.D. 2011. Isolation and identification of Entomopathogenic nematodes from citrus orchards in South Africa and their biocontrol potential against the false codling moth. *Journal of Invertebrate Pathology* 108: 115-125.

MOORE, S.D., KIRKMAN, W. & STEPHEN, P. 2004. Cryptogran: a virus for the biological control of false codling moth. *South African Fruit Journal* 3:35-39.

POINAR, G.O. 1990. Taxonomy and biology of Steinernematidae and Heterorhabditidae. In: Gaugler, R., Kaya, H.K. (eds.), Entomopathogenic nematodes in biological control. CRC Press, Boca Raton, 23-61.

SCHWARTZ, A. 1981. 'n Bydrae tot die biologie en beheer van die valskodlingmot, *Cryptophlebia leucotreta* (Meyr.) (Lepidoptera: Eucosmidae) op nawels. Proefskrif ingelewer vir die graad Doktor in die Wysbegeerte (Landbou) aan die Universiteit van Stellenbosch, Stellenbosch.

SPAULL, V.W. 1988. A preliminary evaluation of entomogenous nematodes to control the African sugarcane stalk borer *Eldana saccharina* (Lepidoptera: Pyralidae). *Proceedings of the South African Sugar Technologists Association* 62: 120-123.

SPAULL, V.W. 1990. Field test to control the pyralid, *Eldana saccharina*, with an entomogenous nematode, *Heterorhabditis* sp. *Proceedings of the South African Sugar Technologists Association* 64: 103-106.

SPAULL, V.W. 1991. *Heterorhabditis* and *Steinernema* species (Nemata: Rhabditida) for the control of a sugar cane borer in South Africa. *Phytophylactica* 23: 213-215.

WANG, J.X. & LUI, Z.M. 1983. The safety of the nematode, *Neoaplectana glaseri* Steiner, to invertebrates. II. A test on rabbits. *Natural Enemies of Insects* 5(4): 240-242.

WANG, J.X., QUI, L.H. & LUI, Z.M. 1983. The safety of the nematode *Neoaplectana glaseri* Steiner to vertebrates. I. A test on rats. *Natural Enemies of Insects* 5(1): 39-41.

WANG, J.X., HUANG, J.T. & CHEN, Q.S. 1984. The safety of the nematode *Neoaplectana glaseri* Steiner to vertebrates. III. A test on monkeys, Macaca mulatta. *Natural Enemies of Insects* 6(1): 41-42.