Entomopathogenic nematodes (EPNs) for the control of false codling moth *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) in avocado orchards in South Africa

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ABSTRACT

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* occur naturally in soil throughout the world where they parasitize different life stages of various soil-inhabiting insects.

The nematodes are symbiotically associated with bacteria and together they kill and utilise their insect host. Infective juveniles (IJs) are the only free-living stage of nematodes found in the soil and carry the bacteria in their intestines, releasing them once the body of the host is penetrated. The main interest in these nematodes is their potential as biological control agents in integrated pest management systems due to the fact that they are able to kill their hosts within 48 hours. These nematodes can be produced commercially and applied with standard spraying equipment or through certain types of irrigation systems.

A survey was undertaken in the north-eastern parts of the country to establish whether any endemic EPNs can be found in these areas. Soil samples were collected in litchi, macadamia, avocado, mango, granadilla and guava orchards in the different production areas. Soil samples from undisturbed natural soils were also collected. Samples were transported to the laboratory where the soil was baited with mealworms to trap nematodes from the soil. Mealworm larvae showing signs of EPN infection after 7 to 14 days have been placed on White traps to collect the IJs emerging from the cadavers. Nematodes were sent to the Stellenbosch University for molecular and morphological identification. To date, 47 samples have been collected in the Nelspruit area of the Mpumalanga Province and in the Hoedspruit area of the Limpopo Province. Of the seven samples taken from avocado orchards, five have tested positive for the presence of EPNs. The survey will continue with samples also being taken in the production areas of KwaZulu-Natal and in the Eastern Cape provinces.

INTRODUCTION

The false codling moth, *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae), is a pest on avocado in all major avocado producing areas (Van den Berg, 2001) (Fig. 1). Relative low rates of infestation are typical of general infestation patterns of these moths on subtropical fruit. However, in a number of cases where conditions are favourable, tortricid moths are known to become a serious pest (Schoeman & De Beer, 2008). The eggs are oviposited singly on the fruit. Larval entrance holes on the fruit can be spotted by the white exudate and granular excreta (Du Toit

et al., 1979; Du Toit & De Villiers, 1990). Resulting lesions reduce the market value of fruit due to culling (Fig. 2). Larvae usually do not complete the life cycle in avocado fruit on the tree, but in other subtropical fruit, larvae exit the fruit at pupation (Erichsen & Schoeman, 1992; Newton, 1998; Grovè *et al.*, 2000). Last instar larvae (Fig. 3) drop to the ground and pupate on the soil surface or beneath leaf litter.

The South African avocado industry is interested in gaining access to new markets. The United States' Department of Agriculture has conducted a pest risk analysis and identified, among others, false codling moth as a pest of quarantine importance.

If the export market is expanded to new countries, the South African industry needs to ensure that the fruit is false codling moth free as false codling moth is a quarantine pest for many of the new markets. In South Africa, the avocado industry currently employs a combination of cultural, chemical and microbial control techniques to suppress insect pests like false codling moth. However, none of these measures target the soil-borne stages of the false codling moth. As soil is the natural habitat of EPNs, the last instar false codling moth larvae which fall onto the soil, as well as the pre-pupae, pupae and emerging moths, offer a window of opportunity for the use of EPNs as bio-control agents against this moth pest.

Objectives of this study

The main objectives of this study are:

- To isolate EPNs especially from South African macadamia, avocado and litchi orchards.
- To mass rear EPNs found in samples for use in laboratory bio-assays.
- 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 bio-assays.
- To evaluate EPNs in field trials where the effects of concentration, temperature, humidity and other environmental conditions will be determined on the efficacy of the EPNs.

MATERIALS AND METHODS

Soil samples

Soil samples were collected randomly from litchi, macadamia, avocado and other sub-tropical fruit orchards as well as undisturbed soils in the Mpumalanga and Limpopo provinces during 2014. Each of the soil samples of approximately 2 kg comprised of three sub-samples taken at a depth of up to 20 cm in an area of 3 m^2. The samples were placed in polyethylene bags (450 mm x 300 mm) to minimize dehydration. The bags were marked clearly and the GPS points per sample were determined. Other data recorded at each sampling site includes, height above sea level, crop, cultivar and age of the trees. The soil samples were transported in an insulated cooler to the laboratory at the Agricultural Research Council's Institute for Tropical and Subtropical Crops (ARC-ITSC) in Nelspruit, Mpumalanga. The samples were stored at room temperature in the laboratory and processed within the first week of collection.

Figure 1. The false codling moth, *Thaumatotibia leucotreta* (Meyrick).

Figure 2. Feeding damage of false codling moth larvae on an avocado fruit. Note the granular excreta of the larvae protruding from the fruit.

Figure 3. Final instar larvae of the false codling moth.

Nematode recovery

The insect baiting technique of Bedding & Akhurst (1975) was used to recover the nematodes from the soil. Each soil sample was thoroughly mixed and two 1ℓ plastic containers were each filled with 900 mℓ of soil. Five mealworm (*Tenebrio molitor* (L.)) larvae were placed on the soil surface of each container (Fig. 4), covered with a lid and placed in a growth chamber for 7 to 14 days at 25°C. Thereafter, the dead larvae

were removed, rinsed with filtered water and placed on a moistened filter paper in a Petri dish (30 mm x 10 mm). After 2 to 3 days in the Petri dish, larvae showing signs of infection by EPNs were placed on a modified White trap (White, 1927) for the collection of the emerging infective juveniles (IJs) (Fig. 5). The modified White trap consisted of the bottom part of a Petri dish (85 mm diameter) placed in a glass Petri dish (140 mm diameter) (Fig. 6). The *T. molitor* cadavers were placed on a moist piece of filter paper (80 mm diameter) in the bottom part of a plastic Petri dish. The outer glass Petri dish was filled with 20 mℓ filtered water. The IJs crawled into this part soon after emerging from the insect cadavers. Infective juveniles were harvested during the first week of emergence (Fig. 7) and send to Dr. Antoinette Malan at the University of Stellenbosch for identification. The rest of each soil sample was send to the ARC-ITSC soil laboratory for a routine soil analysis.

RESULTS AND DISCUSSION

Soil samples

Thirty eight samples from the Mpumalanga Province

Table 1. Soil samples collected from the Mpumalanga Province during 2014. Samples marked purple have tested positive for entomopathogenic nematodes.

Sample	Area	Location / Farm name	Nearest town	Latitude	Longitude	Height above sea level	Crop type	Positive / Negative for EPNs
MP 1	Nelspruit	ARC-Farm	Nelspruit	25°21.385'S	30°58.082'E	602 m	Litchi	Positive
MP 2	Nelspruit	Friedenheim	Nelspruit	25°26.654'S	30°59.126'E	673 m	Macadamia	Negative
MP 3	Nelspruit	ARC-Farm	Nelspruit	25°27.921'S	30°58.387'E	640 m	Avocado	Positive
MP 4	Nelspruit	ARC-Farm	Nelspruit	25°27.672'S	30°58.491'E	648 m	Macadamia	Negative
MP 5	Nelspruit	ARC-Farm	Nelspruit	25°28.736'S	30°58.493'E	657 m	Undisturbed soil	Positive
MP 6	Nelspruit	ARC-Farm	Nelspruit	25°28.682'S	30°58.193'E	666 m	Litchi	Positive
MP 7	Nelspruit	Boschrand	Nelspruit	25°30.368'S	30°56.651'E	718 m	Avocado	Positive
MP 8	Nelspruit	Boschrand	Nelspruit	25°25.387'S	30°55.853'E	720 m	Litchi	Negative
MP 9	Nelspruit	Friedenheim	Nelspruit	25°30.927'S	30°58.681'E	701 m	Litchi	Positive
MP 10	Kiepersol	ARC-Farm	Hazyview	25°06.129'S	31°04.065'E	790 m	Avocado	Positive
MP 11	Kiepersol	ARC-Farm	Hazyview	25°06.357'S	31°05.193'E	768 m	Macadamia	Positive
MP 12	Kiepersol	ARC-Farm	Hazyview	25°06.461'S	31°04.267'E	768 m	Undisturbed soil	Negative
MP 13	Kiepersol	ARC-Farm	Hazyview	25°06.420'S	31°04.129'E	784 m	Litchi	Negative
MP 14	Legogote	Quartzberg	White River	25°10.864'S	31°02.075'E	1085 m	Avocado	Negative
MP 15	Legogote	Quartzberg	White River	25°10.681'S	31°01.921'E	1120 m	Avocado	Negative
MP 16	Legogote	Quartzberg	White River	25°10.876S	31°02.097'E	1095 m	Grenadilla	Negative
MP 17	Nelspruit	ARC-Farm	Nelspruit	25°27.380'S	30°58.244'E	648 m	Mango	Positive
MP 18	Nelspruit	ARC-Farm	Nelspruit	25°26.971'S	30°58.670'E	670 m	Grenadilla	Negative
MP 19	Nelspruit	ARC-Farm	Nelspruit	25°27.156'S	30°58.426'E	731 m	Guava	Negative
MP 20	Ngodwana	Elandshoek	Kaapsehoop	25°29.701'S	30°42.255'E	850 m	Undisturbed soil	Negative
MP 21	Legogote	Quartzberg	White River	25°10.815'S	31°01.972'E	1084 m	Blueberries	Negative
MP 22	Legogote	Quartzberg	White River	25°10.781'S	31°02.032'E	1098 m	Macadamia	Negative
MP 23	Legogote	Quartzberg	White River	25°10.776'S	31°02.032'E	1093 m	Undisturbed soil	Negative
MP 24	Legogote	Quartzberg	White River	25°10.692'S	31°01.725'E	1110 m	Grenadilla	Negative
MP 25	Hazyview	Eldrie Boerdery	Hazyview	25°02.673'S	31°04.895'E	549 m	Guava	Negative
MP 26	Hazyview	Eldrie Boerdery	Hazyview	25°02.660'S	31°04.847'E	540 m	Undisturbed soil	Negative
MP 27	Hazyview	Perry's Bridge	Hazyview	25°02.103'S	31°07.565'E	492 m	Macadamia	Negative
MP 28	Hazyview	Perry's Bridge	Hazyview	25°02.107'S	31°07.597'E	491 m	Litchi	Negative
MP 29	Hazyview	Perry\s Bridge	Hazyview	25°02.056'S	31°07.638'E	484 m	Mango	Negative
MP 30	Hazyview	Perry's Bridge	Hazyview	25°01.939'S	31°07.935'E	457 m	Undisturbed soil	Negative
MP 31	Nelspruit	ARC-Farm	Nelspruit	25°26.987'S	30°58.228'E	658 m	Litchi	Negative
MP 32	Nelspruit	ARC-Farm	Nelspruit	25°26.971'S	30°58.363'E	651 m	Litchi	Negative
MP 33	Nelspruit	ARC-Farm	Nelspruit	25°27.151'S	30°58.302'E	662 m	Litchi	Negative
MP 34	Nelspruit	ARC-Farm	Nelspruit	25°27.167'S	30°58.291'E	663 m	Avocado	Positive
MP 35	Nelspruit	ARC-Farm	Nelspruit	25°27.291'S	30°58.162'E	664 m	Macadamia	Negative
MP 36	Nelspruit	ARC-Farm	Nelspruit	25°27.401'S	30°58.187'S	654 m	Avocado	Positive
MP 37	Nelspruit	ARC-Farm	Nelspruit	25°27.543'S	30°58.380'E	671 m	Macadamia	Positive
MP 38	Nelspruit	ARC-Farm	Nelspruit	25°27.426'S	30°58.103'E	669 m	Undisturbed soil	Positive

(Table 1) and 9 from the Limpopo Province (Table 2) have been collected. Of the total of 47 samples, 14 samples tested positive for EPNs. This represents a 30% recovery rate. Seven of the samples were taken from avocado orchards and five of those samples tested positive for EPNs. This is a recovery rate of 71.4% for avocados. The positive samples are currently at the University of Stellenbosch for DNA identification to identify the species. Soil samples from KwaZulu-Natal Province and more samples from the Limpopo Province are currently in the baiting process at the ARC-ITSC.

The current EPN survey is the first systematic survey conducted to assess the presence and diversity of EPNs occurring on a specific crop or crop type (avocado, macadamia and litchi) in the north-eastern parts of South Africa (Mpumalanga, Limpopo, KwaZulu-Natal and Eastern Cape). The few other surveys done in South Africa focused mainly on the Western Cape Province (De Waal, 2008; Hatting *et al*., 2009; Malan *et al.*, 2006; Malan *et al*., 2011) and on different crops such as apples, pears and citrus.

The 30% recovery rate of EPNs from 47 soil samples during the current survey is high when compared to surveys conducted by De Waal (2008) and Hatting *et al.* (2009). De Waal (2008) collected 200 soil samples in the Western Cape and 20 of the samples tested positive for entomopathogenic nematodes, thus a recovery rate of only 10%. Hatting *et al*. (2009) collected 1 506 samples from different

Figure 4. Meal worm, *Tenebrio molitor* larvae on top of the soil in the container.

Figure 5. Entomopathogenic nematodes (infective juveniles) emerging from a wax moth larva.

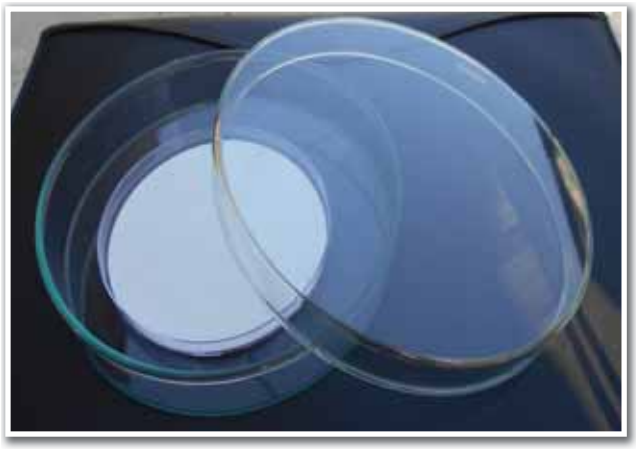

Figure 6. A modified White trap consisting of a Petri dish (85 mm diameter) placed in a glass Petri dish (140 mm diameter).

Table 2. Soil samples collected from the Limpopo Province during 2014. Samples marked purple have tested positive for entomopathogenic nematodes.

Sample	Area	Location / Farm name	Nearest town	Latitude	Longitude	Height above sea level	Crop type	Positive / Negative for EPNs
L 1	Hoedspruit	Mohlatsi	Hoedspruit	24°28.824'S	30°51.063'E	604 m	Mango	Positive
L 2	Hoedspruit	Mohlatsi	Hoedspruit	24°28.513'S	30°50.722'E	528 m	Grenadilla	Negative
L 3	Hoedspruit	Mohlatsi	Hoedspruit	24°28.497'S	30°50.746'E	588 m	Undisturbed soil	Negative
L 4	Hoedspruit	Mohlatsi	Hoedspruit	24°29.008'S	30°51.278'E	611 m	Guava	Negative
L 5	Hoedspruit	Bijamoya	Hoedspruit	24°27.787'S	30°27.787'E	556 m	Guava	Negative
L 6	Hoedspruit	Bijamoya	Hoedspruit	24°27.811'S	30°50.135'E	567 m	Undisturbed soil	Negative
L 7	Hoedspruit	Bavaria	Hoedspruit	24°23.665'S	30°53.064'E	514 m	Mango	Negative
L 8	Hoedspruit	Bavaria	Hoedspruit	24°24.346'S	30°53.049'E	519 m	Mango	Negative
L 9	Hoedspruit	Bavaria	Hoedspruit	24°24.354'S	30°53.044'E	526 m	Undisturbed soil	Negative

Figure 7. Thousands infective juveniles, seen as a milky substance in the outer glass Petri dish.

habitats in seven geographic regions of South Africa and only achieved a 5% recovery rate. The recovery rate for the avocado samples alone in this study is 71.4%, also much higher than the total of 10% collected in 2008 by De Waal.

Soil samples during the current survey were also taken from undisturbed natural soils which was included in the amount of samples taken. In contrast to human modified areas, natural habitats are more likely uncontaminated by introduced nematodes and therefore offer a better chance for finding native species.

Research into the biological control of insects has shown that no single biocontrol method, including the use of EPNs, can, by itself, effectively replace pesticide usage. Research into EPNs in South Africa has mostly been directed toward the control of insect pests on a commercial scale. To integrate nematodes into an integrated pest management system, it is important to conduct research under local climatic conditions for a specific crop. Especially for commercial application, the unique environmental conditions in the various production areas need to be assessed to allow for the effective use of various nematode species. Research into endemic EPNs, mainly targeting the two key South African lepidopteran pests, codling moth (*Cydia pomonella*) on apples and pears, and false codling moth (*Thaumatotibia leucotreta*) on citrus, forms the current bulk of our knowledge. This research expands the current knowledge base on EPNs.

FUTURE RESEARCH

- Continue soil sampling in the different production areas including Mpumalanga, Limpopo, KwaZulu-Natal and the Eastern Cape provinces.
- Bio-assays in laboratory for determining the potential of EPN species found and identifying the most virulent species.
- Evaluate the most virulent species in field trials together with the commercially available product, Cryptonem™, from River Bioscience to determine the efficacy of these EPNs on false codling moth.

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REFERENCES

BEDDING, R.A. & AKHURST, R.J. 1975. A simple technique for detection of insect parasitic rhabditid nematodes in soil. *Nematologica* 21: 109-110.

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.

DU TOIT, W.J., DE VILLIERS, E.A. & TUFFIN, A. 1979. The identification of causes of typical surface lesions on avocado fruit. *South African Avocado Growers' Association Yearbook* 3: 52-53.

DU TOIT, W.J. & DE VILLIERS, E.A. 1990. Identifisering van avokadovrugletsels wat deur insekte veroorsaak word. *South African Avocado Growers' Association Yearbook* 13: 56-60.

ERICHSEN, C. & SCHOEMAN, A. 1992. Economic losses due to the insect pests on avocado fruit in the Nelspruit/Hazyview region of South Africa during 1991. *South African Avocado Growers' Association Yearbook* 15: 49-54.

GROVé, T., STEYN, W.P. & DE BEER, M.S. 2000. Host status of 'Hass' avocado fruit for the false codling moth, *Cryptophlebia leucotreta* (Meyrick) (Lepidoptera: Tortricidae). *South African Avocado Growers' Association Yearbook* 23: 99-102.

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.

MALAN, A.P., NGUYEN, K.B. & ADDISON, M.F. 2006. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from the southwest 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.

NEWTON, P.J. 1998. False codling moth *Cryptophlebia leucotreta* (Meyrick). pp. 192-200. In: Bedford, E.C.G., Van Den Berg, M.A. & De Villiers, E.A. (Eds) *Citrus Pests in the Republic of South Africa.* Dynamic Ad, Nelspruit. 288 pp.

SCHOEMAN, P.S. & DE BEER, M.S. 2008.Control of the false codling moth with Isomate mating disruption technology. *South African Avocado Growers' Association Yearbook* 31: 42-43.

VAN DEN BERG, M.A. 2001. False codling moth, *Cryptophlebia leucotreta* (Meyrick). Van den Berg, M.A., De Villiers, E.A. & Joubert. P.H. (Eds.) *Pests and Beneficial Arthropods of Tropical and Non-citrus Subtropical Crops in South Africa*. ARC-Institute for Tropical and Subtropical Crops, Nelspruit, p. 320-325.

WHITE, G.F. 1927. A method for obtaining infective nematode larvae from culture. *Parasitology* 4: 147-154.