Distribution, isolation and diversity of entomopathogenic nematodes from the north-eastern parts of South Africa and their biocontrol potential against the false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae)

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ABSTRACT

A survey was conducted to determine the diversity and frequency of endemic entomopathogenic nematodes (EPN) in subtropical fruit tree crops in the Mpumalanga, Limpopo and KwaZulu-Natal provinces of South Africa. A total of 136 soil samples were randomly collected from cultivated and uncultivated habitats, including subtropical fruit tree crops (avocado, litchi, macadamia, mango and guava) and natural vegetation. EPNs were isolated from 14 samples (10.3%) by baiting with the larvae of *Tenebrio molitor* (mealworm). *Heterorhabditis* was the most common genus isolated from 12 samples, while only two *Steinernema* species were isolated. The most common *Heterorhabditis* isolated were *Heterorhabditis noenieputensis* and *H. zealandica*, which were both isolated from four different localities. Other species recovered were two unknown *Heterorhabditis* sp. and two *Steinernema* species, both unknown species. Laboratory bioassays, using 24-well bioassays plates, were conducted to determine the potential of local EPNs to control the false codling moth (FCM). Last instar larvae of FCM were screened for susceptibility to seven nematode species. Six of the nematode species were obtained during the survey and one, *S. yirgalemense,* was obtained from the nematode collection of the University of Stellenbosch. Last instar FCM larvae were found to be most susceptible to *S. yirgalemense*, an unidentified *Steinernema* sp. (WS9) and *H. zealandica* (WS23), causing 100%, 94% and 94% mortality respectively.

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 leave the fruit at pupation (Erichsen & Schoeman, 1992; Newton, 1998; Grove *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 their 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 entomophagous nematodes (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 entomopathogenic nematodes as bio-control agents against this moth pest.

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

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

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

Objectives of this study

The main objectives of this study is:

- To isolate EPNs especially from South African macadamia, avocado and litchi orchards.
- To mass rear EPN isolates found in samples for use in laboratory bioassays.
- To determine the potential of the EPN isolates found in these soils for control of the soil stages of the target insect and identifying the most promising isolate by means of bioassays.
- To evaluate most promising EPN isolate 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 Mpumalanga-, Limpopo- and KwaZulu-Natal provinces during 2014/15. 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 minimise 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 Campus for Tropical and Subtropical Crops (ARC-TSC) in Nelspruit, Mpumalanga. The samples were initially stored at room temperature in the laboratory and processed within the first week of collection.

Nematode recovery

The insect baiting technique (Bedding & Akhurst, 1975) was used to recover the nematodes from the soil. Each soil sample was thoroughly mixed and two 1 ℓ 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, covered with a lid and placed in a growth chamber for 7-14 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 2-3 days in the Petri dish, larvae showing

Figure 4. A modified White trap consisting of a Petri dish (85 mm diameter) placed in a glass Petri dish (140 mm diameter).

Figure 6. 12 wells in a 24-well bioassays plate lined with filter paper.

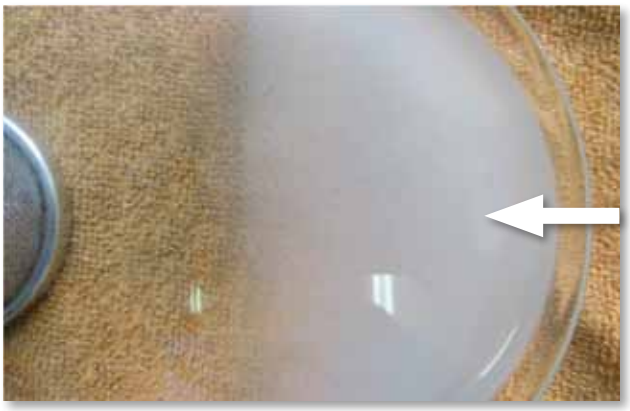

Figure 5. Thousands infective juveniles, seen as a milky substance.

Figure 7. Last instar false codling moth larvae is placed in each of the 12 wells.

signs of infection by EPNs were placed on a modified White trap (White, 1927) for the collection of the emerging infective juveniles (IJs) (Fig. 4). 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. 5). 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. 5) The IJs were send to Dr. Antoinette Malan at the University of Stellenbosch for identification. The rest of each soil sample was send to the ARC-TSC soil laboratory for a routine soil analysis.

Laboratory bioassays

Twenty-four-well bioassay protocol

24-well bioassay trays were used as the test arena. To obtain even distribution in the plates, every alternate well was lined with a circular piece of filter paper (13 mm diameter), thus using 12 wells per tray and five trays for each treatment (nematode isolate) and five control plates for each treatment (Fig. 6). Each of the 12 wells was inoculated with a specific concentration of IJs in 50 µℓ-filtered tap water. Control plates receive 50 µℓ of water only. A last instar FCM larva was added to each of the wells (Fig. 7). The wells were then covered with a glass pane inside the lid to prevent the FCM larvae from escaping and secured with a rubber band. The wells were closed in a plastic container lined with moistened tissue paper (to ensure high relative humidity) and placed in a growth chamber at 25 ± 2°C for 48 hours. Thereafter, mortality was determined and infection of the insects was confirmed by dissection of insects with the aid of a stereomicroscope.

Source of insects

Mealworm, *Tenebrio molitor* (L) (Coleoptera: Pyralidae), last instar larvae was reared at room temperature in plastic containers on fine wheat bran. To improve humidity, apple slices are laid over the surface of the colony. Last instar mealworm larvae is harvested regularly and kept at 4°C until needed. Last instar false codling moth larvae used for the bioassays were obtained from River Bioscience in Hermitage, Addo, South Africa.

Source of nematodes

Nematode isolates used in this study was obtained from the survey that was done during 2014/15 and maintained at the University of Stellenbosch and the

ARC-TSC. An isolate of *Steinernema yirgalemense* (157-C) was included in the bioassays. The decision to include this isolate in the current bioassays was based on previous studies were *S. yirgalemense* was found to be highly effective against insects. In a laboratory assay, Malan *et al*. (2011) showed *S. yirgalemense* to be highly virulent against FCM larvae. The isolates used include WS7, WS20, WS23, and WS24 for *Heterorhabditis* and WS9, WS22 and 157-C for *Steinernema*. For each of the bioassays, nematode inoculum was freshly prepared, using *Tenebrio molitor* (mealworm) larvae. Nematodes were harvested within the first week of emergence from the modified white traps (White, 1927) and stored horizontally at 14°C, in 500 mℓ vented culture flasks and used within one month after harvesting. The flasks were shaken weekly to improve aeration and nematode survival. Nematode concentrations were calculated according to the technique of Navon and Ascher (2000).

RESULTS AND DISCUSSION

Soil samples

During the survey the focus was to take soil samples on subtropical crops where Lepidopteran pests is a problem. Samples were therefore taken from avocado, litchi and macadamia orchards as well as undisturbed soils in the production areas of these crops in the north eastern parts of the country. A total of 136 soil samples were taken from the different production areas: 38 from Mpumalanga, 57 from Limpopo and 41 from KwaZulu-Natal. Of the 136 samples taken, 14 of the samples tested positive for EPNs. This represents a 10.3% recovery rate. The EPN species identified were *Heterorhabditis noenieputensis*, *H. zealandica,* two unidentified *Heterorhabditis* sp. and two unidentified *Steinernema* species. Steinernematids are generally recovered more often than heterorhabditids during non-targeted surveys (Hominick, 2000). Previous work done in South Africa also found *Steinernema* to be more prevalent than *Heterorhabditis* (Spaull, 1990; 1991; Molotsane *et al*., 2007; Hatting *et al*., 2009). However, contrasting results were obtained during this study. Heterorhabditids were more common than steinernematids. These results mirror those obtained from a random survey conducted in the south-western parts of South Africa, where Malan *et al*. (2006) and De Waal (2008) also found *Heterorhabditis* to be the dominant genus. Of the heterorhabditids recovered from the present survey, *H. noenieputensis* and *H. zealandica* were most often recovered. Only two *Steinernema* isolates were recovered, both unknown species of EPN from South Africa. The one *Steinernema* species is a new species not known to South Africa. This species is currently being described as a new EPN species for South Africa and the other *Steinernema* species is an isolate of another new species for South Africa, but have been previously found on wattle trees in the Piet Retief area of Mpumalanga. Very little is yet known about these two species regarding its distribution, virulence and host range as no studies have been conducted on this new species.

Laboratory bioassays

Results showed that the control plates from all seven isolates had low mortality rates ranging from 0% to 3.4%. This is due to the natural death of the insects. Last instar FCM larvae were found to be susceptible to all of the seven nematode isolates used in the bioassays, with mean percentage mortalities ranging between 42% and 100% (Fig. 8). Data from the two test dates were pooled and analysed using one-way ANOVA that showed a significant effect of the treatment on the percentage larval mortality.

Steinernema yirgalemense (SY) gave the best control (100%), followed by *Steinernema* sp. 1 (WS9) and *H. zealandica* (WS23), both with 94% control. *Steinernema yirgalemense*, WS9 and WS23, did not differ significantly from each other. *Heterorhabditis* sp. (WS7), *H. zealandica* (WS20), *Steinernema* sp. (WS22) and *H. zealandica* (WS24) differed significantly from *S. yirgalemense*, WS9 and WS23 in their percentage control. WS7 did not gave good results with the lowest percentage control of 42%. *Heterorhabditis zealandica* (WS20) (77%) and *Steinernema* sp. 2 (WS22) (65%) also differed significantly from the others (Fig. 8).

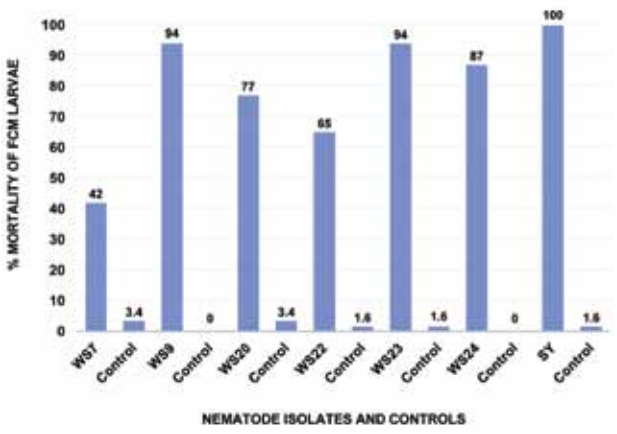

Figure 8. Percentage mortality of last instar FCM larvae inoculated with 50 IJs per insect for the different isolates after 48 h at 25°C.

This EPN survey was the first survey conducted to assess the presence and diversity of EPNs occurring on a specific crop or crop type in the north-eastern parts of South Africa (Mpumalanga, Limpopo, and KwaZulu-Natal). The few other surveys done in South Africa, focused mainly in 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.

Soil samples during the survey were also taken from undisturbed natural soils. 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.

FUTURE RESEARCH

Evaluate the most virulent isolate in field trials together with the commercially available product from River Bioscience to determine the efficacy of these EPNs on false codling moth in avocado orchards.

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