Entomopathogenic nematodes (EPNs) – Update of research on avocados and the way forward

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ABSTRACT

A survey was conducted to determine the diversity and frequency of endemic entomopathogenic nematodes (EPNs) in subtropical fruit tree crops in the Mpumalanga, Limpopo and KwaZulu-Natal provinces of South Africa. A total of 136 soil samples were randomly taken 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 from two samples. 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 unknown *Steinernema* 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. These three isolates, together with an EPN product, Nematop 50®, containing *Heterorhabditis bacteriophora*, which was imported from Germany, were evaluated in a semi-field trial in an avocado orchard at the ARC-TSC to determine their efficacy and persistence on last instar FCM larvae. Results indicated that *S. yirgalemense* was the best performing isolate with mortalities ranging from 74.4% at 2 days after application to 40.6% at 28 days after application.

INTRODUCTION

The false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Fig. 1) is a pest on avocado in all major avocado producing areas (Van den Berg, 2001). 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 serious pests (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) (Fig. 2). Resulting lesions reduce the market value of fruit due to culling.

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 avocado industry needs to ensure that their fruit is false codling

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 protruding from the fruit on the right.

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

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 supress insect pests like false codling moth. However, none of the control measures currently employed by the industry 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 (Fig. 3) 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. Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* are symbiotically associated with bacteria and together they kill and utilise their insect host within 48 hours.

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 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 blood of the insect, the EPN infective juvenile releases a highly specialized 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.

Objective of this study

The main objective of the current study was to evaluate the most virulent isolates from the previously done bioassays in a semi-field trial in an avocado orchard at the ARC-TSC in Nelspruit.

MATERIALS AND METHODS

Source of insects

Last instar false codling moth larvae were used in the semi-field trial and were obtained from River BioScience in Addo, South Africa.

Source of nematodes

Infective juveniles (IJs) of the nematode isolates obtained from the survey study and the Stellenbosch University were maintained at the ARC-TSC and were used in the semi-field trial. Nematodes were cultured *in vivo* on mealworm larvae, Tenebrio molitor (L) in a growth chamber at 25 °C. IJs were harvested and stored in 150 mℓ filtered water in horizontally placed, vented 600 mℓ corning flasks at 14 °C, and used within 2 weeks of harvesting. Concentrations of nematodes were quantified in the laboratory using procedures described by Glazer & Lewis (2000).

Baseline trapping for EPNs and soil analysis

Soil samples were taken from underneath each of the 40 avocado data trees used in the field trial at the ARC-TSC, Nelspruit. The samples (500 g) were taken using a garden trowel. Each soil sample was placed in a 1 ℓ plastic container, 10 mealworms were added, the containers closed and left at 25 °C. The containers were inspected for EPN-infected mealworm 14 days later. A 1 kg subsample was mixed from the 40 soil samples and a soil analysis was undertaken by the ARC-TSC soil laboratory, to determine the physical soil properties (percentage of sand, silt and clay).

Semi-field trial application, layout and evaluation protocol

A semi-field trial was conducted in an avocado orchard (various cultivars) at the ARC-TSC in the Nelspruit area of Mpumalanga province, South Africa. The efficacy of EPN isolates, WS9 (*Steinernema litchii*), WS23 (*Heterorhabditis zealandica*) and SY (*Steinernema yirgalemense*), the best performing isolates from the previously done bioassays as well as a commercial product, Nematop50®, imported from Germany, was evaluated under field conditions to control final-instar FCM larvae according to a trial layout, application and evaluation protocol. The nematodes were applied at a concentration of 30 IJs/cm^2 (150 000 IJs/0.5 m^2). To apply the nematode concentrations, a 2 ℓ adjustable pressure hand held sprayer (Garden Master SA)

was used (Fig. 4). The nematodes were applied in 500 mℓ of filtered water in an area of 0.5 m^2 around the base of the trees (Fig. 4) just before the first loaded cages were buried at treated trees. The mulch in the 0.5 m^2 area was removed at the time of spraying and replaced after nematodes were applied. Water only, without any nematodes, was applied to the control trees. The cages were left in the soil for two days before they were retrieved. The cages were then refilled with soil and 20 FCM larvae and were buried at the same data trees 7, 14, 21, 28, 35 and 42 days respectively after the application of the nematodes.

A semi-field trial was conducted in an avocado orchard (various cultivars) at the ARC-TSC in the Nelspruit area of Mpumalanga province, South Africa. The temperature in the top 4 cm of the soil in the orchards for the duration of each was monitored using ibuttons® (Maxim ibuttons [DS1922E] temperature loggers, Coldchain Thermo Dynamics, Johannesburg, South Africa) buried in the soil beneath a tree located in the middle of each of the treatment rows. The ibuttons were set to record the temperature every 30 minutes for the duration of the trial. The orchard was irrigated every two days by means of micro-jets placed in the 0.5 m^2 area beneath each data tree. In case of sufficient rainfall no irrigation water was given.

Cylindrical wire-mesh (45 mesh/425 µm aperture size) cages, based on the design by Duncan *et al.* (2003), were constructed by rolling 12 X 9 cm pieces of wire mesh around a glass cylinder. The glass cylinder was then removed. A plastic poly top cap on the one end was glued shut (Fig. 5) with a glue gun, while the other end of the cylinder was left unglued, to grant access to the cage. Each of the cages measured 11 x 3 cm in diameter. After being filled with sifted soil from the trial orchard, 20 false codling moth last instar larvae were placed in each cage and cages were closed with another plastic poly top cap and secured by placing Parafilm™ around the edges (Fig. 5). The cages loaded with the soil and FCM larvae were placed in plastic containers for 24 h to ensure that the cages were

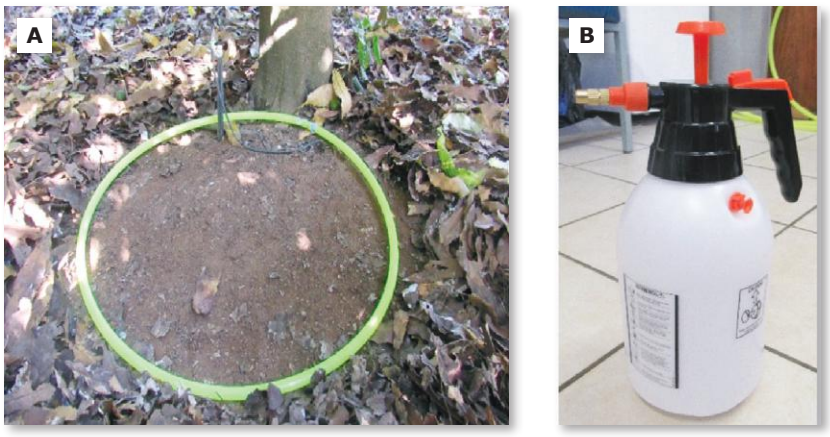

Figure 4: (A) Nematodes were applied in a 0.5 m^2 area beneath each data tree with (B) 2 ℓ adjustable pressure hand held sprayers.

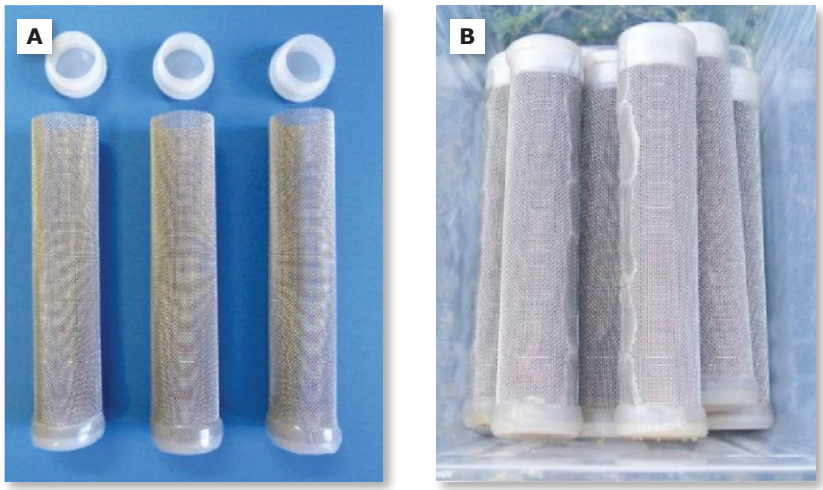

Figure 5: (A) Wire-mesh cages with plastic poly top caps glued onto one side of the cage and (B) loaded cages filled with soil from the trial orchard together with 20 final-instar FCM larvae.

secured in such a way as to prevent FCM larvae from escaping. One loaded cage was buried horizontally, 15 cm away from the tree stem, just beneath the soil surface (Fig. 6). Eight cages, each containing 20 last instar FCM larvae (n=160) were used per treatment, with a total of 800 larvae being used for the trial period (at application of EPNs, 7, 14, 21, 28, 35 and 42 days after application of EPN). A different coloured flag for each treatment (8 per treatment) was planted into the soil at each data tree next to the cage to facilitate easy retrieval and burying of the cages during the trial period (Fig. 6).

The experimental design was a complete randomised design and consisted of five treatments (four EPN isolates and a control) with eight trees for each treatment, with two buffer trees between treated trees and one buffer row between treatment rows in the trial. Extreme care was taken to use separate spades to dig up the cages from the different treatments to avoid contamination. When the cages were retrieved, the soil was removed from each cage and washed through a sieve to allow the retrieval of FCM cocoons and larvae. Cocoons were opened carefully with the aid of a stereomicroscope to ensure that the pupae and larvae inside remain intact. Larvae and pupae retrieved were placed on moist filter paper in a petri dish (90 mm diameter). The petri dishes containing the insects of each treatment were enclosed in a plastic container, lined at the bottom with moist paper towels. They were then returned to a growth chamber for 4 days at 25 °C. Thereafter each insect was evaluated for mortality, and infection with EPN was confirmed by dissection under a stereomicroscope.

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Figure 6: One loaded cage was buried just beneath the soil surface underneath each data tree in the 0.5 m^2 area where nematodes were applied. Different coloured flags planted at each burying site helped to identify where the cages were buried.

RESULTS

No natural occurring EPNs were found in the soil samples taken from each of the 40 data trees in the trial orchard. The soil from the orchard had high percentage sand (84%), with 1% silt and 15% clay as well as 4.58% organic matter. The soil temperature for the duration of the avocado trial was recorded using ibuttons®. The average soil temperature recorded in the avocado orchard during the trial (14 November 2017 – 28 December 2017) was 21 °C (15.5 – 25.6 °C). The average ambient temperature recorded was 23.1 °C (12.8 – 38.5 °C) and the average relative humidity (RH) recorded was 67.58% (18.5 – 94.6%). The total rainfall recorded for the duration of the avocado trial was 206.5 mm. Results for the mortality of FCM larvae from the avocado trial were analysed by means of a repeated measurement of ANOVA with the days after application as subplot factor, showing significant differences between the treatments (nematode isolates), the control and the days after application of the nematodes (Fig. 7).

Results indicated that *S. yirgalemense* was the best performing isolate during the field trial. These results mirror the results obtained from the bioassays where SY proofed to be the best isolate (100% mortality). It gave the best percentage mortality of the FCM larvae from day 2 up to day 28 after application of the EPNs. At day 35 and 42 there were very little control of the FCM larvae. Although WS23 and HB did not perform as well as SY, they still gave good results up to day 21 after application. WS9 (*Steinernema* sp.), which was one of the best performing isolates during the bioassays (93.5%), did not perform as well during the semi-field trial (Fig. 7).

DISCUSSION

Since false codling moth is a key pest of citrus in South Africa and it is fast becoming an economically important pest on avocados, EPNs should be tested for their field efficacy in an IPM system. False codling moth is a multivoltine species, offering year round availability as a host for EPN, thus increasing the possibility of persistence in avocado orchards. As currently, no soil treatment for false codling moth is employed, thus making the use of EPNs a potential additional tool for the reduction in false codling moth populations in avocado orchards.

From the semi-field trial, it is clear that *Steinernema yirgalemense* (SY) performed the best under field conditions. These results mirror the results obtained from previously done bioassays where SY proofed to be the most virulent against FCM larvae (100% mortality). Although not significantly different from WS23 and HB on 2 days after application, it was the isolate with the highest percentage mortality of FCM larvae. SY continued to give high percentage mortality from 2 days (74.4%) up to day 21 (66.8%) days after application, which indicates high persistence of this nematode species in the soil for up to 3 weeks after application. Although HB and WS23 did not perform as well as SY during the semi-field trial, their results were still very good since they provided mortality of 50% and higher for FCM larvae at 2, 7, 14 and 21 days after application of the nematodes. WS9, which was the second best isolate from the bioassays, did not perform well in the field trial with a mortality of only 40%, 2 days after application, after which it dropped with each time interval and provided mortality of 11.8%, 21 days after application of the nematodes, which did not differ significantly from the control treatment.

According to Nguyen *et al*. (2006), EPNs with large infective juveniles (IJs) (>1000 µm) can be advantageous in terms of their persistence in the field. Even though WS9 was expected to give enhanced persistence due to its IJ size (1054 µm), such expectations were not realised under field conditions. SY (635 µm), WS23 (685 µm) and HB (570 µm) all have IJs <1000 µm and results therefore indicate that body size is not the only reason for making an EPN more or less effective. Other factors, such as soil temperature, soil moisture and relative humidity, can also play an important role in the biological control potential of EPN 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. 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.

Furture research

- Semi-field trial will be repeated in a litchi orchard during 2018.

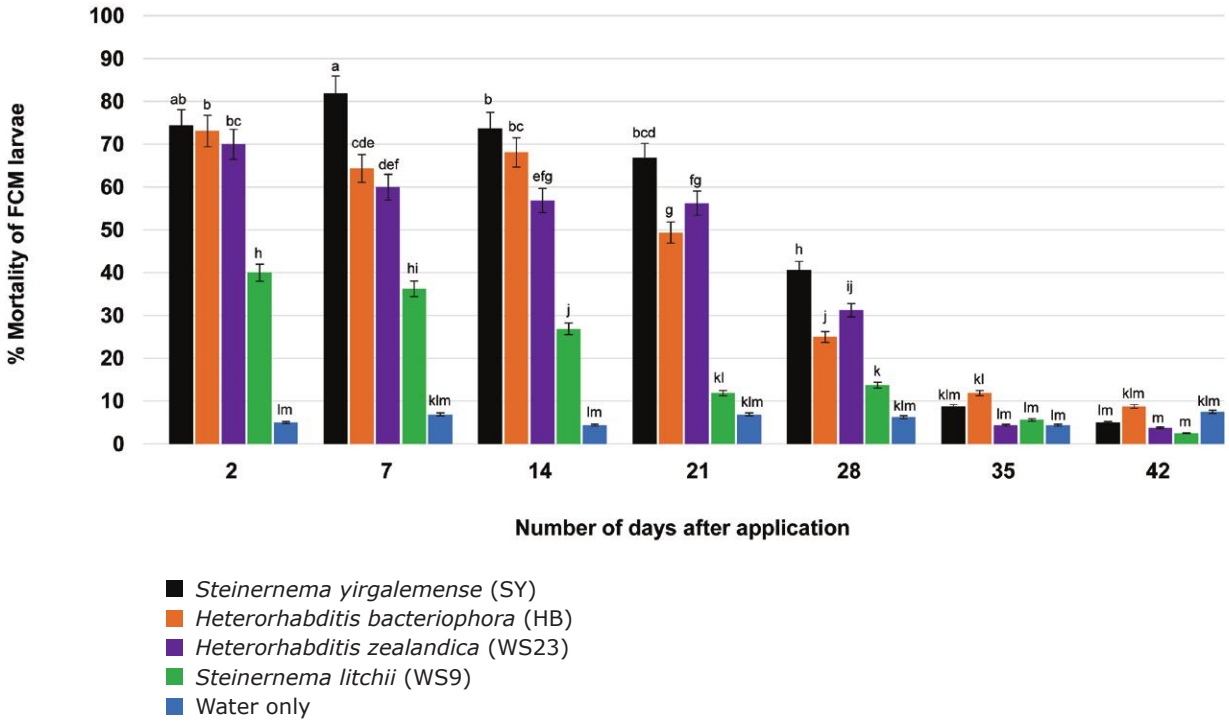

Figure 7: Mean percentage mortality for late instar false codling moth larvae: 2, 7, 14, 21, 28, 35 and 42 days after application of water only, *Steinernema yirgalemense* (157-C), *Heterorhabditis bacteriophora* (Nematop 50®), *Heterorhabditis zealandica* (WS23) and *Steinernema litchii* (WS9) in an avocado orchard at ARC-TSC, Nelspruit at concentrations of 30 IJs/cm^2. Different letters above vertical bars indicate statistically significant differences.

- Best performing isolate from the 3 semi-field trials will be selected and large scale field trials in all 3 the production areas of avocados will be done.

Acknowledgements

The authors wish to thank SAAGA and the ARC-TSC for their financial support, as well as River Bioscience for supplying the FCM larvae for the semi-field trial.

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