Controlling the false codling moth, *Thaumatotibia leucotreta* (Meyrick) with entomopathogenic nematodes

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ABSTRACT

False codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is an endemic and indigenous pest to Africa, generally south of the Sahara, and mostly in tropical and subtropical areas. FCM has been found in most sub-Saharan areas of Africa and on adjoining islands in the Atlantic and Indian oceans, as well as from Israel. Apart from FCM being an important citrus pest in South Africa, it is fast becoming an economically important pest species on subtropical fruit tree crops such as avocado, litchi and macadamia. In South Africa, the subtropical fruit crop industries currently employ a combination of cultural, chemical and microbial control techniques to suppress insect pests like FCM. However, no control measure that is currently in place targets the soilborne stages. Semi-field trials were conducted in avocado, litchi and macadamia orchards, using entomopathogenic nematodes (EPNs) viz. *Steinernema yirgalemense*, *Heterorhabditis bacteriophora*, *Heterorhabditis zealandica* and *Steinernema litchii*. The effect on FCM mortality, directly after application (2 days), as well as on persistence for 7, 14, 21, 28, 35 and 42 days post application, for all EPN species in avocado, litchi and macadamia orchards, were determined. Results from the field trials using an application concentration of 30 IJs/cm^2 of *S. yirgalemense* showed the highest control of 86% directly after application, followed by *H. bacteriophora, H. zealandica* and with *S. litchii* being the least effective of the four EPN species, with 63% control. High persistent mortality of >50% was found for all species except for *S. litchii* after 21 days, with a steep decline thereafter until day 28. All local South African EPN species, including the imported *H. bacteriophora*, clearly have great potential for the biological control of the soil stages of FCM, with the added advantage of persistence.

INTRODUCTION

The false codling moth (FCM), *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 & De Villiers, 1990) (Fig. 2). Resulting lesions reduce the market value of fruit due to culling.

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.

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, FCM 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 FCM free, as the 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 FCM. However, none of the control measures currently employed by the industry, target the soil-borne stages of FCM. As soil is the natural habitat of entomophagous nematodes (EPNs), the last instar FCM 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, 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 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.

Objective of this study

The main objective of the current study was to evaluate the most virulent isolates from the previously done bioassays together with an imported EPN product in semi-field trials in an avocado, litchi and macadamia 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

A formulated product, Nematop 50®, containing infective juveniles (IJ) of *H. bacteriophora*, was obtained from River Bioscience, Eastern Cape Province, imported from e-nema, Schwentinental, Germany in 2017 (permit no: P0076779). Nematodes from the formulated product were recycled *in vivo* in last instar mealworm larvae and were used as such in subsequent semi-field trials. Infective juveniles of the nematode isolates obtained from the survey study and the Stellenbosch University were maintained at the ARC-TSC. IJs were harvested and stored in 150 mℓ filtered water in horizontally placed, vented 600 mℓ corning flasks at 14°C, and used within two 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, litchi and macadamia data trees used in the field trials 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.

Semi-field trial application, layout and evaluation protocol

Semi-field trials were conducted in avocado, litchi and macadamia orchards at the ARC-TSC in the Nelspruit area of Mpumalanga Province, South Africa, during 2018. 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®, obtained from River Bioscience which was 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 S.A.) 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 data 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.

Figure 4. (A) Nematodes were applied with 2 ℓ adjustable hand held sprayers (B) In a 0.5 m² area beneath each data tree.

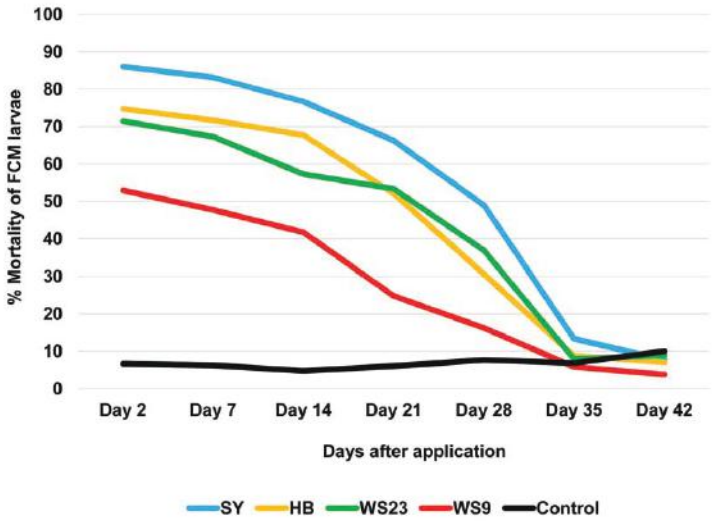

Figure 5. Mean percentage mortality (95% confidence level) recorded for last instar false codling moth larvae after exposure to *Steinernema yirgalemense* (SY), *Heterorhabditis bacteriophora* (HB), *Heterorhabditis zealandica* (WS23), *Steinernema litchii* (WS9) and water as a control treatment for all 3 semi-field trials combined at ARC-TSC, Mbombela, with concentrations of 30 IJ/cm² (repeated measures ANOVA); ($F = 107.20$; $df = 24$; $P = 0.001$).

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 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. The cages loaded with the soil and FCM larvae were placed in plastic containers for 24 h to ensure that the cages were 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. 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.

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.

Analysis of data

Statistical analysis of data was done using GenStat® (PC/Windows 8). Data from each of the three semi-field trials were analysed by means of repeated measures of ANOVA with the days after application as subplot factor. The data from the three separate trials were also pooled, irrespective of the crop type and date of each trial and was analysed by means of a repeated measures of ANOVA with the days after application as subplot factor. Treatment means were separated, using Fisher's protected t-test least significant difference at the 5% level of significance (Snedecor & Cochran, 1991).

RESULTS

Baseline trapping for EPNs and soil analysis

No natural occurring EPNs were found in the soil samples taken from each of the 40 data trees in each of the three trial orchards.

Three semi-field trials combined

Steinernema yirgalemense (SY) provided the highest mortality compared to the other isolates tested (Fig. 5). Not only did it outperformed the other isolates at every evaluation date, it was also able to control FCM larvae with more than 50% until 28 days after application, in contrast to the other isolates. *Heterorhabditis bacteriophora* (HB) were only able to ensure 50% mortality of FCM larvae until day 21 after application, whereas *S. litchii* (WS9) was only able to provide 50% mortality at day 2 after application. All nematode isolates differed significantly from the control treatment for each trial at each time interval up to day 28 after application of the nematodes, after which no control was observed anymore. *S. yirgalemense* (SY) was the best performing isolate in the three field trials, with an average percentage mortality of 50.18% for the avocado trial, 59.82% for the litchi trial and 53.75% for the macadamia trial. *H. bacteriophora* (HB) and *H. zealandica* (WS23) provided similar results for the litchi and macadamia trial, but showed a significant difference in the avocado trial with *H. bacteriophora* (HB) giving a better result than *H. zealandica* (WS23). *Steinernema litchii* (WS9) did not perform well during the field trials and differed significantly from the other isolates in percentage control of FCM larvae during each trial, with on average a percentage control of only 27%.

DISCUSSION

Results from the three semi-field trials using 30 IJ/cm^2 as soil application showed that all nematode species evaluated showed some degree of biological control potential against last instar FCM larvae under field conditions. *Steinernema yirgalemense* provided the highest mortality compared to the other isolates tested. Not only did it outperformed the other isolates at every evaluation date, it was also able to cause mortality of FCM larvae of more than 50% up till 28 days after application, in contrast with the other species. *Heterorhabditis zealandica* and *H. bacteriophora* were only able to ensure 50% mortality of FCM larvae up till day 21. *Steinernema litchii* was only able to provide more than 50% mortality at day 2. This species did not perform well in these field trials. Except for *S. litchii*, which has never been tested in a field experiment before, the other isolates proved in previous studies to have various degrees of virulence against insect pests, which confirm to the current results. *Steinernema yirgalemense* was the best performing isolate in each semi-field trial during the current study.

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.

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

Given the performance of *S. yirgalemense* during the current study and its persistence in the field, future research should be aimed at large scale field trials with this nematode species with different application techniques and in the different production areas of avocado, litchi and macadamia to confirm its efficacy.

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