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FINAL REPORT

2014

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Project Information

Research Organisation Project number	EPN 01
Project title	An investigation into the use of entomopathogenic nematodes for the control the grapevine mealybug (*Planococcus ficus*) in wine and table grapes

Fruit kind(s)	Grapes		
Start date (mm/yyyy)	02/2012	**End date** (mm/yyyy)	03/2014

Project keywords	Entomopathogenic nematodes, biological control, soil application

Approved by Research Organisation Programme leader (tick box) X

THIS REPORT MUST INCLUDE INFORMATION FROM THE **ENTIRE** PROJECT

Executive Summary

Entomopathogenic nematodes (EPNs), belonging to the families Heterorhabditidae and Steinernematidae, have been identified as lethal insect pathogens and their insecticidal action, towards a variety of insect pests, has proven them to be valuable and effective biocontrol agents.

Laboratory bioassays, to determine the ability of eight different EPN isolates to infect and kill *P. ficus,* were conducted. Six of the isolates were indigenous species and the other two, *Heterorhabditis bacteriophora* and *Steinernema feltiae,* were produced in Germany and are commercially available in South Africa. *Planococcus ficus* was highly susceptible to two indigenous species, *Heterorhabditis zealandica and Steinernema yirgalemense;* responsible for 96% ± 2% and 65% ± 10% mealybug mortalities, respectively. Biological studies illustrated that both *H. zealandica* and *S. yirgalemense* are able to complete their life cycles within adult female *P. ficus.* There was no significant difference in the pathogenicity of commercially produced *H. bacteriophora,* recycled through an insect host, and those from the formulated commercial product. However, commercially produced *S. feltiae* individuals, that were recycled through an insect host, were statistically significantly more effective than those that were not. The LC_{50} and LC_{90} values for *H. zealandica,* in the current study, were 19 and 82 infective juveniles (IJs) respectively, which were similar to the LC_{50} and LC_{90} values for *S. yirgalemense* at 13 and 80, respectively. The LC_{50} and $LC_{90,}$ for commercially available *H. bacteriophora,* were greater than they were for both *H. zealandica* and *S. yirgalemense*, with values of 36 and 555, respectively. Such results indicate that there is a definite positive relationship which exists between the concentration of IJs of all three nematode species, used for inoculation, and the percentage mortality of *P. ficus.* Sand column tests resulted in *S. yirgalemense* outperforming *H. zealandica* significantly, with average mortalities of 95% ± 1.4% and 82% ± 4.1%, respectively. As a result *S. yirgalemense* was chosen for further studies in the field.

IJs of commercially produced *H. bacteriophora* and *S. feltiae* were exposed to imidacloprid in laboratory bioassays to determine the effect on survival and infectivity. This study established the fact that these two EPN species can be applied, in combination with imidacloprid, in an integrated pest management scheme. Soil application field trials at Welgevallen and Nietvoorbij, using *S. yirgalemense* and mealybugs in Eppendorf tubes, buried 15 cm in the soil, resulted in 50% ± 10% and 52% ± 12% mealybug mortalities, respectively, when applying IJs at a concentration of 80 IJs/cm^2. These results exceptionally good taking into account that the mealybugs were 15 cm below the soil and only a 48 hour exposure period was allowed. Also no significant difference was found between mealybug mortalities as a result of the three IJ concentrations applied (20, 40 and 80 IJs/cm^2) for both vineyards. Persistence trials indicated that after four months post application, *Cydia pomonella* larval (used indicator insect for persistence of the nematodes in the soil) mortalities showed no significant reduction in infectivity on the Welgevallen vineyard, while on the Nietvoorbij vineyard there were no larval mortalities.

Tests to establish whether or not *S. yirgalemense* and *H. zealandica* produced ant deterrent factors, showed no significant differences between the number of intact cadavers for both nematode species and for cadavers that were either four or six days old. There is, however, indication that deterrent factors may be in action in cadavers that were used six days after inoculation with 60% and 49% remaining intact for *H. zealandica* and *S. yirgalemense* infected cadavers respectively. All freeze killed cadavers were consumed by *Linepithema humile* (Mayr) (Argentine ant).

The effects of low temperatures on EPN movement and infectivity were tested for *H. zealandica* and *S. yirgalemense* in the laboratory. The mortality of *P. ficus* at 14˚C, as opposed to 25˚C, for *S. yirgalemense* and *H. zealandica* were found to be 9% and 2% respectively. Vertical sand column tests were also conducted at 14˚C for *S. yirgalemense* and *H. zealandica,* which produced low mealybug mortalities of 3% and 9% respectively. This illustrates the low infectivity of the two local species at low temperatures. Laboratory persistence trials, conducted over a period of four months with *S. yirgalemense,* showed steady persistence of 100%, while *H. zealandica* had a statistically significant decrease of codling moth mortalities to 44%.

A three armed olfactometer was designed to establish if *S. yirgalemense* responds and moves towards chemical cues in the soil. A significant greater average number of IJs moved towards the grape vine roots (246I Js), than to the mealybugs (133 IJs) and to the control (4 IJs). This demonstrates that *S. yirgalemense* does actively seek out its hosts and that volatile cues produced by damaged grape vine roots, are more attractive to the EPN than cues produced by the insect itself.

This study illustrates that *S. yirgalemense* has great potential as a biopesticide for controlling *P. ficus* in the soil of South African grape vineyards. Emphasis was placed on soil application of *S. yirgalemense* in the field, which produced exceptionally good results, while laboratory tests indicate the potential for further aerial field application trials on grape vines. As the EPNs are not negatively affected by the agrochemical imidacloprid, the simultaneous use and combined action of both agents will potentially provide the farmer with excellent control against *P. ficus*. Further field- and aerial application studies will complement the current study and hopefully provide positive results for the efficient control of *P. ficus* on grapevine.

Problem identification and objectives

Planococcus ficus (Signoret) (Hemiptera: Pseudococcidae), the vine mealybug, is of economic importance to the wine and table grape industries, as it characteristically causes more damage than other mealybug species. Mealybug infestations contaminate grapes with their waxy secretions, egg-sacs and honeydew production, on which sooty mould grows, resulting in the fruit being unmarketable. Many export grapes are rejected, prior to shipment, as a result of infestations and phytosanitary concerns with regard to mealybug infestations. They are also vectors for various plant viruses. Up to date, the most common method of mealybug control in South Africa has been the use of chemical insecticides. Unfortunately, mealybugs are difficult to control chemically, due to their secretive/hidden lifestyle, where chemicals do not reach them. Of great concern is the ability of mealybugs to rapidly build up resistance to insecticides as well as the negative environmental effects associated with chemical pesticide use.

The overall aim of the current study was to generate knowledge of, and awareness of how to control P. ficus in South African vineyards by using EPNs as a biological control agent.

The objectives of the study were:

- to determine which species and isolate of EPNs is the most effective in controlling *P. ficus* in the laboratory*;*
- to determine the compatibility of EPNs with agrochemicals used in the soil and the efficacy of controlling *P. ficus* that is found on the roots of grapevine.
- to determine the effects of environmental factors that may influence the movement and efficacy of EPNs as a biological control agent.

Workplan (materials and methods)

Source of nematodes and insects

IJs were reared at room temperature, using codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), according to the methods developed by Kaya and Stock (1997). IJs from codling moth cadavers were harvested within the first week of emerging using White traps (White, 1927). The IJs were stored horizontally in 500 ml vented culture flasks, containing approximately 150 ml of distilled water in a fridge at 14°C. To prevent the use of potentially weak and unhealthy nematodes, the EPNs were used within a month after harvesting. To aid in nematode survival during storage, culture flasks were shaken weekly to increase aeration. The two local EPN species used, namely *H. zealandica* (SF 41) and *S. yirgalemense* (157-C), were originally obtained from previous local surveys and are maintained and stored in the Stellenbosch University nematode collection.

A laboratory colony of *P. ficus* was established and reared on butternuts at 25°C, while *C. pomonella* eggs and diet were obtained from Entomon Technologies (Pty) Ltd, Stellenbosch. These were reared to last instar larvae under diapausing conditions [photoperiod 10:14 (L: D)], 25˚C, and 60% humidity and were stored in the diet in a closed container in a cold room at 5˚C until needed.

Bioassay protocol

For the test arena 24-well bioassay trays (Flat bottom, Nunc™, Cat. No. 144530, Thermo Fisher Scientific (Pty) Ltd., Gauteng, Johannesburg, South Africa) were used. The bottom of each alternate well was lined with a circular (13-mm-diameter) piece of filter paper. Five trays were used for each treatment, as well as five trays for the control. A single adult female *P. ficus* was placed in each well and inoculated with the desired IJ concentration per 50 µl, using an Eppendorf micropipette. The *P. ficus* individuals in the controls received 50 µl of water only. To prevent the insects from escaping, each plate was covered with a glass pane inside the lid, and held closed with a rubber band. The trays were placed in plastic

containers, lined with moistened paper towels, and closed with a lid, to ensure high humidity levels (RH ± 95%), in a dark growth chamber at 25 ± 2°C for 48 h. After two days, mortality was determined and dead individuals from the treatment were removed and rinsed of external IJs, using water, then placed in small Petri dishes, lined with moist filter paper, sealed with PARAFILM®, and placed back in the growth chamber at 25 ± 2°C for another 48 h. A water drop was then placed on each of the cadavers for dissection, and viewed with the aid of a stereomicroscope to validate that the deaths concerned had been due to nematode infection. Cadavers with visible nematodes were recorded as having been infected, while others (in both the control and treatments) were recorded as having died from natural causes.

The survival of *S. feltiae* and *H. bacteriophora* with the insecticide imidacloprid (Confidor®) was tested. The pesticide was prepared at twice the recommended dose. Suspensions of both nematode species were prepared at a concentration of 4000 IJs/ml. Treatments contained a mixture of 1 ml of nematode suspension in water and 1 ml of the pesticide solution, which was added to a Petri dish, sealed with PARAFILM® and left in a growth chamber at 25 ± 2ºC. Controls contained 1 ml of nematode suspension and 1 ml of water. Five treatment and five control Petri dishes were prepared for both nematode species. Nematode survival was estimated by means of repeatedly collecting 10 µl samples from each Petri dish, until 50 IJs were counted, of which individuals were recorded as being either dead or alive. Samples were taken directly after preparation (0 h), and then again after 6, 12, and 24 h.

To establish the virulence of both nematode species post exposure to the insecticide, 5 ml at 4000 IJs/ml of each treatment was prepared and kept in a growth chamber for 24 h at 25 ± 2ºC. After 24 h the 5 ml nematode/pesticide solution was diluted in 1 L of distilled water, in a measuring cylinder. After the nematodes had been allowed to settle to the bottom, excess liquid was then siphoned off, leaving 10 ml behind. The remaining 10 ml was used to inoculate five Petri dishes, containing ten codling moth larvae each, at a concentration of 100 IJs/insect. Five control Petri dishes, containing 10 codling moth larvae each, received water only, and both were left for 48 h in a growth chamber at 25 ± 2ºC. The codling moth larvae were then assessed for infection. The experiment for both nematode species was repeated on a different date.

In-field soil application and infectivity of Steinernema yirgalemense

Two study sites, Welgevallen and Nietvoorbij (ARC)-Infruitech-Nietvoorbij, in Stellenbosch were selected to conduct field trials. Welgevallen is situated at 33˚ 56' 28" S; 18˚ 51' 46" E, at an elevation of 117 meters in the foothills of the surrounding mountains, while Nietvoorbij is situated at 33˚ 54' 27" S; 18˚ 52' 12" E at an elevation of 236 meters, facing in a south-east direction. For both sites, a randomised design was performed using four treatments with concentrations of 0, 20, 40 and 80 IJs/cm^2. There were eight vines per treatment (n = 32 vines), with six rows of vines, of which the first and last vine, and the first and the last row, were left untreated to avoid any possible edge effects. Eight vines from each row were selected, ensuring that every second vine was left untreated to prevent cross-contamination of treatments. Two perforated (using a heated surgical needle) 0.2-ml Eppendorf tubes were tied together with cotton thread. After placing 5 adult female mealybugs in each tube, the respective lids were closed, and the tubes were buried 15 cm deep in the soil, as close to each treatment vine trunk as possible. The thread was left extending above the ground to enable easy detection and withdrawal. An area of 80 × 100 cm was measured around each treatment vine.

The desired number of S. *yirgalemense* for each treatment was prepared in 200 ml of water and sprayed as evenly as possible onto the soil using a handheld spray bottle. Once the mealybugs in the tubes and four i-buttons were buried, each treatment vine was watered with 10 L of water, and the nematode solutions applied. One day later they were watered again (if there was no natural rainfall or irrigation), and after 48 h the mealybugs were removed from the soil. The mealybugs were rinsed with water, placed in small Petri dishes lined with moist filter paper, sealed with PARAFILM®, and placed in a growth chamber at 25 ± 2°C, where they were left to incubate at 25˚C for 24 h. The mealybugs were then dissected to confirm infection.

In-field soil persistence of Steinernema yirgalemense

The same sites at Welgevallen and Nietvoorbij were used to conduct persistence trials. Instead of using *P. ficus,* codling moth larvae, with a high susceptibility to *S. yirgalemense* (De Waal *et al*., 2011), were used. Five perforated 0.2 ml Eppendorf tubes were tied together with cotton thread, with a single larva being placed in each tube. The tubes were buried as close as possible to the treatment vine trunks, with the thread extending above the soil. Larvae were left for five days in the soil, watered on the first day with 10 L of water, and then retrieved and placed on moist filter paper in a Petri dish. The dish was sealed with

PARAFILM® and left to incubate for 24 h at 25˚C. After which, the individuals were dissected to confirm infection. This process was repeated in time intervals of one week, two weeks, a month, and three months after the date of EPN application. For each date, four i-buttons were buried to record soil temperatures.

Ant deterrent factors of Heterorhabditis zealandica *and* Steinernema yirgalemense

Ten *C. pomonella* larvae were placed in five 13-cm Petri dishes, and lined with moist filter paper. Petri dishes were inoculated with 100 IJs/larvae (1000 IJs in total), sealed with PARAFILM®, and left in a dark growth chamber at 25 ± 2℃ for four and six days. This was repeated for both *S. yirgalemense* and *H. zealandica*, while control larvae were freeze killed. Two shaded Argentine ant *Linepithema humile* (Mayr) nests were identified and used as study sites. At each nest, 25 codling moth larvae cadavers infected with *H. zealandica*, 25 infected with *S. yirgalemense* and 25 freeze-killed larvae (control) were individually placed in single perforated PCR 0.2-ml tubes. They were all linked (alternating between cadaver types, orange caps containing *S. yirgalemense*, black caps containing *H. zealandica* and clear caps containing controls) together by a thread (Fig. 3.2) and left for 24 hours. The procedure was repeated on a different date, with a single i-button used at each nest, for the duration of the experiment. The cadavers were taken back to the laboratory, where they were categorised into ‘intact’, ‘bitten’, and ‘consumed’. The tubes and the tiny holes burnt in them ensured that only ants could reach the cadavers.

Effect of low temperature on the mortality of adult females of P. ficus

For this bioassay 24-well bioassay trays (Flat bottom, Nunc™, Cat. No. 144530, Thermo Fisher Scientific (Pty) Ltd., Gauteng, Johannesburg, South Africa) were used to test the ability of *S. yirgalemense* and *H. zealandica* to infect adult female *P. ficus* at 14℃. To reduce possible edge effects and to ensure an even distribution in the plate, the bottom of each alternate well was lined with a circular (13-mm-diameter) piece of filter paper. Thus only 12 wells were occupied per tray. Five trays were used for each treatment, as well as five trays for the control. A single adult female *P. ficus* was placed in each well and inoculated with 100 IJ/ 50 µl within the 14℃ chamber. The *P. ficus* individuals, in the controls, received 50 µl of 14℃ water only. To prevent the insects from escaping, each plate was covered with a glass pane inside the lid and held closed with a rubber band. The trays were placed in plastic containers, the lids closed and left in a dark chamber at 14℃ for 48 hours. After the two days the number of dead individuals from the treatment and controls were recorded and infection confirmed by dissection. The procedure was repeated on a separate date with a fresh batch of nematodes.

Effect of low temperature on vertical movement of nematodes in sand

The ability of *S. yirgalemense* and *H. zealandica* to detect and infect adult female *P. ficus* in vertically placed sand columns at 14℃ was tested. Five mealybugs were placed in a single perforated PCR 0.2 ml tube. The tube was then placed at the bottom of a 15 × 1.5 cm centrifuge tube. For each nematode species, 20 tubes (therefor in total 100 mealybugs) were used. To sterilise the river sand, it was frozen at -40℃, and dried in an oven overnight at 50℃. To ensure the correct moisture level and temperature, a ratio of 1:10 v/v of water and sand was mixed and left to cool for 12 h at 14℃ in the fridge. Each centrifuge tube was filled with the sand to the top. A 13-mm-diameter piece of filter paper was inoculated with 500 IJs/ 50 µl. The inoculated side was placed downward and left on top of the sand. The lid of each centrifuge tube was screwed on and they were placed vertically in a dark chamber at 14℃ for 48 h. After the two days dead individuals from the treatment and controls were recorded. The experiment was repeated on a separate date with a fresh batch of nematodes.

Laboratory persistence of S. yirgalemense *and* H. zealandica

Sterilised river sand, prepared at a ratio of 1:10 v/v of water and sand, was added to 250 ml plastic tubs and each tub was then inoculated with 10 IJ/cm^2, which equated to 1040 IJs a tub that were placed at the centre of each tub. This was done for both *S. yirgalemense* and *H. zealandica.* Once the tubs were inoculated they were then placed in a large plastic container, lined at the bottom with moist paper, closed and left in a room at 25℃ for the duration of the experiment. Baiting was conducted by using ten codling moths per tub at 0, 1, 2, 3 and 4 months after initial IJ inoculation. Each bating session comprised of two batches with five tubs for each batch, for both species and the control tubs (which received no IJs), thus a total of 30 tubs. The codling moth larvae in the tubs were left in a dark growth chamber at 25 ± 2℃ for 48 h. Dead codling moth larvae were immediately dissected and checked for infection, while living individuals

were rinsed and left in a dark growth chamber at 25 ± 2℃ for another 48 h and then checked for infection, if they were dead. The experiment was repeated on a separate date with a fresh batch of nematodes. Data from the two batches were then pooled and analysed.

Cue attraction response for S. yirgalemense

A three armed olfactometer was assembled. Three small holes were made in the sides of a 7-cm-diameter petri dish and one small hole, to fit the tip of an Eppendorf pipette tip, in the centre of the lid. Three disposable plastic pipettes were cut to remove the centre column; the front 4 cm and the bulb were then re-connected. The petri dish and the three modified pipettes were filled with sterilised river sand, prepared at a ratio of 1:10 v/v of water and sand. The lid was placed on the petri dish and sealed with PARAFILM®. The tips of the three pipettes were inserted into the holes of the petri dish. Of the three bulbs one contained nothing (control), the other 15 *P. ficus* adults and the third 0.5 g of *V. vinifera* roots, collected from a grapevine and cut into small pieces (Fig. 4.1). The experimental setup, containing the study subjects, was left for 6 h to allow a chemical gradient to develop in the sand. Approximately 2000 IJs of *S. yirgalemense,* concentrated in 100 µl water, were pipetted through a small hole made in the centre of the lid of the petri dish after which the hole was sealed using BostikPrestik®. The apparatus was left in a dark growth chamber at 25 ± 2℃ for 24 h, after which the contents of each pipette arm were emptied separately into a petri dish, the mealybugs was removed, followed by rinsing the inside of the arm and bulb. Water was added and the IJs were counted with the aid of a Leica stereomicroscope. The mealybugs were then placed on moistened filter paper and left for a further 48 h in the incubator, after which each was dissected for developed nematodes, which were counted and added to the total, found in the olfactometer arm.

Data analysis

All statistical analyses were done using STATISTICA version 11 (StatSoft Inc. 2012). All data, except for the concentration trials, were corrected, in order to compensate for natural deaths, using Abbott's formula (Abbott, 1925). An analysis of variance (ANOVA) was used to analyse the data. A post-hoc comparison of means was done using Bonferroni's method, or, when residuals were not normally distributed, a bootstrap multi-comparison of means was conducted, with 95% confidence intervals (Efron & Tibshirani, 1993). Data from different test dates were pooled. A probit analysis was performed using Polo PC (LeOra Software 1987) to determine lethal nematode dosages (Finney, 1971). For the olfactometer test an ANOVA was done using a generalised non-linear model (GLZ), assuming a Poisson distribution of the variables (counting variables) with a log link function. The Wald test was used as the test statistic.

Results and discussion

The overall aim of this thesis was to generate information to assist in the control of vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) in South African wine and table grape vineyards by using entomopathogenic nematodes (EPNs) (Rhabditida: Heterorhabditidae and Steinernematidae). The main objectives of the study were to initially establish which species and isolates of EPNs would be the most effective in controlling adult female *P. ficus* in the laboratory. This was established by using six local EPN species and two commercially available species. Secondly the compatibility of the two selected EPNs with the agrochemical, imidacloprid (Confidor®), to which the EPNs would very likely be exposed to in an IPM programme, was evaluated. The third objective was to establish the efficacy of the selected EPN to control subterranean *P. ficus* on the roots of grapevines in the field. Lastly various ecological factors were tested to establish their different effects on the efficacy of EPNs as a biological control agent.

The first section of the study focussed entirely on laboratory work, whereby six South African nematode species and two commercially available species were screened to identify the two candidates, which show the greatest potential for controlling *P. ficus. Heterorhabditis zealandica* (SF 41) and *Steinernema yirgalemense* (157-C) produced the greatest mealybug mortalities, out competing the two commercially produced species. This reason alone should discourage the use of exotic nematode species and encourage research and funding directed towards commercial production of local, South African EPN species. From these results, *H. zealandica* and *S. yirgalemense* were then selected for further studies in the laboratory. Both were able to complete their life cycles inside adult female *P. ficus* cadavers and both were able to move through sand columns, infect and kill *P. ficus* adults at 25℃. An EPN concentration trial showed a definite positive relationship that exists between the concentration of the nematode species used for inoculation and the percentage mortality of *P. ficus*. From this study the LC_{50} and LC_{90} values

were established, where *H. zealandica* and *S. yirgalemense* produced similar, competitive results. The results obtained from the laboratory bioassays done in Chapter 2, illustrated the great potential of both the selected EPN species for controlling *P. ficus* in the field.

The compatible use of EPNs and agrochemicals as well as the field performance of *S. yirgalemense,* for controlling *P. ficus* adult females underground were approached. Both the commercially produced EPNs *Steinernema feltiae* and *Heterorhabditis bacteriophora* indicated no negative side effects related to the pesticide imidacloprid. Neither the nematode's survival nor virulence was compromised. This is encouraging as tank mixing EPNs and agrochemicals is convenient and helps to save farmers time and money when applying the two products together in an IPM scheme. The two vineyards chosen, Welgevallen and Nietvoorbij, to conduct the field trials were characteristically different. The Welgevallen vineyard is found at the foothills of the Stellenbosch mountains, protected from the wind and comprised of dark moist soil, while Nietvoorbij, on the other hand, is situated at the top of a hill, exposed to the characteristic strong South easterly and North westerly winds and has light coloured, rocky dry soil. The differences of the two vineyards did not affect the mean mortality results of 50% ± 10% at Welgevallen and 52% ± 12% at Nietvoorbij of adult female *P. ficus* mortalities at a concentration of 80 IJs/cm^2. Such results are promising when considering that the mealybugs were buried 15 cm below the soil surface and were only given 48 h to detect and infect them. Differences for the two vineyards came about when testing the persistence of *S. yirgalemense*. At Nietvoorbij there was no indication of the nematode still persisting after 12 weeks, while at Welgevallen the codling moth larval mortalities (indicative of *S. yirgalemense* persistence) were still above 70% at 12 weeks post application, indicating good persistence and insecticidal activity. Such a test illustrates the uniqueness of each farm and how different conditions will affect EPNs in either positive or negative ways.

Inadvertently an experiment developed through the findings of the field persistence trials, which was to determine whether or not *H. zealandica* and *S. yirgalemense* were able to produce scavenger deterrent factors that would protect the infected cadavers in the field. The argentine ant, being a very predominant species in the Western Cape vineyards, was selected at the scavenger arthropod. Unfortunately no significant results in the experimental setup were produced, but a clear trend was visible illustrating that these two EPN species may produce deterrent factors. However, control cadavers (freeze killed) were all entirely consumed while 60% ± 17% of *H. zealandica* and 49% ± 11% of *S. yirgalemense* infected cadavers (6 day old) were left intact. The value in such a study lies with the fact that if *H. zealandica* and *S. yirgalemense* produce such insect deterrent factors, then their ability to recycle and persist in the applied vineyard will increase, thus increasing their efficacy as a biological control agent.

The last objective, of establishing the effects of environmental factors on the efficacy of EPNs as a biological control agent for *P. ficus*, involved repeating the bioassay and sand column tests in the laboratory, but at 14°C instead of at 25°C. The results for both species for both tests indicated the low efficacy of both species to perform at this temperature. The results of the study could indicate potential difficulties of controlling overwintering mealybugs in the field. This does not, however, mean that the EPNs are killed at this temperature, but that once temperatures rise to favourable levels the EPNs will become active once again.

The extensive data available on the persistence of applied EPNs involves many different parameters for each species, when concerning the strains and environmental conditions tested, making the comparison of results difficult. The laboratory persistence trial for both *H. zealandica* and *S. yirgalemense* was conducted in the hope that comparable results would be produced for current and future South African studies and to help draw conclusions from the infield persistence trials conducted in Chapter 3. For example, field persistence studies were not able to distinguish between the persistence of the release population of EPNs or the recycling and continuation of their offspring through available hosts. The laboratory persistence trials indicated a drop in the levels of *H. zealandica* after four months, while *S. yirgalemense* still continued steadily, which was a similar finding at the Welgevallen vineyard, but not at Nietvoorbij. Reasons could range from the Nietvoorbij vineyard being so exposed to the winds and sun, the presence of many arthropod scavengers, the soil type and possibly the management practices applied by the farmer.

Other environmental factors that affect nematodes are those of a biotic nature. In this instance plants are able to produce volatile organic compounds which serve to communicate with other surrounding plants and microorganisms. The three-armed olfactometry test established that both *P. ficus* and damaged grape vine roots produce chemicals that are attractive to *S. yirgalemense*. The test also confirmed that *S. yirgalemense* actively moves towards the source of these chemicals. Over and above these two findings, damaged vine root attracted a significantly higher number of IJs than that of the mealybugs. This indicates that there may be tritrophic levels of interaction between the plant, insect pests and EPNs in the field. Thus the grapevine roots are not a passive victim to insect herbivores, but instead utilises EPNs by attracting them to the insect pest.

Unfortunately this study did not include studies focussed at controlling *P. ficus* colonies living above ground. The difficulties faced with spraying EPNs onto the aerial parts are well known with regard to desiccation and ultra violet (UV) radiation being factors restricting the efficacy of using EPNs as a biocontrol agent. Despite this, effective adjuvants have been tested that reduce the negative effects of desiccation and UV. Considering the vertical movement of *P. ficus* colonies and the deciduous nature of grapevine, logic suggests that in the cooler months when the leaves have been shed, the colonies will be more grouped and 'concentrated' on the branches, cordons and trunk of the vine. This would be an opportune time to spray both the aerial parts of the grapevine, when there is a smaller surface area to the plant (having no leaves and grape bunches) and when the colonies are grouped together. The difficulty emerges when developing an un-biased method for sampling the mealybugs on the trunks and branches after the EPNs have been applied. The hidden behaviour, very small body size of *P. ficus* and the complex architecture of the grape vine trunk and branch surfaces lend themselves to be very difficult and challenging aspects of the study when considering the efficacy of aerial application. Hypothetically, if one were to spray EPNs to a vineyard infested with *P. ficus*, the researcher would need to sample as objectively as possible. To do this when a large part of the colony is not visible would be exceptionally challenging. Thus those sampled would be the visible individuals, when the individuals hiding under bark and deep within crevices are not sampled. These may be the individuals that are most highly impacted by the biocontrol. The micro habitats in such areas of the plant are also more conducive to the survival and insecticidal activity of the EPNs, due to higher humidity and protection from UV rays. Aerial semi-field trials could be an option to avoid such a bias, but in the instance of this study, pilot study attempts failed due to the movement, tiny size and inconspicuous behaviour of *P. ficus*. An unexplored option as an aerial containment method will be to use cylindrical mesh cages filled with grapevine bark and Eppendorf tubes with mealybugs contained inside.

As a result this study needs to be extended to aerial applications, ensuring that the whole population (aerial and subterranean) of *P. ficus* has been tested and exposed to the applied EPNs. This will provide information describing the biocontrol agent's to full potential for controlling *P. ficus* in the field within an IPM scheme. The search for new EPN strains needs to continue in the hope to find those that are more effective at cooler temperatures and more resistant to harsh aerial conditions.

Complete the following table

Milestone	Target Date	Extension Date	Date Completed	Achievement
1. Laboratory bioassays	Dec 2012	None	2012	Scientific publication in the SAJEV
2.Field trials	Sep 2013	None	2013	Complete two field trials at Welgevallen and at Nietvoorbij
3.Confidor tests	2012	None	2012	Determine effect of Confidor on nematode mortality
4.Bio-ecological factors influencing EPN application	2013	None	2013	Biology aspects of a grapevine influence the persistence and control of insects with nematodes
5. Journal publication/s – final milestone	2013	None	Dec 2013	MSc thesis, three scientific, one popular publication

Accumulated outputs

2012 – Present a poster at the 34th Congress of the SA Society of Enology and Viticulture at at Allée Bleue, 14-16 November. Awarded with the price of the best student poster

2013 – Publish an overview of the project in SAJEV

2013 – Publish a research paper on laboratory bioassays in the SAJEV

2013 – Oral presentation at the 35th Congress of the South African Society for Enology and Viticulture at Lord Charls Hotel, Somerset Wes

2013 – Awarded the best student presentation at the Annual Research Day of the Department Conservation Ecology and Entomology of the Stellenbosch University

2013 – Obtain MSc degree with *cum laude*

2014 – Popular publication in the SA Fruit Journal: Insect-killing nematodes as an environmentally friendly option to control the vine mealybug

2014 – Manuscript accepted for publication in SAJEF on the future prospects of using EPN to control the vine mealybug

Conclusions

Basic information is provided for future research on the foliar use of EPN to control the vine mealybug in an IPM system

Technology development, products and patents

None

Suggestions for technology transfer

Protocol for testing the immediate effect of nematodes after application to the soil on the mealybug population

Human resources development/training

Student level (BSc, MSc, PhD, Post doc)	Cost to Project
1. MScAgric student	R130 000

Publications (popular, press releases, semi-scientific, scientific)

Le Vieux, P. & Malan, A.P. (2013). An overview of the vine mealybug (*Planococcus ficus*) in South African vineyards and the use of entomopathogenic nematodes as potential biocontrol agents. *South African Journal of Enology and Viticulture* 34 (1): 108-118.

Le Vieux, P. & Malan, A.P. (2013). The Potential use of Entomopathogenic Nematodes to Control *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae). *South African Journal of Enology and Viticulture* 34 (1): 296-305.

Le Vieux, P.D. (2013). Entomopathogenic nematodes for the control of the vine mealybug (*Planococcus ficus*) in South African wine and table grapes. MScAgric thesis, Department of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, 110 pp.

Le Vieux, P.D. & Malan, A.P. (2014). Insect-killing nematodes as an environmentally friendly option to control the vine mealybug. *South African Fruit Journal* (Feb/Mar): 44-46.

Le Vieux, P.D. & Malan, A.P. (2014). Prospects for using entomopathogenic nematodes to control the vine mealybug*, Planococcus ficus*, in South African Vineyards. *South African Journal of Enology and Viticulture* (Accepted for publication).

Presentations/papers delivered

Le Vieux, P.D. & Malan, A.P. 2012. Poster presentation: 34th Congress of the South African Society for Enology and Viticulture at Allée Bleue, 14-16 November. (Prize for best student poster).

Le Vieux, P.D., 2013. Potensial of entomopathogenic nematodes to control the soil stages of the vine mealybug, *Planococcus ficus*. Stellenbosch University Research Day, 24 May 2013. (Prize for best MScAgric student presentation).

Le Vieux, P.D. & Malan, A.P., 2013. An investigation into the use of entomopathogenic nematodes to control the vine mealybug in wine and table grapes. International SASEV Conference, Lord Charles Hotel, Somerset West, South Africa, 13-14 November

Total cost summary of the project

TOTAL COST IN REAL TERMS	COST	CFPA	DFTS	Deci-duous	SATI	Winetech	THRIP	OTHER	TOTAL
YEAR 1	R180000					R120000	R60000		R180 000
YEAR 2	R180000					R120000	R60000		R180 000
YEAR 3									
YEAR 4									
YEAR 5									
TOTAL	R360000					R240000	R120000		**R360 000**