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RESEARCH ARTICLE

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The occurrence of entomopathogenic fungi in apple orchards and their biocontrol potential against *Eriosoma lanigerum*

LL Mathulwe*, AP Malan and NF Stokwe

Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch, South Africa

Eriosoma lanigerum (Hausmann) (Hemiptera: Aphididae), woolly apple aphid (WAA) is an important pest on apples globally and a key pest of apple production in South Africa. The aphid has developed some level of resistance against several chemical insecticides. Entomopathogenic fungi (EPF) have been identified as promising biological control agents against a wide array of insect pests. The main aim of this study was to conduct a survey in local apple orchards in the Western Cape province for EPF and to use isolates to test the susceptibility of WAA under optimum laboratory conditions. Soil samples were collected from apple orchards and baited using larvae of *Galleria mellonella* and *Tenebrio molitor* to isolate EPF. Six EPF species: *Beauveria bassiana, Cordyceps fumosorosea, Metarhizium brunneum, M. pinghaense, M. robertsii* and *Purpureocillium lilacinum* were identified from the soil samples. The results from bioassays showed that *Metarhizium robertsii* and *M. pinghaense* isolates were the most effective with an average percentage mortality of > 90%. Metarhizium brunneum also proved to be effective when used against the insect with an average percentage mortality of > 80%, while *B. bassiana* and *C. fumosorosea* were the least effective with average percentage mortality of 52% and 48%, respectively. The lethal time required to kill 50% and 90% of the colony at a concentration of 107 conidia/ml over five days, LT50 and LT90, of *M. robertsii* (2.12 and 4.19 days, respectively) and *M. pinghaense* (2.05 and 4.45 days, respectively) showed to require similar mortality time in days of *E. lanigerum*. The results obtained in the study have provided an insight into the diversity of EPF species across apple orchards of the Western Cape and shown the efficacy of the *Metarhizium* isolates as potential biological control agents of the *E. lanigerum*.

CORRESPONDENCE
LL Mathulwe

EMAIL
lukimathulwe@gmail.com

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INTRODUCTION

Apple, *Malus domestica* (Borkhausen), production is one the most economically important horticultural crop practices in temperate regions across the world (Harris et al. 2002; Stoeckli et al. 2008). However, apple production is subjected to attack by several insect pests that negatively affect productivity, resulting in significant crop damage (Beers et al. 2003). *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae) or woolly apple aphid (WAA) is a major crop pest that attacks apple trees globally, resulting in significant economic losses to the apple-producing industry (Blackman and Eastop 2000; Abu-Romman and Ateyyat 2014).

WAA is of serious concern to the main apple production areas of South Africa, as it has become widespread within apple orchards, with it being considered as one of the main insect pests that attack apple trees (Damavandian 2000; Heunis & Pringle 2003). Infestation of apple trees by root-based colonies of WAA induces formation of hypertrophic galls on the root system, and the presence of galls impedes the flow of plant nutrients and water throughout the plant (Brown et al. 1995). The root colonies of WAA are the main source of arboreal infestation, as the first instar nymphs, which are also known as crawlers, continuously migrate from the roots to the arboreal parts of the plant (Pringle et al. 1994; Mols and Boers 2001; Damavandian & Pringle 2007; Lordan et al. 2015). Crawlers are produced by adult female aphids which reproduce parthenogenetically and overwinter on the roots (Thwaite and Bower 1983; Pringle et al. 1994).

Arboreal colonies of WAA form densely packed colonies, covered by a white, waxy filamentous secretion which protects the colonies from unfavourable environmental conditions, attacks by parasites and predators, and from chemical insecticides (Smith 1999; Moss et al. 2006). Arboreal colonies mostly infest and develop on vulnerable parts of the apple trees such as on pruning cuts and spits that have resulted from heavy cropping (Nicholas et al. 2005). In cases of severe infestation, the presence of the WAA can result in yearly reduction of the quality and quantity of the fruit that is produced. This is due to the destruction of buds, shoots and fruit-bearing wood, which eventually affects production capacity (Brown and Schmitt 1990; Brown et al. 1995).

Management of WAA in South African apple orchards, and across the world, generally relies on the combined use of biological and chemical control with other alternative methods like the use of resistant apple cultivars (Pringle 1998; Timm 2003; Stoeckli et al. 2008; Gontijo 2011; Gresham 2013). For biological control, during the 20th century, the main parasitoid of WAA, *Aphelinus mali* (Heldemann) (Hymenoptera: Aphelinidae) was introduced from North America into apple orchards globally. In the South African apple-growing areas, the parasitoid was introduced in 1920 (Cross et al. 1999b; Short 2003; Dedryver et al. 2010). However, *A. mali* parasitises and controls mainly the arboreal colonies of WAA, and not the root colonies, which contributes to the current failure of the parasitoid to control the WAA (Heunis and Pringle 2003). Hence, the parasitoid alone was found to be ineffective at controlling and preventing outbreaks of WAA (Heunis and Pringle 2006).

The most common method of controlling WAA infestation in apple orchards has, for the past several decades, been the application of high doses of chemical insecticides (Vega et al. 2009; Bhatia et al. 2011). Chemical pesticides including vamidothion and other organophosphates, have been used since the 1960s to control WAA in apple orchards (Damavandian 2000; Nicholas 2000; Damavandian and Pringle 2002; Christians 2003). However, many apple growers globally have reported that vamidothion failed to provide effective control of WAA in their orchards, as different biotypes of WAA have been shown to have developed some level of resistance to the insecticide (Pringle et al. 1994; Nicholas 2000; Timm 2003). Pringle et al. (1994) also found a vamidothion-resistant biotype of WAA in South Africa, across two apple-producing areas in the Elgin and Grabouw area. The aphid has also developed some level of resistance against a wide array of chemical insecticides that were used in the past for its control (Christians 2003).

The development of resistance against chemical insecticides by pests such as WAA, as well as concerns regarding the deleterious effects of chemicals on the environment, have provided a strong drive for the development of microbial agents to be used in controlling insect pests (Inglis et al. 2001). Entomopathogenic fungi (EPF) have the potential to be used for biological control of sap-sucking insects such as the WAA, which cannot easily be controlled using chemical pesticides and other biological control means (Chandler et al. 1997; Wraight et al. 1998; Cross et al. 1999a; Pu et al. 2005). The potential of EPF for biological control was first noticed during the 19th century by Metchnikoff, in 1879, and by Krassilstschik, in 1888, when they mass-produced *Metarhizium anisopliae* (Metchn.) Sorokin, and tested the fungus for control of the wheat cockchafers and the sugar beet curculionid (Gillespie and Moorhouse 1989).

The fungal division Ascomycota contains the Hypocreales fungal species including the PARB clade of the *M. anisopliae* complex and the *Beauveria* species, of which some are commercially produced and used globally for biological control against a variety of agricultural pests in agroecosystems (De Faria and Wraight 2007; Quesada-Moraga et al. 2007; Hatting et al. 2019). Fungal species belonging to the *M. anisopliae* species complex, including the species *Metarhizium brunneum* Petch, *Metarhizium robertsii* (Metchnikoff) Sorokin, *Metarhizium pinghaense* Chen & Guo, and *M. anisopliae* are cosmopolitan soil-dwelling entomopathogens that have been well studied in terms of biological control (Rehner and Kepler 2017). Entomopathogenic fungi cause mycosis in many different taxa of arthropods and in almost every order of insects, with them having the ability to infect all the life stages of insects (Dedryver et al. 2010; Shahid et al. 2012).

Stokwe (2016) explored the efficacy of entomopathogenic nematodes (EPN) and fungi as biological control agents of WAA, under South African conditions. Results from the study indicate that EPN cannot be used as a biocontrol agent against WAA, as the associated bacteria do not grow in the haemocoel of WAA (Stokwe and Malan 2016, 2017), however the use of commercial fungal isolates, derived from *Beauveria bassiana* (Bals.-Criv) Vuill. (Hypocreales: Cordycipitaceae) and *Metarhizium anisopliae* (Metchnikoff) Sorokin, showed the potential for use against this insect species, under laboratory conditions. No previous research has been conducted in testing the potential of South African fungi isolated from apple orchards, for the control of WAA.

The aim of this study was to survey for EPF in the local apple orchards of the Western Cape province, and to assess their potential as biological control agents of the WAA, under laboratory conditions. This was accomplished by the collection of soil samples from apple orchards and baiting for EPF using susceptible insects. All isolated fungi were identified to species level, using both morphological and molecular techniques. The susceptibility of female WAA against identified EPF isolates was screened, and the best candidates used in a dose- and time-response bioassay.

MATERIALS AND METHODS

Isolation and identification of EPF

Collection of soil samples

A total of 48 soil samples, with each sample comprising of five subsamples, were collected across six apple farms in the Grabouw, Elgin and Vyeboom areas located in the Western Cape province, South Africa. At each farm, two apple orchards were selected and from each orchard four soil samples were collected. Each apple orchard was divided into four quadrants and from each quadrant five apple trees were randomly selected, and soil subsamples collected from a depth of 15 cm under each tree canopy (Inglis et al. 2012). The five soil subsamples collected from each quadrant were pooled in clear labelled plastic bags and transported to the laboratory in a cooler box.

Baiting procedure

Each combined soil sample was mixed and sieved through a mesh sieve (4 mm) to remove the rock and leaf material and transferred to 1-l containers. For aeration, the 1-l containers were filled with soil samples leaving 10 cm space on top. All soil samples were baited for EPF after collection, and in the case of the soil samples that were dry, distilled water was used to moisten the soil to maintain enough humidity during the baiting process (Meyling and Eilenberg 2007; Goble et al. 2010). Ten larvae of both *Galleria mellonella* L. (Lepidoptera: Pyralidae) and *Tenebrio molitor* (Coleoptera: Tenebrionidae) were added to the surface of the soil samples in the plastic containers which were then closed and incubated in the dark at a controlled room temperature of ± 25 °C (Zimmermann 1986; Meyling 2007). Larvae of both *G. mellonella* and *T. molitor* used as EPF bait insects were reared in the laboratory following methods by Van Zyl and Malan (2015).

To ensure that the insects made good contact with the soil, the containers were inverted daily (Goble et al. 2010). After 7 and 14 days the dead larvae were removed. The dead larvae were surface sterilised by first being dipped in distilled water to remove the soil and then dipped first in 70% ethanol and then in distilled water. To isolate the EPF, each dead larva with symptoms of fungal infection, characterised by fungal growth or by hardening of the cadaver was placed on a Petri dish fitted with a filter paper that was moistened with distilled water (Goble et al. 2010). The Petri dishes were then placed in 2-l plastic containers with paper towels moistened with distilled water. The plastic containers were closed and incubated in a controlled room temperature of ± 25 °C, in the dark.

Every four days, overt mycosed insects were removed and the fungi were scraped from the surface of the larva cadaver and placed on Sabouraud dextrose agar plates (9 mm diam.) with 1 g of yeast (SDAY) plates, supplemented with 200 µl of penicillin-streptomycin, to prevent bacterial contamination. The fungal cultures on the agar plates were then incubated at a controlled temperature of ± 25 °C in a growth chamber and checked for fungal growth every four days. The fast-growing saprophytic fungi were discarded, and the contaminated plates were re-plated. Fungal plates were grouped according to morphological characteristics of growth patterns and spore structure.

Confirming insect susceptibility to fungi

To determine the pathogenicity of the isolated fungi, one fungal agar plate was selected from each of the morphologically similar groups of fungal culture plates. The conidia, 2–3 week-old, were

scraped from the growth medium plates using a sterile scalpel. The collected fungal spores were transferred into sterile 1.5-ml micro-centrifuge tubes, containing 1.0 ml sterilised distilled water and a single drop of 0.05% v/v Tween 20. The micro-centrifuge tubes were closed and vortex-mixed for 60 s to produce a homogenous conidial suspension (of *T. molitor* were dipped in each conidial (Goble et al. 2010). Five final instars of *G. mellonella* and five final instars solution for a period of 5 s. This was used to confirm insect susceptibility to the collected fungal isolates against insects in general. The dipped larvae were then transferred to Petri dishes (90 mm diam.), each fitted with a piece of filter paper moistened with distilled water. The Petri dishes were placed in 2 l plastic containers that were then closed with a lid and incubated at a controlled room temperature of ± 25 °C in the dark. After two to three days, the Petri dishes were checked for dead larvae, which were removed from the Petri dishes and surface sterilised. The cadavers were surface sterilised by first dipping in 70% ethanol, thereafter in distilled water, followed by being dipped in 5% bleach and three times in distilled water to conclude the process (Lacey and Solter 2012). Each cadaver was placed on a paper towel to dry, after which it was positioned on a SDAY plate which was sealed with Parafilm and incubated at room temperature for a period of three to four days to verify the death of the larvae by overt mycosis.

Identification of isolated EPF

DNA was extracted from the 12 selected morphologically different EPF isolates using the Zymo Research Quick-DNA fungal/bacterial miniprep kit (Zymo Research Corporation), according to the supplied manufacturer protocol. The polymerase chain reaction (PCR) process was undertaken for the molecular identification process for the selected EPF strains, using the KAPA2G ReadyMix PCR Kit (Labotec). The DNA extract of each of the selected EPF from the DNA extraction process was used for the process. Internal transcribed spacer (ITS) primers, ITS 1 [forward primer (5'-TCCGTAGGTGAACCTGCGG-3')] and ITS 4 [reverse primer (5'-CTCCTCCGCTTATTGATATGC-3')], and the elongation factor-1alpha gene (EF-1α) sequencing primers, as well as EF1F [forward primer (5'-GTCGGTGGTATCGACAAGCGT-3')] and EF2R [reverse primer (5'-AGCATGTTGTCGCCGTTGAAG-3')], were used for the PCR procedure. The PCR thermocycle conditions consisted of the first denaturing step being undertaken at 94 °C for 10 min, followed by 36 cycles of 94 °C for 30 s, at 56 °C for 30 s (for the ITS primers), or at 53 °C for 30 s (for the EF primers) and then at 72 °C for 1 min. The PCR reaction was completed with a final extension at 72 °C for 7 min, after which cooling off took place, with the substance being held at 4 °C. The thermocycle conditions were adapted from Abaajeh and Nchu (2015).

The PCR products were visualised on an agarose gel using ethidium bromide. The un-purified PCR products were sent to the Central Analytical Facility (CAF), DNA Sequencing Unit, Department of Genetics at Stellenbosch University, for post-PCR clean-up and sequencing reaction. DNA sequences were aligned and edited using the software program, CLC Main Workbench, version 6. Sequences were blasted against the NCBI (National Centre for Biotechnology Information) GenBank database for species identification.

Pathogenicity of conidial suspensions

Preparation of conidial suspensions

Selected EPF isolates were screened for their pathogenicity against the WAA, by harvesting conidia from 2–3-week-old surface cultures, grown on Saboraud Dextrose Agar (SDA) plates, by scraping with a sterile blade. Collected fungal spores were suspended in 20 ml of sterile distilled water supplemented with 0.05% v/v Tween 20, in McCartney (28 ml) glass bottles. The bottles containing conidia were sealed, and vortex-mixed for 2 min to produce a homogenous suspension. The conidial suspension was poured into a sterile 100-ml glass beaker, through an organza fabric, to remove the fungal hyphae and mycelium, before being poured back into the McCartney bottle, vortex-mixed for 2 min, and used as the conidial stock (Mathulwe et al. 2022). One ml of the conidial stock was transferred into a McCartney (28 ml) glass bottles containing 9 ml of sterile distilled water, and vortex mixed for 3–4 min to produce a homogenous suspension. To determine the conidial concentration of the suspensions, 100 µl of the suspension was pipetted on both ends of a haemocytometer under a coverslip and the total number of spores was counted using a Zeiss Axiolab 5 light microscope. An average number of spores was determined, after counting the total number of spores on both ends of the haemocytometer, and to quantify the conidial concentration per unit volume, methods by Inglis et al. (2012) were followed. Serial dilutions were conducted to obtain the desired conidial concentrations.

The conidial viability of the produced diluted suspensions for each EPF isolate was checked by spread plating 100 µl of the suspension on three SDA plates, at a conidial concentration of 10^7 conidia/ml (Ekesi et al. 2002). The percentage germination of the conidia on the plates was checked 24 h post incubation in the dark at a controlled room temperature of ±25 °C, by 100 spore count from each plate. The conidia were counted as viable if they developed a germ tube, and those without a germ tube were counted as non-viable. The average number of viable conidia were calculated from the three plates, and only conidial suspensions with percentage viability of >85% were used in the screening bioassay (Mathulwe et al. 2022).

Screening of entomopathogenic fungi

Collected EPF isolates were screened for their pathogenicity against the WAA. All isolates were tested at a standard infective conidial concentration of 1×10^7 conidia/ml, and insects in the control treatment were treated with sterile distilled water supplemented with 0.05% v/v Tween 20. A total of 60 adult female WAA (12 insects in five 24-well bioassay plates) per treatment were used to test the pathogenicity of each EPF isolate. Each insect was placed in an alternate well of a 24-well bioassay plate, which was fitted with a filter disc paper, and inoculated using 50 µl of the produced conidial suspension. To prevent settling of conidia at the bottom of the inoculum, the conidial suspensions were regularly vortex mixed for 20–30 s during the inoculation process (Mathulwe et al. 2022). To prevent escape of insects from the wells, each plate was closed with a glass cover. The bioassay plates were transferred to 2-l plastic containers fitted with moistened paper towels and incubated at controlled temperature of ± 25 °C. Insect mortality was recorded five days post treatment. To determine the cause of insect mortality, insect cadavers were surface sterilised using 70% ethanol and sterile distilled water. Insect cadavers were placed on water agar and incubated at a temperature of ± 25 °C. Four to five days following incubation, overt mycosis was recorded. The bioassay trials were repeated for each EPF isolate using fresh conidia-inoculum, on a different date.

Dose-response bioassays

The two most effective fungal isolates, *M. robertsii* (6EIKEN) and *M. pinghaense* (5HEID), based on the screening bioassays, were used. Conidial suspensions were prepared following the same procedure detailed above. The conidial viability of suspensions was conducted following the same process detailed above. Four conidial concentrations for both *M. robertsii* (6EIKEN) and *M. pinghaense* (5HEID), and a control treatment were tested (0, 10^4, 10^5, 10^6 and 10^7 conidia/ml). The same number of insects (n = 60) were used, following the same experimental

methodology as detailed above for each concentration used. The mortality of WAA and the cause of mortality was assessed five days post-treatment for each conidial concentration following the same procedure outlined above, to determine the lethal concentration required to kill 50% and 90% of the colony, LC_{50} and LC_{90}. The experiment was repeated for each EPF isolate and conidial concentration using fresh inoculum, on a different date.

Exposure-time-response bioassays

The two effective isolates, *M. robertsii* (6EIKEN) and *M. pinghaense* (5HEID), were used for the exposure – time-response bioassays. Conidial suspensions were prepared following the procedure described above. Insects were exposed to the EPF isolates at a concentration of 10^7 conidia/ml, over five different periods (1, 2, 3, 4, and 5 days). Insect mortality was recorded after each time period, from each bioassay plate. The same inoculation and experimental procedure outlined above was followed, to calculate the lethal time required to kill 50% and 90% of the colony at a concentration of 10^7 conidia/ml over time, LT_{50} and LT_{90} values.

Statistical analysis

Analysis of the obtained data was done using the statistical software, STATISTICA Version 13.5.0.17 (TIBCO Software Inc. 2018). Data was analysed using ANOVA, and the normality of the residuals was checked using normal probability plots. To check the assumption that the variances were homogeneous, the Levene's test was used. LSD (least significant difference) tests and post-hoc tests at 95% confidence intervals were conducted. For the exposure-time-response bioassays, a Logit analysis was conducted to calculate the LT_{50} and LT_{90} values. A Probit analysis was conducted, using the statistical software NCSS 2020 version 20.0.2 (Hintze 2007) and IBM SPSS statistics data editor, version 28, to determine the lethal concentration dose.

RESULTS

Molecular identification of EPF

The information related to the various fungal isolates that were collected across the six farms in the Grabouw, Elgin and Vyeboom areas is indicated (Table 1) (Figure 1). Molecular identification of the isolated EPF using ITS region indicated that the 13 selected morphologically different indigenous isolates belonged to four different EPF species. The EPF species included an isolate of *Cordyceps fumosorosea* (Wize) Kelper, Shrestha and Spatafora, an isolate of *Purpureocillium lilacinum* (Thom) Luangsa-ard, Hou-braken, Hywel-Jones and Samson, two isolates of *B. bassiana* and nine isolates belonging to the *M. anisopliae* species-complex (Table 1). The EF-1α gene region identified the *Metarhizium* species as falling in the PARB clade of the *M. anisopliae* species complex. From the EF-1α region, two were *M. robertsii*, two were *M. pinghaense* and five of the isolates were *M. brunneum* (Table 1). One fungal isolate, not known to be an EPF, namely *Aspergillus tamarii* (Kita) Centralbl. Bakteriol. (MT340979), was also collected during the survey.

Screening of EPF against WAA

Overt mycosis on EPF-infected cadavers of *E. lanigerum* occurred five days following exposure of the insects to the five local EPF isolates (Figure 2). A significant difference in percentage insect mortality due to infection by the EPF isolates was observed (ANOVA; $F_{5,54} = 224.89$; $p < 0.001$) (Figure 3). The highest average percentage infection of *E. lanigerum* was obtained using *M. robertsii* (6EIKEN) (96.7% ± 4.3%), mean ± standard deviation. No significant difference ($p > 0.05$) was observed between *M. robertsii* (6EIKEN) and *M. pinghaense* (5HEID) (91.7% ± 5.6%), and between *M. pinghaense* (5HEID) and *M. brunneum* (3GREY) (87.5% ± 9%). The lowest average percentage mortality was obtained using *C. fumosorosea* (47.5% ± 9.7%). No significant difference was observed between *C. fumosorosea* and *B. bassiana* (51.7% ± 8.6%). The control treatment differed significantly from all treatments. *Beauveria bassiana* and *C. fumosorosea* isolates recorded a significantly lower percentage mortality of *E. lanigerum* than the *Metarhizium* isolates (Figure 3).

EPF dose-response and exposure time bioassays

The *M. robertsii* (6EIKEN) and *M. pinghaense* (5HEID) isolates required similar doses to cause both 50% and 90% insect mortality in colonies of *E. lanigerum*, $LD_{50} = 3.27$ and $LD_{90} = 7.98$, and $LD_{50} = 3.37$ and $LD_{90} = 8.15$, respectively. The probit regression lines for *M. robertsii* (6EIKEN) and *M. pinghaense* (5HEID) were *Y* =

Table 1. Fungal isolates collected during a survey of entomopathogenic fungi (Hypocreales) across six apple farms in the Western Cape province, South Africa.

Farm	GPS reading	No. of isolates	Isolate	Family	Species name	ITS region	EF -1α gene
Grabouw (ARC)	34°08′24.4″S 19°01′14.4″E	4	1ARC	Cordycipitaceae	*Beauveria bassiana*	MT355658	_
			6ARC	Cordycipitaceae	**Cordyceps fumosorosea*	MT333241	_
			4ARC	Ophiocordycipitaceae	***Purpureocillium lilacinum*	MT338935	_
			12ARC	Clavicipitaceae	*Metarhizium robertsii*	MT355666	MT373088
Heideland	34°11′19.9″S 19°02′17.3″E	2	8HEID	Clavicipitaceae	*Metarhizium brunneum*	MT362047	MT373089
			5HEID	Clavicipitaceae	*M. pinghaense*	MT367414	MT895630
Graymead (Fruitways)	34°1′48.29″S 19°7′18.09″E	2	3GREY	Clavicipitaceae	*M. brunneum*	MT374162	MT380848
			6GREY	Clavicipitaceae	*M. brunneum*	–	MT997151
Glen Elgin (Fruitways)	34°8′50.05″S 19°2′24.30″E	1	3GLEN	Clavicipitaceae	*M. brunneum*	MT372486	MT895633
Eikenhof (Fruitways)	34°7′47.75″S 19°2′52.95″E	3	6EIKEN	Clavicipitaceae	*M. robertsii*	MT378171	MT380849
			3EIKEN	Clavicipitaceae	*M. brunneum*	–	MT380847
			12EIKEN	Clavicipitaceae	*M. pinghaense*	–	MT895631
Montheith Trust farm	34°15′45.8″S 19°03′06.1″E	1	2MONT	Cordycipitaceae	*B. bassiana*	MT887635	MT895632

**Cordyceps fumosorosea* (= *Isaria fumosoroseus*)
***Purpureocillium lilacinum* (= *Paecilomyces lilacinus*)

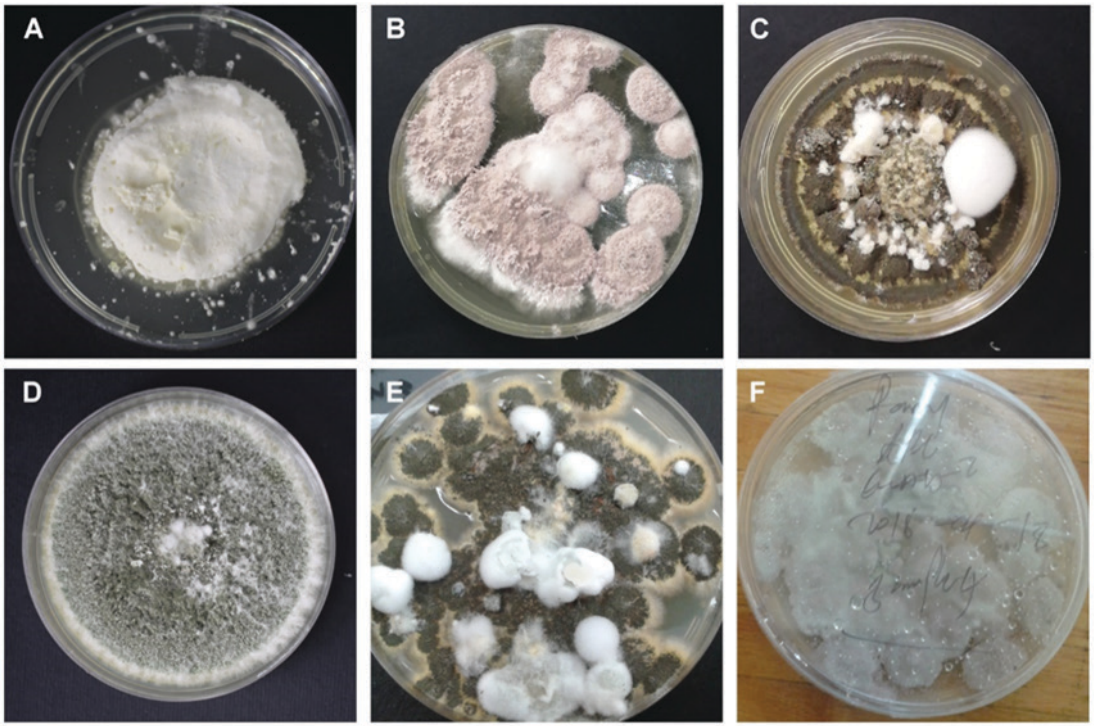

Figure 1. Typical growth, on agar plates, of entomopathogenic fungi isolated from the collected soil samples. **A:** *Beauveria bassiana;* **B:** *Cordyceps fumosorosea;* **C:** *Metarhizium brunneum;* **D:** *Metarhizium robertsii;* **E:** *Metarhizium pinghaense;* **F:** *Purpureocillium lilacinum*

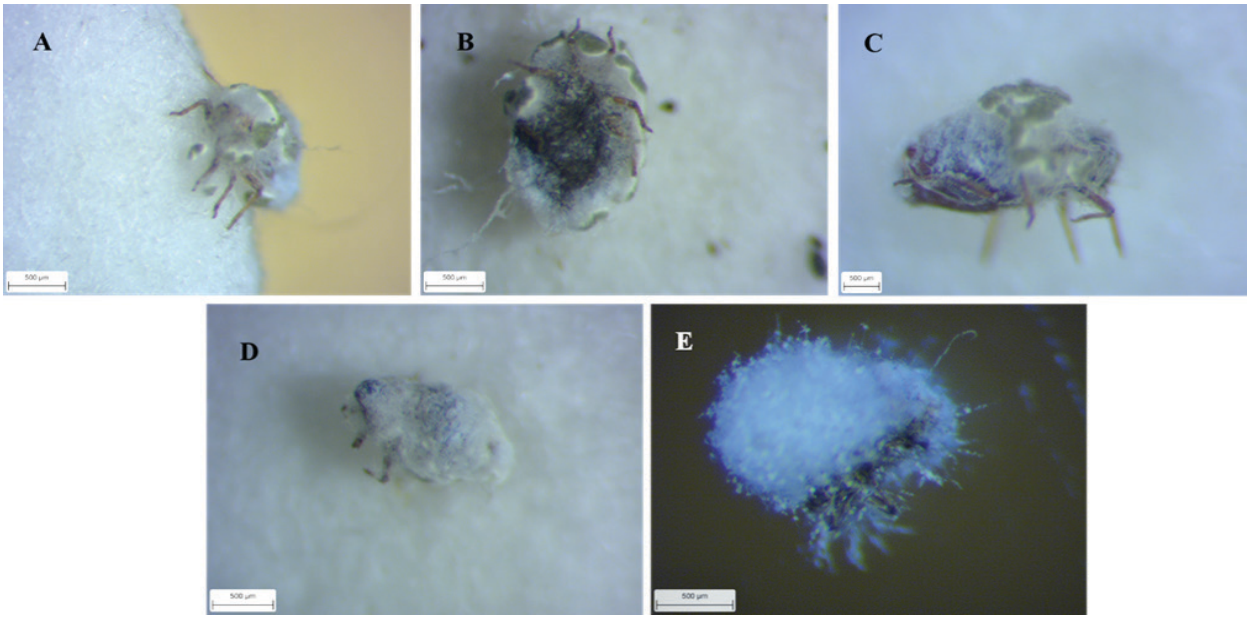

Figure 2. Infected woolly apple aphid cadavers five days post exposure to **(A)** *Metarhizium brunneum,* **(B)** *Metarhizium pinghaense,* **(C)** *Metarhizium robertsii,* **(D)** *Cordyceps fumosorosea*; **(E)** *Beauveria bassiana*

0.5901X + 2.3736 and Y = 0.5995X + 2.2836, respectively, where Y was the probit mortality and X was the log fungal concentration. The data fitted the model well, and a positive correlation was observed between the fungal concentration and insecticidal activity for both EPF isolates (Figure 4). The same amount of time, measured in days, was required to kill 50% of the *E. lanigerum*, for both *M. robertsii* (6EIKEN) (LT_{50} = 2.12) and *M. pinghaense* (5HEID) (LT_{50} = 2.05), at a concentration of 10^7 conidia/ml. To kill 90% of the *E. lanigerum* colonies, the *M. robertsii* required less time (LT_{90} = 4.19) than did *M. pinghaense* (LT_{90} = 4.45).

DISCUSSION

From the study, six different EPF species were obtained across the six apple farms. Collected EPF were all hypocrealean fungal species, which are among the well-known EPF species that are common components of the soil environment microbiota with a cosmopolitan distribution (Chandler et al. 1997; Zimmermann 2007). Among the collected EPF isolates were *C. fumosorosea*, *B. bassiana* and *M. anisopliae* species complex isolates, which are well-known to cause mycosis within various taxa of arthropod, and virtually in all insect orders (Chase et al. 1986; Zimmermann

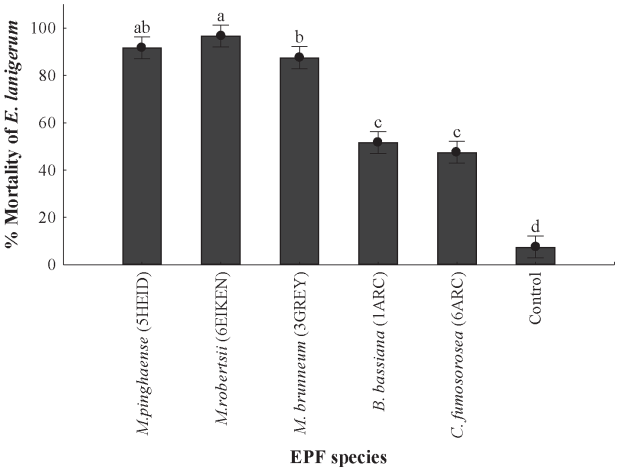

Figure 3. Mean percentage mortality of *Eriosoma lanigerum* (95% confidence interval), using *Metarhizium pinghaense* (5HEID), *M. brunneum* (3GREY), *M. robertsii* (6EIKEN), *Beauveria bassiana* (1ARC) and *Cordyceps fumosorosea* (6ARC) (ANOVA; $F_{5,54}$ = 224.89; $p < 0.001$). Different letters above the bars indicate a significant difference between the different fungal strains and insect mortalities

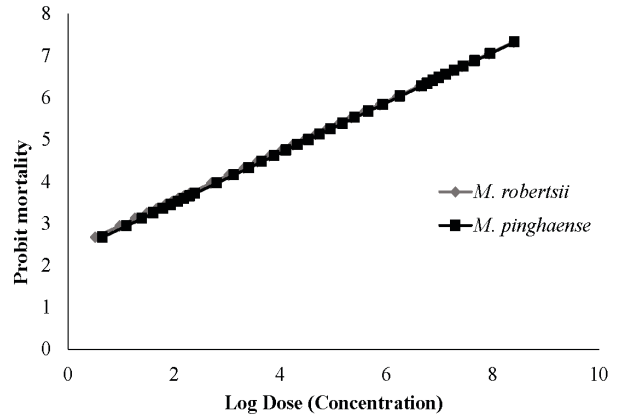

Figure 4. The probit mortality plot of *Eriosoma lanigerum* at five different conidial concentrations (log dose) for *Metarhizium robertsii* (6EIKEN) and *Metarhizium pinghaense* (5HEID), generated using the IBM SPSS statistics data editor, version 28, software program

1986; Meyling and Eilenberg 2007). Similar observations to those that were made in the current study were made in a study conducted by Abaajeh and Nchu (2015), in which fungal isolates belonging to six entomopathogenic fungal species were also successfully isolated from soil samples collected in the Western Cape province, using similar baiting techniques, where collected EPF species included both *M. robertsii* and *P. lilacinum*.

Purpureocillium lilacinum, when previously used against the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), was observed to have a negative effect on the reproduction of this aphid species when present as an endophyte of the plants (Castillo Lopez et al. 2014). Testing of the efficacy of *P. lilacinum* isolate collected during the current study against the WAA was however not possible due to the short survival period of the fungus when grown an artificial media, SDA.

Strains of almost all the collected EPF species have also been commercially developed as mycoinsectides for biological control against various insect pest species across agroecosystems (De Faria and Wraight 2007; Asensio et al. 2003; Shi and Feng 2004). Some of the fungal strains are currently commercialised for use against other insect pests in South Africa (Hatting et al. 2019). Three of the isolated *M. anisopliae* species complex isolates collected during this survey, namely *M. robertsii*, *M. pinghaense* and *M. brunneum*, are known to be of interest as entomopathogens in agricultural ecosystems (Rehner and Kepler 2017; Hatting et al. 2019). In South Africa, five commercial products containing *B. bassiana* are currently registered for the management of insect pests, with the products including BB plus WP®, manufactured by Biological Control Products SA (Pty) Ltd, is registered for use against both aphids and spider mites (De Faria and Wraight 2007). All isolates collected during the current study are also known to affect all the life stages of their host, as they produce toxic metabolites such as beauvericin, oosporein and insecticidal cyclic peptides, known as destruxins (Inglis *et al*. 2001; Zimmermann 2007; Dedryver et al. 2010; Shahid et al. 2012).

Screening of the pathogenicity of the South African EPF isolates at a standard conidial concentration of 10^7 conidia/ml against the WAA, showed the efficacy of local EPF isolates and their potential as possible biological control agents of the WAA. It was observed in this study that the exposure of WAA to the local *Metarhizium* isolates, *M. pinghaense*, *M. robertsii* and *M. brunneum*, resulted in higher percentage mortality of the insects, relative to when the insects were treated using *C. fumosorosea* and *B. bassiana*. This is an indication that the WAA was more susceptible to infection by the *Metarhizium* isolates, relative to other local fungal isolates. Similarly, Mathulwe et al. (2022) when screening South African EPF and EPN isolates for their pathogenicity against the obscure mealybug, *Pseudococcus viburni* (Hemiptera: Pseudococcidae), under laboratory conditions, showed the potential of the same EPF isolates used in the current study, *M. robertsii* (6 EIKEN) and *M. pinghaense* (5HEID), as possible biological control agents against the mealybug, with an average percentage mortality of > 90%, induced by both EPF isolates, tested at an infective concentration 10^7 conidia/ml. Their study also showed high efficacy of two South African *M. brunneum* isolates (DO30 and 3GREY), when used against the insect pest. This indicates that these local South African *Metarhizium* isolates are effective when used against sucking insects and can further be evaluated for their potential as possible biological control agents of the insects under field conditions.

Erler et al. (2014), showed a high efficacy of an isolate of *M. brunneum* (F52) when evaluating the field efficacy of the strain against pear psylla, *Cacopsylla pyri* L. (Hemiptera: Psyllidae), an insect pest of pears that shows some level of resistance against insecticides. In their study, *M. brunneum* F52 was found to be effective against different stages of the pear psylla and was also comparable in efficacy to the standard chemical pesticides used for the management of the insect pest. In other countries, *M. brunneum* has already been successfully commercialised against a variety of insect pests (Rehner and Kepler 2017). *Metarhizium pinghaense*, in a study conducted by Kirubakaran et al. (2018), was found to have some lethal effects when used against the rice leafroller, *Cnaphalocrocis medinalis* (Gunee) (Lepidoptera: Pyralidae), which is a destructive pest of rice crops. Stokwe (2016) also showed the potential of two South African-registered commercial EPF products derived from *B. bassiana* and *M. anisopliae* to be effective against the WAA, resulting in > 70% mortality under laboratory conditions, with no significant difference between the isolates.

Further analyses of the exposure time and the dose-response bioassays, showed no significant difference between the *M. pinghaense* and *M. robertsii* isolates. The results suggest that the *Metarhizium* isolates have similar effect on the WAA colonies, and when applied under field and semi-field conditions they might perform in similar manner, depending on their tolerance to various environmental variables such as changes in temperature, ultra-violet light and humidity. Both

M. pinghaense and *M. robertsii* were also observed to require a short amount of time of about two and four days, to cause 50% and 90% infection and mortality of the WAA, under laboratory conditions, respectively.

In conclusion, the results obtained in the current study provide an insight into the diversity of EPF species across the agricultural lands of the Western Cape province, South Africa. The obtained results of the screening bioassays conducted in the current study also provides useful information by giving an insight into the potential of local EPF isolates as possible biological control agents, which can be used for the control of WAA in apple orchards, specifically *M. brunneum*, *M. robertsii* and *M. pinghaense*. The results from the study also broaden the range of possible biological control agents that can be used in WAA management, since various biotypes of the WAA are well-documented to have developed some level of resistance against a wide range of chemical insecticides, and currently only a few WAA management techniques are available, most of which have proven to be unsuccessful. Further research into the performance of the three fungal species against WAA, under both glasshouse and field conditions, as well as into their persistence under field conditions, should be undertaken.

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ORCID IDs

Letodi L. Mathulwe – https://orcid.org/0000-0002-5118-3578
Antoinette P. Malan – https://orcid.org/0000-0002-9257-0312
Nomakholwa F. Stokwe – https://orcid.org/0000-0003-2869-5652

REFERENCES

Abaajeh AR, Nchu F. 2015. Isolation and pathogenicity of some South African entomopathogenic fungi (Ascomycota) against eggs and larvae of *Cydia pomonella* (Lepidoptera: Tortricidae). Biocontrol Science and Technology 25(7): 828–842. https://doi.org/10.1080/09583157.2015.1019831

Abu-Romman S, Ateyyat M. 2014. Phenotypic and molecular screening of apple genotypes to woolly apple aphid resistance. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 42(1): 99–103. https://doi.org/10.15835/nbha4219460

Asensio L, Carbonell T, López-Jiménez JA, Lopez-Llorca LV. 2003. Entomopathogenic fungi in soils from Alicante province [Spain]. Spanish Journal of Agricultural Research 1(3): 37–45. https://doi.org/10.5424/sjar/2003013-33

Beers EH, Suckling DM, Prokopy RJ, Avilla J. 2003. Ecology and management of apple arthropod pests. In: Ferree D, editor. Apples: Botany, Production and Uses. Wallingford, UK: CABI. p. 489–519. https://doi.org/10.1079/9780851995922.0489

Bhatia V, Uniyal PL, Bhattacharya R. 2011. Aphid resistance in Brassica crops: challenges, biotechnological progress and emerging possibilities. Biotechnology Advances 29(6):879–888. https://doi.org/10.1016/j.biotechadv.2011.07.005

Blackman RL, Eastop VF. 2000. Aphids on the world's crops: An identification and information guide. New York, USA:Wiley.

Brown MW, Schmitt JJ. 1990. Growth reduction in non-bearing apple trees by woolly apple aphids (Homoptera: Aphididae) on roots. Journal of Economic Entomology 83(4): 1526–1530. https://doi.org/10.1093/jee/83.4.1526

Brown MW, Schmitt JJ, Ranger S, Hogmire HW. 1995. Yield reduction in apple by edaphic woolly apple aphid (Homoptera: Aphididae) populations. Journal of Economic Entomology 88(1): 127–133. https://doi.org/10.1093/jee/88.1.127

Castillo Lopez D, Zhu-Salzman K, Ek-Ramos MJ, Sword GA. 2014. The entomopathogenic fungal endophytes *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*) and *Beauveria bassiana* negatively affect cotton aphid reproduction under both greenhouse and field conditions. PLoS One 9(8): e103891. https://doi.org/10.1371/journal.pone.0103891

Chandler D, Hay D, Reid AP. 1997. Sampling and occurrence of entomopathogenic fungi and nematodes in UK soils. Applied Soil Ecology 5(2): 133–141. https://doi.org/10.1016/S0929-1393(96)00144-8

Chase AR, Osborne LS, Ferguson VM. 1986. Selective isolation of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* from an artificial potting medium. Florida Entomology 69(2): 285–292. https://doi.org/10.2307/3494930

Christians GE. 2003. Identification of molecular markers linked to woolly apple aphid (*Eriosoma lanigerum*) (Hausmann) resistance in apple. Ph.D. dissertation, Stellenbosch University, Stellenbosch, South Africa.

Cross JV, Solomon MG, Babandreier D, Blommers L, Easterbrook MA, Jay CN, Jenser G, Jolly RL, Kuhlmann U, Lilley R, et al. 1999b. Biocontrol of pests of apples and pears in northern and central Europe: parasitoids. Biocontrol Science and Technology 9(3): 277–314. https://doi.org/10.1080/09583159929569

Cross JV, Solomon MG, Chandler D, Jarrett P, Richardson PN, Winstanley D, Bathon H, Huber J, Keller B, Langenbruch GA, et al. 1999a. Biocontrol of pests of apples and pears in northern and central Europe: 1. Microbial agents and nematodes. Biocontrol Science and Technology 9(2): 125–149. https://doi.org/10.1080/09583159929721

Damavandian MR, Pringle KL. 2002. Development of a system for sampling population levels of subterranean *Eriosoma lanigerum* (Homoptera: Aphididae) in apple orchards. African Entomology 10(2): 341–344.

Damavandian MR, Pringle KL. 2007. The field biology of subterranean populations of the woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae), in South African apple orchards. African Entomology 15(2): 287–294. https://doi.org/10.4001/1021-3589-15.2.287

Damavandian MR. 2000. Biology of subterranean populations of woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Homoptera: Aphididae), in apple orchards. Ph.D dissertation, Stellenbosch University, Stellenbosch, South Africa.

De Faria MR, Wraight SP. 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biological Control 43(3): 237–256. https://doi.org/10.1016/j.biocontrol.2007.08.001

Dedryver CA, Le Ralec A, Fabre F. 2010. The conflicting relationships between aphids and men: a review of aphid damage and control strategies. Comptes Rendus Biologies 333(6–7): 539–553. https://doi.org/10.1016/j.crvi.2010.03.009

Ekesi S, Maniania N, Lux S. 2002. Mortality in three tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. Biocontrol Science and Technology 12(1): 7–17. https://doi.org/10.1080/09583150120093077

Erler F, Pradier T, Aciloglu B. 2014. Field evaluation of an entomopathogenic fungus, *Metarhizium brunneum* strain F52, against pear psylla, *Cacopsylla pyri*. Pest Management Science 70(3): 496–501. https://doi.org/10.1002/ps.3603

Gillespie AT, Moorhouse ER. 1989. The use of fungi to control pests of agricultural and horticultural importance. In: Whipps JM, Lumsden RD, editors. Biotechnology of Fungi for Improving Plant Growth. Cambridge, UK: University of Cambridge; p. 55–84.

Goble TA, Dames JF, Hill MP, Moore SD. 2010. The effects of farming system, habitat type and bait type on the isolation of entomopathogenic fungi from citrus soils in the Eastern Cape Province, South Africa. BioControl 55(3): 399–412. https://doi.org/10.1007/s10526-009-9259-0

Gontijo LM. 2011. Integrated biological control of woolly apple aphid in Washington State. Ph.D. dissertation. Washington State University, Pullman, WA, USA.

Gresham SDM. 2013. Ecological and logistical considerations toward introducing *Heringia calcarata* to New Zealand. Ph.D. dissertation. Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.

Harris, S.A., Robinson, J.P. & Juniper, B.E. 2002. Genetic clues to the origin of the apple. Trends in Genetics 18(8): 426–430. https://doi.org/10.1016/S0168-9525(02)02689-6.

Hatting JL, Moore SD, Malan AP. 2019. Microbial control of phytophagous invertebrate pests in South Africa: current status and future prospects. Journal of Invertebrate Pathology 165: 54–66. https://doi.org/10.1016/j.jip.2018.02.004.

Heunis JM, Pringle KL. 2003. The susceptibility of *Aphelinus mali* (Haldeman), a parasitoid of *Eriosoma lanigerum* (Hausmann), to pesticides used in apple orchards in the Elgin area, Western Cape province, South Africa. African Entomology 11(1): 91–95.

Heunis JM, Pringle KL. 2006. Field biology of woolly apple aphid, *Eriosoma lanigerum* (Hausmann), and its natural enemy, *Aphelinus mali* (Haldeman), in apple orchards in the Western Cape province. African Entomology 14(1): 77–86.

Inglis GD, Enkerli J, Goettel MS. 2012. Laboratory techniques used for entomopathogenic fungi: Hypocreales. In: Lacey LA, editor. Manual of Techniques in Invertebrate Pathology. 2nd ed. Cambridge, MA, USA: Academic Press. p. 189–253. https://doi.org/10.1016/B978-0-12-386899-2.00007-5

Inglis GD, Goettel MS, Butt TM, Strasser H. 2001. Use of hyphomycetous fungi for managing insect pests. In: Butt TM, Jackson C, Magan N, editors. Fungi as Biocontrol Agents: Progress, Problems and Potential. Wallingford, UK: CAB International. p. 23–69. https://doi.org/10.1079/9780851993560.0023

Kirubakaran SA, Abdel-Megeed A, Senthil-Nathan S. 2018. Virulence of selected indigenous *Metarhizium pingshaense* (Ascomycota: Hypocreales) isolates against the rice leaffolder, *Cnaphalocrocis medinalis* (Guenèe) (Lepidoptera: Pyralidae). Physiological and Molecular Plant Pathology 101: 105–115. https://doi.org/10.1016/j.pmpp.2017.06.004

Krassilstschik J. 1888. Industrial production of entomopathogens for the destruction of harmful insects. Bulletin Scientifique de la France et de la Belgique 19: 461–472.

Lacey LA, Solter LF. 2012. Initial handling and diagnosis of diseased invertebrates. In: Manual of Techniques in Invertebrate Pathology. 2nd ed. p. 1–14. Cambridge, MA, USA: Academic Press. https://doi.org/10.1016/B978-0-12-386899-2.00001-4

Lordan J, Alegre S, Gatius F, Sarasúa MJ, Alins G. 2015. Woolly apple aphid *Eriosoma lanigerum* Hausmann ecology and its relationship with climatic variables and natural enemies in Mediterranean areas. Bulletin of Entomological Research 105(01): 60–69. https://doi.org/10.1017/S0007485314000753.

Mathulwe LL, Malan AP, Stokwe NF. 2022. Laboratory screening of entomopathogenic fungi and nematodes for pathogenicity against the obscure mealybug, *Pseudococcus viburni* (Hemiptera: Pseudococcidae). Biocontrol Science and Technology 32: 397–417. https://doi.org/10.1080/09583157.2021.2010653

Meyling NV, Eilenberg J. 2007. Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control. Biological Control 43(2): 145–155. https://doi.org/10.1016/j.biocontrol.2007.07.007

Meyling NV. 2007. Methods for Isolation of Entomopathogenic Fungi from the Soil Environment – Laboratory Manual. Denmark: University of Copenhagen.

Mols PJM, Boers JM. 2001. Comparison of a Canadian and a Dutch strain of the parasitoid *Aphelinus mali* (Hald) (Hym. Aphelinidae) for control of woolly apple aphid *Erisoma lanigerum* (Haussmann) (Hom. Aphididae) in the Netherlands: a simulation approach. Journal of Applied Entomology 125(5): 255–262. https://doi.org/10.1046/j.1439-0418.2001.00543.x.

Moss R, Jackson RR, Pollard SD. 2006. Mask of wax: secretions of wax conceal aphids from detection by spider's eyes. New Zealand Journal of Zoology. 33(3): 215–220. https://doi.org/10.1080/03014223.2006.9518448.

Nicholas AH. 2000. The pest status and management of woolly aphid in an Australian apple orchard IPM program. Ph.D. dissertation, University of Western Sydney, Sydney, Australia.

Nicholas AH, Spooner-Hart RN, Vickers RA. 2005. Abundance and natural control of the woolly aphid *Eriosoma lanigerum* in an Australian apple orchard IPM program. BioControl 50(2): 271–291. https://doi.org/10.1007/s10526-004-0334-2

Pringle KL. 1998. The use of imidacloprid as a soil treatment for the control of *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae). Journal of the Southern African Society for Horticultural Sciences 8: 55–56.

Pringle KL, Giliomee JH, Addison MF. 1994. Vamidothion tolerance in a strain of the woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae). African Entomology 2(2): 123–125.

Pu XY, Feng MG, Shi CH. 2005. Impact of three application methods on the field efficacy of a *Beauveria bassiana* based mycoinsecticide against the false-eye leafhopper, *Empoasca vitis* (Homoptera: Cicadellidae) in the tea canopy. Crop Protection 24(2): 167–175. https://doi.org/10.1016/j.cropro.2004.07.006.

Quesada-Moraga E, Navas-Cortés JA, Maranhao EA, Ortiz-Urquiza A, Santiago-Álvarez C. 2007. Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. Mycological Research 111(8): 947–966. https://doi.org/10.1016/j.mycres.2007.06.006

Rehner SA, Kepler RM. 2017. Species limits, phylogeography and reproductive mode in the *Metarhizium anisopliae* complex. Journal of Invertebrate Pathology 148: 60–66. https://doi.org/10.1016/j.jip.2017.05.008

Shahid AA, Rao QA, Bakhsh A, Husnain T. 2012. Entomopathogenic fungi as biological controllers: new insights into their virulence and pathogenicity. Archives of Biological Sciences 64(1)21–42. https://doi.org/10.2298/ABS1201021S

Shi WB, Feng MG. 2004. Lethal effect of *Beauveria bassiana, Metarhizium anisopliae,* and *Paecilomyces fumosoroseus* on the eggs of *Tetranychus cinnabarinus* (Acari: Tetranychidae) with a description of a mite egg bioassay system. Biological Control 30(2):165–173. https://doi.org/10.1016/j.biocontrol.2004.01.017

Short BD. 2003. Inaugural studies of the life history and predator/prey associations *of Heringia calcarata (Loew) (Diptera: Syrphidae*), a specialist predator of the woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Homoptera: Eriosomatidae). Ph.D. dissertation, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.

Smith RG. 1999. Wax glands, wax production and the functional significance of wax use in three aphid species (Homoptera: aphididae). Journal of Natural History 33(4): 513–530. https://doi.org/10.1080/002229399300227

Stoeckli S, Mody K, Gessler C, Patocchi A, Jermini M, Dorn S. 2008. QTL analysis for aphid resistance and growth traits in apple. Tree Genetics & Genomes 4(4): 833–847. https://doi.org/10.1007/s11295-008-0156-y

Stokwe NF. 2016. Efficacy of entomopathogenic nematodes and fungi as biological control agents of woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae) under South African conditions. PhD dissertation, Stellenbosch University, Stellenbosch, South Africa.

Stokwe NF, Malan AP. 2016. Woolly apple aphid, *Eriosoma lanigerum* (Hausmann), in South Africa: biology and management practices, with focus on the potential use of entomopathogenic nematodes and fungi. African Entomology 24(2): 267–278. https://doi.org/10.4001/003.024.0267

Stokwe NF, Malan AP. 2017. Laboratory bioassays to determine susceptibility of woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae), to entomopathogenic nematodes. African Entomology 25(1): 123–136. https://doi.org/10.4001/003.025.0123

Thwaite WG, Bower CC. 1983. Woolly Aphid. Agfact H4. AE. 3. NSW Agriculture, Orange, Australia.

Tibco Software Inc. 2018. Statistica, version 13.3. https://statistica.io

Timm AAE. 2003. Genetic diversity of root-infesting woolly apple aphid *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae) populations in the Western Cape. Ph.D. dissertation, Stellenbosch University, Stellenbosch, South Africa.

Van Zyl C, Malan AP. 2015. Cost-effective culturing of *Galleria mellonella* and *Tenebrio molitor* and entomopathogenic nematode production in various hosts. African Entomology 23(2): 361–375. https://doi.org/10.4001/003.023.0232

Vega FE, Goettel MS, Blackwell M, Chandler D, Jackson MA, Keller S, Koike M, Maniania NK, Monzón A, Ownley BH, et al. 2009. Fungal entomopathogens: new insights on their ecology. Fungal Ecology 2(4): 149–159. https://doi.org/10.1016/j.funeco.2009.05.001

Wraight SP, Carruthers R, Bradley CA, Jaronski ST, Lacey LA, Wood P, Galaini-Wraight S. 1998. Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silverleaf whitefly, *Bemisia argentifolii*. Journal of Invertebrate Pathology 71(3): 217–226. https://doi.org/10.1006/jipa.1997.4734

Zimmermann G. 1986. The Galleria bait method for detection of entomopathogenic fungi in soil. Journal of Applied Entomology 102(1–5): 213–215. https://doi.org/10.1111/j.1439-0418.1986.tb00912.x

Zimmermann G. 2007. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Science and Technology 17(6): 553–596. https://doi.org/10.1080/09583150701309006