BioControl (2020) 65:197–209
https://doi.org/10.1007/s10526-019-09989-3

Association between chemical properties of vineyard soils and occurrence of entomopathogenic fungi causing different levels of mortality in *Planococcus ficus*

Siphokazi Moloinyane · Pia Addison · Kwaku A. Achiano · Felix Nchu

Received: 3 January 2019 / Accepted: 12 December 2019 / Published online: 2 January 2020

Abstract Global demand for environmentally friendly grapevine cultivation and pest control has necessitated an improved understanding of the relationship between soil properties and beneficial, naturally occurring antagonists such as entomopathogenic fungi (EPF). Sixty six soil samples were collected from 22 vineyards in the Western Cape, South Africa. The association between soil nutrient status and EPF prevalence was examined. Fungi were isolated with methods of insect baiting and selective media. In addition, fungal isolates were tested against a key grapevine pest, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae). Pathogenicity of fungal strains against *P. ficus* was assessed with an immersion bioassay at a concentration of 1×10^8 conidia ml^{-1}. Twenty-three fungal strains were isolated and correspondence analysis of data indicated a positive association between EPF (*Metarhizium robertsii* (Hypocreales: Clavicipitaceae) and *Clonostachys rosea f. catenulata* [Hypocreales: Bionectriaceae]) occurrence and optimum to high levels of soil-based macronutrients (C, N and Ca). Logistic regression revealed that K was positively correlated with *M. robertsii* (estimate = 0.03 ± 0.01; $P < 0.05$; odds ratio = 1.03). Strains of *Beauvaria bassiana* (Hypocreales: Cordycipitaceae) caused the highest mortalities (77.0 ± 2.0% to 87.0 ± 3.0%). This study showed that some soil nutrient properties corresponded to greater occurrence of EPF in grapevine soils.

Keywords Biological control · Entomopathogenic fungi · Grapevine · vine mealybug · Soil nutrient content

Handling Editor: Helen Roy

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10526-019-09989-3) contains supplementary material, which is available to authorized users.

S. Moloinyane · F. Nchu (✉)
Department of Horticultural Sciences, Cape Peninsula University of Technology, Symphony Way, Bellville, P.O. Box 1906, Bellville 7535, South Africa
e-mail: felixnchu@gmail.com

P. Addison
Department of Ecology and Entomology, Faculty of AgriSciences, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa

K. A. Achiano
ARC-Infruitec/Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa

Introduction

Conventional control of arthropod pests in agricultural production, including vineyards, has depended on synthetic chemicals, which have been liable contributors to environmental degradation (Gaskin et al.

2002; Huber et al. 2000). This has necessitated the quest for alternative and more environmentally benign control strategies, which has led to recognition of biological control agents such as entomopathogenic fungi, EPF (Epstein and Bassein 2003; Fernandez-Cornejo 1998; Strasser et al. 2000). EPF are generally ubiquitous (Goettel 1984). However, farming systems influence fungal species distribution (Chandler et al. 1997; Goble 2009). For example, Goble (2009) recovered significantly more isolates of the EPFs *B. bassiana* (Hypocreales: Cordycipitaceae) from organically farmed soils than conventionally farmed soils of citrus orchards in the Eastern Cape Province of South Africa. Farming activities, such as grapevine cultivation, disrupt natural biotic and abiotic conditions, causing ecological disequilibrium, which can affect insect occurrences as well as those of their natural enemies, including EPF (Bruck 2010; Crous et al. 2017; Pell et al. 2010).

Rhizospheric soil EPF can influence plant performance and nutrient cycling and may be used in agroecological systems to improve crop production (Bruck 2009; Raya-Dáaz et al. 2017). However, the abundance of soil microorganisms is affected by soil nutrient content in space and time (Koorem et al. 2014). For example, Koorem et al. (2014) observed that abundance of arbuscular mycorrhizal fungi was negatively related to soil phosphorus and positively influenced by soil nitrogen content. Studies such as those by Klingen et al. (2002) and Quesada-Moraga et al. (2007) have already found a positive correlation between the level of soil organic matter content and the presence of EPF species, such as *B. bassiana* and *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae), which they attributed to the release of nitrogen during the mineralization of soil organic matter. Sharma et al. (2018b) demonstrated that high C/N ratio and lower exchangeable K^+ favor higher abundance of entomopathogenic *Fusarium oxysporum* in soils from Douro Vineyards in Portugal and adjacent hedgerows. More recently, Uzman et al. (2019) recorded an increase of *Metarhizium* spp. with bioavailable copper content, increasing C/N ratio and conventional management in vineyards in Germany. These findings suggest that soil chemical properties influence occurrence of EPF. The ecological relationship between soil nutrient status and EPF needs to be further understood (Safavi et al. 2007). Given the current heightened appreciation of the integral role of EPF in agronomic production, it is expected that nutrient management guidelines for many crops, including grapevine, should not only enable optimum grapevine yield, but also promote optimum soil EPF occurrence. It was, therefore, hypothesized that EPF occurrence is associated with the prescribed optimum ranges of soil nutrients in the Cape Winelands of South Africa. Hence, we investigated which soil nutrients and soil nutrient levels were associated with the occurrence of soil-borne EPF in grapevines of the Cape Winelands.

The Cape Winelands is the main grapevine-growing region in South Africa, and it is characterized by a Mediterranean climate with intense sunlight, dry heat in summer and cold, wet winters (du Plessis and Schloms 2017; Jones and Davis 2000). Most of the vineyards in this area use conventional cultivation approaches to achieve high yields, and control insects and phytopathogens. Nevertheless, insect pests, such as the grapevine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), still remain serious obstacles to achieving optimum crop production. It is a key pest in vineyards in many countries including South Africa, causing direct crop losses to table and wine grapes, and it is a vector of the notorious grapevine leafroll-associated virus (Daane et al. 2012; Walton and Pringle 2004). Although Sharma et al. (2018a) has examined the insect associated fungi occurring inside naturally mycosed vine mealybug, to the best of our knowledge this is the first study that examined the occurrence of EPF in the Cape Winelands of southern Africa and the relationships between EPF presence and soil nutrient content on grapevine farms. The objectives of this study were to (1) survey the occurrence of EPF in vineyard soils in the Cape Winelands, (2) explore the relationship between soil chemical properties and presence of EPF in the Cape Winelands, and (3) ascertain their pathogenicity against the grapevine mealybug.

Materials and methods

Soil sampling

A total of 66 soil samples were collected from 22 vineyards in the Western Cape, South Africa in the spring (October to November) of 2016, when minimum temperatures were between 12 and 16 °C and

maximum temperatures 22–26 °C. These selected vineyards were located in five of the Western Cape's major wine production areas, namely Stellenbosch, Constantia, Franschoek, Paarl and Worcester (Table 1). At each vineyard, three blocks of grapevine were randomly selected, 200 m apart. Soil samples were collected with a garden spade at a depth of 15–20 cm after removal of surface debris. Soil samples, 300 g and 1 kg in weight, were put into transparent plastic containers and paper bags, respectively. The plastic containers and bags were sealed with rubber bands. The 1 kg bags of soil were sent to Bemlab Pty. Ltd. (Somerset West, Western Cape, South Africa) for soil analysis and the 300 g soil samples were transported to the laboratory, air-dried and sieved using a 2-mm sieve, and then used in the process of fungal isolation within 24 h of collection.

Isolation of entomopathogenic fungi

Fungi were isolated from the soil samples by baiting with fifth instar larvae of *Cydia pomonella* (Lepidoptera: Tortricidae) obtained from a continuous laboratory culture held at Entomon Pty. Ltd. (Stellenbosch, Western Cape, South Africa) and fed on an artificial diet mixture described by Stenekamp (2011). Fifth instar larvae had been previously used to isolate 39 EPF isolates from soils in various locations of the Western Cape Province of South Africa (Abaajeh and Nchu 2015). Fifty grams of each soil sample was passed through a metal sieve with a mesh size of 4 mm and transferred to a plastic container, and then sprayed with sterile distilled water until moist. Fifteen fifth instar *C. pomonella* larvae were placed on the surface of each soil sample in the plastic container and incubated in the dark at 25 °C. Containers were briefly inverted and returned to upright position once per day for the first week to increase contact between insects and soil particles and checked for dead larvae every three to four days for three weeks. Dead larvae were surface sterilized with 70% (v/v) ethanol for 30 s, rinsed with sterile distilled water for 1 min, and then placed on moistened filter paper and incubated at 25 °C. Sporulating larvae were placed on a selective media of half strength potato dextrose agar (PDA, Sigma-Aldrich Pty. Ltd., South Africa) (19.5 g l^{-1}) supplemented with 0.02 g l^{-1} penicillin and 0.04 g l^{-1} streptomycin (Tuininga et al 2009; Gouli et al. 2013). Fungi were also isolated using a plating soil method (Tkaczuk et al. 2014). A mixture of soil (100 mg) and sterile 0.05% (v/v) Tween 80 (Sigma-Aldrich Pty. Ltd., South Africa) was agitated for 10 min to form a suspension. The suspension (50 µl) was plated on medium in Petri dishes (9 cm diameter) made-up of half strength PDA (19.5 g l^{-1}) supplemented with 0.02 g l^{-1} penicillin and 0.04 g l^{-1} streptomycin per soil sample. Each soil sample had five replicates.

Fungal identification

To obtain single spore isolates the method described by Ho and Ko (1997) was followed. Conidia from the cultures of each fungal isolate were harvested and surface cultured on half strength PDA at 25 ± 2 °C and 70 ± 2% RH for three to four weeks. Twenty-three fungal cultures were transferred to the Molecular Biology Laboratory in the Department of Microbiology, Stellenbosch University, South Africa for a more precise identification. All strains were plated on malt extract agar, Czapek yeast agar and oatmeal agar for initial grouping. The morphology was determined from structures mounted in lactic acid, using a Nikon Eclipse E800 light microscope. Isolates were first identified based on morphological and cultivation characteristics as described in the literature (Anwar et al. 2018; Bischoff et al. 2009; Kepler et al. 2014; Nelson et al. 1983). For molecular characterization, DNA was extracted from fresh cells using the ZR fungal/bacterial DNA kit (Zymo Research, California, USA) and the presence of genomic DNA confirmed on a 1% (w/v) agarose gel, stained with ethidium bromide. PCR reactions were performed using a GeneAmp PCR System 9700 (Applied Biosystems, USA). The reaction mixture contained 0.5 µl (± 50 ng μl^{-1}) of the purified genomic DNA, 500 nM of each primer and 5 µl of 2X Kapa Taq Ready mix (Kapa Biosystems, South Africa) in a total volume of 10 µl. Genomic loci amplified in this study included the internal transcribed spacer region of the fungal 28S ribosomal DNA (ITS) (ITS1 [5′-TCCGTAGGTGAACCTTGCGC-3′; forward primer] and ITS4 [5′-TCCTCCGCTTATTGATATGC-3′; reverse primer]) (White et al. 1990), and the β-tubulin gene (Bt2a [5′-GGTAACCAAATCGGTGCTGCTTTC-3′; forward primer] and Bt2b [5′-ACCCTCAGTGTAGTGACCCTTGGC-3′; reverse primer]) (Glass and Donaldson 1995). The PCR conditions for the ITS region

Table 1 Locations within the Western Cape Winelands, South Africa where soils were sampled, fungal species isolated and Abbott-corrected insect mortality means ± SE (%) caused by selected fungal species and isolates (1×10^8 spores ml^{-1}) against adult females of *Planococcus ficus* at four days post-treatment

Location	Site	GPS coordinates	*Fungal isolate	Isolation method I (Insect bait) S (Soil dilution)	*Fungal species	Abbott-corrected mortality ± SE
Stellenbosch	Site 1	18.492800°–33.532048°	SM3	I	*Beauveria bassiana*	87.0 ± 3.0
			CPUT15	S	*Metarhizium robertsii*	44.0 ± 5.0
			SM4A	S	*Clonostachys rosea f. catenulata*	46.0 ± 1.0
	Site 2	18.749890°–34.015281°	SM6	S	*Clonostachys rosea f. catenulata*	69.0 ± 10.0
			SMA	I	*Beauveria bassiana*	77.0 ± 2.0
			SM2	I	*Fusarium falciforme*	–
	Site 3	18.873310°–33.923300°	–	–	–	–
	Site 4	19.280972°–34.236746°	SM7	I	*Fusarium inflexum*	–
			SM5	S	*Talaromyces sayulitensis*	
	Site 5	18.767340°–33.957130°	CPUT14	S	*Talaromyces pinophilus*	–
			CPUT17	S	*Talaromyces ruber*	–
	Site 6	18.913177°–34.009773°	–	–	–	–
Constantia	Site 7	18.426574°–34.043625°	CPUT16	I	*Metarhizium robertsii*	23.0 ± 6.0
			CPUT20	S	*Talaromyces pinophilus*	–
	Site 8	18.411317°–34.045568°	–	–	–	–
	Site 9	18.404093°–34.034385°	CPUT19	I	*Metarhizium robertsii*	46.0 ± 1.0
			SM8	S	*Clonostachys rosea f. catenulata*	52.0 ± 1.0
	Site 10	19.320406°–34.059153°	SMB	I	*Fusarium solani*	44.0 ± 1.0
	Site 11	19.320306°–34.062953°	SM9	S	*Clonostachys rosea f. catenulata*	18.0 ± 1.1
Paarl	Site 12	19.002860°–33.720840°	–	–	–	–
	Site 13	19.117831°–33.918309°	–	–	–	–
	Site 14	18.958800°–33.766000°	SM10	I	*Clonostachys rosea f. catenulatata*	50.0 ± 2.0
			SM13	I	*Beauveria bassiana*	82.0 ± 2.0

Table 1 continued

Location	Site	GPS coordinates	*Fungal isolate	Isolation method I (Insect bait) S (Soil dilution)	*Fungal species	Abbott-corrected mortality ± SE
Malmesbury	Site 15	20.014027°–33.988441°	CPUT18	S	*Clonostachys rosea f. catenulatata*	32.0 ± 2.0
	Site 16	19.987705°–33.991239°	–	–	–	–
	Site 17	19.987805°–33.991239°	SM1	S	*Fusarium solani*	56.0 ± 1.0
	Site 18	20.127851°–33.887736°	–	–	–	–
Franschoek	Site 19	20.127851°33.887636°	–	–	–	–
	Site 20	19.117514°–33.907261°	SM4B	S	*Aspergillus oryzae*	–
	Site 21	18.413525°–34.015845°	SM12	I	*Aspergillus laciniosus*	–
	Site 22	19.167682°–33.551830°	CPUT22	I	*Metarhizium robertsii*	31.0 ± 0.6
Logistic regression (logit link function)			$\chi^2 = 151.7$; df = 14		$\chi^2 = 56.2$; df = 3	
			$P < 0.001$		$P < 0.001$	

*Denotes insect mortality is significantly associated with fungal species and fungal isolate at $P < 0.05$ following logistic regression analysis

consisted of an initial denaturing step at 94 °C for 5 min followed by 30 cycles each of denaturing at 94 °C for 30 s, annealing at 56 °C for 30 s and elongation at 72 °C for 45 s. The PCR conditions for the amplification of the β-tubulin (BTub) gene consisted of an initial denaturing step at 94 °C for 5 min, followed by 35 cycles each of denaturing at 94 °C for 45 s, annealing at 56 °C for 45 s and elongation at 72 °C for 1 min. The reactions were completed with a final elongation for 7 min at 72 °C, and then held at 4 °C. PCR samples were separated on a 1% (w/v) agarose gel, stained with ethidium bromide and visualized using ultraviolet light. The amplicons from the PCR reactions were sequenced on an ABI 3010xl Genetic Analyser at the Central Analytical Facility (CAF, Stellenbosch University, South Africa). DNA sequences were submitted to BLASTn (nucleotide BLAST; https://www.ncbi.nlm.nih.gov) to identify potential hits for the isolates. Sequence alignment was performed using Clustal X and phylogenetic trees were generated using PAUP*. Distance analyses were performed using the neighbour-joining method, with the substitution model set to TrN + G and the branch support was calculated with a 1000 bootstrap repetitions. DNA sequences of the fungal isolates were submitted to NCBI GenBank and accession numbers obtained (Supplementary Table 1). Fungal cultures are kept in the germplasm of CPUT and Department of Microbiology, Stellenbosch University, South Africa.

Soil analysis

Soil was air dried and sieved (2 mm sieve) prior to tests. Total P, K, Ca, Mg were determined as described in Campbell and Plank (1998) with slight modifications, and the pH was measured as described by The Non-Affiliated Soil Analyses Work Committee (1990). Optical total C and N were determined using total combustion through the use of a LecoTruspec® C/N analyzer. A guide containing the recommended nutrient levels of each nutrient needed for optimum cultivation of grapevine in the Western Cape Province

of South Africa was used to categorize nutrient levels in soil into low, optimum and high. This guide was obtained from Bemlab Pty. Ltd., a commercial testing laboratory. Bemlab is accredited by the South African National Accreditation System (SANAS) and complies with ISO/IEC 17,025 standards of SANAS. Optimum C/N ratio level were adapted from Cooke (1968).

Assessing pathogenicity against *Planococcus ficus*

Prior to assessing pathogenicity, the viability of conidia was determined by spread-plating 0.1 ml of conidia suspension, titrated to 3×10^6 conidia ml^{-1} on PDA plates. Two replicated sterile microscope cover slips were placed on each plate and incubated at 26 ± 2 °C. Plates were then examined after 24 h and percentage germination determined from 100 spore counts under each cover slip. The germination percentage was over 90%.

An immersion bioassay was used to assess the pathogenic effect of 15 EPF isolates (belonging to four species selected on the basis of their reported pathogenicity on insects) collected from the sampled soils against *P. ficus*. Adult female *P. ficus* were obtained from the ARC Infruitec-Nietvoorbij (Agricultural Research Council) Stellenbosch, South Africa courtesy of Dr. K.A. Achiano. The mealybugs were reared on butternut squash in a darkroom at 25 °C and 60% RH. The mealybug cultures were maintained in darkness to prevent crawlers from walking off the butternut squash because they are attracted to light. Three to four week-old aerial conidia of the different fungal isolates were harvested from the single-conidium cultures by scraping, and then suspended separately in 50 ml centrifuge tubes containing 25 ml of sterile 0.05% (v/v) Tween 80 and glass beads. Each suspension was mixed vigorously using a vortex shaker for 5 min to homogenize it. The conidial concentration was determined using an improved Neubauer haemocytometer. The fungal isolates were tested at a standardized conidial concentration of 1×10^8 conidia ml^{-1}. The control solution had sterile 0.05% (v/v) Tween 80. Insects were dipped individually into 3 ml conidial suspensions or the control solution for 30 s. The mealybugs were handled with care by means of fine forceps. For each treatment, ten adult female insects were placed in a 9 cm diameter Petri dish containing a disk (6 cm in diameter) of sterilized leaves of *Vitis vinifera* 'Cabernet Sauvignon', replicated five times. The Petri dishes were kept at 25 °C, 60% RH and L:D 12:12 in a growth chamber. Mortality data was recorded on the fourth day because insect mortality in the control treatment was often higher than 20% beyond four days post-treatment. Insect cadavers were surface sterilised in 70% ethanol for 5 s, rinsed in sterile distilled water for 1 min and incubated at 25 ± 2 °C and 90 ± 5% RH, and mycosis confirmed under a dissecting microscope. No mycosis was observed on the control insects. Generally, high occurrence of mycosis was observed on the cadavers of fungus treated insects.

Statistical analysis

Data on insect mortality in the immersion bioassay were corrected following Abbott (1925). The effects of fungal species and fungal isolates on insect mortality were determined using logistic regression with a logit link function. The statistical level of significance was fixed at P = 0.05. The relationship between soil nutrient content level and EPF (*B. bassiana*, *Metarhizium robertsii* and *C. rosea f. catenulata*) presence was determined with a correspondence analysis (CA) using the statistical software PAST (Hammer et al. 2001). This was followed by logistic regression analysis, with a logit link function, to establish the association between the concentrations of the different nutrients (independent and continuous variable) and the occurrence (presence or absence) of fungi (dependent and dichotomous variable) isolated from soil samples using the Statistical Package for Social Sciences (SPSS) software (version 16, SPSS, Inc., Chicago, IL, USA).

Results

Isolation and identification of entomopathogenic fungi

A total of 23 fungal isolates were obtained from 63.0% of sampled vineyards comprising 11 different species with some sites yielding multiple isolates (Table 1). The isolated fungal species were: *M. robertsii* (Hypocreales: Clavicipitaceae), *Fusarium inflexum*, *Fusarium falciforme*, *Fusarium solani* (Hypocreales: Nectriaceae), *B. bassiana* (Hypocreales:

Cordycipitaceae), *C. rosea f. catenulata* (Hypocreales: Bionectriaceae), *Talaromyces pinophilus*, *Talaromyces ruber*, *Talaromyces sayulitensis* (Eurotiales: Trichocomaceae), *Aspergillus oryzae* and *Aspergillus laciniosus* (Eurotiales: Trichocomaceae). The predominant species isolated from vineyard soil was *C. rosea f. catenulata*, which was recorded at 26.0% (six out of 23 isolates) and these isolates were mostly obtained by the soil dilution method (Table 1). On the other hand, isolates of the two well-known EPF species (*M. robertsii and B. bassiana*) were frequently collected using the insect bait method (Table 1). The BLASTn results based on ITS and BTub primers are presented in Supplementary Table S1. Neighbour-joining trees (based on ITS and BTub sequences) showing relationships in the different genera are presented in Supplementary Fig. S1.

Influence of soil nutrient status on the occurrence of entomopathogenic fungi

Soil nutrient levels and pH for all sites are presented in Table 2. The nutrient data were categorized into high, optimum and low levels, and these were used in a CA to establish the relationship between soil nutrient level and fungal occurrence in the different sites. The first two axes of the CA explained 45.0% of the variation of the data (Fig. 1). Based on the CA loadings, there was a positive association between optimum to high soil nutrient content (Ca, C and N) and fungal (*M. robertsii* and *C. rosea f. catenulata*) occurrence (Fig. 1). Logistic regressions (overall model fit) were significant for the relationship between N ($\chi^2 = 4.9$; df = 1; $P = 0.03$), P ($\chi^2 = 4.5$; df = 1; $P = 0.03$) or K ($\chi^2 = 8.7$; df = 1; $P = 0.003$) and occurrence of *C. rosea f. catenulata*, and soil C/N ($\chi^2 = 5.7$; df = 1; $P = 0.02$), K ($\chi^2 = 7.6$; df = 1; $P = 0.01$), Ca ($\chi^2 = 9.7$; df = 1; $P = 0.002$) and *M. robertsii*. Logistic regression further revealed that only soil K had a significant positive association with the occurrence of *M. robertsii* (estimate = 0.03 ± 0.01; $P < 0.05$; odds ratio = 1.03).

Pathogenicity of EPF against mealybug

Broadly, insect mortality was significantly influenced by fungal species ($\chi^2 = 56.2$; df = 3; $P < 0.001$) and fungal isolate ($\chi^2 = 151.7$; df = 14; $P < 0.001$). All the 15 selected fungal isolates were pathogenic against adult females of *P. ficus*, with four-day mortalities ranging from 18.0 to 87.0% (Table 1), but isolates of *B. bassiana* (SM3, SMA, SM13) and *C. rosea f. catenulata* (SM6) caused the greatest *P. ficus* mortalities, 87.0 ± 3.0%, 77.0 ± 2.0%, 82.0 ± 2.0% and 69.0 ± 10.0%, respectively. The lowest levels of mortality were observed primarily among isolates of *M. robertsii* and *C. rosea f. catenulata* (Table 1).

Discussion

A better understanding of the ecology of EPF could lead to optimal exploitation and integration of EPF in grapevine cultivation. The potential benefits of entomopathogens in agro-ecological systems, including grapevine farms, are many and include natural regulation of insect pest populations, enhancement of plant growth and antagonism of plant pathogens. Fertilizer inputs can influence soil physicochemical properties and, consequently, soil microbial biodiversity (Lazcano et al. 2012). This study revealed, remarkably, that high Ca and optimum C and N were associated with the presence of *C. rosea f. catenulata* and *M. robertsii*, and K was positively correlated with *M. robertsii* in soils of the sampled vineyards.

Of the 23 fungal isolates collected from the sampled vineyards, 26.0% belonged to the species *C. rosea f. catenulata*, closely followed by *M. robertsii* (22.0%) and *B. bassiana* (17.0%). On the basis of this study and a previous study by Abaajeh and Nchu (2015), it appears that *M. robertsii* is the predominant *Metarhizium* species in the Cape Winelands region. Also, it seems that *M. robertsii* and *C. rosea* proliferate better in disturbed and conventionally cultivated habitats than in uncultivated habitats (Quesada-Moraga et al 2007; Uzman et al. 2019). For example, *C. rosea f. rosea* was the most encountered species (30.1 ± 3.4%), followed by *B. bassiana* (12.6 ± 2.4%) in Portuguese Douro Vineyards (cultivated habitat) and adjacent hedgerows (semi-natural habitat) (Sharma et al. 2018b). In countrywide surveys carried out throughout South Africa, 1506 soil samples collected and baited with *Galleria mellonela* yielded 441 isolates of EPF, 81% of which were *B. bassiana* and 13% were *M. anisopliae* (Hatting et al. 2004). In an earlier study, 62 EPF strains, representing 21% occurrence, were isolated from soils in the Sunday's River Valley citrus producing region (Eastern Cape

Table 2 Soil nutrient and pH levels (mean ± SE) in soil samples obtained from selected sites in the Cape Winelands, South Africa, and recommended critical values of nutrients

Location	Site	pH	Phosphorus (mg kg^{-1})	Potassium (mg kg^{-1})	Magnesium (mg kg^{-1})	Calcium [cmol(+) kg^{-1}]	Sulphur (mg kg^{-1})	Nitrogen [cmol(+) kg^{-1}]	Carbon [cmol(+) kg^{-1}]	C/N ratio
Stellenbosch	Site 1	5.7 ± 0.4	52.0. ± 42.0	189.0 ± 25.0	207.0 ± 13.0	1094.0 ± 209.0	7.0 ± 2.0	1180.0 ± 69.0	13266.0 ± 1159.0	11.0:1.0
	Site 2	5.7 ± 0.4	87.0 ± 21.0	152.0 ± 31.0	173.0 ± 32.0	879.0 ± 109.0	9.0 ± 3.0	1253.0 ± 30.0	14866.0 ± 1795.0	11.0:1.0
	Site 3	5.7 ± 0.2	25.0 ± 4.0	132.0 ± 30.0	216.0 ± 17.0	1026.0 ± 136.0	8.0 ± 2.0	1176.0 ± 200.0	14533.0 ± 763.0	12.0:1.0
	Site 4	6.4 ± 0.2	116.0 ± 21.0	23.0 ± 6.7	109.0 ± 34.0	729.0 ± 101.0	10.0 ± 1.7	600.0 ± 81.6	12400.0 ± 100.0	17.0:1.0
	Site 5	5.6 ± 0.1	86.0 ± 30.0	29.0 ± 12.0	123.0 ± 68.0	596.0 ± 372.0	14.0 ± 2.0	700.0 ± 360.0	9866.0 ± 5216.0	12.0.:1.0
	Site 6	6.2 ± 0.1	139.0 ± 36.0	37.0 ± 15.0	162.0 ± 51.0	918.0 ± 207.0	13.0 ± 5.0	733.0 ± 305.0	15300.0 ± 8056.0	20.0:1.0
Constantia	Site 7	6.2 ± 0.9	58.0 ± 44.0	75.0 ± 24.0	57.0 ± 20.0	900.0 ± 462.0	7.0 ± 2.0	1080.0 ± 405.0	10733.0 ± 1975.0	9.0:1.0
	Site 8	6.2 ± 0.4	75.0 ± 8.6	85.0 ± 21.0	119.0 ± 17.0	926.0 ± 32.0	13.0 ± 9.0	1346.0 ± 180.0	12533.0 ± 4479.0	9.0:1.0
	Site 9	6.0 ± 0.6	41.0 ± 4.9	127.0 ± 41.0	95.0 ± 42.0	2214.0 ± 1445.0	21.0 ± 8.0	2370.0 ± 708.0	29600.0 ± 9778.0	12.0:1.0
	Site 10	5.8 ± 0.2	138.0. ± 93.0	26.0 ± 22.0	156.0 ± 58.0	793.0 ± 137.0	11.0 ± 3.0	566.0 ± 57.0	15100.0 ± 3736.0	26.0:1.0
	Site 11	5.5 ± 0.0	17.0 ± 12.0	45.0 ± 35.0	237.0 ± 34.0	1056.0 ± 245.0	15.0 ± 13.0	2800.0 ± 339.0	23300.0 ± 3104.0	23.0:1.0
Paarl	Site 12	5.7 ± 0.4	144.0. ± 32.0	29.0 ± 11.0	98.0 ± 23.0	1234.0 ± 292.0	13.0 ± 4.0	1166.0 ± 550.0	17600.0 ± 6055.0	14.0:1.0
	Site 13	5.4 ± 0.3	101.0 ± 36.0	26.0 ± 21.0	38.0 ± 9.0	535.0 ± 99.0	7.0 ± 5.0	1423.0 ± 635.0	17000.0 ± 5670.0	11.0:1.0
	Site 14	5.7 ± 0.5	90.0 ± 56.0	20.8 ± 12.0	24.0 ± 20.0	274.0 ± 35.2	9.0 ± 2.0	1690.0 ± 673.0	15933.0 ± 4129.0	9.0:1.0
Malmesbury	Site 15	5.8 ± 0.1	62.0 ± 6.0	16.9 ± 1.8	74.0 ± 8.0	388.0 ± 76.0	9.2 ± 0.9	333.0 ± 152.0	7866.0 ± 1184.0	22.0:1.0
	Site 16	6.2 ± 0.5	29.0 ± 5.0	20.0 ± 2.0	79.0 ± 20.0	578.0 ± 211.0	10.0 ± 0.4	466.0 ± 57.0	7433.0 ± 1643.0	16.0:1.0
	Site 17	5.6 ± 0.2	98.0 ± 20.0	20.0 ± 5.0	99.0 ± 67.0	476.0 ± 359.0	13.0 ± 2.0	566.0 ± 288.0	9266.0 ± 6615.0	15.0:1.0
	Site 18	5.6 ± 0.6	58.0 ± 4.0	31.0 ± 10.0	203.0 ± 75.0	694.0 ± 251.0	11.0 ± 3,0	633.0 ± 152.0	13700.0 ± 2773.0	21.0:1.0

Table 2 continued

Location	Site	pH	Phosphorus (mg kg^{-1})	Potassium (mg kg^{-1})	Magnesium (mg kg^{-1})	Calcium [cmol(+) kg^{-1}]	Sulphur (mg kg^{-1})	Nitrogen [cmol(+) kg^{-1}]	Carbon [cmol(+) kg^{-1}]	C/N ratio
Franschoek	Site 19	6.2 ± 0.3	114.0 ± 27.0	27.0 ± 3.0	171.0 ± 62.0	1140.0 ± 298.0	12.5 ± 5.1	666.0 ± 208.0	16400.0 ± 7049.0	24.0:1.0
	Site 20	5.4 ± 0.4	116.0 ± 102.0	29.0 ± 18.0	137.0 ± 89.0	626.0 ± 344.0	12.0 ± 6,0	733.0 ± 450.0	12400.0 ± 8510.0	17.0:1.0
	Site 21	5.7 ± 0.4	84.0 ± 52.0	180.0 ± 9.0	213.0 ± 83.0	169.0 ± 79.5	6.0 ± 2.0	1530.0 ± 496.0	23500.0 ± 8286.0	15.0:1.0
	Site 22	5.9 ± 0.3	158.0 ± 31.0	227.0 ± 15.0	139.0 ± 21.0	2066.0 ± 345.0	7.0 ± 3.0	1773.0 ± 105.0	20100.0 ± 113.0	11.0:1.0
Optimum ranges[a]		5.5–7.5	25.0–170.0	70.0–120.0	60.0–240.0	400.0–1200.0	20.0–200.0	600.0–1500.0	8000.0–15000.0	10.0:1.0

[a]Ranges are published by Bemlab Pty. Ltd. (http://www.bemlab.co.za/uploads/GENERIC%20SOIL%20ANALYSIS%20NORMS_a.pdf)

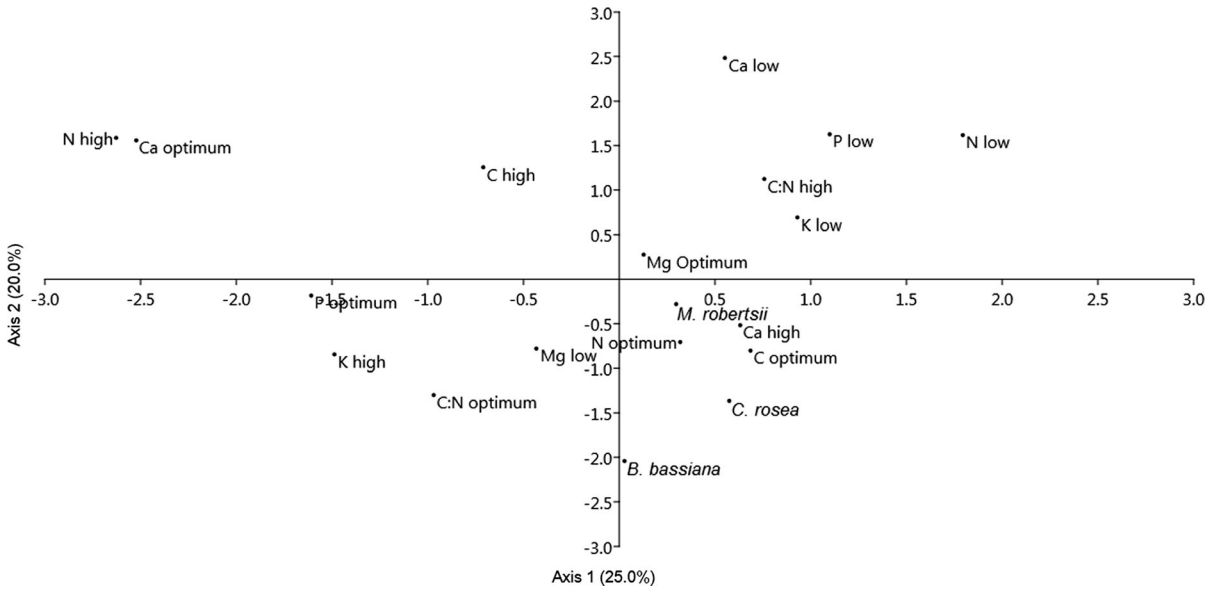

Fig. 1 Correspondence analysis showing association between *M. robertsii* and *C. rosea f. catenulata* occurrence and optimum (N and C) and high (Ca) levels in sampled soils of grapevines in the Cape Winelands of South Africa — their positions in the plot are relatively more closely associated compared to the other variables

Province, South Africa) and *B. bassiana* and *M. anisopliae* var. *anisopliae* were the most frequently isolated (Goble 2009). However, these results should be interpreted with caution. For instance, Goble (2009) had relied on morphological characteristics for fungal identification, which might not be robust enough in separating closely related *Beauveria* spp. and *Metarhizium* spp. (Bischoff et al. 2009; Rehner and Buckley 2005). Species identification in *Beauveria* is difficult due to a lack of a suitable taxonomic morphologically based framework (Rehner and Buckley 2005). It is probable that, if molecular and phylogenetic analyses had been used in that study, some of the fungal isolates that were identified as *M. anisopliae* var. *anisopliae* were, in fact, *M. robertsii*. In this study, while the majority of the isolated *M. robertsii* and *B. bassiana* strains were obtained using the insect bait method, most of the isolates of *C. rosea f. catenulata* were collected by soil dilution method. These results are in agreement with the argument that EPF isolation methods are not equivalent for the determination of the occurrence and distribution of EPF (Medo and Cagáň 2011). Sharma et al. (2018b) demonstrated that the isolation of *M. robertsii* was associated both with *Tenebrio molitor* (Coleoptera: Tenebrionidae) baiting and cultivated habitats, while *B. bassiana* was linked with *G. mellonella* baiting only. A combination of methods is, therefore, necessary for an optimal isolation of EPF from the environment.

Sharma et al. (2018a) isolated EPF from 58 of 183 field collected vine mealybug and went on to argue that fungi are important biocontrol agents. Fifteen strains of four well-known EPF (*B. bassiana*, *M. anisopliae*, *C. rosea f. catenulata* and *F. solani*) were assessed for pathogenicity against the grapevine mealybug *P. ficus*. As expected, the results obtained in this study suggest that the fungus-induced insect mortality level is not only species-dependent but also strain-dependent. *Clonostachys rosea f. catenulata* isolates yielded mortality ranging from 18.0 to 82.0% when used in bio-assays against *P. ficus*. *Clonostachys rosea f. catenulata* is known as a mycoparasitic fungus, which is usually used as a biocontrol agent for plant pathogens, and it has also been observed as a facultative pathogen of nematodes (Sutton et al. 2002; Zhang et al. 2008). In the present study, *B. bassiana,* a widely researched species, showed high insect mortality (77.0–87.0%). *Beauvaria bassiana* is used as an active ingredient for numerous mycopesticides worldwide (Feng et al. 1994; Ownley et al. 2004; Pu et al. 2005). Generally, *M. roberstii* strains induced lower

mortalities in the current study. In fact, a few studies have reported that *B. bassiana* are more virulent to *Planococcus* spp. than *M. robertsii* and other well-known EPF species (Amutha and Banu 2017; Mohamed 2016). Mohamed (2016) argued that the higher virulence of *B. bassiana* might be due to a faster rate of penetration and colonization. It is worth noting that while the *Metarhizium* isolates we collected may not be effective against grapevine mealybug, *B. bassiana* should be considered as a potential biocontrol agent for the pest because of its higher virulence.

We further demonstrated that the macronutrient potassium was significantly associated with the occurrence of *M. robertsii*. The finding that there is a strong positive correlation between K concentration and frequency of isolated *M. robertsii* is particularly interesting because soils in the Cape Winelands are notoriously rich in potassium-containing minerals (Bargmann 2003, 2005). Sharma et al. (2018b) had demonstrated that lower exchangeable K^+ favor higher abundance of entomopathogenic *F. oxysporum* in soils from Douro Vineyards in Portugal. The results in this study contrast the findings of Jabbour and Barbercheck (2009) that showed no relationship between *Metarhizium* detection and soil nutrients, such as Ca, K, and P. Nevertheless, while soil nutrient content is important for microbial abundance in soil, physical and biological factors are equally important drivers of microbial abundance (Frac et al. 2018). This may explain the inconsistent findings obtained in different studies dealing with the relationship of soil nutrient content and EPF. Other related studies have mainly focused on nutrient source use in in vitro culturing of fungi (Mishra and Malik 2012; Zhu et al. 2008) and little information is available on specific nutrient levels required for fungal growth under field conditions. Although nutritional requirements and tolerances may vary with species/strains, generally microorganisms require significant quantities of carbon, nitrogen and phosphorus, which are the essential ingredients of growth factors like amino acids, purines and pyrimidines and vitamins. In a study by Tsvuura et al. (2017), mycelial biomass more than tripled with the addition of nitrogen into grassland plots.

In conclusion, in terms of nutrient status, the recommended optimum N and C levels and high Ca level correlated with the occurrence of *M. robertsii* and *C. rosea f. catenulata* in grapevine of the Cape Winelands. Potassium had a significant positive relationship with the occurrence of *M. robertsii* in this study. This information is valuable for future development of ecological approaches involving creating conditions for enhancement of the occurrence of EPF on grapevine farms. This study provides a basis from which to study EPF ecology and test them in further field-scale trials in different agricultural settings.

Acknowledgements The authors of this paper would like to convey their thanks and appreciation to the National Research Foundation, South Africa and Cape Peninsula University of Technology (University Research Fund; Grant No. R166) for funding this study. Prof, Karin Jacobs at the University of Stellenbosch contributed to fungal identification. We thank Dr. Marilize Le Roes-Hill (Biocatalysis and Technical Biology Research Group, CPUT) for comments that greatly improved the manuscript. The authors thank Entomon Technologies (Pty.) Ltd. in Stellenbosch for supplying the different life stages of codling moths.

Compliance with ethical standards

Conflicts of interest The authors declare no conflict of interest.

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 Sci Technol 25:828–842

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18:265–267

Amutha M, Banu JG (2017) Variation in mycosis of entomopathogenic fungi on mealybug, *Paracoccus marginatus* (Homoptera: Pseudococcidae). Proc Natl Acad Sci India B 87(2):343–349

Anwar W, Ali S, Nawaz K, Iftikhar S, Javed MA, Hashem A, Alqarawi AA, Abd_Allah EF, Akhter A (2018) Entomopathogenic fungus *Clonostachys rosea* as a biocontrol agent against whitefly (*Bemisia tabaci*). Biocontrol Sci Technol 28(8):750–760

Bargmann CJ (2003) Geology and Wine 7. Geology and wine production in the Coastal Region, Western Cape Province. South Africa. Geoscience Can 30(4):161–182

Bargmann CJ (2005) Geology and wine in South Africa. Geoscientist 15:4–8

Bemlab (2016) Soil analysis norms. Available via DIALOG. http://www.bemlab.co.za/uploads/GENERIC%20SOIL%20ANALYSIS%20NORMS_a.pdf. Accessed 19 July 2016

Bischoff JF, Rehner SA, Humber RA (2009) A multilocus phylogeny of the *Metarhizium anisopliae* lineage. Mycologia 101:512–530

Bruck DJ (2009) Fungal entomopathogens in the rhizosphere. In: Roy HE, Vega FE, Chandler D, Goettel MS, Pell J, Wajnberg E (eds) The ecology of fungal entomopathogens. Springer, Dordrecht, pp 103–112

Bruck DJ (2010) Fungal endophyte in the rhizophere. BioControl 55:103–112

Campbell CR, Plank CO (1998) Preparation of plant tissue for laboratory analysis. In: Kalra YP (ed) Handbook of reference methods for plant analysis. CRC Press, Boca Raton, pp 37–49

Chandler D, Hay D, Reid AP (1997) Sampling and occurrence of entomopathogenic fungi and nematodes in UK soils. Appl Soil Ecol 5(2):133–141

Cooke WB (1968) Carbon/nitrogen relationships of fungus culture media. Mycopathologia 34(3):305–316

Crous CJ, Burgess TI, Le Roux JJ, Richardson DM, Slippers B, Wingfield MJ (2017) Ecological disequilibrium drives insect pest and pathogen accumulation in non-native trees. AoB Plants 9:plw081

Daane KM, Almeida RPP, Bell VA, Walker JTS, Botton M, Fallahzadeh M, Mani M, Miano JL, Sforza R, Walton VM, Zaviezo T (2012) Biology and management of mealybugs in vineyards. In: Bostanian NJ, Vincent C, Isaacs R (eds) Arthropod management in vineyards: pests, approaches, and future directions. Springer, New York, pp 271–307

du Plessis JA, Schloms B (2017) An investigation into the evidence of seasonal rainfall pattern shifts in the Western Cape, South Africa. J S Afr Inst Civ Eng 59(4):47–55

Epstein L, Bassein S (2003) Patterns of pesticide use in California and the implications for strategies for reduction of pesticides. Annu Rev Phytopathol 41:351–375

Feng MG, Poprawski TJ, Khachatourians GG (1994) Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control—current status. Biocontrol Sci Technol 4:3–34

Fernandez-Cornejo J (1998) Environmental and economic consequences of technology adoption: IPM in viticulture. Agric Econ 18:145–155

Frac M, Hannula SE, Bełka M, Jędryczka M (2018) Fungal biodiversity and their role in soil health. Front Microbiol 9:707

Gaskin RE, Manktelow DW, Elliott GS (2002) New adjuvant technology for pesticide use on wine grapes. NZ Plant Prot 55:154–158

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61:1323–1330

Goble TA (2009) Investigation of entomopathogenic fungi for control of false codling moth, *Thaumatotibia leucotreta*, Mediterranean fruit fly, *Ceratitis capitata* and Natal fruit fly, *C. rosea* in South African Citrus. Master's thesis, Rhodes University, Grahamstown

Goettel MS (1984) A simple method for mass culturing entomopathogenic hyphomycete fungi. J Microbiol Methods 3:15–20

Gouli V, Gouli S, Marcelino JAP, Margaret S, Parker BL (2013) Entomopathogenic fungi associated with exotic invasive insect pests in Northeastern forests of the USA. Insects 4:631–645

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological statistics software package for education and data analysis. Palaeontol Electron 4(1):9. Available via DIALOG. https://folk.uio.no/ohammer/past/. Accessed 16 Dec 2018

Hatting J, Hazir S, Macucwa G, Jooste H, Jankielsohn A (2004) Isolation of entomopathogens from South African soils using the *Galleria mellonella*-bait technique. In: Proceedings of the 37th annual meeting of the Society for Invertebrate Pathology

Ho WC, Ko WH (1997) A simple method for obtaining single-spore isolates of fungi. Bot Bull Acad Sin 38:41–44

Huber A, Bach M, Frede HG (2000) Pollution of surface waters with pesticides in Germany: modeling non-point source inputs. Agric Ecosyst Environ 80:191–204

Jabbour R, Barbercheck ME (2009) Soil management effects on entomopathogenic fungi during the transition to organic agriculture in a feed grain rotation. Biol Control 51:435–443

Jones GV, Davis RE (2000) Climatic influences on grapevine phenology, grape composition, and wine production and quality for Bordeaux, France. Am J Enol Vitic 51:249–261

Kepler RM, Humber RA, Bischoff JF, Rehner SA (2014) Clarification of generic and species boundaries for *Metarhizium* and related fungi through multigene phylogenetics. Mycologia 106:811–829

Klingen I, Eilenberg J, Meadow R (2002) Effects of farming system, field margins and bait insect on the occurrence of insect pathogenic fungi in soils. Agric Ecosyst Environ 91:191–198

Koorem K, Gazol A, Öpik M, Moora M, Saks Ü, Uibopuu A, Sõber V, Zöbel M (2014) Soil nutrient content influences the abundance of soil microbes but not plant biomass at the small-scale. PLoS ONE 9(3):e91998

Lazcano C, Gómez-Brandón M, Revilla P, Domínguez J (2012) Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function: a field study with sweet corn. Biol Fertil Soils 49:723–733

Medo J, Cagáň L (2011) Factors affecting the occurrence of entomopathogenic fungi in soils of Slovakia as revealed using two methods. Biol Control 15(2):200–208

Mishra S, Malik A (2012) Nutritional optimization of a native *Beauveria bassiana* isolate (HQ917687) pathogenic to housefly *Musca domestica* L. J Parasit Dis 37(2):199–207

Mohamed GS (2016) Virulence of entomopathogenic fungi against the vine mealy bug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae). Egypt J Biol Pest Control 26:47–51

Nelson PE, Toussom TA, Marasas WFO (1983) *Fusarium* species: an illustrated manual for identification. Pennsylvania State University Press, University Park

Non-Affiliated Soil Analysis Work Committee (1990) Handbook of standard soil testing methods for advisory purposes. Soil Science Society of South Africa, Pretoria

Ownley BH, Pereira RM, Klingeman WE (2004) *Beauveria bassiana*, a dual purpose biocontrol organism, with activity against insect pests and plant pathogens. In: Lartey RT, Caesar A (eds) Emerging concepts in plant health management. Research Signpost, Thiruvananthapuram, pp 255–269

Pell JK, Hannam JJ, Steinkraus DC (2010) Conservation biological control of entomopathogens. BioControl 55:187–198

Pu XY, Feng MG, Shi CH (2005) Impact of three application methods on the field efficacy of a *Beauveria bassiana*-based myco-insecticide against the false-eye leafhopper, *Empoasca vitis* (Homoptera: Cicadellidae) in the tea canopy. Crop Prot 24:167–175

Quesada-Moraga E, Navas-Cortés JA, Maranhao EAA (2007) Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. Mycol Res 111:947–966

Raya-Dáaz S, Sánchez-Rodríguez AR, Segura-Fernández JM, del Campillo MdC, Quesada-Moraga E (2017) Entomopathogenic fungi-based mechanisms for improved Fe nutrition in sorghum plants grown on calcareous substrates. PLoS ONE 12(10):e0185903

Rehner SA, Buckley E (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps teleomorphs*. Mycologia 97(1):84–98

Safavi SA, Shah FA, Pakdel AK (2007) Effect of nutrition on growth and virulence of the entomopathogenic fungus *Beauveria bassiana*. FEMS Microbiol Lett 270:116–123

Sharma L, Gonçalves F, Oliveira I, Torres L, Marques G (2018a) Insect-associated fungi from naturally mycosed vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae). Biocontrol Sci Technol 28(2):122–141

Sharma L, Oliveira I, Torres L, Marques G (2018b) Entomopathogenic fungi in Portuguese vineyards soils: suggesting a "*Galleria-Tenebrio*-bait method" as bait-insects *Galleria* and *Tenebrio* significantly underestimate the respective recoveries of *Metarhizium* (*robertsii*) and *Beauveria* (*bassiana*). MycoKeys 38:1–23

Stenekamp D (2011) Optimization of a mass-rearing system to produce codling moth, *Cydia pomonella*, for a Sterile Insect Release programme in South Africa. Dissertation presented for the Degree of Doctor of Philosophy, Faculty of AgriSciences, Stellenbosch University

Strasser H, Vey A, Butt TM (2000) Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of *Metarhizium*, *Tolypocladium and Beauveria species*? Biocontrol Sci Technol 10:717–735

Sutton JC, Liu W, Huang R, Owen-Going N (2002) Ability of *Clonostachys rosea* to establish and suppress sporulation potential of *Botrytis cinerea* in de-leafed stems of hydroponic greenhouse tomatoes. Biocontrol Sci Technol 12:413–425

Tkaczuk C, Król A, Majchrowska-Safaryan A, Nicewicz Ł (2014) The occurrence of entomopathogenic fungi in soils from fields cultivated in a conventional and organic system. J Ecol Eng 15:137–144

Tsvuura Z, Avolio ML, Kirkman KP (2017) Nutrient addition increases biomass of soil fungi: evidence from a South African grassland. S Afr J Plant Soil 34:71–73

Tuininga AR, Miller JL, Morath SU, Daniels TJ, Falco RC, Marchese M, Sahabi S, Rosa D, Stafford KC III (2009) Isolation of entomopathogenic fungi from soils and *Ixodes scapularis* (Acari: Ixodidae) ticks: prevalence and methods. J Med Entomol 46(3):557–565

Uzman D, Pliester J, Leyer I, Entling MH, Reineke A (2019) Drivers of entomopathogenic fungi presence in organic and conventional vineyard soils. Appl Soil Ecol 133:89–97

Walton VM, Pringle KL (2004) Vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), a key pest in South African vineyards. A review. S Afr J Enol Vitic 25:54–62

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols. A guide to methods and applications. Academic, San Diego, pp 315–322

Zhang L, Yang J, Niu Q (2008) Investigation on the infection mechanism of the fungus *Clonostachys rosea* against nematodes using the green fluorescent protein. Appl Microbiol Biotechnol 78:983–990

Zhu Y, Pan J, Qiu J, Guan X (2008) Optimization of nutritional requirements for mycelial growth and sporulation of entomogenous fungus *Aschersonia aleyrodis* Webber. Braz J Microbiol 39(4):770–775

Siphokazi Moloinyane researches in the area of integrated pest management. This research is part of her MSc. It was dedicated to the occurrence of entomopathogenic fungi in vineyards of the Cape Winelands, South Africa and their bioactivities against the grapevine mealybug.

Pia Addison is an entomologist with particular interests in insect taxonomy, insect ecology and integrated management of insect pests.

Kwaku A. Achiano research focuses on insect semiochemicals and their potential use in integrated pest management.

Felix Nchu works on the ecology of arthropod pathogenic fungi and their bioactivities against insects and ticks. This study is part of a larger research project on developing biorational approaches for control of arthropod pests at Cape Peninsula University of Technology.