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Resporulation of *Metarhizium anisopliae* granules on soil and mortality of *Tenebrio molitor*: Implications for wireworm management in sweetpotato

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Abstract

In Australia, sweetpotato (*Ipomoea batatas* L.) is vulnerable to root feeding insect pests such as wireworms (e.g., *Agrypnus* spp.). The number of registered insecticides to control these insect pests is limited and often pest pressure, for example by wireworms, is severe close to harvest, further limiting what insecticides can be applied. Incorporating biological control agents such as entomopathogenic fungi (e.g., *Metarhizium anisopliae*) into integrated pest management programmes may be feasible in sweetpotato. *M. anisopliae* has been shown to be effective in controlling more than 200 insects and it is able to reside and grow in the rhizosphere and rhizoplane, suggesting that *M. anisopliae* could be a promising candidate against soil insect pests. In the study presented here, *M. anisopliae* was formulated into calcium alginate granules fortified with nutrients. The resporulation of the fungal granules was tested on four different soil types in the laboratory. The biocontrol efficacy of the resulting fungal growth was also examined using larval mealworms, *Tenebrio molitor* as a model insect in the laboratory and the glasshouse. Our results indicated that sterilised soil favoured optimal fungal resporulation, although different soil types did not have a significant effect on fungal resporulation. The resulting fungal resporulation and growth on sterilised soil caused high mortality (up to 76%) of larval mealworms in the glasshouse, whereas the fungal granules applied to non-sterile soil demonstrated poor resporulation that led to low mortality (13%) of larval mealworms. The result of this study indicates that the manipulation of microbial populations in field soil is required to enhance the fungal growth and potential insect control against wireworms in the field.

KEYWORDS
biological insect control, calcium alginate granule, entomopathogenic fungi, soil insects

1 | INTRODUCTION

Sweetpotato (*Ipomoea batatas* L.) is one of the major root-vegetable crops in Australia, generating AU$ 100 million annually (Australian Sweetpotato Growers Inc., 2021) with 90% of the national production concentrated in Queensland, particularly in Bundaberg (Australian Sweetpotato Growers Inc., 2021). An estimated 89% of total production is supplied to the domestic fresh market (Hort Innovation, 2021).

Being a root crop, sweetpotato is predisposed to attack from root herbivores, which generally leads to unmarketable produce. Root herbivores such as sweetpotato weevil (*Cylas formicarius*), root-knot nematodes (e.g., *Meloidogyne javanica* and *M. incognita*) and wireworms (both Elateridae and Tenebrionidae family) have been identified as important pests of sweetpotato in Australia, causing considerable damage to the storage roots, and consequently, leading to market rejection (McCrystal, 2010). These pests are also problematic in sweetpotato production globally (Arrington, Kennedy, & Abney, 2016; Hue & Low, 2015; Mukhopadhyay, Chattopadhyay, Chakraborty, & Bhattacharya, 2011; Pasche, Taylor, David, & Gudmestad, 2014; Seal, Chalfant, & Hall, 1992).

Wireworm damage on sweetpotato storage roots results in economic losses to growers, especially in developed countries like Australia where the produce is either rejected (with moderate to severe damage) or downgraded from premium (with minor damage). As a result, growers in Australia apply soil insecticides, for example, Talstar® (Bifenthrin) or Regent® (Fipronil), to control wireworms (Australian Pesticides and Veterinary Medicines Authority, 2021); however, crop losses of up to 21% are still observed. Wireworm activity is typically prevalent in the weeks leading up to harvest and the efficacy of soil insecticides generally does not persist in soil throughout the cropping period (McCrystal, 2014). Thus, treating a field with soil insecticide for wireworm control before planting is often ineffective. Additionally, the tough exoskeleton of wireworm, coupled with its ability to evade the chemical-treated zones by moving deeper into the soil profile, limits the effectiveness of the insecticides (Parker et al., 1996). A study conducted by McCrystal (2014) showed that the intermittent application of chemical insecticide (Regent®) via drip irrigation in sweetpotato root zones until the late stage of crop growth completely protected the crop against wireworm infestation, but the food safety of the chemical-treated produce for human consumption is still questionable. Ongoing economic losses for sweetpotato growers in Australia from continuous pest pressure highlight the need for an alternative to the current pest-control practices (McCrystal, 2014). The adoption of alternative methods such as biological control using entomopathogenic fungi has been proposed as a promising alternative that could be incorporated into an integrated pest management programme (IPM) (Khun, Ash, Stevens, Huwer, & Wilson, 2020; Skinner, Parker, & Kim, 2014).

Entomopathogenic fungi (EPF) are soil-resident microorganisms, which are able to survive saprophytically in the soil and naturally infect soil insects (Stone & Bidochka, 2020; Zimmermann, 2007). For example, *Metarhizium brunneum* has been isolated from wireworm (Kabaluk, Li-Leger, & Nam, 2017) and various genera, for example, *Metarhizium*, *Beauveria*, *Isaria* and *Lecanicillium*, have been developed as biopesticides for insect control (De Faria & Wraight, 2007). *Metarhizium* is one of the most studied genera, displaying pathogenicity against more than 200 insects, particularly coleopteran insects (Pilz, Enkerli, Wegensteiner, & Keller, 2011). Moreover, some species of *Metarhizium* are root endophytes and even colonise the rhizosphere (Krell, Jakobs-Schoenwandt, Vidal, & Patel, 2018; Vega, 2018). These endophytes may play a role in crop protection by deterring attacks from root herbivores (Parsa, Ortiz, & Vega, 2013). Because of these traits, there has been a longstanding endeavour to exploit *Metarhizium* species as a biological control agent for soil insect control (Roberts & Leger, 2004). In conventional practice, fungal propagules have been inundatively applied to soils to control soil-dwelling insects, but fungal viability sharply declines following application (Ekesi, Maniania, & Mohamed, 2010; Gašić & Tanović, 2013). In some cases, the fungus takes substantial time for its establishment and further colonisation following its application in soil, which limits the efficacy of the fungus to protect crops when they experience high pest pressure, especially annual field crops like sweetpotato (Pilz et al., 2011). Entomopathogenic fungal colonisation in fields may be expedited by adopting a modified formulation, in which the fungal propagules can be combined with the food sources to benefit the EPF. Lack of appropriate food resources in the vicinity of the fungal propagules in soils is one of the constraints that potentially limits fungal colonisation in soil (Jackson, Dunlap, & Jaronski, 2010; Jaronski, 2010). As a result, the notion of co-application of fungal inocula combined with exogenous food sources has been proposed. For example, this approach has been used for *M. anisopliae* conidiated on rice grains (Kabaluk, 2014), on rice bran (Moslim, Kamarudin, & Wahid, 2009) and on millet (Rath, Worledge, Anderson, & Carr, 1995). The co-application of fungal inocula with food additives may be further improved using a sodium alginate polymer (Humbert, Przyklenk, Vemmer, & Patel, 2017). Sodium alginate is a positively charged polysaccharide extracted from marine algae and matrices (for example calcium alginate) are normally generated by cross-linking with divalent cations (for example calcium chloride) in an ionotropic gelation reaction (Shah et al., 1998). In fact, sodium alginate is not detrimental to any microorganism, thus it is often used for carrying several beneficial microorganisms including biocontrol fungi (Lewis, Lumsden, & Locke, 1996).

The fate of *M. anisopliae* in soil has been also linked with soil physical properties, for example, soil moisture, soil texture, pH, cation exchange capacity and organic matter (Wraight, Jackson, & De Kock, 2001). A study conducted by Vänninen, Tyni-Juslin, and Hokkanen (2000) in Finland noted that both *M. anisopliae* and *B. bassiana* persisted longer in clay soil than in peat, although the peat allowed for better fungal penetration and persistence at greater soil depths. Detection of *M. anisopliae* was reduced when exposed to soil with high moisture and organic content (Jabbour & Barbercheck, 2009). Another study by Jaronski, Fuller-Schaeffer, Jung, Majumdar, and Boetel (2007) showed that the infectivity but not viability of *M. anisopliae* is influenced by soil moisture and soil texture; in clay (but not other soil types), increasing moisture content adversely affected infectivity. The effect of soil types on fungal persistence and proliferation is not clear yet as the soil is such a milieu with multiple levels of interactions usually rendering confounding results (Garrido-Jurado, Ruano, Campos, & Quesada-Moraga, 2011; Rath, Koen, & Yip, 1992). Further studies investigating the effect of different soil samples on the growth and persistence of entomopathogenic fungi are needed.

There are various estimations in terms of fungal application rate into soil for soil insect control, for example, $>10^6$ spores cm^{-3} soil against wireworm control in soil (Kabaluk, Vernon, & Goettel, 2007) or approximately 10^5–10^6 colony forming units (CFU) cm^{-3} or g^{-1} soil (Jaronski, 2010). However, the estimation with exact value is not possible as the fungal efficacy may be impacted by soil characteristics. The conventional recommended rate may be reduced by using encapsulated

granules as it is anticipated that the fungus may produce secondary resporulation in the soil. A study by Przyklenk, Vemmer, Hanitzsch, and Patel (2017) demonstrated that the encapsulation of *M. brunneum* conidia at 0.01%, fortified with corn starch and dead baker's yeast as nutritive additives can increase the fungal resporulation up to 1,000 times and is referred to as microfermentation. Nutrient sources containing carbon and nitrogen are considered as primary nutrients for the fungal mycelial growth and conidial development (Jackson et al., 2010). These resporulated conidia produced on fungal granules are considered a primary source of infection for soil insects that encounter the resporulated fungal granules. The success of encapsulated fungal granules for soil insect control relies on the resporulation efficacy of fungal granules once they are applied to the soil. However, there has been a significant paucity of knowledge about the infectivity of soil resporulated fungal granules against soil insects.

In this study, we evaluated the infectivity of conidia formed on fungal granules following their application on soil against larval mealworms in laboratory and glasshouse conditions.

2 | MATERIALS AND METHODS

2.1 | Metarhizium anisopliae

Metarhizium anisopliae strain QS155 was originally isolated from the soil at Mapuru, Northern Territory, and is maintained at the New South Wales Department of Primary Industries Herbarium with the accession number DAR 82480 (Dotaona, Wilson, Stevens, Holloway, & Ash, 2015). Cultures of *M. anisopliae* were grown on Sabouraud dextrose agar amended with 1% yeast extract (SDAY) (Merck KGaA, Germany). To produce conidia, *M. anisopliae* isolate QS155 was grown on SDAY at 27°C with a 12:12 h light and dark photoperiod for 21 days. The conidia were harvested using a sterile scalpel by gentle scraping the colony and dried on Petri dishes (Ø, 9 cm) in a biohazard cabinet (Esco class II BSC) for 2 h. The air-dried conidia were stored in sterile plastic 50 mL tubes and sealed with a lid at 5°C for 7 days until the conidia were prepared for formulation.

Before all experiments, the conidia viability was assessed by inoculating 20 μL of a conidial suspension (10^6 conidia per mL) over a thin layer of SDAY medium (1.5 cm × 1.5 cm × 0.5 cm) on a glass microscope slide, which was covered with a coverslip and placed inside a Petri dish (Ø, 9 cm) containing Whatman® filter paper moistened with sterile distilled water. The Petri dish was sealed with Parafilm® and incubated at 27°C and 12:12 h dark and light photoperiod. Following 14 h of incubation, 200 conidia were assessed at ×400 using a compound microscope (Olympus, Model BX53). Only samples with >98% germination were used for further experimentation.

2.2 | Preparation of *M. anisopliae* granules

To prepare the calcium-alginate formulation, 2% (w/v) sodium alginate (Chem-Supply Pty Ltd., Australia) was dissolved in 0.05% sterile Tween®80 (VWR Chemicals) in water; the resultant suspension was heated with continuous agitation for 30 min before the suspension was autoclaved at 121°C for 6 min only, to prevent chemical denaturation of sodium alginate (Vemmer & Patel, 2013). The conidia of *M. anisopliae* QS155 were mixed into the sodium alginate, in combination with nutritive additives: 20% w/w corn starch (Sigma–Aldrich) and 20% w/w compressed baker's yeast (Lesaffre Australia Pacific Pty Ltd). The above nutritive additives, which were autoclaved at 121°C for 15 min, were suspended in the sterile sodium alginate solution and homogenised thoroughly using a stirrer for 10 min. Fresh conidia of *M. anisopliae* QS155 (1% w/w) were then added into the suspension and stirred using a stirring rod for 5 min. The homogenised suspension was immediately dripped into sterile 2% (w/w) calcium chloride solution (ICN Biomedicals Inc.) using a syringe (Norm-Ject®, drain tube Ø = 4 mm, length = 10 mm). The droplets of the suspension remained immersed in the calcium chloride solution for 30 min with continuous agitation for complete gelatinisation (Vemmer et al., 2016). Granules were separated from the calcium chloride solution by collecting them on a sterile Buchner funnel. Granules were rinsed twice with sterile water before being dried for 14 h inside a laminar flow cabinet (Labec Laboratory Equipment) at room temperature (22–24°C). Following the drying, 55% of moisture was removed from the granules, which were then sealed in a 100 mL container and stored at 5°C until the experiment commenced. These fungal granules are referred to as $CAG_{Ma+Cs+By}$ (Ø = 3.5 mm, weight 25 mg per granule, ~9×10^6 conidia per granule) henceforth where 'CAG' is defined as calcium alginate granule, 'Ma' is *M. anisopliae*, 'Cs' is corn starch and 'By' is baker's yeast (Figure 1). For the control treatment, calcium alginate granules only contained the nutritive additives (20% w/w corn starch and 20% w/w baker's yeast) without the fungal inocula and are referred to as food granules (CAG_{Cs+By}).

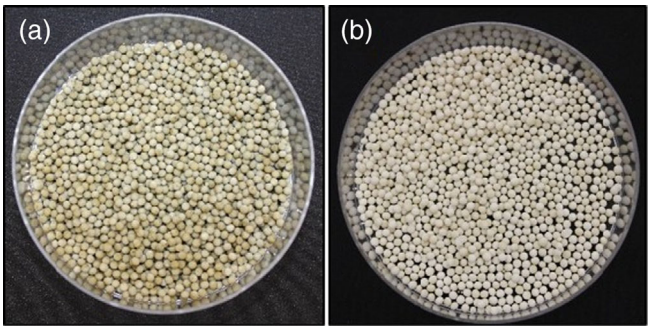

FIGURE 1 Fungal granules, $CAG_{Ma+Cs+By}$ used as a treatment for mealworm mortality (a) and food granules, CAG_{Cs+By} used as a control for mealworm mortality (b)

2.3 | Insects

The target insect for this study was wireworm (Coleoptera: Elateridae and Tenebrionidae), which are subterraneous insects causing feeding damage to the underground plant parts of various crops including

TABLE 1 Soil properties

Soil ID	Clay (%)	Silt (%)	Sand (%)	pH	EC (mS/m)	C (%)	N (%)	Crop history
Soil 1	75	15	10	5.7	33	1.53	0.20	Sweetpotato
Soil 2	10	75	15	6.2	77	0.51	0.05	Sweetpotato
Soil 3	13	48	40	6.4	89	0.53	0.05	Sweetpotato
Soil 4	60	20	20	6.6	7	3.22	0.22	Barley

sweetpotato. Wireworms are a sporadic insect pest on sweetpotato; however, wireworm infestation is impacted by the season, soil moisture, soil temperature and vegetation cover and is commonly referred to as a cryptic insect (Barsics, Haubruge, & Verheggen, 2013). Because of these factors, wireworms are not always attracted to grain or sweetpotato-based baits, which are placed 5–10 cm deep in the soil (S. Shah., pers. observation; anonymous sweetpotato grower pers. communication, 2018). In addition, wireworms exist as a cryptic species complex in fields that challenge the wireworm collection as a cohort and homogenous species. For these reasons, the yellow mealworm (*Tenebrio molitor*, Coleoptera: Tenebrionidae) was used as a model insect in this study to evaluate the fungal infectivity. Both false wireworms and yellow mealworms belong to the same family, Tenebrionidae, making them a suitable model insect in our study (Lestari & Rao, 2016). In addition, larval mealworms are broadly used as a bioassay tool to evaluate the infectivity of entomopathogenic fungi as these insects are regularly available and are easy to rear and maintain in the laboratory (Karabörklü, Altin, & Keslin, 2019; Praprotnik, Loncar, & Razinger, 2021). A study by Bharadwaj and Stafford (2011) indicated that larval mealworms showed more resistance than adult *Ixodes scapularis* to *M. brunneum*, further bolstering the fact that using larval mealworms as a bioassay probe is appropriate for insect hosts. Acquisition and maintenance of host insects usually necessitate specific expertise and substantial resources, thus using larval mealworms as a model insect is worthwhile especially for preliminary studies investigating the efficacy of novel fungal formulation or infectivity of novel strains. Thus, larval mealworms were used in our study that was supplied by Bio Supplies (https://biosupplies.net.au), Yagoona, NSW. The mealworms were reared in the laboratory of the University of Southern Queensland, Toowoomba at room temperature (20–22°C) in a diurnal light regime and were supplied with wheat germ and sweetpotato roots as a food source.

2.4 | Effect of different soil samples on the resporulation of fungal granules

In this experiment, the resporulation of fungal granules was tested on four different soil samples collected from agricultural fields in Australia (Table 1). Each soil sample was further treated as non-sterile, pasteurised and sterilised. Three soil samples were collected from sweetpotato fields in Bundaberg (GPS coordinate: 24°86′70″ S, 152°21′4″ E) Queensland, identified as 'soil 1', 'soil 2' and 'soil 3'. The fourth soil sample, identified as 'soil 4' was collected from an agricultural field of University of Southern Queensland (USQ),

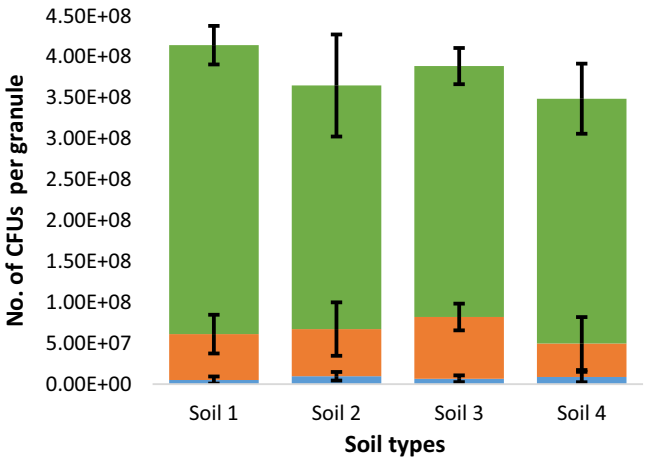

FIGURE 2 Fungal CFUs (mean ± SE, replicates = 3, $p = .05$) were compared among four different soil samples, which were collected from various locations. Soil samples are further categorised into non-sterile (blue), pasteurised (orange) and sterilised soil (green)

Toowoomba (GPS coordinate: 27°36′33″S, 151°55′55″E) Queensland, Australia. After the soil collection, samples were immediately transported to the laboratory, where the samples were air-dried, homogenised and graded, by passing them through a 10 mm sieve before storage at 10°C. Analysis of soil properties from these soil samples was also conducted at the soil laboratory of the University of Southern Queensland. Equal size soil samples were pasteurised (oven-dried at 105°C for 24 h), sterilised (autoclaved twice at 121°C for 1 h) or left untreated (non-sterile) (Figure 2).

For each soil sample, three freshly prepared fungal granules ($CAG_{Ma+Cs+By}$) or food granules (CAG_{MA+Cs}) were inoculated onto a Petri dish (Ø = 9 cm, 1.5 cm deep) containing 50 g of either non-sterile, sterilised or pasteurised soil. The soils were moistened with 10 mL of distilled water per Petri dish. The Petri dishes were then sealed with Parafilm® and incubated at 25°C in the dark in a growth chamber and arranged in a randomised complete block design (RCBD). This experiment consisted of 12 treatments with three granules per Petri dish.

At 28 days post-incubation, the fungal granules from the soil surface in the Petri dishes were individually removed using a sterilised scalpel for assessment of conidia. Each individually removed granule was transferred to a 50 mL tube containing 10 mL of sterile 0.05% Tween®80 solution and homogenised for 1 min using a vortex (Select Vortexer). Six serial dilutions (×10 dilution factor) were made from the stock suspension, and each dilution was replicated thrice. A

100 μL aliquot of soil suspension from each dilution was spread over SDAY amended with 0.01% chloramphenicol in a Petri dish, sealed with Parafilm® and incubated (25°C and a 12:12 h light and dark photoperiod) in a growth chamber (Conviron MP6010) for 48–72 h (Castro et al., 2016). Fungal colonies established on the medium were visualised using a stereomicroscope (Olympus S251) and the colonies were counted. Hyphal and spore morphologies were taken into account for the confirmation of species, as described by Humber (2012).

2.5 | Laboratory assessment of fungal infectivity

The experimental was set up as a factorial (2 × 3), with two treatments (fungal granules and food granules) and three soil treatments (non-sterile soil, simulated solarised soil and sterilised soil). Before the insect release, either fungal granules ($CAG_{Ma+Cs+By}$) as the treatment or food granules (CAG_{Cs+By}) as the control were added to the soil. All soil samples were collected from the USQ agriculture field, Toowoomba. For the simulated solarised soil, soil samples were heated at 45°C for 14 days in an oven (Steridium) and for the sterilised soil, the soil was heated at 105°C for 72 h in an oven. Transparent plastic containers (500 mL) were filled with 150 g of either non-sterile, simulated solarised or sterilised soil, and then inoculated with fungal granules at a rate equivalent to 3.8×10^6 conidia g^{-1} soil; individual fungal granules contained an average of 10^7 conidia.

At 28 days post-incubation, a cohort of 30 mealworm larvae (mean body length 2.65 cm; body weight 0.1 g; and eight abdominal rings) was released into granule-inoculated soils of individual containers. The containers were then inverted thrice. All containers containing soil were moistened with an additional 15 mL of sterile, distilled water per container. To feed the mealworms, corn was autoclaved and air-dried for 2 h in a laminar flow cabinet, then 1 g of corn was placed into a separate Petri dish (Ø = 2 cm) within the container to minimise contact with soil (minimising contaminating fungal growth from the corn). The containers were then sealed with a perforated lid to facilitate the aeration for mealworms, and all containers were reincubated (22°C, 80% RH, in the dark) in the growth chamber.

Assessments of mortality began at 7 days after insect exposure (DAIE) and continued every day. Following the mortality assessment on 20 DAIE, an additional 15 mL of sterile water was added to each soil container to compensate for the moisture loss during the incubation period. Dead mealworms recovered at the assessments were immediately surface sterilised, placed onto a moistened filter paper and incubated at 25°C to check for mycosis.

2.6 | Glasshouse assessment of fungal infectivity

The experiment was conducted in a glasshouse (Agriculture Science and Engineering Precinct, USQ) at 18–30°C and 60% relative humidity from May until August 2019. A plastic pot (Ø = 25 cm, h = 23.5 cm) containing 6 kg of either non-sterile or sterilised soil was inoculated with preconditioned fungal granules (granules were placed at 25°C for 24 h before the inoculation to activate the conidial germination) or non-preconditioned fungal granules at the rate of 2 g of granules (Ø = 3.5 mm) per pot, equivalent to 10^5 conidia cm^{-3} of soil. Each of the four treatments was replicated six times resulting in 24 pots. For the controls, food granules (CAG_{Cs+By}) were used following their conditioning at 25°C for 24 h before the inoculation referred to as preconditioned food granules, and CAG_{Cs+By} were used without conditioning referred to as non-preconditioned food granules. This experiment comprised eight treatments including: (i) non-sterile soil plus non-preconditioned fungal granule; (ii) non-sterile soil with non-preconditioned food granule (control); (iii) sterilised soil with non-preconditioned fungal granule; (iv) sterilised soil treated with non-preconditioned food granule (control); (v) non-sterile soil with preconditioned fungal granule; (vi) non-sterile soil with preconditioned food granule (control); (vii) sterile soil with preconditioned fungal granule and (viii) sterile soil with preconditioned food granules (control). All pots were arranged in a randomised complete block design in the glasshouse.

Each pot was first filled two-thirds with soil, then a presprouted Bellevue sweetpotato root ('Bellevue' sweetpotato [*Ipomoea batatas* (L.) Lam.]) was planted horizontally (mean weight = 600 g, mean Ø = 8 cm), and the root with the remaining one-third of the soil, which was premixed with fungal granules at the rate noted above. After planting, soil in each pot was irrigated with 200 mL of tap water, while the disparity of moisture content lying between sterilised soil (0% moisture content w/w) and non-sterile soil (20% moisture content w/w) was equalised by adding waterin the pots to weight, which was allowed to soak overnight before the inoculation.

On the 30th day after the inoculation of granules, a cohort of 40 larval mealworms (mean body length 2.65 cm; body weight 0.1 g; and 8 abdominal rings) was added to each pot. The soil surface was then covered with 2 cm presterilised (105°C for 24 h) sugarcane mulch to act as a shade for the mealworms. During the experimental period, sweetpotato shoots longer than 20 cm in length were pruned twice, on the 20th day and the 40th day after planting to avoid the vines touching one another.

2.6.1 | Post-harvest and data collection

On the 30th day after the insect release into the inoculated soil, the glasshouse experiment was terminated by harvesting the experimental pots. Pot harvesting was carried out by removing the shoots first, and then the roots were separated from the soil. Mealworms were recovered from the soil and their mortality status was assessed. The number of dead or live mealworms recovered from the individual pots were recorded, with the life stage of mealworms recovered from the soil recorded, that is, larva, pupa or adult. The health status of live mealworms was further assessed classifying them into vigorous vs moribund. Similarly, the dead insects (cadavers) were categorised into mycosed and non-mycosed based on conspicuous fungal outgrowth over the cadaver. Non-mycosed cadavers were placed over a moist

FIGURE 3 Non-damaged sweetpotato storage root after 30 days of exposure to mealworms on sterilised soil with preconditioned food granules (control treatment) (a) and mealworm damage on a sweetpotato storage root (inside the yellow circle) after 30 days exposure to mealworms on sterilised soil with preconditioned fungal granules (b)

chamber and incubated at 25°C to stimulate the fungal conidiation on the cadavers.

Live mealworms that were recovered after the harvest were further examined to confirm whether infection could be forced in a smaller volume of soil. Collected insects were placed into a 50 mL plastic container containing 40 g of soil sampled from individual pots. After the insects were transferred, the soil in the container was moistened with 5 mL of tap water and sealed with a perforated lid. The soil in the container was inverted 5 times and 5 corn seeds (autoclave sterilised) were added as food for the insects. The containers were incubated in the same glasshouse (18–30°C and 60% RH) and after 18 days, mealworm mortality was assessed. Non-conidiated cadavers were transferred to a Petri dish lined with a moist filter paper and incubated at 25°C (photoperiod 12:12 h day and night) for 7 days to encourage resporulation from the cadavers.

The sweetpotato storage roots were assessed for feeding damage caused by mealworms. Roots with any number of feeding holes were rated as damaged and roots without holes were rated as non-damaged roots (Figure 3).

2.7 | Data analysis

All statistical analyses were performed using the software IBM SPSS version 24 (SPSS). All data were checked for normality and homogeneity of variance using the Shapiro–Wilk and Levene test, respectively. All datasets satisfied the criteria of normality and homogeneity of variance and were, therefore, analysed through parametric tests. For the experiment of 'Effect of soil samples on the resporulation of fungal granules', the data analysis was performed using a Two-way ANOVA. For the experiment on the fungal infectivity test, the mortality data were converted to percentages and then further corrected using Abbot's formula (Abbott, 1925). The percentage data were analysed using a two-way ANOVA to determine the factorial interaction. There was not any mortality effect of food granules, regardless of soil level. Therefore, the effect of fungal granules in three different soil levels was analysed using an ANOVA analysis ($p \leq .05$). For the experiment of 'glasshouse assessment of fungal infectivity', the mortality data were converted to percentages and further corrected the mortality percentages using Abbot's formula. The corrected data were analysed using an ANOVA analysis. The correlation between the damaged sweetpotato roots and recovered live mealworms was assessed using the Pearson Correlation test ($p \leq .05$).

3 | RESULTS

3.1 | Effect of different soil samples on resporulation of fungal granules

No significant interaction was observed between soil samples and soil treatment ($p = .453$). The effect of soil samples on fungal resporulation was not significant ($p = .422$). However, the effect of soil treatment, namely non-sterile soil, pasteurised soil and sterilised soil on fungal resporulation was significant ($p = .001$), regardless of soil type. The greatest number of fungal CFU were obtained from fungal granules on sterilised soil, whereas the lowest fungal CFU were evident on non-sterile soil.

3.2 | Laboratory assessment of fungal infectivity

There was a significant ($p < .05$) effect of using fungal granules over food granules on mealworm mortality. Fourteen days after the insects were released, the following morality was recorded: 52% (±21) in non-sterile soil, 8% (±7) in simulated solarised soil, and 13% (±5) for sterilised soil, all of which were significantly ($p < .05$) different from one another. Significant ($p < .05$) mortality was also observed 20 days after insects were released: 79% (±21) mortality for non-sterile soil, at 32% (±18) for simulated solarised soil and 27% (±17) for sterilised soil. The insect mortalities among non-sterile soil, simulated solarised soil and sterilised soil were significantly different ($p < .05$). Twenty-five days after insect release, the mealworm mortalities were also found to be significantly different between simulated solarised (78% (±17)), sterilised soils (67% (±24)) and non-sterile soil (92% (±8)). At 30 days after the insects were released, mealworm mortalities were 96% (± 5), 91% (±14) and 84% (±17) in non-sterile soil, simulated-solarised soil and sterilised soil respectively; these were not significantly different from one another.

More than 95% of the dead mealworms recovered during the assessments that were not found with mycosis had fungal growth after they were placed in a moist chamber. Out of all the dead mealworms found in *M. anisopliae*-granule treated soils, 75% of the cadavers remained as larvae, 13% were pupae and 2% were adult beetles. At 30 days after insects were released, only two adult mealworms were recorded, although they presented as deformed adults, possibly because of poor nutrition. A total of 12 larval mealworms were observed to be moribund and displayed little movement. Across all the replicates, five live pupae were also found. In contrast, the original larval mealworms released on soil with food granules (control) had predominantly metamorphosed into adults (75%), and 10% of them had emerged into pupae and 5% remained as larvae. Pupae remained highly vulnerable to cannibalism by adult mealworms. Dead larval or pupal mealworms (~5%) were also randomly recovered in the control-treated soil, particularly in sterilised soil; the cadavers later developed into *Metarhizium*-like conidiation following their placement in a moist chamber.

3.3 | Glasshouse assessment of fungal infectivity

Thirty days after the mealworms were added to the pots the experiment was terminated. Exposure of mealworms to the preconditioned fungal granules in sterilised soil and non-sterile soil resulted in 60.82% (±15.62) and 17.96% (±7.58) mealworm mortality, respectively. Mealworms exposed to the non-preconditioned fungal granules inoculated in sterilised soil and non-sterile soil resulted in 42.18% (±14.60) and 13.63% (±10.65) respectively ($p = .001$). Likewise, the main effect of fungal granules (non-preconditioned and preconditioned) was also significantly different ($p = .036$) (Figure 4a). However, no significant ($p = .178$) interaction was found between two factors, that is, fungal granule type and soil treatment. Fungal-derived mealworm mortalities were significantly greater in sterilised soil than in non-sterile soil, irrespective of the types of fungal granules, that is, preconditioned or non-preconditioned fungal granules ($p < .05$). No significant difference ($p = .556$) in terms of mealworm mortality was observed between preconditioned and non-preconditioned fungal granules inoculated in the non-sterile soil, whereas a significant ($p = .018$) difference was found between the preconditioned and non-preconditioned fungal granules inoculated in sterilised soil.

Mealworms introduced into sterilised soil with preconditioned food granules and non-preconditioned food granules during the glasshouse study succumbed to the fungal-induced death at 3% (±4.45) and 4% (±5.5) respectively, which were significantly lower than those mortalities on soil with fungal treatments ($p < .05$), but preconditioned and non-preconditioned food granules on non-sterile soil did not cause any fungal related mealworm mortalities.

No positive correlation was observed between the damaged sweetpotato roots and the number of live mealworms recovered ($p > .05$). Following the glasshouse experiment, the recovered live mealworms were further assessed to check for latent infection by the fungus. After exposing live mealworms to experimental soil in confined containers, mortality was reassessed. Mealworms added to fungal granule-treated soil had significantly greater mortality than those exposed to food granule-treated soil ($p < .05$). Both preconditioned and non-preconditioned fungal granules inoculated in sterilised soil resulted in 81% (±8.1), and 81% (±3.8) mealworm mortality respectively, while mealworm mortalities at 58% (±8.4) and 59% (±11.34) were achieved in non-preconditioned fungal granules and preconditioned fungal granules inoculated on non-sterile soil, respectively (Figure 4b). The mealworm mortality observed in sterilised soil inoculated with fungal granules was significantly ($p < .05$) greater than that observed in non-sterile soil, irrespective of fungal granule type.

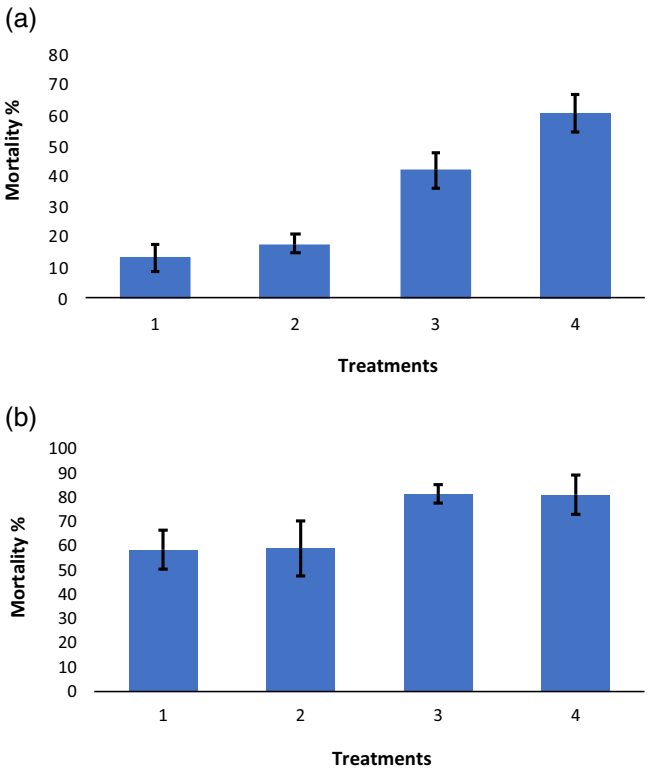

FIGURE 4 Mealworm mortality (mean ± SE, replicates = 6, $F = 7.919$, $p = .05$) from non-preconditioned fungal granules on non-sterile soil (1), preconditioned fungal granules on non-sterile soil (2), non-preconditioned fungal granules on sterilised soil (3) and preconditioned fungal granules on sterilised soil (4) during the glasshouse conditions (a) and post-glasshouse bioassay in laboratory conditions (b)

4 | DISCUSSION

This fungal resporulation study conducted on four different soil samples did not demonstrate any significant difference among the three soil types, despite the contrasting soil characteristics among the four different soil samples. The fungal resporulation was significantly affected when the fungal granules were treated on three different soil levels, namely sterilised soil, pasteurised soil and non-sterile soil. Our result showed that sterilised soil favoured the greatest fungal

resporulation compared to that observed in pasteurised and non-sterile soil. Effective soil insect control has been linked to the fungal abundance and persistence of the infective fungal colonies in soil (Ekesi, Maniania, Mohamed, & Lux, 2005). Fungal colonisation in crop fields can ensure high fungal density with good infectivity, which eventually confers the protection of a host plant against soil insects (Mayerhofer, Enkerli, Zelger, & Strasser, 2015). To encourage the rapid growth in soil, the fungal inoculum can be supplemented with nutrient additives (Knudsen, Eschen, Dandurand, & Wang, 1991). Our study showed that the number of fungal CFU recovered from sterilised soil statistically outnumbered the number of fungal CFUs recovered from pasteurised or non-sterile soil. This may be attributed to microbial suppression during the soil sterilisation, which enabled the encapsulated *M. anisopliae* to utilise the coencapsulated foods in sterilised soil, culminating in vigorous mycelial growth and subsequent resporulation (Jaronski, 2010). Non-sterile soil naturally contains a diverse range of soil microbes such as bacteria, archaea and fungi and these soil microbes inextricably interact with each other to maintain the balance status of the ecosystem. Saprotrophic fungi, particularly *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma* and *Mucor* are natural decomposers of plant and animal debris (Aislabie, & Deslippe, 2013; Lestan & Lamar, 1996). For this reason, they quickly colonise coencapsulated food sources leading to competitive fungistasis (Jaronski, 2007). *Metarhizium anisopliae* is considered a weaker saprotroph than the other soil saprophytes, which likely outcompete the encapsulated *M. anisopliae* for food (Zimmermann, 2007). Moreover, these saprotrophs release several types of enzymes and metabolites which induce the antibiosis against nearby soil microbes, for example, *Penicillium urticae* causes antibiotic fungistasis to other microbes by releasing the toxin patulin as a metabolite (Jaronski, 2010). Based on this information, the lack of germination from the conidia extracted from the fungal granules inoculated on non-sterile soil may be attributed to the antibiosis imposed by the presence of growth of contaminating saprotrophs on the granules. Bacterial growth was also observed on the granules applied to non-sterile soil. In vitro studies showed that volatile and non-volatile metabolites produced by bacteria, such as *Bacillus*, *Pseudomonas* and *Streptomyces* can be detrimental to the viability of the externally applied entomopathogenic fungi (Jaronski, 2007). Interestingly, actinomycetes, for example, *Streptomyces* spp. can produce a broad-spectrum antibiotic against other soil microbes (Aislabie, & Deslippe, 2013). Some studies elucidated that the phenomena of soil mineralisation, for instance, the conversion of organic nitrogen into ammonia (NH_4), the increased bioavailability of soil elements, for example, manganese (Mn), and alteration of soil pH occurring during soil sterilisation may favour soil microbes in the exponential growth phase when they are released into sterilised soil (Kitur & Frye, 1983). However, since the fungal granules that we used in our experiment already contained the nutrient additives to support its growth in soil, the fungus *M. anisopliae* likely did not rely on soil nutrients alone for its growth, implying that inherent soil nutrients might not have a significant impact on the *Metarhizium* growth. On the contrary, we observed the rapid and extensive growth of saprotrophic fungi over the food granules (control granules) when food granules were placed onto sterilised soil, whereas such extensive growth was not seen in non-sterile soil. Regarding the impact of edaphic factors on *M. anisopliae* growth, there is still ambiguity. A study by Rath et al. (1992) showed that soil physical properties such as soil texture, pH, electrical conductivity and cation exchange capacity do not induce any significant effect on the growth and development of EPF. Our study is also in agreement with the latter claim because no variability in terms of fungal colonies was evident among four different soil types, despite the contrasting soil physical properties.

Significant mealworm mortality observed on non-sterile soil (96% ±5) or simulated-solarised soil (91% ±14) or sterilised soil (84% ±17) with fungal inoculation within a 30-day period may be attributed to the conducive arrangements in the laboratory. Ideal temperature and relative humidity, which could contribute to the optimal fungal granule resporulation potential resulted in *M. anisopliae* being more competitive against the soil microbes. However, such favourable conditions do not naturally exist in fields that potentially restrict the fungal growth, while soil microbes show their great thermal plasticity making them more competitive than EPF in terms of nutritive food utilisation (Crowther & Bradford, 2013). In context to our target insect pest wireworms, the significant level of fungal resporulation is necessary because wireworm is characterised with tough cuticle which is further strengthened by the microbial symbiosis defending the host insect against entomopathogens (Kabaluk et al., 2017). Instead of wireworms, our study used mealworms as a model insect that limited the scope of our study, thus further testing on those specific target insects (wireworms) is required to determine if the EPF have a role in their management, while larval mealworms are widely used as a host for EPF studies (Batta, Murdoch, & Mansfield, 2010; Lestari & Rao, 2016; Przyklenk et al., 2017). However, the climate conditions where sweetpotato is grown in Australia are likely to favour the persistence and resporulation of applied biopesticides, especially as targeted irrigation along the rows keeps the soil moist in times of no rainfall. These conditions are likely to maintain fungal resporulation from the fungal granules, especially near the crop root zone, which will potentially reduce the feeding damage in sweetpotato (host crop) roots. However, we know that indigenous soil microbes and their fungistasis activity can inhibit EPF resporulation and we also know that the greater fungal resporulation found on sterilised or pasteurised soil was possibly linked to the reduced soil microbes. A practice of soil disinfestation, for example, soil fumigation is used by some commercial sweetpotato growers in Australia to control soil-borne pests and pathogens. If used, fumigation is applied to the soil when the soil for bedding roots is prepared and has been used occasionally to manage soil fungi like scurf (*Monilochaetes infuscans*), which causes substantial cosmetic damage to storage roots. Apart from controlling soil-borne pathogens and pests, Mazzola (2007) showed that soil fumigation caused a broad range of microbial suppression, providing the competitive advantage to the entomopathogenic fungus like *M. anisopliae* if it is applied on post-fumigated soil.

Our glasshouse study showed that both preconditioned and non-preconditioned fungal granules on non-sterile soil caused 17.96%

±7.58 and 13.63% ±10.65 mealworm mortalities, respectively. Based on this evidence, it may be implied that the fungal inocula applied on non-sterile soil are likely to be inhibited by soil microbes. A study conducted by Rogge, Mayerhofer, Enkerli, Bacher, and Grabenweger (2017) demonstrated that the fungal granules (calcium alginate encapsulated *M. brunneum* with autoclaved baker's yeast as food for the fungus) applied in soil could not enhance their density in soil, nor safeguard potato tubers against wireworm damage. Field soil containing a multitude of native microbes has been usually blamed for obstructing the fungal sporulation in soil (Garbeva, Hol, Termorshuizen, Kowalchuk, & De Boer, 2011). Fungistasis seems quite possible, especially in the nutrient-fortified fungal granules (Bonanomi, Gaglione, Incerti, & Zoina, 2013). In our observation, when the fungal granules were applied to non-sterile soil, opportunistic saprotrophs living in the soil, such as *Penicillium*, *Aspergillus*, *Mucor*, *Rhizopus* and *Trichoderma*, started to rapidly grow over the fungal granules by exploiting the coencapsulated food substrates (Inglis, Enkerli, & Goettel, 2012). When saprotroph growth occurs over the fungal granules, the viability of encapsulated *M. anisopliae* is impaired potentially because of the antibiotic effect of saprotrophs (Lingg & Donaldson, 1981). A follow-up viability test of fungal conidia, which were extracted from saprotroph-grown fungal granules, failed to revive *M. anisopliae* (data not shown). The second plausible reason behind the low mealworm mortality on non-sterile soil is that an unintended glasshouse temperature spike (up to 49°C for at least 7 days intermittently) occurred during the fungal sporulation period impaired the fungal growth and development. Insect mortality is dose-dependent (Ansari, Pope, Carpenter, Scholte, & Butt, 2011) and the fungal density of at least 10^6 conidia g^{-1} soil is required for wireworm infection by *M. anisopliae* in the field (Kabaluk et al., 2007). In the work presented here, the fungal granules applied on non-sterile soil produced, by far, less fungal density than that needed to result in mealworm to cause the mortality on non-sterile soil. Moreover, the relatively low overall rate of death in this experiment irrespective of treatment (soil) suggests that the concentration of conidia was insufficient, or that the mealworms effectively avoided soil containing EPF, opting to shelter safely under the provided sugarcane mulch instead. The recovery of some EPF infected mealworm cadavers on sterilised soil with food granules (control treatment) has been attributed to cross-contamination inside the glasshouse, likely because of the fan moving air and subsequent airborne conidia of *M. anisopliae* QS155.

Both preconditioned and non-preconditioned fungal granules showed 60.82% ±15.62 and 42.18% ±14.60 mealworm mortalities on sterilised soil in the glasshouse. The greatest mealworm mortality shown in this study has been linked to the reduced fungistasis on sterilised soil, as soil sterilisation, a widely adopted method to remove fungistasis, could allow the fungal granules to resporulate into the fullest capacity by exploiting the food substrates. A study conducted by Susurluk (2007) confirmed that insect infectivity by entomopathogenic nematodes (*Heterorhabditis bacteriophora* or *Steinernema feltiae*) against larval mealworms (*T. molitor*) was significantly higher in sterilised soil than in non-sterile soil. Some studies suggest that an actively growing microorganism before its inoculation into the soil can resist the antagonism of indigenous soil microbes, to some extent (Lestan & Lamar, 1996). Our study agrees with this because the preconditioned granules profusely resporulated in both sterilised and non-sterile soil, subsequently causing greater mealworm mortality than that resulting from non-preconditioned granules. A study by Mayerhofer et al. (2017) highlighted that *M. brunneum* conidiated on autoclaved barley kernels did not allow the growth of soil saprotrophs because the fungus has already consumed the nutrients available in the barley kernels. In our case, the preconditioned granules were incubated for 24 h before soil inoculation, providing a longer period of activation to achieve the profuse fungal growth over the granules. Moreover, homogeneous infection is unlikely in the soil environment, as opposed to the enforced inoculation conducted in a laboratory (Bruck, Snelling, Dreves, & Jaronski, 2005), because insects might evade the infectious fungal propagules in soil (Jaronski, 2010). Mycosed cadavers were found in the preconditioned inoculated soil which could contribute to long-term insect control by providing an additional source of inoculum, however, such consideration was beyond the scope of our study. Despite the inoculation of fungal granules in soil, the planted storage roots still incurred some feeding damage by larval mealworms, probably because the root was challenged by 100 individuals in a small volume of soil and entomopathogen-based insecticides alone cannot prevent damage with such high insect pressure (Mayerhofer et al., 2015). We aimed at targeting the control of soil insects like wireworm in sweetpotato. Wireworm naturally tends to be attracted to the host roots by following the CO_2 gradient. In our observation, mealworms tend to live at the soil surface or subsurface underneath the mulch we provided, whereas mealworms feeding in deep soil profiles were found with notable mortality because of optimal soil moisture in deep soil. Following the laboratory bioassay, live mealworms that were recovered at the time of pot harvest succumbed to fungal-induced death, confirming that mealworms that were recovered at the end of the experiment carried asymptomatic infections. Some studies also revealed that fungal asymptomatic infection in insect hosts has been linked to adverse impacts of egg-laying, hatchability, longevity and feeding efficacy of host insects (Jarrahi & Safavi, 2016; Quesada-Moraga, Santos-Quiros, Valverde-García, & Santiago-Álvarez, 2004).

This glasshouse study has indicated that the existing level of fungal resporulation on non-sterile soil from the fungal granules may not be able to safeguard sweetpotato against wireworm infestation because of the failure of optimal resporulation from the fungal granules. However, since the results with high mealworm mortalities appeared in sterilised soil, the efficacy of fungal granules can be enhanced by integrating the inoculation of fungal granules with a soil disinfecting method, for example, soil fumigation in very high pest populations, or with existing registered insecticides of sweetpotato, for example, Talstar® or Regent®. *Metarhizium anisopliae* has shown compatibility with various insecticides acting synergistically against insect control, for example, *M. anisopliae* and spinosad (insecticide derived from actinomycete toxin) giving a synergistic effect for wireworm control (Ericsson, Kabaluk, Goettel, & Myers, 2007), and similarly, the farm registered insecticides for macadamia in Australia such as certain concentrations of Lancer® (acephate) and Avatar®

(indoxacarb) was synergistic with *M. anisopliae* against macadamia seed weevil (*Kuschelorhynchus macadamiae*) under both laboratory and glasshouse conditions (Khun et al., 2020). Thus, future studies could be oriented towards the compatibility study between the registered sweetpotato insecticides and *M. anisopliae*. Moreover, the use of the microsclerotia or blastoconidia of *M. anisopliae* as a propagule for the fungal granules is also an area to be explored in the future, as microsclerotia are less likely to be affected by fungistasis (Jackson, & Jaronski, 2012), and the germination of blastoconidia is faster than conidia (Jackson, McGuire, Lacy, & Wraight, 1997). Additionally, the sporulation of fungal granules can be further assessed in soils pretreated with fumigants because microbial suppression in soil resulting from soil fumigation could be conducive for the fungal resporulation. Optimising the preconditioning of fungal granules before their application into the soil can be also included in future studies.

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REFERENCES

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, *18*(2), 265–267.

Aislabie, J., & Deslippe, J. R. (2013). Soil microbes and their contribution to soil services. In J. R. Dymond (Ed.), *Ecosystem services in New Zealand—Conditions and trends* (pp. 143–161). Lincoln, New Zealand: Manaaki Whenua Press.

Ansari, M. A., Pope, E. C., Carpenter, S., Scholte, E.-J., & Butt, T. M. (2011). Entomopathogenic fungus as a biological control for an important vector of livestock disease: The *Culicoides* biting midge. *PLoS One*, *6*(1), e16108.

Arrington, A. E., Kennedy, G. G., & Abney, M. R. (2016). Applying insecticides through drip irrigation to reduce wireworm (Coleoptera: Elateridae) feeding damage in sweet potato. *Pest Management Science*, *72*(6), 1133–1140. https://doi.org/10.1002/ps.4089

Australian Pesticides and Veterinary Medicines Authority. (June 10, 2021). *PubCRIS database*. Retrieved from https://apvma.gov.au/node/10831

Australian Sweetpotato Growers Inc. (October 14, 2021). *Sweetpotato growing in Australia*. Retrieved from http://aspg.com.au

Barsics, F., Haubruge, E., & Verheggen, F. J. (2013). Wireworms' management: An overview of the existing method, with particular regards to *Agriotes* spp. (Coleoptera: Elateridae). *Insects*, *4*, 117–152. https://doi.org/10.3390/insects4010117

Bharadwaj, A., & Stafford, K. C., III (2011). Potential of *Tenebrio molitor* (Coleoptera: Tenebrionidae) as a bioassay probe for *Metarhizium brunneum* (Hypocreales: Clavicitaceae) activity against *Ixodes scapularis* (Acari: Ixodidae). *Journal of Economic Entomology*, *104*(6), 2095–2098. https://doi.org/10.1603/EC11170

Batta, Y., Murdoch, G., & Mansfield, S. (2010). Investigations into the formulation and efficacy of entomopathogenic fungi against larvae of yellow mealworm (*Tenebrio molitor* L., Coleoptera: Tenebrionidae). *General and Applied Entomology: The Journal of the Entomological Society of New South Wales*, *39*, 5–8.

Bonanomi, G., Gaglione, S. A., Incerti, G., & Zoina, A. (2013). Biochemical quality of organic amendments affects soil fungistasis. *Applied Soil Ecology*, *72*, 135–142.

Bruck, D. J., Snelling, J. E., Dreves, A. J., & Jaronski, S. T. (2005). Laboratory bioassays of entomopathogenic fungi for control of *Delia radicum* (L.) larvae. *Journal of Invertebrate Pathology*, *89*(2), 179–183. https://doi.org/10.1016/j.jip.2005.02.007

Castro, T., Mayerhofer, J., Enkerli, J., Eilenberg, J., Meyling, N. V., de Andrade Moral, R., … Delalibera, I. (2016). Persistence of Brazilian isolates of the entomopathogenic fungi *Metarhizium anisopliae* and *M. robertsii* in strawberry crop soil after soil drench application. *Agriculture, Ecosystems & Environment*, *233*, 361–369.

Crowther, T. W., & Bradford, M. A. (2013). Thermal acclimation in widespread heterotrophic soil microbes. *Ecology Letters*, *16*(4), 469–477. https://doi.org/10.1111/ele.12069

De Faria, M. R., & Wraight, S. P. (2007). Mycoinsecticides and mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. *Biological Control*, *43*(3), 237–256.

Dotaona, R., Wilson, B. A. L., Stevens, M. M., Holloway, J., & Ash, G. J. (2015). Screening of tropical isolates of *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) for virulence to the sweet potato weevil, *Cylas formicarius* (Coleoptera: Brentidae). *International Journal of Tropical Insect Science*, *35*(4), 153–163. https://doi.org/10.1017/s1742758415000211

Ekesi, S., Maniania, N. K., & Mohamed, S. A. (2010). Efficacy of soil application of *Metarhizium anisopliae* and the use of GF-120 spinosad bait spray for suppression of *Bactrocera invadens* (Diptera: Tephritidae) in mango orchards. *Biocontrol Science and Technology*, *21*(3), 299–316. https://doi.org/10.1080/09583157.2010.545871

Ekesi, S., Maniania, N. K., Mohamed, S. A., & Lux, S. A. (2005). Effect of soil application of different formulations of *Metarhizium anisopliae* on African tephritid fruit flies and their associated endoparasitoids. *Biological Control*, *35*(1), 83–91.

Ericsson, J. D., Kabaluk, J. T., Goettel, M. S., & Myers, J. H. (2007). Spinosad interacts synergistically with the insect pathogen *Metarhizium anisopliae* against the exotic wireworms *Agriotes lineatus* and *Agriotes obscurus* (Coleoptera: Elateridae). *Journal of Economic Entomology*, *100*(1), 31–38. https://doi.org/10.1603/0022-0493(2007)100[31:siswti]2.0.co;2

Garbeva, P., Hol, W. G., Termorshuizen, A. J., Kowalchuk, G. A., & De Boer, W. (2011). Fungistasis and general soil biostasis—A new synthesis. *Soil Biology and Biochemistry*, *43*(3), 469–477.

Garrido-Jurado, I., Ruano, F., Campos, M., & Quesada-Moraga, E. (2011). Effects of soil treatments with entomopathogenic fungi on soil dwelling non-target arthropods at a commercial olive orchard. *Biological Control*, *59*(2), 239–244. https://doi.org/10.1016/j.biocontrol.2011.07.001

Gašić, S., & Tanović, B. (2013). Biopesticide formulations, possibility of application and future trends. *Pesticidi i Fitomedicina*, *28*(2), 97–102.

Hort Innovation Australia Limited. (20 Nov 2021). *Australian horticulture statistics handbook*. Retrieved from https://horticulture.com.au

Hue, S. M., & Low, M. Y. (2015). An insight into sweet potato weevils management: A review. *Psyche: A Journal of Entomology*, *2015*, 849560.

Humber, R. A. (2012). Identification of entomopathogenic fungi. In L. A. Lacey (Ed.), *Manual of techniques in invertebrate pathology* (2nd ed., pp. 151–187). Amsterdam: Elsevier.

Humbert, P., Przyklenk, M., Vemmer, M., & Patel, A. V. (2017). Calcium gluconate as cross-linker improves survival and shelf life of encapsulated and dried *Metarhizium brunneum* and *Saccharomyces cerevisiae* for the application as biological control agents. *Journal of Microencapsulation*, *34*(1), 47–56.

Inglis, G. D., Enkerli, J., & Goettel, M. S. (2012). Laboratory techniques used for entomopathogenic fungi: Hypocreales. In L. A. Lacey (Ed.), *Manual of techniques in invertebrate pathology* (pp. 189–253). New York: Elsevier.

Jabbour, R., & Barbercheck, M. E. (2009). Soil management effects on entomopathogenic fungi during the transition to organic agriculture in a feed grain rotation. *Biological Control*, *51*(3), 435–443. https://doi.org/10.1016/j.biocontrol.2009.08.004

Jackson, M. A., Dunlap, C. A., & Jaronski, S. T. (2010). Ecological considerations in producing and formulating fungal entomopathogens for use in insect biocontrol. *BioControl*, *55*(1), 129–145.

Jackson, M. A., & Jaronski, S. T. (2012). Development of pilot-scale fermentation and stabilisation processes for the production of microsclerotia of the entomopathogenic fungus *Metarhizium brunneum* strain F52. *Biocontrol Science and Technology*, *22*(8), 915–930.

Jackson, M. A., McGuire, M. R., Lacy, L. A., & Wraight, S. P. (1997). Liquid culture production of desiccation tolerant blastospores of the bioinsecticidal fungus *Paecilomyces fumosorosus*. *Mycological Research*, *101*(1), 35–41.

Jaronski, S. T. (2007). Soil ecology of the entomopathogenic ascomycetes: A critical examination of what we (think) we know. In S. Ekesi, & N. K. Maniania (Eds.), *Use of entomopathogenic fungi in biological pest management* (pp. 91–143). Trivandrum, India: Research Signpost.

Jaronski, S. T. (2010). Ecological factors in the inundative use of fungal entomopathogens. *BioControl*, *55*(1), 159–185.

Jaronski, S. T., Fuller-Schaeffer, C., Jung, K., Majumdar, A., & Boetel, M. (2007). Challenges in using *Metarhizium anisopliae* for biocontrol of sugarbeet root maggot, *Tetanops myopaeformis*. *IOBC WPRS Bulletin*, *30*(7), 119.

Jarrahi, A., & Safavi, S. A. (2016). Fitness costs to *Helicoverpa armigera* after exposure to sub-lethal concentrations of *Metarhizium anisopliae* sensu lato: Study on F1 generation. *Journal of Invertebrate Pathology*, *138*, 50–56. https://doi.org/10.1016/j.jip.2016.05.008

Kabaluk, J. T., Vernon, R. S., & Goettel, M. S. (2007). Mortality and infection of wireworm, *Agriotes obscurus* (Coleoptera: Elateridae), with inundative field applications of *Metarhizium anisopliae*. *Phytoprotection*, *88*(2), 51–56 WOS:000253805400002.

Kabaluk, T. (2014). Targeting the click beetle *Agriotes obscurus* with entomopathogens as a concept for wireworm biocontrol. *BioControl*, *59*(5), 607–616. https://doi.org/10.1007/s10526-014-9603

Kabaluk, T., Li-Leger, E., & Nam, S. (2017). *Metarhizium brunneum* – An enzootic wireworm disease and evidence for its suppression by bacterial symbionts. *Journal of Invertebrate Pathology*, *150*, 82–87. https://doi.org/10.1016/j.jip.2017.09.012

Karabörklü, S., Altin, N., & Keslin, Y. (2019). Native entomopathogenic fungi isolated from Duzce, Turkey & their virulence on the mealworm beetle (*Tenebrio molitor* L) (Coleoptera: Tenebrionidae). *Philippine Agricultural Scientist*, *102*(1), 82–89.

Khun, K. K., Ash, G. J., Stevens, M. M., Huwer, R. K., & Wilson, B. A. (2020). Interactions of fungal entomopathogens with synthetic insecticides for the control of *Kuschelorhynchus macadamiae* (Coleoptera: Curculionidae). *Journal of Applied Entomology*, *145*(6), 553–566.

Kitur, B., & Frye, W. (1983). Effects of heating on soil chemical properties and growth and nutrient composition of corn and millet. *Soil Science Society of America Journal*, *47*(1), 91–94.

Knudsen, G., Eschen, D., Dandurand, L., & Wang, Z. (1991). Method to enhance growth and sporulation of pelletized biocontrol fungi. *Applied and Environmental Microbiology*, *57*(10), 2864–2867.

Krell, V., Jakobs-Schoenwandt, D., Vidal, S., & Patel, A. V. (2018). Encapsulation of *Metarhizium brunneum* enhances endophytism in tomato plants. *Biological Control*, *116*, 62–73.

Lestan, D., & Lamar, R. T. (1996). Development of fungal inocula for bioaugmentation of contaminated soils. *Applied and Environmental Microbiology*, *62*(6), 2045–2052.

Lestari, A. S., & Rao, S. (2016). Laboratory bioassay of *Metarhizium* spp and *Beauveria* spp against *Tenebrio molitor* larvae. *Proceedings of the International Symposium for Sustainable Humanosphrere*, Bogor.

Lewis, J. A., Lumsden, R. D., & Locke, J. C. (1996). Biocontrol of damping off diseases caused by *Rhizoctonia solani* and *Pythium ultimum* with Alginate prills of *Gliocladium virens*, *Trichoderma hamatum* and various food bases. *Biocontrol Science and Technology*, *6*(2), 163–174. https://doi.org/10.1080/09583159650039368

Lingg, A., & Donaldson, M. (1981). Biotic and abiotic factors affecting stability of *Beauveria bassiana* conidia in soil. *Journal of Invertebrate Pathology*, *38*(2), 191–200.

Mayerhofer, J., Eckard, S., Hartmann, M., Grabenweger, G., Widmer, F., Leuchtmann, A., & Enkerli, J. (2017). Assessing effects of the entomopathogenic fungus *Metarhizium brunneum* on soil microbial communities in *Agriotes* spp. biological pest control. *FEMS Microbiology Ecology*, *93*(10), fix117. https://doi.org/10.1093/femsec/fix117

Mayerhofer, J., Enkerli, J., Zelger, R., & Strasser, H. (2015). Biological control of the European cockchafer: Persistence of *Beauveria brongniartii* after long-term applications in the Euroregion Tyrol. *BioControl*, *60*(5), 617–629. https://doi.org/10.1007/s10526-015-9671-6

Mazzola, M. (2007). Manipulation of rhizosphere bacterial communities to induce suppressive soils. *Journal of Nematology*, *39*(3), 213–220.

McCrystal, R. (2010). *Improving Sweetpotato soil insect pests*. Australia: Horticulture Innovation.

McCrystal, R. (2014). *Integration of crop and soil insect management in sweetpotato (VG09052)*. Australia: Australian Sweetpotato Growers.

Moslim, R., Kamarudin, N., & Wahid, M. B. (2009). Pathogenicity of granule formulations of *Metarhizium anisopliae* against the larvae of the oil palm rhinoceros beetle. *Oryctes rhinoceros* (L.). *Journal of Oil Palm Research*, *21*, 602–612.

Mukhopadhyay, S. K., Chattopadhyay, A., Chakraborty, I., & Bhattacharya, I. (2011). Crops that feed the world 5. Sweetpotato. Sweetpotatoes for income and food security. *Food Security*, *3*(3), 283–305.

Parker, W. E. (1996). The development of baiting techniques to detect wireworms (Agriotes spp., Coleoptera: Elateridae) in the field, and the relationship between bait-trap catches and wireworm damage to potato. *Crop Protection*, *15*(6), 521–527.

Parsa, S., Ortiz, V., & Vega, F. E. (2013). Establishing fungal entomopathogens as endophytes: Towards endophytic biological control. *Journal of Visualized Experiments*, *74*, e50360.

Pasche, J. S., Taylor, R. J., David, N. L., & Gudmestad, N. C. (2014). Effect of soil temperature, injection depth, and metam sodium rate on the management of verticillium wilt of potato. *American Journal of Potato Research*, *91*(3), 277–290. https://doi.org/10.1007/s12230-013-9348-6

Pilz, C., Enkerli, J., Wegensteiner, R., & Keller, S. (2011). Establishment and persistence of the entomopathogenic fungus *Metarhizium anisopliae* in maize fields. *Journal of Applied Entomology*, *135*(6), 393–403.

Praprotnik, E., Loncar, J., & Razinger, J. (2021). Testing virulence of different species of insect associated against yellow mealworm (Coleoptera: Tenebrionidae) and other potential growth stimulation to maize. *Plants*, *10*(11), 2498. https://doi.org/10.3390/plants10112498

Przyklenk, M., Vemmer, M., Hanitzsch, M., & Patel, A. (2017). A bioencapsulation and drying method increases shelf life and efficacy of *Metarhizium brunneum* conidia. *Journal of Microencapsulation*, *34*(5), 498–512.

Quesada-Moraga, E., Santos-Quiros, R., Valverde-García, P., & Santiago-Álvarez, C. (2004). Virulence, horizontal transmission, and sublethal reproductive effects of *Metarhizium anisopliae* (Anamorphic fungi) on the German cockroach (Blattodea: Blattellidae). *Journal of Invertebrate Pathology*, *87*(1), 51–58. https://doi.org/10.1016/j.jip.2004.07.002

Rath, A., Koen, T., & Yip, H. (1992). The influence of abiotic factors on the distribution and abundance of *Metarhizium anisopliae* in Tasmanian pasture soils. *Mycological Research*, *96*(5), 378–384.

Rath, A. C., Worledge, D., Anderson, G. C., & Carr, C. J. (1995). Virulence of the entomogenous fungi *Metarhizium anisopliae* (Metschnikoff) Sorokin, *M. flavoviride* Gams and Rozsypal and *Beauveria bassiana* (Balsamo) Vuillemin to *Adoryphorus couloni* (Burmeister) (Coleoptera, Scarabaeidae). *Journal of the Australian Entomological Society*, *34*, 181–186 WOS:A1995RT25100002.

Roberts, D. W., & Leger, R. J. S. (2004). *Metarhizium* spp., cosmopolitan insect-pathogenic fungi: Mycological aspects. *Advances in Applied Microbiology*, *54*, 1–70.

Rogge, S. A., Mayerhofer, J., Enkerli, J., Bacher, S., & Grabenweger, G. (2017). Preventive application of an entomopathogenic fungus in cover crops for wireworm control. *BioControl*, *62*, 613–623. https://doi.org/10.1007/s10526-017-9816-x

Seal, D. R., Chalfant, R. B., & Hall, M. R. (1992). Effectiveness of different seed baits and baiting methods for wireworms (Coleoptera: Elateridae) in sweetpotato. *Environmental Entomology*, *21*(5), 957–963.

Shah, P. A., Aebi, M., & Tuor, U. (1998). Method to immobilize the aphid-pathogenic fungus *Erynia neoaphidis* in an alginate matrix for biocontrol. *Applied and Environment Microbiology*, *64*(11), 4260–4263.

Skinner, M., Parker, B. L., & Kim, J. S. (2014). Role entomopathogenic fungi in integrated pest management. In D. P. Abrol (Ed.), *Integrated pest management* (pp. 169–191). London: Academy Press. https://doi.org/10.1016/B978-0-12-398529-3.00011-7

Stone, L. B., & Bidochka, M. J. (2020). The multifunctional lifestyles of *Metarhizium*: Evolution and applications. *Applied Microbiology and Biotechnology*, *104*(23), 9935–9945.

Susurluk, A. (2007). Effectiveness of the entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema feltiae* against *Tenebrio molitor* (yellow mealworm) larvae in different soil types at different temperatures. *Turkish Journal of Biology*, *30*(4), 199–205.

Vänninen, I., Tyni-Juslin, J., & Hokkanen, H. (2000). Persistence of augmented *Metarhizium anisopliae* and *Beauveria bas*siana in Finnish agricultural soils. *BioControl*, *45*(2), 201–222.

Vega, F. E. (2018). The use of fungal entomopathogens as endophytes in biological control: A review. *Mycologia*, *110*(1), 4–30. https://doi.org/10.1080/00275514.2017.1418578

Vemmer, M., & Patel, A. V. (2013). Review of encapsulation methods suitable for microbial biological control agents. *Biological Control*, *67*(3), 380–389. https://doi.org/10.1016/j.biocontrol.2013.09.003

Vemmer, M., Schumann, M., Beitzen-Heineke, W., French, B. W., Vidal, S., & Patel, A. V. (2016). Development of a CO_2-releasing coformulation based on starch, *Saccharomyces cerevisiae* and *Beauveria bassiana* attractive towards western corn rootworm larvae. *Pest Management Science*, *72*(11), 2136–2145.

Wraight, S., Jackson, M., & De Kock, S. (2001). Formulation of fungal biocontrol agents. In T. M. Butt, C. Jackson, & N. Magan, (Eds.), *Fungi as biocontrol agents: Progress, problems and potential* (p. 253). Wallingford, UK: CAB International.

Zimmermann, G. (2007). Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. *Biocontrol Science and Technology*, *17*(9–10), 879–920. https://doi.org/10.1080/09583150701593963

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