# Improving biocontrol of black vine weevil (*Otiorhynchus sulcatus*) with entomopathogenic fungi in growing media by incorporating spent mushroom compost

Ralph Noble · Andreja Dobrovin-Pennington · Jean D. Fitzgerald · Katherine Dew · Craig D. Wilson · Kyle Ross · Colin J. Perkins

**Abstract** Amending a peat-based growing medium with 10 %v/v spent mushroom compost, a source of fungal chitin and other nutrients, prolonged the persistence of entomopathogenic fungi (*Metarhizium brunneum* Petsch and *Beauveria bassiana* (Balsamo) Vuillemin; Hypocreales: Clavicipitaceae). This resulted in improved efficacy of *M. brunneum* against black vine weevil, *Otiorhynchus sulcatus* F. (Coleoptera: Curculionidae) larvae compared with using inoculum without spent mushroom compost. *B. bassiana* only controlled larvae when used in combination with spent mushroom compost (75±7% reduction in live larvae). Mixing entomopathogenic fungal inoculum with spent mushroom compost and growing medium was as effective in controlling black vine weevil larvae as using spent mushroom compost colonised with *M. brunneum* or *B. bassiana* in the growing medium (80±12% reduction in live larvae). The former method is preferable since it does not require production and storage of colonised spent mushroom compost, or registration of new substrate formulations of *M. brunneum* or *B. bassiana*.

**Keywords** Coleoptera, Curculionidae, Hypocreales, Clavicipitaceae, *Metarhizium brunneum*, *Beauveria bassiana*

## Introduction

Black vine weevil (BVW), *Otiorhynchus sulcatus* F. (Coleoptera: Curculionidae) is a significant pest of ornamental and fruit crops. Larval feeding on roots and underground plant

parts can stunt plant growth and with serious infestations, cause plant death. Although chemical insecticides can provide effective control (Cross and Burgess 1986), they have gradually been withdrawn due to environmental and safety concerns and increased the demand for biological alternatives such as entomopathogenic fungi (EPF). Several species of EPF are common in the soil environment but the introduction and persistence of high levels of colony forming units (CFU) in the soil has been shown to be critical in achieving good control of soil dwelling insect pests (Ansari et al. 2007; Parker et al. 2015). Pilz et al. (2011) showed that *Metarhizium brunneum* Petsch (Hypocreales: Clavicipitaceae) (until recently *Metarhizium anisopliae* var. *anisopliae*) applied to soil as fungal-colonised barley kernels was able to establish in the field and reduce populations of *Diabrotica virgifera* LeConte in maize, but the number of CFU of *M. brunneum* in the soil then declined if no insect host was present. Bruck (2005) also showed a gradual decline in the number of CFU of *M. brunneum* in a peat-based growing medium (GM) after inoculation. However, there has been little work on which soil factors are responsible for longevity of EPF populations (rhizosphere competence), and how this can be extended (Roberts and St Leger 2004). In experiments designed to understand the behaviour of *M. brunneum* in the soil after application, the highest numbers of CFU were found in the rhizosphere (Hu and St Leger 2002; Bruck 2005; Klingen et al. 2015).

Attempts have been made to supply nutrients to sustain EPF in the absence of insect hosts, either in axenic culture or in soil in the presence of competing microorganisms. EPF such as *M. brunneum* and *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) produce extracellular chitinases to penetrate the chitin cuticle of insects (St Leger et al. 1986). The EPF *Beauveria brongniartii* (Saccardo) Petch, *B. bassiana*, *Lecanicillium psalliotae* (Treschew) Zare and *M. brunneum* have been successfully grown on axenic media containing chitin or chitosan (St Leger et al. 1986; Palma-Guerrero et al. 2007).

Chitin-rich substrates including silkworm chrysalis, shellfish wastes and yeast extract, a source of fungal chitin, have been used to axenically culture several EPF (Suresh and Chandrasekaran 1998; Matsumoto et al. 2004; Ypsilos and Magan 2005; Mishra and Malik 2012; Rustiguel et al. 2012;). Amendment of GM with chitinous material, crab meal, has been used to support the growth and persistence of inoculated *M. brunneum* for BVW control (Bruck 2005). Various other carbon and nitrogen sources including cornsteep solid, almond mesocarp, maize, millet, rice and wheat grains, whey, seeds, palm leaves, vegetable and coffee wastes, sugar cane bagasse, fish meal extract, feather powder and urea, have been used as nutrient sources for EPF in axenic culture (Mohan and Pillai 1982; Dorta et al. 1990; Lopez-Llorca et al. 1999; Ypsilos and Magan 2005; Santa et al. 2005; Soundarapandian and Chandra 2007; Sahayaraj and Namasivayam 2008; Kassa et al. 2008; Rodriguez Marcondes et al. 2008). Parker et al. (2015) found that *B. bassiana* and *M. brunneum* persisted longer in forest soil when applied as a millet formulation than as a peanut formulation. The persistence of *B. bassiana* and *M. brunneum* inocula and their pest control efficacy have been shown to improve by the amendment of soil with composts, plant wastes and animal manures (Lopez-Llorca et al. 1999; Sabbour 2006), although not to our knowledge, spent mushroom compost (SMC).

SMC from button mushroom, *Agaricus bisporus* (Lange) Imbach cultivation is a source of fungal chitin, as well as other organic and inorganic nutrients (Gerrits 1988; Nitschke et al. 2011). SMC is widely used in GM (Chong et al. 1991) and as a soil amendment as a source of organic matter and inorganic nutrients (Stofella and Kahn 2001); globally over 30 Mt are produced annually (Gerrits 1988; Anon. 2014). It has not been established whether SMC is a suitable substrate for EPF, although mushroom wastes and SMC have been shown to sustain mycoparasites in the biocontrol of soil-borne fungal plant pathogens (Coventry et al. 2006; Trillas et al. 2006; Gupta et al. 2009; Lopez-Mondejar et al. 2012). SMC frequently contains

fungi such as *Trichoderma harzianum* Rifai (Visscher 1988) which may compete with EPF, so that it requires pasteurisation before use. Colonising pasteurised substrates with EPF in enclosed incubation would reduce microbial competition before mixing into the soil or GM, but increase costs. However, SMC is often steamed at 60-70 ºC for several hours before being emptied from mushroom sheds (Gerrits 1988), thereby avoiding the need for subsequent pasteurisation if it can be used promptly after emptying.

The aim of this study was to evaluate the effect of SMC has on *M. brunneum* and *B. bassiana* persistence and virulence towards BVW in a peat-based GM. The hypothesis that SMC would affect persistence and virulence was evaluated in replicated factorial design experiments. To test this hypothesis, *M. brunneum* and *B. bassiana* were added to the GM with or without SMC, both in short-term incubations (20 ºC) and in longer-term cool winter glasshouse conditions using potted strawberry plants.

## Materials and Methods

### EPF inocula

*M. brunneum* strain F52 was obtained as an axenic rice grain inoculum containing a manufacturer specified 2 %w/w *M. brunneum* or $9 \times 10^8$ CFU $g^{-1}$ (Met52® Novozymes Biologicals BioAg Group, Bagsvard, Denmark). *B. bassiana* strain ATCC 74040 was obtained as an axenic liquid inoculum containing a manufacturer specified 7.16 %w/w *B. bassiana* or $2.3 \times 10^7$ CFU $ml^{-1}$ (Naturalis L®, Belchim Crop Protection Ltd, Cambridgeshire, UK). To determine the actual numbers of CFU of *M. brunneum* or *B. bassiana* in EPF inocula or substrates used in the experiments, samples (1 g) were mixed with 9 ml sterile water in each of three replicate universal tubes. The resulting extracts were then serially diluted with sterile water so that dilutions of $10^0$ to $10^{-5}$ were made. Aliquots (0.1) ml of each

dilution were pipetted into sterile Petri dishes containing potato dextrose agar (Oxoid, Fisher Scientific, 39 g $l^{-1}$) + chlortetracycline (Sigma Aldrich, 2ml $l^{-1}$) + Triton X-100 (Fisher Scientific, 2 ml $l^{-1}$), which were then incubated at 22 ˚C. The numbers of actively growing colonies of *M. brunneum* and *B. bassiana* were recorded with a binocular microscope after 3, 5 and 7 d, which were then used to calculate the number of CFU per g fresh weight of inoculum or substrate. To ensure that the colonies counted were *M. brunneum* or *B. bassiana*, the plate cultures were allowed the sporulate and then identified according to their macro- and micro- characteristics (Domsch et al. 1980) and by comparison with axenic cultures of similar age.

Substrates

Non-steamed SMC was obtained within one week of mushroom cultivation from G's Fresh, May Farm, Littleport, Cambridgeshire, UK. The compost was originally prepared from wheat straw-based horse manure, poultry manure and gypsum, and was colonised by mushroom mycelium. Casing material, a peat and lime mixture used to cover the compost for mushroom production, was removed due to its alkalinity which reduces its suitability as a GM. GM (Bulrush Professional Multi-purpose Compost, Bulrush Peat Co., Magherafelt, Co. Derry, N. Ireland), based on about 60 %v/v peat and about 40 %v/v wood fibre, and containing proprietary 3-month controlled release fertiliser and base fertiliser was used for the experiments. Substrates were analysed for pH, electrical conductivity and moisture content (Coventry et al. 2006).

SMC was pasteurised at 65 ºC for two hours in 354 ml glass jars (microcosms) each containing 250 ml (90 g) SMC. After cooling to ambient, the SMC in the microcosms was inoculated with either *M. brunneum* or *B. bassiana*. Met52 granules were added to substrates (SMC or GM) at the recommended rate of 0.5 g $l^{-1}$ (Anon. 2016) producing an initial

substrate CFU count of 6.5 ±0.5 × $10^6$ CFU $l^{-1}$. Naturalis L was applied to substrates at a rate of 1 ml $l^{-1}$, which in preliminary tests (data not shown) produced a similar initial substrate CFU count to that used for Met52. Microcosms of pasteurised SMC without EPF inoculum were also prepared. The microcosms had loosely fitting metal lids allowing gas exchange but minimising desiccation and contamination of the substrate.

The microcosms were incubated at 20 ±1 °C in darkness for 18 d before the number of CFU of *M. brunneum* or *B. bassiana* in the substrates were determined. Samples of the *M. brunneum* and *B. bassiana* inocula used in each experiment were analysed for CFU with the same procedure. The incubated and/or EPF colonised SMC samples were used for the following experiments within two weeks.

Microcosm experiment 1: Persistence of EPF in peat-based GM

GM (250 ml, 90 g) containing the following additives were filled into microcosms described above ($n$ = 3): (a) none, control (b) uninoculated fresh SMC, 10 % v/v (c) *M. brunneum* Met52 inoculum, 0.5 g $l^{-1}$ (d) *B. bassiana* Naturalis L inoculum, 1 ml $l^{-1}$ (e) SMC colonised with *M. brunneum*, 10 % v/v (f) SMC colonised with *B. bassiana*, 10 % v/v.
The microcosms were then kept in an incubator at 21 ±1 ºC in darkness. Samples of the microcosm contents were analysed for the populations of *M. brunneum* and *B. bassiana* CFU after 0, 11, 24, 41, 78 and 189 d as described above.

Microcosm experiment 2: Control of BVW larvae

Microcosms containing the same treatments as in microcosm experiment 1 were prepared. The following treatments where EPF inocula were added to SMC at the time of filling of

microcosms were also prepared: (g) SMC, 10% v/v, inoculated with *M. brunneum* Met52 0.5 g $l^{-1}$ (h) SMC, 10 % v/v, inoculated with *B. bassiana* Naturalis L 1 ml $l^{-1}$.

Between 6 and 10 late instar BVW larvae (Fig. 1), obtained from infested strawberry (*Fragaria* × *ananassa* Duchesne ex Rozier) plants were inserted around the surface of the substrate in each microcosm to a depth of 25 mm. Small pieces of carrot as food for the larvae and moisture were added to the surface of the substrate and then replaced or replenished weekly. Microcosms were kept at 20 ±1 ºC in darkness for three weeks, the contents were then removed and sorted and the viability of the larvae assessed. Samples of substrate were removed from 25 mm depth after 0, 5, 8, 11 and 21 d and analysed for the populations of *M. brunneum* and *B. bassiana* CFU as described above. The experiment consisted of four similar consecutive batches of microcosms, each with three replicate microcosms of each treatment (a) to (h).

Glasshouse strawberry pot experiments

Two similar glasshouse strawberry pot experiments with a duration of 125 d were set up on 3 October 2014 and 5 October 2015. GM containing treatments (a) to (h) described above were prepared and filled into 1.5 l plastic pots (size 14T, Plantpak, Waalwijk, Netherlands) with a single strawberry plant (cv. Flamenco) grown in 7 cm square pots containing a peat-based GM. Four second or third instar BVW larvae, obtained from infested strawberry plants, were inserted in the substrate at a depth of 40 mm at opposite sides of the pot, 30 mm from the edge. Similar pots were prepared without BVW larvae. There were ten replicates of each treatment, and 20 replicate pots of treatment (a). The pots were placed in plastic saucers and spaced on a glasshouse bench in a randomised block design, each block containing a single replicate of each of treatments (b) to (h) and two replicates of treatment (a) (Fig. 2). The pots

were regularly watered to maintain a moisture content of 72 ±3 %w/w in the substrates. The glasshouse minimum air temperature was 2 ±0.5 ºC; ventilation was set at an air temperature of 20 ºC. The pots were lit continuously with high intensity sodium discharge grow lamps (Master Son-T PIA Plus 400W, Philips) at 4.25 W $m^{-2}$.

Substrate samples were taken at the start of the glasshouse experiments and from the pots after 59 and 109 d and assessed for populations of *M. brunneum* and *B. bassiana* CFU. At the end of the experiments, before any adult BVW had emerged, the substrate was removed and inspected for the numbers of viable and dead BVW larvae and pupae. The remaining substrate was carefully removed and washed from the roots and surface moisture removed from the plants. The plants were then weighed before and after drying at 70 ºC.

Statistical analysis

For analyses of EPF CFU in samples taken from different GM treatments and at different time points in the above microcosm and glasshouse experiments, we used one- and two-way ANOVA. A $\log_{10}$ transformation of numbers of EPF CFU in GM samples was used to homogenize the variances in the data. Numbers of surviving BVW larvae and pupae and final plant weights were initially analysed separately for glasshouse experiments 1 and 2 by ANOVA. Since there was no significant effect of the blocking structure on any of the measurements in either experiment, the treatment means were then included in a combined ANOVA of both experiments 1 and 2. The effect of treatments and time sampling points was assessed by conducting two-sided t-tests on means obtained from the ANOVA analyses. Results were analysed by GenStat Version 13.1. Throughout, $\alpha \leq 0.05$ was used to determine statistical significance.

## Results

### Properties of SMC, GM and EPF inocula

The GM and SMC used in the experiments had similar pH values and moisture contents but the SMC had a much higher electrical conductivity (Table 1). Addition of 10 % v/v SMC to GM resulted in a ×2 increase in the electrical conductivity compared with non-amended GM.

The average EPF populations in the axenic inoculum products determined were 1.28 (±SE 0.73) × $10^9$ CFU $g^{-1}$ for *M. brunneum* in Met52 and 3.37 (±SE 1.24) × $10^8$ CFU $ml^{-1}$ for *B. bassiana* in Naturalis L. These values are similar to the manufacturer product specifications stated earlier. The mean EPF populations in the colonised SMC used in the incubated microcosm and glasshouse strawberry pot experiments were 3.98 (±SE 1.88) × $10^8$ CFU $g^{-1}$ for *M. brunneum* and 5.97 (±SE 1.75) × $10^8$ CFU $g^{-1}$ for *B. bassiana*.

### Incubated microcosms

As expected, no *M. brunneum* or *B. bassiana* could be detected in the SMC or GM used in the experiments without the addition of Met52 or Naturalis L. Treatments (a) GM only and (b) GM + SMC were therefore removed from the statistical analysis of numbers of CFU. In microcosm experiment 1, there were differences in CFU between the other GM amendment treatments ($F_{3,24}$ = 39.52; $p < 0.001$), the time sampling points ($F_{5,24}$ = 11.13; $p < 0.001$), and the interaction of GM treatment × time of sampling ($F_{15,24}$ = 3.92; $p$ = 0.001). The population of *B. bassiana* CFU in GM declined rapidly after the addition of Naturalis L, and could not be detected after 41 d (Fig. 3). Where *B. bassiana* was added with 10 %v/v colonised SMC, the population of CFU declined more gradually and was still detectable after 189 d although lower than at the start ($t_{24}$ = 2.54; $p$ = 0.018). Conversely, there was no significant reduction in the population of *M. brunneum* CFU after addition of Met52 granules or 10 %v/v

colonised SMC to GM ($t_{24}$ = 0.787 or 1.593; $p$ = 0.439 or 0.124) (Fig. 3).

In microcosm experiment 2, there were differences in CFU between the EPF and SMC amendment treatments in the GM ($F_{5,30}$ = 3.30; $p$ = 0.017), the time sampling points ($F_{4,30}$ = 5.56; $p$ = 0.002), and the interaction of GM treatment × time of sampling ($F_{20,30}$ = 2.82; $p$ = 0.005). Where *B. bassiana* was added to GM as Naturalis L liquid inoculum without SMC or with colonised SMC, the number of CFU did not change significantly during the experiment ($t_{30}$ = 1.21 or 0.06; $p$ = 0.236 or 0.953). Similarly, the addition of *M. brunneum* to GM, either as Met52 granules or as colonised SMC, did not result in a significant change in the number of CFU during the experiment ($t_{45}$ = 0.95 or 0.05; $p$ = 0.347 or 0.960). Addition of *M. brunneum* or *B. bassiana* with uncolonised SMC to GM resulted in an initial increase in CFU followed by stable numbers in the substrate; i.e. differences between days 0 and 5 were significant ($t_{30}$ = 2.85 or 4.27; $p$ = 0.008 or <0.001) but no significant differences between days 5 and 21 ($t_{30}$ = 0.79 or 0.25; $p$ = 0.436 or 0.804) (Fig. 4).

At the end of incubated microcosm experiment 2, the majority of live BVW were retrieved as late instar larvae although a small number of larvae had pupated due to the warm incubation period. Of the retrieved dead BVW larvae, 82 % were visibly colonised with mycelium of *M. brunneum* or *B. bassiana*. There were differences between GM amendment treatments in the percentage of retrieved live larvae and pupae ($F_{7,24}$ = 5.27; $p$ < 0.001). Addition of EPF with 10 %v/v colonised SMC to GM resulted in fewer live BVW larvae at the end of the experiment than the untreated or SMC treated GM ($t_{24}$ > 2.09; $p$ < 0.047) (Fig. 5). The addition of *M. brunneum* to GM resulted in fewer live BVW larvae and pupae when applied in colonised SMC than when Met52 granules were used alone at ×10 rate (0.5 g $l^{-1}$) ($t_{24}$ = 3.14; $p$ = 0.004). Addition of Met52 granules at 0.05 g $l^{-1}$ to GM with SMC at the time of potting resulted in a final percentage of live BVW larvae and pupae that was intermediate between and not significantly different to those obtained with the above two methods of

applying *M. brunneum* to GM, ($t_{24} < 1.71$; $p > 0.100$). The effects of addition of *B. bassiana* to GM, either alone at ×10 rate (Naturalis L at 1 ml $l^{-1}$) or with SMC at the time of potting at 0.1 ml $l^{-1}$, on the percentage of live BVW larvae and pupae were not significant when compared with the untreated control GM ($t_{24} < 1.46$; $p > 0.157$).

Glasshouse strawberry pot experiments

The minimum, average and maximum glasshouse air temperatures were 3.8, 12.0 and 24.6 ºC in experiment 1 and 1.9, 12.1 and 22.0 ºC in experiment 2. At the times of the final assessments, the roots of the strawberry plants had grown through the potting substrates and were visible at the sides and base of the pots.

No EPF CFU were found in the strawberry pots containing only GM or GM with 10 %v/v SMC. Treatments (a) GM only and (b) GM + SMC were therefore removed from the statistical analysis of numbers of CFU. There were differences in CFU between the other GM amendment treatments ($F_{5,18} = 5.83$; $p = 0.002$) and the interaction of GM treatment × time of sampling ($F_{10,18} = 4.01$; $p = 0.005$). In pots where EPF and SMC were combined with GM at the time of potting of strawberry plants, the initial populations of EPF CFU were lower than in the other EPF treatments ($t_{18} > 3.77$; $p = 0.001$) (Fig. 6). However, by day 59 of the experiments, these populations had increased and were not significantly different to those of the other EPF treatments ($t_{18} < 1.07$; $p > 0.298$). Where Naturalis L was added to GM without SMC, the *B. bassiana* CFU population declined, and by the end of the experiments was significantly lower than in the other EPF treatments ($t_{18} > 2.59$; $p < 0.019$) (Fig. 6).

In both of the glasshouse strawberry pot experiments, small natural infestations of BVW larvae were recorded in pots that were not artificially infested. Of pots that did not have an initial artificial infestation of larvae, 37 % in glasshouse experiment 1 and 3 % in glasshouse experiment 2 had larvae at the end. It can be assumed that similar percentages of artificially

infested pots also had a similar natural infestation of BVW larvae since they were randomly positioned among pots without an artificial infestation.

At the end of the glasshouse strawberry pot experiments, the majority of live BVW were retrieved as late instar larvae although a small number of pupae were also retrieved. Based on the initial artificial and subsequent natural infestations of larvae, 89% of dead BVW larvae had disintegrated and could not be found although 11% of dead larvae and pupae were retrieved. Of these retrieved dead larvae and pupae, 76% were visibly colonised with mycelium of *M. brunneum* or *B. bassiana*. There were significant differences between GM amendment treatments in the percentage of retrieved live larvae and pupae ($F_{7,16} = 2.99$; $p = 0.033$). Addition of 10 %v/v SMC or *B. bassiana* as Naturalis L at 1 ml $l^{-1}$ to GM did not significantly affect the final percentage of live BVW larvae and pupae in pots compared with the untreated GM control ($t_{16} = 0.758$ or 0.155; $p = 0.460$ or 0.879) (Fig. 7). Compared with the untreated control, addition of SMC with EPF inocula to GM at a rate of 0.05 g $l^{-1}$ (Met52) or 0.1 ml $l^{-1}$ (Naturalis L) resulted in reductions in the percentage of live BVW larvae and pupae ($t_{16} > 2.08$; $p < 0.048$), which were not significantly different to that obtained with adding Met52 alone to GM at 0.5 g $l^{-1}$ ($t_{16} < 0.43$; $p > 0.673$).

Across all treatments in the two glasshouse experiments, dry weight and fresh weight of strawberry plants were closely correlated ($R^2 = 0.928$ and 0.882; $p < 0.001$). The effects of BVW larvae on plant dry weight were assessed by noting which uninfested plants did not have a natural infestation of larvae, either dead or alive at the end. However, it is possible that some of these plants had a natural infestation but due to disintegration of dead larvae, these were not detected at the end of the experiment. Overall, the presence of BVW larvae resulted in a reduction in plant dry weight (mean values 9.0 g and 10.5 g with and without larvae present) ($F_{1,16} = 5.19$; $p = 0.037$). In the absence or presence of BVW larvae, there were no significant effects of SMC or EPF treatments on plant dry weight compared with plants

growing in unamended GM ($F_{7,16} < 2.27$; $p > 0.097$).

## Discussion

This work shows that SMC enables EPF to persist in a GM in the absence of an insect host, possibly by providing an alternative nutrient source such as chitin. This resulted in improved efficacy of EPF inoculum compared with using EPF inoculum without SMC. For *M. brunneum*, SMC reduced the amount of EPF inoculum needed to obtain the same BVW larvae kill by at least tenfold, and/or improved the kill of BVW larvae. *B. bassiana* was only effective in killing BVW larvae when incorporated in GM with SMC. *M. brunneum* persisted better than *B. bassiana* in the absence of SMC, possibly due to the nutrition provided by rice grain in the Met52 formulation. The initial increases in numbers of CFU of *M. brunneum* and *B. bassiana* following addition to GM with uncolonised SMC were probably due to the nutrition provided by the SMC. Where inoculum was added as colonised SMC there was no further increase above the initial level of CFU. Mixing the EPF inoculum with SMC at the time of blending with GM is preferable to using SMC colonised with EPF in GM since it does not require production and storage of colonised SMC, or registration of new SMC substrate formulations of EPF. The cost of axenic EPF inoculum is typically >\$50 $kg^{-1}$ (e.g. http://www.evergreengrowers.com; http://www.growershouse.com); at a recommended rate of a commercial formulation of *M. brunneum*, Met52 of 500 g $m^{-3}$, this adds >\$25 $m^{-3}$ to the cost of a GM. However, by incorporation with SMC, the effective rate and cost of EPF inoculum can be reduced by 90 %. Bruck and Donohue (2007) showed that for a granular formulation of *M. brunneum* F52, an application rate 600 g $m^{-3}$ GM provided slightly better control of BVW than 300 g $m^{-3}$. The level of BVW control they achieved corresponded with that obtained here with a similar, recommended Met52 rate of 500 g $m^{-3}$ GM (Anon. 2016).

In cool temperate soil over one year, rhizosphere competence of Norwegian isolates of

*Beauveria pseudobassiana* Rehner & Humber and *B. bassiana* was better than that of an exotic isolate *M. brunneum* resulting in greater long term control of BVW larvae (Klingen et al. 2015). Parker et al. (2015) found that the persistence of *B. bassiana* isolates was better than that of *M. brunneum* isolates following inoculation in Canadian forest soil. When EPF were applied with SMC in incubated microcosms here, *M. brunneum* was more effective in controlling BVW larvae in the short-term and sustained higher populations of CFU in the longer-term than *B. bassiana*. However, in glasshouse experiments at lower temperature, both EPF sustained similar populations of CFU and were equally effective in BVW larvae control in the longer-term.

Klingen et al. (2015) found that *B. pseudobassiana*, *B. bassiana* and *M. brunneum* were all effective in controlling BVW larvae at 18 ºC whereas at 6 ºC, only the *Beauveria* species had efficacy, although the level of control was poor. Amendment of soil or GM with SMC may improve the biocontrol efficacy of EPF at low temperatures, which has been a significant challenge (Klingen et al. 2015).

The strawberry plant material used for the glasshouse experiments was raised in a peat-based GM that was not protected against BVW. After repotting into larger pots containing GM with EPF and/or SMC treatments, the original potting medium in the centre of the pot may have allowed some BVW survival, particularly from a natural infestation which was not necessarily placed in the surrounding GM. The inclusion of EPF + SMC in the strawberry propagation GM may provide a greater level of protection against BVW throughout the crop.

The chitin content of mycelium of *A. bisporus* is around 5 %w/w on a dry matter basis (Nitschke et al. 2011) so it is possible that the fungal chitin in SMC provided a selective nutrient source for EPF in the same way as insect cuticle chitin. There are also nutrient sources for EPF in SMC other than mushroom mycelium since both *M. brunneum* and *B. beauveria* grew well on sterilised compost before mushroom cultivation, although growth on

pasteurised compost without mushroom mycelium was poor (Fitzgerald and Noble 2013). Further work is needed using different sources of SMC to determine if there is a relationship between the content of chitin and/or other nutrient sources and the growth of EPF. Further work is also needed to determine if effective and economic control of BVW and other soil dwelling pests can be achieved in the field at low application rates of SMC + EPF inoculum. However, this work has already demonstrated that SMC can provide a benefit in the biocontrol of BVW larvae and potentially other soil-dwelling pests in containerised plant production, thereby valorising this by-product.

**References**


Anonymous (2014) FAO Statistics. Mushrooms and truffles. Rome: Food and Agriculture Organization of the United Nations http://www.fao.org/faostat/en/#search/mushrooms%20and%20triffels (Cited 1 May 2017)

Anonymous (2016) Met52® Bioinsecticide for your Integrated Pest Management (IPM) program. http://www.monsantobioag.com/global/emea/Products/Documents/5Met52/Met52_flyer_EN1015.pdf (Cited 1 May 2017)

Ansari MA, Shah FA, Whittaker M, Prasad M, Butt TM (2007) Control of western flower thrips (*Frankliniella occidentalis*) pupae with *Metarhizium anisopliae* in peat and peat alternative growing media. Biol Control 40: 293–297

Bruck DJ (2005) Ecology of *Metarhizium anisopliae* in soilless potting media and the rhizosphere: implications for pest management. Biol Control 32: 155–163

Bruck DJ, Donohue KM (2007) Persistence of *Metarhizium anisopliae* incorporated in soilless potting media for control of the black vine weevil *Otiorhynchus sulcatus* in container-grown ornamentals. J Invertebrate Path 95: 146–150

Chong C, Cline RA, Rinker DL (1991) Growth and mineral nutrient status of containerized woody species in media amended with spent mushroom compost. J Amer Soc Hort Sci 116: 242–247

Coventry E, Noble R, Mead A, Marin FR, Perez JA, Whipps JM (2006) Allium white rot suppression with composts and *Trichoderma viride* in relation to sclerotia viability. Phytopathol 96: 1009–1020

Cross JV, Burgess CM (1986) Localised insecticide treatment for the control of vine weevil larvae *(Otiorhynchus sulcatus)* on field-grown strawberry. Crop Protection 6: 565–574

Domsch KH, Gams W, Anderson TH (1980) Compendium of soil fungi, Volume 1. Academic Press, London

Dorta B, Bosch A, Arcas JA, Ertola RJ (1990) High level of sporulation of *Metarhizium anisopliae* in a medium containing by-products. Appd Microbiol Biotechnol 33: 712–715

Fitzgerald JD, Noble R (2013) Improving the biocontrol potential of entomopathogenic fungi for soil dwelling arthropod pests. Final Report Defra Project PS2138. http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=2&ProjectID=18587 (Cited 1 May 2017)

Gerrits JPG (1988) Nutrition and compost. In: van Griensven LJLD (ed) The cultivation of mushrooms. Darlington Mushroom Laboratories Ltd, Sussex, UK, pp. 29–72.

Gupta VP, Sharma DD, Mahadevawamy H, Chandrashekar DS (2009) *Trichoderma pseudokoningii* for hastening the decomposition of various sericultural wastes and impact of enriched composts on disease suppression in mulberry (*Morus* spp.). Archiv Phytopathol Plant Protection 42: 603–609

Hu G, St Leger RJ (2002) Field studies using a recombinant mycoinsecticide (*Metarhizium anisopliae*) reveal that It Is rhizosphere competent. Appd Env Microbiol 68: 6383–6387

Kassa A, Brownbridge M, Parker BL, Skinner M, Gouli V, Gouli S, Guo M, Lee F, Hata T (2008) Whey for mass production of Beauveria bassiana and *Metarhizium anisopliae*. Mycol Res 112: 583–591

Klingen I, Westrum K, Meyling NV (2015) Effect of Norwegian entomopathogenic fungal isolates against *Otiorhynchus sulcatus* larvae at low temperatures and persistence in strawberry rhizospheres. Biol Control 81:1–7

Lopez-Llorca LV, Carbonell T, Salinas J (1999) Colonization of plant waste substrates by entomopathogenic and mycoparasitic fungi - a SEM study. Micron 33: 325–333

Lopez-Mondejar R, Blaya J, Obiol M, Ros M, Pascual JA (2012) Evaluation of the effect of chitin-rich residues on the chitinolytic activity of *Trichoderma harzianum*: In vitro and greenhouse nursery experiments. Pesticide Biochem Physiol 103: 1–8

Matsumoto Y, Saucedo-Castaneda G, Revah S, Shirai K (2004) Production of β-*N*-acetylhexosaminidase of *Verticillium lecanii* by solid state and submerged fermentations utilizing shrimp waste silage as substrate and inducer. Process Biochem 39: 665–671

Mishra S, Malik A (2012) Comparative evaluation of five *Beauveria* isolates for housefly (*Musca domestica* L.) control and growth optimization of selected strain. Parasitol Res 111: 1937–1945

Mohan KS, Pillai GB (1982) A method for laboratory-scale mass cultivation of *Metarhizium anisopliae*. Folia Microbiologica 27: 281–283

Nitschke J, Altenbach H-J, Malolepszy T, Mölleken H (2011) A new method for the quantification of chitin and chitosan in edible mushrooms. Carbohydrate Res 346: 1307–1310

Palma-Guerrero J, Jansson H-B, Salinas J, Lopez-Llorca LV (2007) Effect of chitosan on hyphal growth and spore germination of plant pathogenic and biocontrol fungi. J Appd Microbiol 104: 541–553

Parker BL, Skinner M, Gouli SY, Gouli VV, Tobi D, Kim JS (2015) Persistence of *Beauveria bassiana* sensu lato and *Metarhizium anisopliae* sensu lato in Vermont (USA) forest soil. Biocontrol Sci Technol 25: 768–788

Pilz C, Enkerli J, Wegensteiner R, Keller S (2011) Establishment and persistence of the entomopathogenic fungus *Metarhizium anisopliae* in maize fields. J Appd Entomol 135: 393–403

Roberts DW, St Leger RJ (2004) *Metarhizium* spp., Cosmopolitan Insect-Pathogenic Fungi: Mycological Aspects. Adv Appd Microbiol 54: 1–70

Rodrigues Marcondes N, Ledesma Taira C, Cirena Vandresen D, Estivalet Svidzinski TI, Kadowaki MK, Peralta RM (2008) New feather-degrading filamentous fungi. Microbial Ecol 56: 13–17

Rustiguel CB, Jorge JA, Guimaraes LHS (2012) Optimisation of the chitinase production of different *Metarhizium anisopliae* strains under solid state fermentation with silkworm chrysalis as substrate using CCRD. Adv Microbiol 2: 268–276

Sabbour MM (2006) Effect of some fertilizers mixed with bioinsecticides on the potato tuber moth *Phthorimaea operculella* infesting potato in the field and store. Pakistan J Biol Sci 9: 1929–1934

Sahayaraj K, Namasivayam SKR (2008) Mass production of entomopathogenic fungi using agricultural products and by products. Afr J Biotechnol 7: 1907–1910

St Leger RJ, Cooper RM, Charnley AK (1986) Cuticle-degrading enzymes of entomopathogenic fungi: Regulation of production of chitinolytic enzyme. J Gen Microbiol 132: 1509–1517

Santa HSD, Santa ORD, Brand D, Vandenberghe LPDS, Soccol CR (2005) Spore production of *Beauveria bassiana* from agro-industrial residues. Brazilian Archiv Biol Technol 48: Issue SPEC. ISS. 51–60

Soundarapandian P, Chandra R (2007) Mass production of entomopathogenic fungus *Metarhizium anisopliae* (Deuteromycota; Hyphomycetes) in the laboratory. Res J Microbiol 2: 690–695

Stofella PJ, Kahn BA (2001) Compost utilization in horticultural cropping systems. CRC Press LLC, Boca Raton, FA

Suresh PV, Chandrasekaran M (1998) Utilization of prawn waste for chitinase production by the marine fungus *Beauveria bassiana* by solid state fermentation. Wrld J Microbiol Biotechnol 14: 655–660

Trillas MI, Casanova E, Cotxarrera L, Ordovás J, Borrero C, Avilés B (2006) Composts from agricultural waste and the *Trichoderma asperellum* strain T-34 suppress *Rhizoctonia solani* in cucumber seedlings. Biol Control 39: 32–38

Visscher H (1988) Casing soil. In: van Griensven LJLD (ed) The cultivation of mushrooms. Darlington Mushroom Laboratories Ltd, Sussex, UK, pp. 73–88.

Ypsilos IK, Magan N (2005) Characterisation of optimum cultural environmental conditions for the production of high numbers of *Metarhizium anisopliae* blastospores with enhanced ecological fitness. Biocontrol Sci Technol 15: 683–699

Table 1. Mean properties (±SEs) of spent mushroom compost (SMC) and peat-based growing medium (GM) used in the experiments; $n = 5$

| Substrate | pH | Elec. conductivity $\mu S\ cm^{-1}$ | Moisture, % w/w |
|---|---|---|---|
| SMC | 6.21 ±0.45a | 7475 ±1125c | 55.7 ±5.1a |
| GM | 6.02 ±0.60a | 655 ±100a | 60.5 ±2.0a |
| GM + 10%v/v SMC | 6.04 ±0.43a | 1370 ±175b | 59.1 ±3.1a |

Electrical conductivity was determined on a 1:5 v/v suspension in distilled water and measured in microsiemens $cm^{-1}$. Values in the same column followed by the same letter are not significantly different ($\alpha = 0.05$)

**Fig. 1** Vine weevil larvae used for microcosms experiments

**Fig. 2** Glasshouse strawberry pot experiment

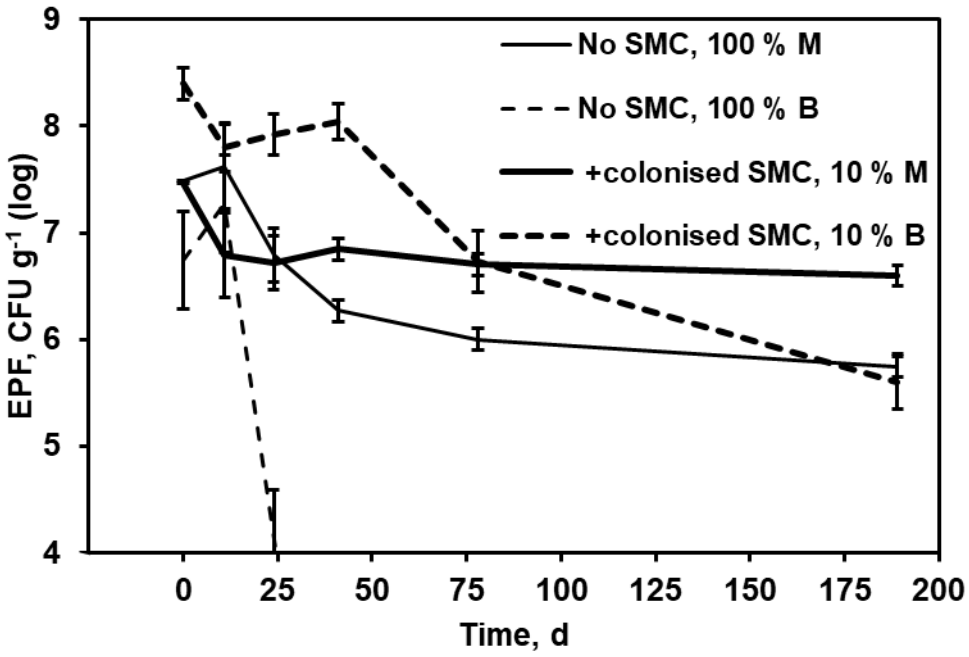


**Fig. 3** Mean population (±SE) of entomopathogenic fungi (EPF; Mb, *M. brunneum* Met52; Bb, *B. bassiana* Naturalis L) in growing medium (GM) with and without 10 % v/v spent mushroom compost (SMC) in microcosms, $n = 3$. At 100 %, EPF rates per l GM were 0.5 g for Mb and 1 ml for Bb. No EPF were detected in microcosms containing uninoculated GM, with or without SMC, or after 41 d in microcosms containing GM + 100% Bb without SMC

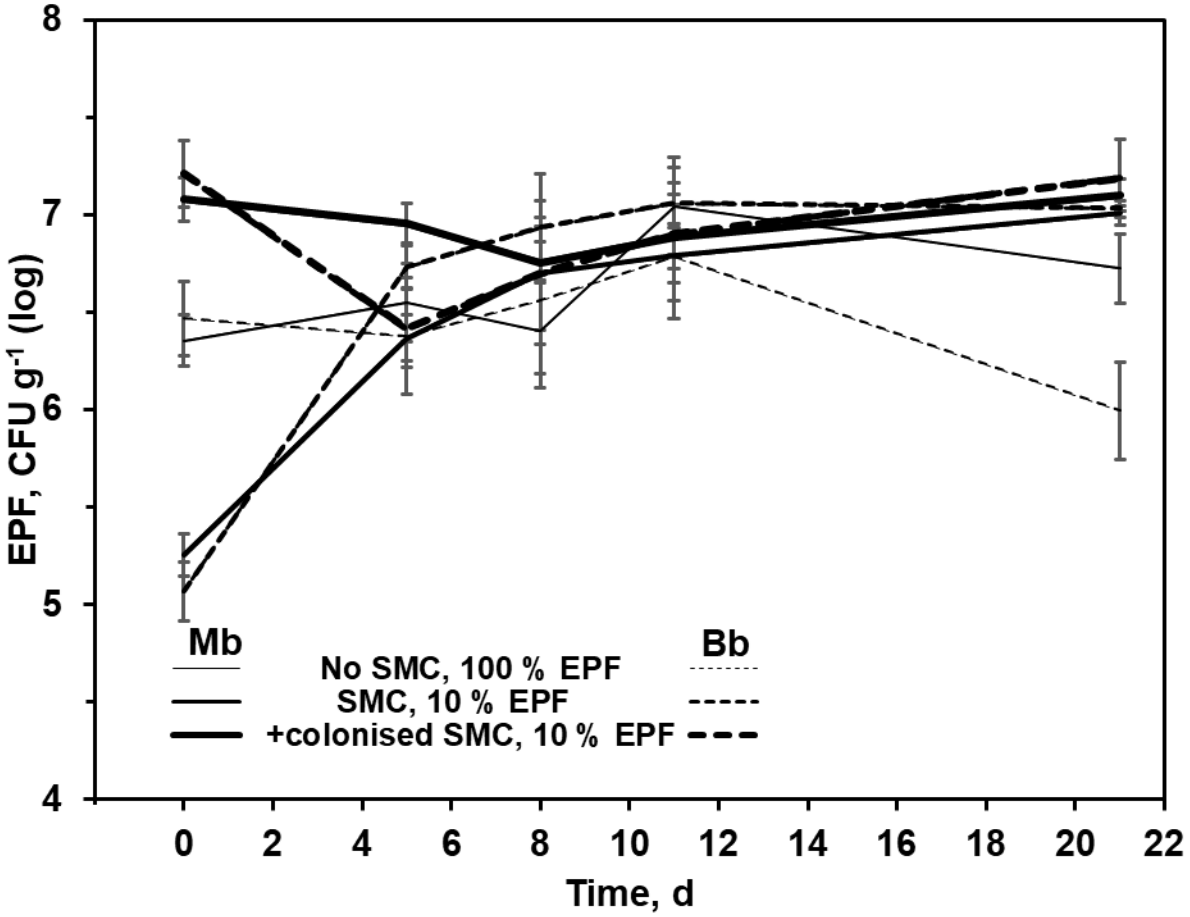


**Fig. 4** Mean population (±SE) of entomopathogenic fungi (EPF; Mb, *M. brunneum* Met52; Bb, *B. bassiana* Naturalis L) in growing medium (GM) with and without 10 % v/v spent mushroom compost (SMC) across four batches of microcosms; *n* = 3 in each batch. At 100 %, EPF rates per l GM were 0.5 g for Mb and 1 ml for Bb. No EPF were detected in microcosms containing uninoculated GM, with or without SMC

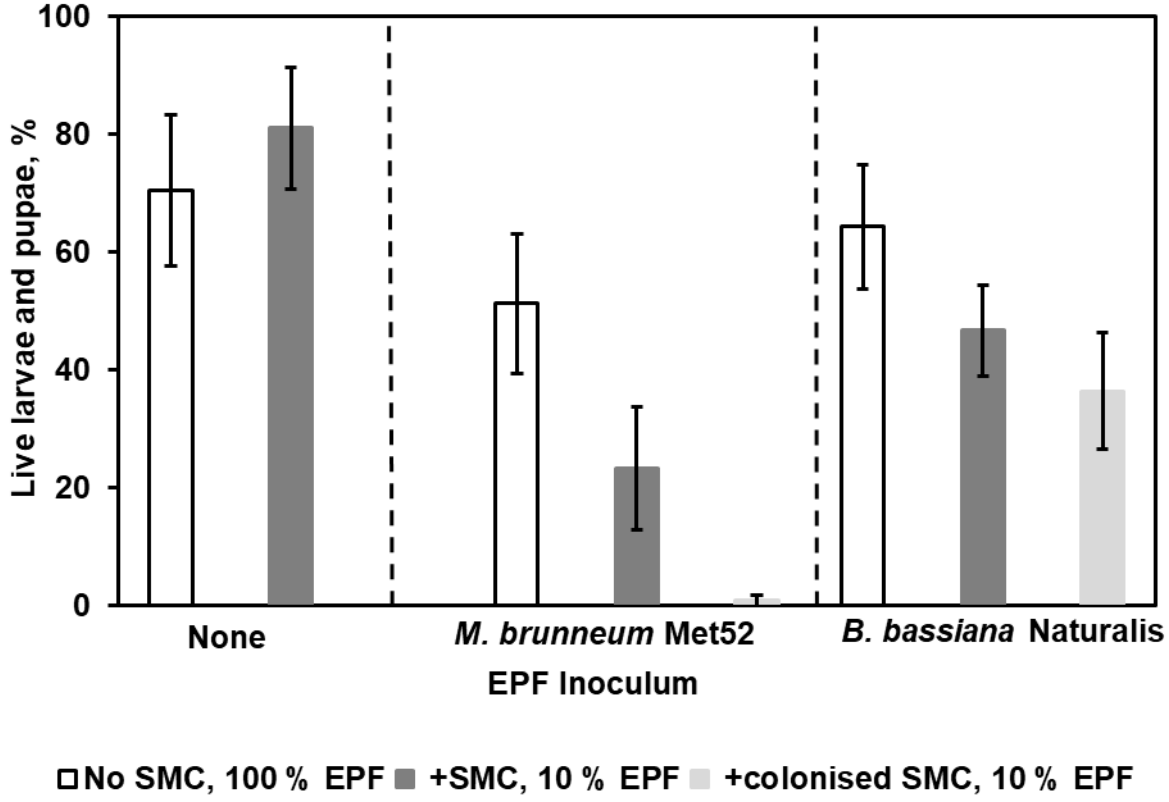


**Fig. 5** Mean final percentage (±SE) of live black vine weevil larvae and pupae in growing medium (GM) with and without 10 % v/v spent mushroom compost (SMC) and entomopathogenic fungi (EPF) across four batches of microcosms initially containing 6 to 10 larvae, $n = 3$ in each batch. Any of the eight bars with the same letter are not significantly different ($\alpha = 0.05$). At 100 %, EPF rates per l GM were 0.5 g for *M. brunneum* Met52 and 1 ml for *B. bassiana* Naturalis L

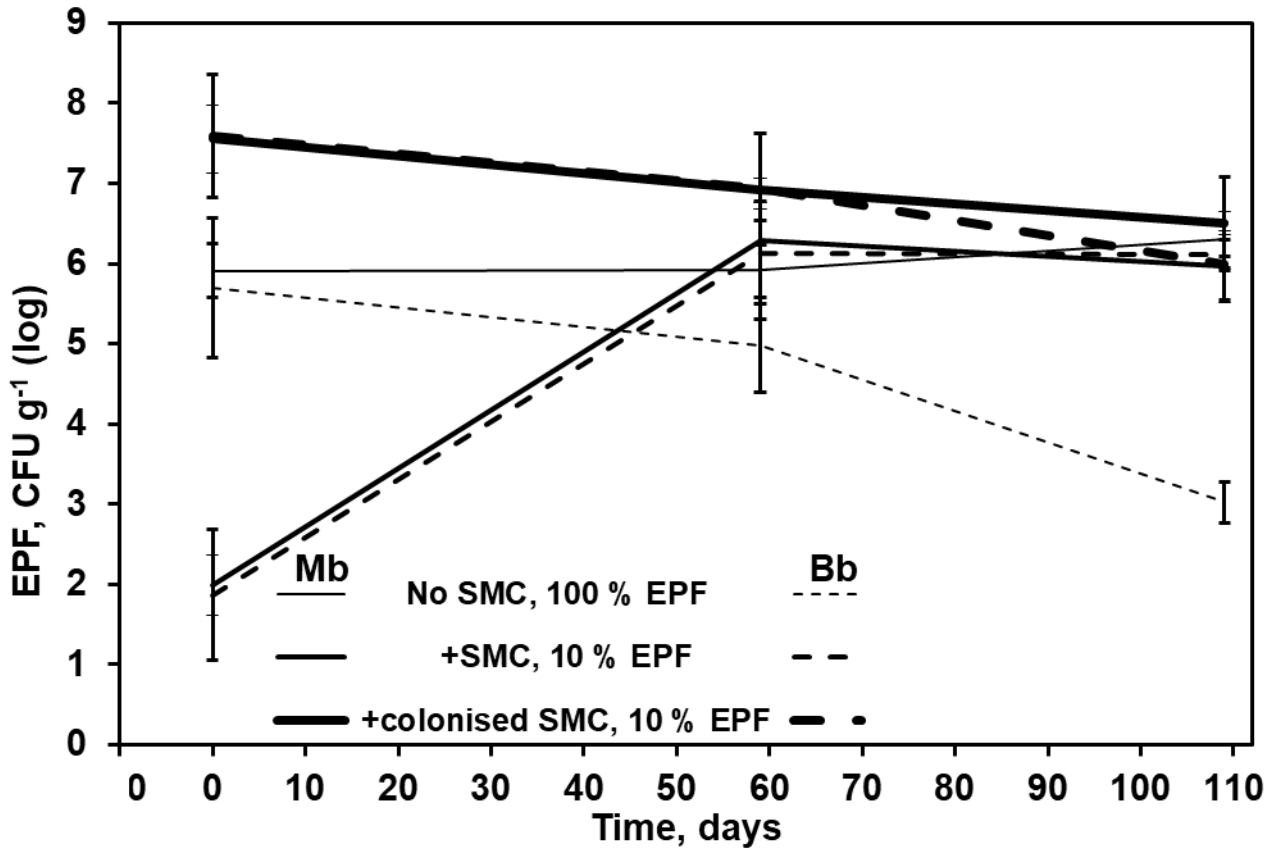


**Fig. 6** Mean population (±SE) of entomopathogenic fungi (EPF; Mb, *M. brunneum* Met52; Bb, *B. bassiana* Naturalis L) in growing medium (GM) with and without 10 % v/v spent mushroom compost (SMC) following potting of strawberry plants in two experiments; $n = 3$ in each experiment. At 100 %, EPF rates per l GM were 0.5 g for Mb and 1 ml for Bb. No EPF were detected in pots containing uninoculated GM, with or without SMC

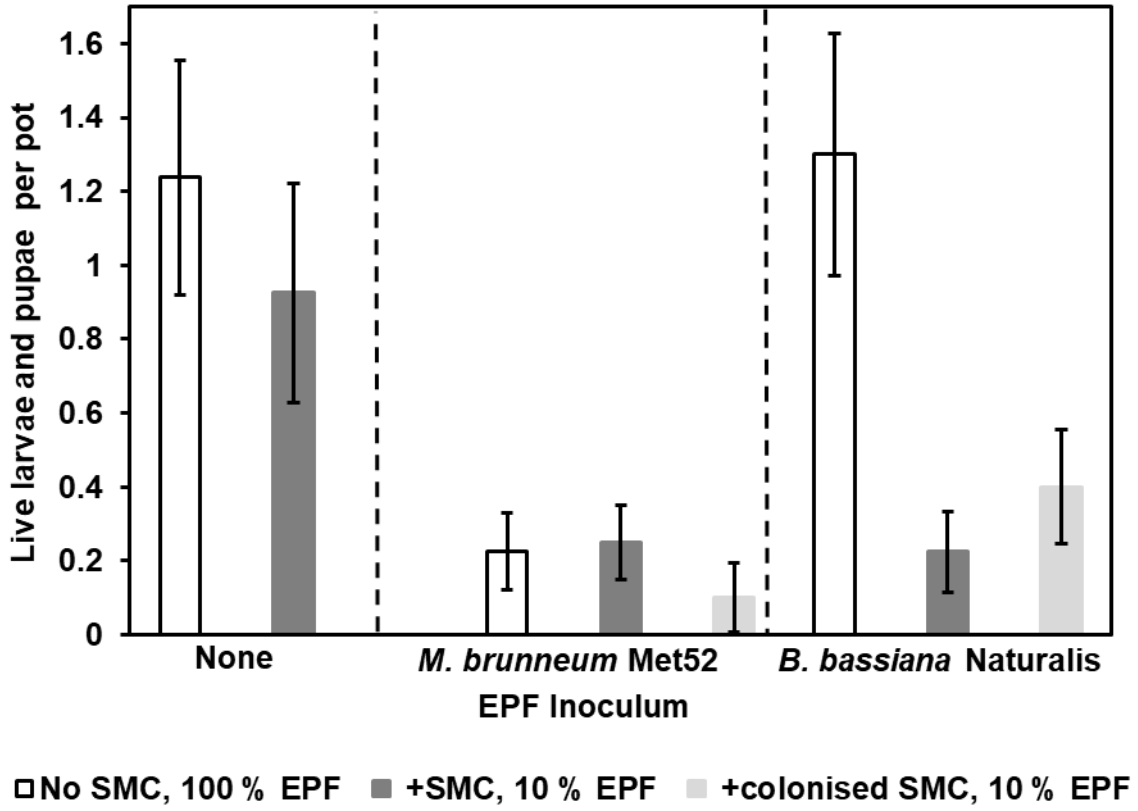


**Fig. 7** Mean final number (±SE) of live black vine weevil (BVW) larvae and pupae in growing medium (GM) with and without 10 % v/v spent mushroom compost (SMC) and entomopathogenic fungi (EPF) across two strawberry pot experiments; *n* = 10 and 20 for untreated controls in each experiment. There were low natural background BVW larvae infestations of pots in both experiments. Values are the means of pots with and without an initial artificial infestation of four BVW larvae. Any of the eight bars with the same letter are not significantly different (α = 0.05). At 100 %, EPF rates per l GM were 0.5 g for *M. brunneum* Met52 and 1 ml for *B. bassiana* Naturalis L