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| **Project Title**: | Evaluation of *Metarhizium anisopliae* for control of black vine weevil larvae in field grown strawberries |
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| **Date Project Commenced**: | 1st April 2009 |
| **Date Project completed/ date completion due**: | 31st December 2010 |

‘The results and conclusions in this report are based on an investigation conducted over a 20 month period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations’.

# Contents

# GROWER SUMMARY

## Headline

- The insect-pathogenic fungus *Metarhizium anisopliae* is highly efficacious against black vine weevil larvae in sandy and clay loam soils.

## Background and expected deliverables

The black vine weevil (BVW), *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) is a major pest of strawberries and other soft fruit (e.g. raspberries, blackcurrants). BVW spend part of their life cycle in soil feeding on plant roots. Targeting of the subterranean stages (larval stages) would significantly reduce crop damage and improve crop yield. A strain of the insect pathogenic fungus, *M. anisopliae* V275 (Novozymes F52 = BIPESCO 5), has already been identified which shows much promise as a biological alternative for the control of BVW larvae in hardy nursery stock and strawberries in soil-less media (HNS 133, HortLink project, HL 0171; Shah *et al*., 2007).

The overall aim of this project was to evaluate two formulations of *M. anisopliae* in controlling BVW larvae in field grown strawberries in different soil types in the UK. It offers a biological alternative to chemical insecticides that are currently at risk of being phased out or of resulting in chemical residues in the fruit. The specific objectives were to:

1. Determine the optimum dose of two formulations (granular and conidial powder) of *M. anisopliae* for BVW control in field grown strawberries.
2. Determine the efficacy *M. anisopliae* on a grower holding (i.e. commercial setting).

## Summary of the project and main conclusions

The objective of this study was to evaluate two formulations of the insect-pathogenic fungus, *Metarhizium anisopliae* Met 52, MAPP 15168, measured by infectivity against black vine weevil (BVW) larvae. The trials were conducted in field grown strawberries in sandy and clay loam soil at two different locations of Haygrove Fruits, Ledbury, Gloucestershire, UK in April 2009 and May 2010. The three application methods tested included a granular formulation (122 kg/ha) incorporated into soil before planting of strawberries commenced, a suspension formulation applied as a drench and the same suspension formulation used as a slurry treatment of the bare roots at planting. Fungal efficacy was then determined.

All three application methods were effective in controlling BVW in the different soil types and locations. The highest dose tested ($1 \times 10^{14}$ conidia/ha = 122 kg product/ha) provided significantly better control than the intermediate ($1 \times 10^{13}$ conidia/ha = 12 kg/ha) or low ($1 \times 10^{12}$ conidia/ha = 1.2 kg/ha) doses. BVW larval control at the high, intermediate and low doses was 71-96%, 40-75% and 6-11%, respectively. Premixing, drench or bare root treatment with *M. anisopliae* gave similar levels of BVW control. The high dose rate gave the best control irrespective of the application method or soil types. Significantly high larval control was achieved (78-97%) when chlorpyrifos was applied at planting rather than 8 weeks post planting (53%).

Additional studies were conducted to determine if entomopathogenic nematodes would increase the efficacy of *M. anisopliae*. Two doses of *Heterorhabditis bacteriophora* and *Steinernema kraussei* at the rate of 12,500 and 25,000 nematodes/plant were applied alone or the same doses were applied 5 months after plants were treated with *M. anisopliae* ($1 \times 10^{14}$ conidia/ha). There were significant differences in BVW control between *M. anisopliae* (88%) and *H. bacteriophora* (20-29%) or low dose of *S. kraussei* (39%) when applied alone. When used together, low dose of *S. kraussei* and *M. anisopliae* provided 100% control of the BVW larvae 6 weeks after nematode application. No significant differences were observed between the high dose of *S. kraussei* alone or in combination with *M. anisopliae*.

In conclusion, our preliminary observations suggest that Novozymes granular formulation of *M. anisopliae* can be premixed into soil or alternatively conidia can be applied as a drench or used as a slurry treatment of the bare roots. **Only the granular growing media incorporated method is currently commercially available**. When used at 122 kg product per hectare, *M. anisopliae* is efficacious in the prophylactic control of BVW larvae and offers an environmentally benign alternative to chemical pesticides.

## Financial benefits

An estimated cost comparison has been provided in Table 1 for control of BVW larvae in strawberry. The values in the table show that the cost of the granular formulation of the *Metarhizium* product is 2-3 times higher than the cost of the nematode product currently in use; however, the *Metarhizium* gives better results as indicated in this report. Both the fungus and nematode products are more costly than the currently recommended chemical insecticides. It is anticipated that the cost of the *Metarhizium* product will be much lower than the nematode product once application methods have been evaluated by growers. For example, the cost of treating bare roots with *Metarhizium* would be much more cost effective than premixing or drenching. However, considerable further research is required to establish the feasibility of treating bare roots with *Metarhizium*.  A comparison of the different control methods and treatments is summarised in the table below.

**Table 1**. Comparison of different agents to control black vine weevil larvae in field grown strawberries.

| **Products** | **Equivalent product or conidia/ha** | **Product cost/ha** | **Application cost/ha** | **Total cost/ha** |
|---|---|---|---|---|
| *Metarhizium anisopliae* F52- granular formulation | 1 × $10^{14}$ CFU (= 50-100 kg) | £26/kg | No application cost - granular product can be incorporated in soil through strawberry BedMaker (Haygrove Fruits, UK) | £1300-2600 |
| Nematodes- drench (Nemasys L™) (*Steinernema kraussei*) | 25,000 nematode/plant | £0.07/plant = £224/ha × 2-3 times = £672 | No application cost - product can be mixed in irrigation tank (general practice) | £500-700 |
| Nematodes- drench (nematop®) (*Heterorhabditis bacteriophora*) | 25,000 nematode/plant | £300/ha × 2-3 times = £600-900 | No application cost - product can be mixed in irrigation tank (general practice) | £600-900 |
| Chlorpyrifos- drench | 2 l AI<br>285-570 ml/plant | £250 | Cost of drench application is about £400-500/ha | £650-750 |

Approximately 32,000 strawberries plants/ha (source: Hargrove Fruits, Ledbury, Gloucestershire, UK. AI = Active ingredient).

## Action points for growers

- A granular formulation of *Metarhizium anisopliae* F52 (Met52, MAPP 15168) is available in the UK. The product launched 1st February 2011, is produced by Novozymes and distributed through Fargro Ltd, Littlehampton, UK.

- This project shows that growers have a choice of delivery for the inoculum (**currently only the granular formulation is commercially available**).

- There is no additional cost for application of this product because granular formulations can be premixed in the soil during the strawberry bed preparation.

- Once other formulations become available, growers should evaluate different application systems to ascertain which best suits their production methods.

- Growers should acquaint themselves of the strengths and weaknesses of *Metarhizium* products and learn to use these products effectively.

# SCIENCE SECTION

## Introduction

Black vine weevil (BVW), *Otiorhynchus sulcatus* is a major pest of ornamental nursery stock and soft fruit (Cross and Burgess, 1997; Moorhouse *et al*., 1992, 1993; van Tol *et al*., 2004). Adult weevils feed on leaves causing mostly cosmetic damage whilst the larvae feed on plant root systems, which can lead to plants being stunted, or collapsing and dying. Current control is dependent on the use of chemical insecticides (e.g. imidacloprid, chlorpyrifos) or insect pathogenic nematodes. However there is considerable interest to reduce the input of chemical pesticides because of the potential risks they pose to humans and the environment. Furthermore, recent EU directives (e.g. Directive 128/2009/EC) make it obligatory for member states to develop and implement benign pest control strategies.

An earlier DEFRA HortLink project (HL0171, HNS 133) showed that the *M. anisopliae* V275 was highly efficacious in controlling BVW larvae and western flower thrips (*Frankliniella occidentalis*) pupae in hardy ornamental plants grown in a range of soil-less plant growing media (Ansari *et al*., 2007, 2008a; Shah *et al*., 2007). This strain was also effective in controlling BVW larvae in potted *Euonymus* and strawberries produced in grow bags; its efficacy was enhanced when used in combination with low dose chemicals (Shah *et al*., 2007) or insect-pathogenic nematodes, *Heterorhabditis bacteriophora* or *Steinernema kraussei* (Ansari *et al*., 2008b, 2010), respectively.

To date, most studies have focused on evaluating the efficacy of two formulations (granular *versus* wettable power) of *M. anisopliae* in soil-less media. The **overall aim** of the current project was to evaluate the efficacy of *M. anisopliae* for the control of BVW larvae in field grown strawberries as a biological alternative to chemical pesticides.

### Specific objectives:

1 Determine the optimum dose of two formulations (granular and conidial powder) of *M. anisopliae* for BVW control in field grown strawberries.

2 Determine the efficacy of *M. anisopliae* on a grower holding (i.e. commercial setting).

## Materials and Methods

*First year field trials- 2009*

*Dose determination study*

**Fungus, insecticide and strawberry plants**

A granular formulation of *M. anisopliae* strain F52 (Met52 MAPP 15168), was supplied by Novozymes. The product contains $9.0 \times 10^8$ conidia/g rice (= $9.0 \times 10^{11}$ conidia/kg). This was stored at 5ºC until required. The conidium is the infective unit of *M. anisopliae*. One viable conidium will germinate on a suitable substrate to give rise to a single colony forming unit (CFU). Bare root strawberry plants cultivar 'Elsanta' were purchased from Hargreaves Plants Ltd, Lincolnshire, UK. The chemical insecticide Cyren® (chlorpyrifos 46% w/w, MAPP 11028) was kindly provided by Cheminova, Denmark.

**Trial 1 (2009) Newent Site**

This trial was conducted in a polytunnel on a commercial strawberry farm at Newent, Gloucestershire, UK. The soil at this site was sandy loam. None of the sites had been treated with insecticides during the previous year. No natural populations of entomopathogenic nematodes and fungi were detected at the trial sites as determined using the "*Galleria* bait method". This method entails incubation of larvae of the greater wax moth (*Galleria mellonella*) in the soil where these readily succumb to entomopathogenic fungi and nematodes if present. This method provides a convenient way of studying natural and introduced populations of entomopathogenic fungi and nematodes.

The plots measured 1.5 × 0.9 m with a 0.3 m buffer and were arranged in a complete randomized block design with 4 replicates per treatment (EPPO standard PP 1/181). The different treatments listed in Table 2, were applied on April 28, 2009. It was a partially cloudy day with the air and soil (at 7 cm depth) temperatures being 20°C and 17°C, respectively. The different doses of *M. anisopliae* were applied as premixed, drench or bare root treatments. The latter entailed dipping bare roots in a fungal suspension immediately prior to planting. Plants were destructively assessed on October 12, 2009 (air temperature 15°C; soil temperature at 5 cm depth14°C), 5 months after treatment.

### Inoculation of BVW eggs

Each plant was to be artificially inoculated with 20 BVW eggs but this was not possible during the summer of 2009 due to the lack of sufficient numbers of commercially produced eggs for trials. Plants were instead exposed to natural levels of BVW larvae in the plots. Two months after planting, a few plants from each plot and trial site (treated and untreated) were checked for natural infestation of BVW larvae. There was an average of 2 BVW larvae per plant (ca. ≤ 30 larvae/plot). Trials were allowed to proceed because there were significant natural BVW larval infestations of strawberry plants.

### Application of *M. anisopliae* and insecticide

*Please note that the drench and bare root treatments outlined below are not Novozymes' recommendations for their granular product but are experimental treatments.*

***Premixed application:*** *M. anisopliae* was premixed into the soil using three doses 0.165g (= 1.35 × $10^8$ conidia/plot = 1 × $10^{12}$ conidia/ha), 1.65 g (= 1.35 × $10^9$ conidia/plot = 1 × $10^{13}$ conidia/ha) and 16.5 g (= 1.35 × $10^{10}$ conidia/plot = 1 × $10^{14}$ conidia/ha). The granular formulated product was first mixed in 1.0 litre of soil then vigorously shaken to dislodge the spores from the rice grain and spread over the soil surface before being mixed into the top 5cm of soil (the recommended application method is simply to incorporate in to the top 5cm of soil). The rates were in accordance with the manufacturer's recommended rate for BVW control which ranges between 30. to 122/ha of product = 4.5 × $10^{13}$ to 1.35 × $10^{14}$ CFUs/ha (the UK approved rate is up to 61 kg/ha. of crop, **not treating paths and non-crop area**).

***Drench application:*** The granular product was suspended in 3 litres of 0.03% Aq Tween 80 and transferred to 0.5 litre plastic bottles then vigorously shaken to dislodge the spores from the rice grain. The spore suspension was pooled and filtered through cheesecloth and the required doses prepared and validated using a haemocytometer. A 200 ml spore suspension was applied around the base of each plant with the application rates corresponding to 1 × $10^{12}$, $10^{13}$ and $10^{14}$ Conidia/ha.

***Bare root treatment:*** The granular product (0.165g, 1.65 g and16.5 g/plot) was suspended in 0.5 litre of 0.03% Aq. Tween, filtered through cheesecloth to separate the spores from the grain and spore doses validated as described above. Plant roots were dipped in the spore suspension prior to plantation.

***Other treatments:*** 300 ml of chlorpyrifos (recommended rate 2 litres of product/1000 litres of water; apply 285-570 ml/plant) was applied as a drench around the base of each plant for BVW control while the untreated plots received water only.

During the trials, all plants received regular irrigation through capillary tubes. Plants were exposed to the same biotic (pest pressure, plant diseases, etc.) and abiotic (light intensity, temperature, water availability, etc) factors as the commercially grown plants at each location. Trials were conducted between June-October 2009. Each treatment was replicated 4 times with 15 plants per replicate. Treatments were arranged in a randomized complete block design with each plant adequately spaced to avoid cross-contamination. Plants were destructively assessed 5 months post-treatment in accordance with EPPO guidelines (EPPO standard PP 1/111 (2).

**Table 2**. Treatments used to control black vine weevil in field grown strawberries in 2009.

| **Treatments** | **Conidia/ha** | **Equivalent product/plot (g)** | **Equivalent Product/ha (kg)** |
|---|---|---|---|
| Untreated control | - | - | - |
| *M. anisopliae* – applied as premixed | $1 \times 10^{12}$ | 0.165 | 1 |
| *M. anisopliae* – applied as premixed | $1 \times 10^{13}$ | 1.65 | 12 |
| *M. anisopliae* – applied as premixed | $1 \times 10^{14}$ | 16.5 | 122 |
| *M. anisopliae* - applied as drench | $1 \times 10^{12}$ | 0.165 | 1 |
| *M. anisopliae* - applied as drench | $1 \times 10^{13}$ | 1.65 | 12 |
| *M. anisopliae* - applied as drench | $1 \times 10^{14}$ | 16.5 | 122 |
| *M. anisopliae* - bare root treatment* | $1 \times 10^{12}$ | 0.165 | 1 |
| *M. anisopliae* - bare root treatment* | $1 \times 10^{13}$ | 1.65 | 12 |
| *M. anisopliae* - bare root treatment* | $1 \times 10^{14}$ | 16.5 | 122 |
| Chlorpyrifos applied as drench at recommended rate at planting | - | - | |

Each treatment was replicated 4 times with 15 plants/treatment.

* = Strawberry bare roots dipped in *M. anisopliae* conidial suspension prior to planting.

**Trial 2 (2009) Ledbury Site**

A second trial was conducted in a polytunnel at the Ledbury site of Haygrove Farm, Gloucestershire, UK. Treatments were as described above except this site had a **clay loam** soil and treatments were applied on May 20, 2009 (air temperature 18°C; soil temperature at 7 cm depth 16°C; cloudy, light shower). The plots measured 1.5 × 0.9 m with a 0.3 m buffer; these were arranged in a complete randomized block design with 4 replicates per treatment. Plants were destructively assessed on October 13, 2009 (air temperature 14°C; soil temperature at 5 cm depth13°C), 5 months after treatment.

**Second year field trials-2010**

In the first year trials, the highest dose (1 × $10^{14}$ conidia/ha; hereafter referred as **optimum dose**) of *M. anisopliae* provided higher control of BVW than the intermediate (1 × $10^{13}$ conidia/ha) or low (1 × $10^{12}$ conidia/ha) doses in the different soil types (sandy *versus* clay loam). In the second year, trials were repeated with optimum dose of *M. anisopliae* with other higher doses at **Newent** and **Ledbury** sites in sandy and clay soil using the same methods outlined in the first year except additional fungal doses were included to compare optimum dose.

**Trial 1 (2010) Newent Site**

This trial was conducted in a polytunnel on a commercial strawberry farm at Newent, Gloucestershire, UK. The soil at this site was sandy loam. Treatments listed in Table 3 were applied on May 10, 2010 (soil temperature at 7 cm depth 18.5°C; air temperature 19.5°C; sunny, partial cloudy). Each plant was artificially inoculated with 10 BVW eggs around the roots at 2-3 cm deep in soil on August 18, 2010. Immediately after inoculation, each plant received 100 ml of irrigation water to protect eggs from desiccation. Plants were destructively assessed on October 27, 2010 (10 weeks after BVW eggs inoculation). The air and soil temperatures on the 27$^{th}$ November were 13.0°C and 11.0°C, respectively.

**Trial 2 (2010) Ledbury Site**

A second trial was conducted in a polytunnel at the Ledbury site of Haygrove Farm, Gloucestershire, UK. Treatments were the same as described above in Table 3 except this site had a **clay loam** soil and treatments were applied on May 12, 2010 (air temperature 20.5°C; soil temperature at 7 cm depth 18.5°C; sunny; partial cloudy). Each plant was artificially inoculated with 10 BVW eggs around the roots at 2-3 cm deep in soil on September 17, 2010 (air temperature 18.0°C; soil temperature at 7 cm depth 17.0°C; sunny; partial cloudy). Immediately after inoculation, each plant received 100 ml of irrigation water to protect eggs from desiccation.

Unfortunately, the whole of the 2010 Ledbury trial site was accidentally ploughed in due to a misunderstanding between the farm supervisor and workers on 19th November 2010 before the planned assessment. Therefore it was not possible to undertake the destructive assessment.

**Table 3**. Treatments used to control black vine weevil in field grown strawberries in 2010.

| **Treatments** | **Conidia/ha** | **Equivalent product/plot (g)** | **Equivalent product/ha (kg)** |
|---|---|---|---|
| Untreated control | - | - | - |
| *M. anisopliae* – applied as premixed | $1 \times 10^{14}$ | 16.5 | 122 |
| *M. anisopliae* - applied as drench | $1 \times 10^{14}$ | 16.5 | 122 |
| *M. anisopliae* – applied as premixed | $2 \times 10^{14}$ | 33.0 | 244 |
| *M. anisopliae* - applied as drench | $2 \times 10^{14}$ | 33.0 | 244 |
| *M. anisopliae* – applied as premixed | $1 \times 10^{15}$ | 165.0 | 1222 |
| *M. anisopliae* - applied as drench | $1 \times 10^{15}$ | 165.0 | 1222 |
| *M. anisopliae* – premixed + drench | $1 \times 10^{14} + 1 \times 10^{14}$ | 33.0 | 244 |
| Chlorpyrifos applied as drench at recommended rate at planting | - | - | - |
| Chlorpyrifos applied as drench at recommended rate 8 weeks post planting | - | - | - |

**Additional studies- Enhancement of fungal efficacy through the strategic use of reduced rates of entomopathogenic nematodes**

Previously, the combination of reduced rates of entomopathogenic nematodes, *Heterorhabditis bacteriophora* or *Steinernema kraussei* and *M. anisopliae* provided additive or synergistic mortality to third-instar BVW in potted *Euonymus fortunei* Blondy (Celastraceae) (Ansari et al., 2008b) and strawberry grow bags (Ansari et al., 2010) under greenhouse conditions. In the above studies, BVW larvae were exposed to various doses of *M. anisopliae*, and the nematodes were applied simultaneously, or 1 or 2 weeks after application of *M. anisopliae*. Throughout the experiment, the combined application of *M. anisopliae* with nematodes resulted in increased efficacy against BVW larvae compared with when applied alone. However, when the nematodes were applied 1 or 2 weeks after application of *M. anisopliae*, 100% larval mortality was obtained, even when both nematode and fungus were applied at reduced doses.

The aim of this study was to determine if *M. anisopliae* worked synergistically with the entomopathogenic nematodes, *H. bacteriophora* or *S. kraussei* in controlling BVW larvae infesting field grown strawberries in different soil types (sandy *versus* clay soil). The field soil environment is totally different from that of commercial soil-less plant growing media (i.e. commercial composts). Although soil-less media contain a range of microbes, these do not appear to influence the efficacy of *M. anisopliae* (Shah *et al*., 2008). In contrast, field soils contain a wide range of antagonistic organisms which could influence the efficacy of insect pathogenic fungi like *M. anisopliae* (Scheepmaker & Butt, 2010).

## Materials and Methods

### Nematodes

The *S. kraussei* (Nemasys L™) was kindly provided by Becker Underwood, UK and *H. bacteriophora* (Nematop®) was provided from Enema, Germany. Both nematode species are recommended by their producers to be applied at the rate of 25,000 nematodes/plant for BVW larval control in the UK.

### The effect of exposure of BVW larvae to *M. anisopliae* prior to nematode application

This trial was conducted at the Newent site only, as outlined above, except that nematode treatments mentioned in Table 4 were applied 5 months after *M. anisopliae* application. Two doses of *H. bacteriophora* or *S. kraussei* (12,500 and 25,000 nematodes/plants = 0.56 to $1.25 \times 10^9$ nematode/ha) were applied alone or the same doses applied to plants that were previously treated with *M. anisopliae* ($1 \times 10^{14}$ conidia/ha) for 5 months. The nematode suspensions were applied in 100 ml of irrigation water/plant followed by another 100 ml of water to wash the nematodes into the soil. Control plants were treated with 200 ml of water only. Nematodes were applied on October 13, 2010 (air temperature 14°C; soil temperature at 7 cm depth 13°C; partial cloudy, light rain) and plants were destructively assessed on November 23, 2010 (6 weeks after nematode application). The soil and air temperatures on November 23, 2010 were 8.0°C and 11.5°C, respectively.

### Statistical analysis

Plants were assessed destructively and the number of live larvae recovered per plot was recorded. The percentage efficacy of the treatments was calculated using the following formula and data were subjected to analysis of variance (ANOVA) using software for statistical analysis (SPSS v 16, 2007). Differences among treatments were compared using Tukey's mean separation test using $P < 0.05$.

$$\% \text{ Efficacy} = \frac{\text{Number of live larvae in control} - \text{Number of live larvae in treatment}}{\text{Number of live larvae in control}} \times 100$$

**Table 4**. Treatments used to control black vine weevil in field grown strawberries in 2010.

| Treatments | Conidia/ha | Nematode/ Plant* | Equivalent product/plot (g) | Equivalent product/ha (kg) |
|---|---|---|---|---|
| Untreated control | - | - | - | - |
| *M. anisopliae* – applied as premixed | $1 \times 10^{14}$ | - | 16.5 | 122 |
| *M. anisopliae* - applied as drench | $1 \times 10^{14}$ | - | 16.5 | 122 |
| *H. bacteriophora* - applied as drench | - | 12,500 | - | - |
| *H. bacteriophora* - applied as drench | - | 25,000** | - | - |
| *M. anisopliae* - premixed + *H. bacteriophora* applied as drench | $1 \times 10^{14}$ | 12,500 | 16.5 | 122 |
| *M. anisopliae* - drench + *H. bacteriophora* applied as drench | $1 \times 10^{14}$ | 25,000 | 16.5 | 122 |
| *S. kraussei* - applied as drench | - | 12,500 | - | - |
| *S. kraussei* - applied as drench | - | 25,000 | - | - |
| *M. anisopliae* - premixed + *S. kraussei* applied as drench | $1 \times 10^{14}$ | 12,500 | 16.5 | 122 |
| *M. anisopliae* - drench + *S. kraussei* applied as drench | $1 \times 10^{14}$ | 25,000 | 16.5 | 122 |

* = *Heterorhabditis bacteriophora* and *Steinernema kraussei* were applied 5 months after *Metarhizium Anisopliae* application.

** = This dose of nematode is recommended to apply 2-3 times in one crop season (25,000 x 3 = 75,000 nematodes/crop).

## Results

## First year trials-2009

The overall efficacy of *M. anisopliae* against BVW larvae at the two sites differed non-significantly ($P > 0.05$), confirming that *M. anisopliae* was efficacious irrespective of trial site, soil type or application method (premix, drench, bare root treatments). However, larval control differed significantly with the different doses of the fungus ($P < 0.05$). Interactions among trial sites, application methods and doses were not significantly different ($P > 0.05$). The doses corresponding to $1 \times 10^{13}$ and $10^{14}$ conidia/ha consistently gave better control (60-96%) of BVW larvae than the lower ($1 \times 10^{12}$ conidia/ha) dose (6-11%) irrespective of the trial site, application method or soil type. We observed significantly high numbers of conidia colonising roots of strawberries when *M. anisopliae* was applied as drench or bare roots treatment than premixed (data not shown).

## Trial 1 (2009) Newent Site

### Effect of different doses of *M. anisopliae* on BVW larvae

Significantly low numbers of larvae were recovered from plants treated with *M. anisopliae* compared with untreated control (Fig. 1 and Table 5). A similar number of larvae were recovered from untreated plots and those treated with $1 \times 10^{12}$ conidia/ha.

At the high dose, the drench application gave slightly better control (< 5 larvae/plot) than premixed (≤ 5 larvae/plot) or bare root treatments (≥10 larvae/plot). Lower numbers of BVW larvae were recovered from plants treated with premixed or drench application of high dose of *M. anisopliae* than chlorpyrifos.

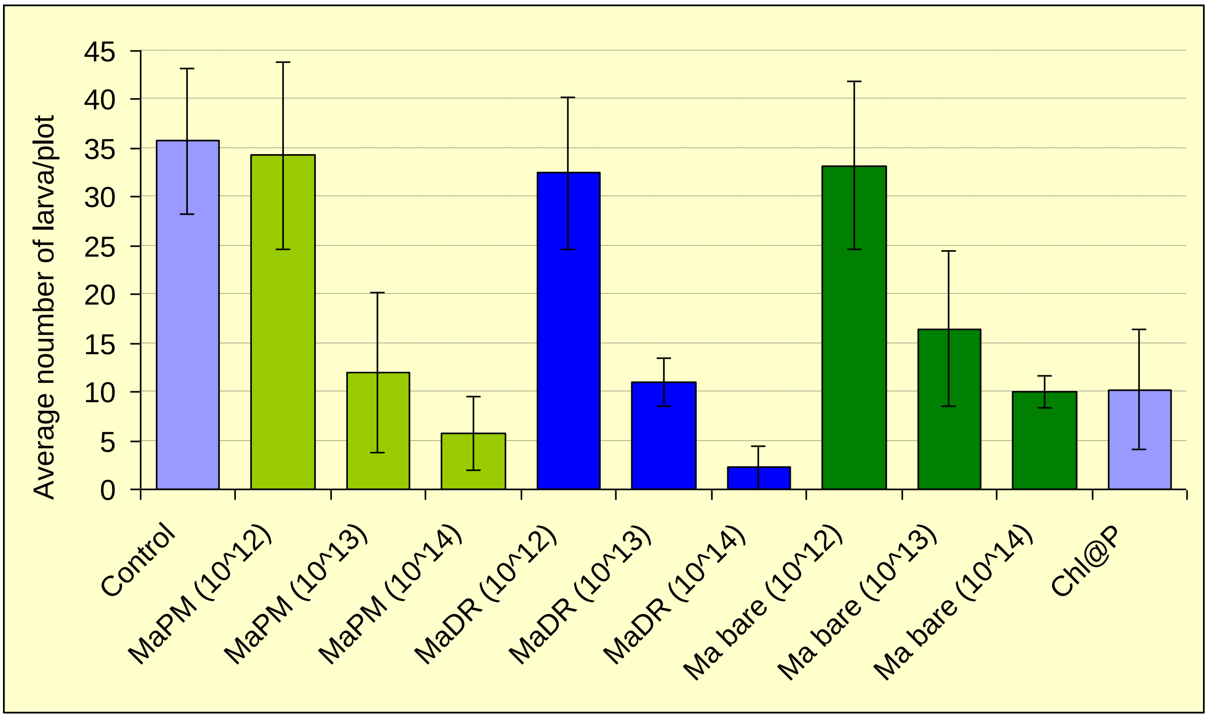



**Figure 1.** Average number of black vine weevil larvae recovered from each treatment. *Metarhizium anisopliae* applied at different doses in soil for the control of a natural infestation of black vine weevil in field grown strawberries. No treatment (control), *M. anisopliae* premixed (MaPM), *M. anisopliae* applied as drench (MaDR), *M. anisopliae*-bare root treatment (Ma bare), chlorpyrifos applied as a drench at the recommended rate at planting (Chl@P). The trial was conducted in **sandy soil** under a polytunnel at Newent, Gloucestershire, UK between June and October 2009. Each strawberry plant was infested with 10 black vine weevil eggs and plants were destructively assessed 10 weeks post inoculation. Each treatment was replicated 4 times with 15 plants/treatment. Treatments are listed in Table 2.

**Table 5.** Average number of black vine weevil larvae recovered from each treatment. *Metarhizium anisopliae* applied at different doses in soil for the control of a natural infestation of black vine weevil in field grown strawberries. The trial was conducted in **sandy soil** under a polytunnel at Newent, Gloucestershire, UK between June and October 2009.

| **Treatments** | **Conidia/ha** | **Average number of larvae/plot** |
|---|---|---|
| Control | - | 35.8 ± 7.4[b] |
| *M. anisopliae* – applied as premixed | $1 \times 10^{12}$ | 34.3 ± 9.6[ab] |
| *M. anisopliae* – applied as premixed | $1 \times 10^{13}$ | 12.0 ± 8.2[ab] |
| *M. anisopliae* – applied as premixed | $1 \times 10^{14}$ | 5.8 ± 3.8[ab] |
| *M. anisopliae* - applied a drench | $1 \times 10^{12}$ | 32.5 ± 7.8[ab] |
| *M. anisopliae* - applied as drench | $1 \times 10^{13}$ | 11.0 ± 2.5[ab] |
| *M. anisopliae* - applied as drench | $1 \times 10^{14}$ | 2.3 ± 2.3[a] |
| *M. anisopliae* - bare root treatment | $1 \times 10^{12}$ | 33.3 ± 8.6[ab] |
| *M. anisopliae* - bare root treatment | $1 \times 10^{13}$ | 16.5 ± 7.9[ab] |
| *M. anisopliae* - bare root treatment | $1 \times 10^{14}$ | 10.0 ± 1.6[ab] |
| Chlorpyrifos applied as drench at recommended rate at planting | - | 10.3 ± 6.1[ab] |

Within a column, values followed by the same letter are not significantly different ($P > 0.05$; Tukey's test). Each treatment was replicated 4 times with 15 plants/treatment.

**Efficacy of *M. anisopliae* against BVW larvae**

Control of BVW larvae differed non-significantly among different application methods ($P > 0.05$), however, larval control differed significantly among different doses of fungus ($P < 0.05$) 5 months after treatment (Fig. 2 and Table 6). Interactions between application methods and doses were not significantly different ($P > 0.641$). Irrespective of application methods, the medium and high doses of *M. anisopliae* were consistently more effective, providing between 60-75% and 71-96% larval control respectively, compared with 10% for the low dose.

Chlorpyrifos provided the same level of larval control (78 %) as the medium and high doses of *M. anisopliae* (Fig. 2).

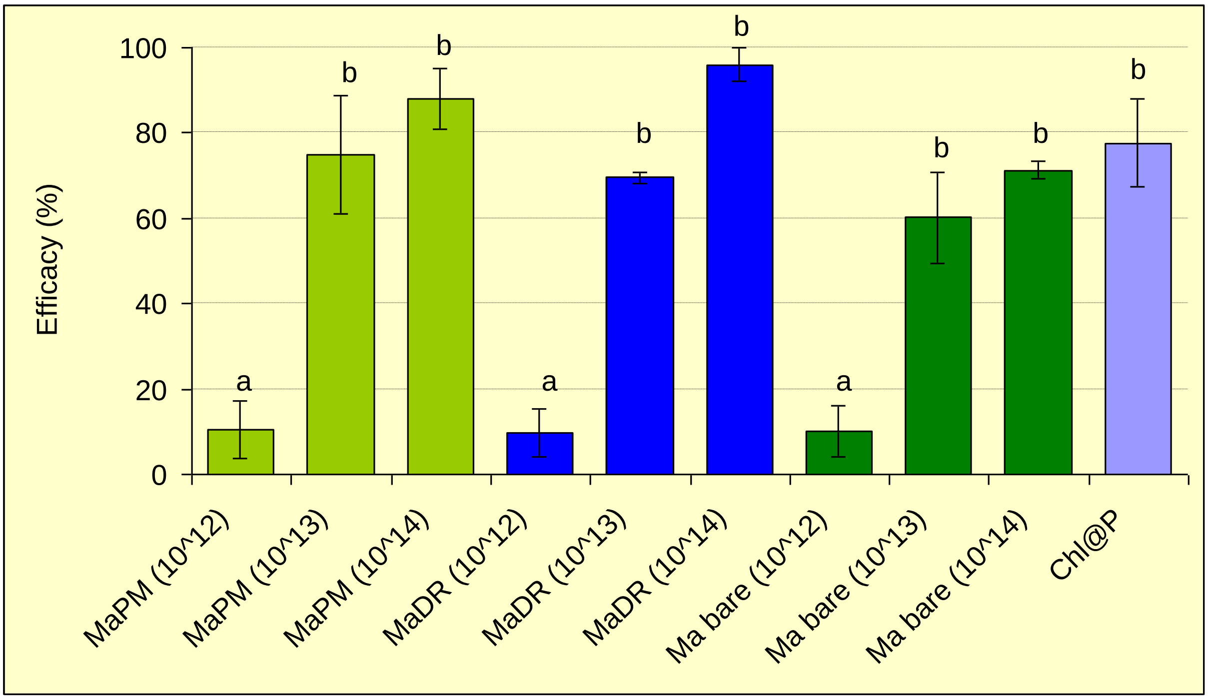



**Figure 2**. Efficacy (%) of *Metarhizium anisopliae*, applied at different doses in soil for the control of a natural infestation of black vine weevil in field grown strawberries. *M. anisopliae* premixed (MaPM), *M. anisopliae* applied as drench (MaDR), *M. anisopliae*-bare root treatment (Ma bare), chlorpyrifos applied as a drench at the recommended rate at planting (Chl@P). The trial was conducted in **sandy soil** under a polytunnel at Newent, Gloucestershire, UK between June and October 2009. Each strawberry plant was infested with 10 black vine weevil eggs and plants were destructively assessed 10 weeks post inoculation. Bars displaying the same letters are not significantly different ($P > 0.05$; Tukey's test). Each treatment was replicated 4 times with 15 plants/treatment. Treatments are listed in Table 2.

**Table 6.** Efficacy (%) of *Metarhizium anisopliae*, applied at different doses in soil for the control of a natural infestation of black vine weevil in field grown strawberries. The trial was conducted in **sandy soil** under a polytunnel at Newent, Gloucestershire, UK between June and October 2009.

| **Treatments** | **Conidia/ha** | **Mean ± SE** |
|---|---|---|
| *M. anisopliae* – applied as premixed | $1 \times 10^{12}$ | 10.5 ± 6.8[a] |
| *M. anisopliae* – applied as premixed | $1 \times 10^{13}$ | 75.0 ± 13.9[b] |
| *M. anisopliae* – applied as premixed | $1 \times 10^{14}$ | 88.0 ± 7.2[b] |
| *M. anisopliae* - applied a drench | $1 \times 10^{12}$ | 9.8 ± 5.6[a] |
| *M. anisopliae* - applied as drench | $1 \times 10^{13}$ | 69.5 ± 1.4[b] |
| *M. anisopliae* - applied as drench | $1 \times 10^{14}$ | 96.0 ± 4.0[b] |
| *M. anisopliae* - bare root treatment | $1 \times 10^{12}$ | 10.1 ± 6.0[a] |
| *M. anisopliae* - bare root treatment | $1 \times 10^{13}$ | 60.2 ± 10.7[b] |
| *M. anisopliae* - bare root treatment | $1 \times 10^{14}$ | 71.2 ± 2.1[b] |
| Chlorpyrifos applied as drench at recommended rate at planting* | - | 77.6 ± 10.3[b] |

Within a column, values followed by the same letter are not significantly different ($P > 0.05$; Tukey's test). Each treatment was replicated 4 times with 15 plants/treatment.

Plants treated with *M. anisopliae* had a well developed root system compared with that of the untreated control plants because the control roots had been eaten by BVW larvae (Fig. 3). Some of the larvae recovered from treated plots were visibly infected with the fungus *M. anisopliae* (Fig 3. bottom right picture).

Preparation for planting

Preparation for premixing of fungus

Trial site before assessment

Destructive assessment

Untreated plant

Treated plants

**Figure 3**. Trial conducted at Haygrove Farms, Gloucestershire, UK to control black vine weevil between May and October 2009.

### Trial 2 (2009) Ledbury Site

#### Effect of different doses of *M. anisopliae* against BVW larvae

Significantly lower numbers of larvae were recovered from plants treated with the two higher rates of *M. anisopliae* than the untreated control (Fig. 4 and Table 7). A similar number of larvae were recovered from untreated plots and those treated with 1 × $10^{12}$ conidia/ha. At the high dose, the drench application gave slightly better control (< 5 larvae/plot) than premixed (≤ 10 larvae/plot) or bare root treatments (≥ 10 larvae/plot).

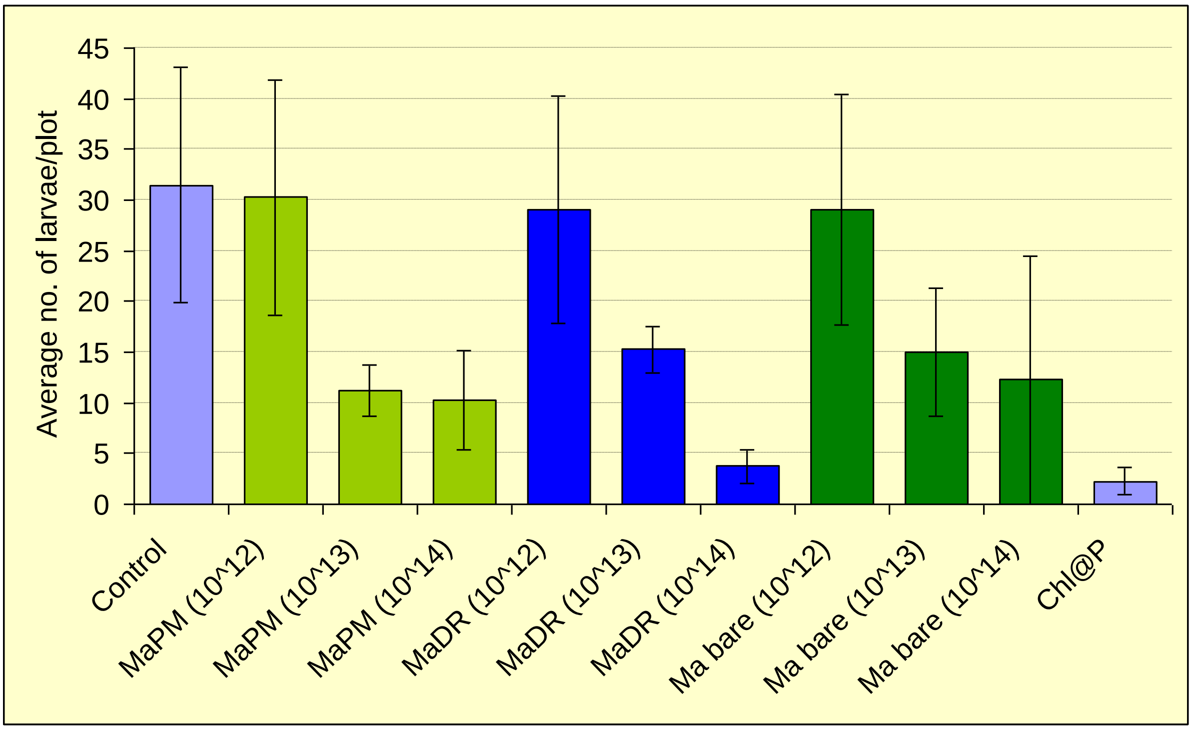



**Figure 4**. Average number of black vine weevil larvae recovered from each treatment. *Metarhizium anisopliae* applied at different doses for the control of a natural infestation of black vine weevil in field grown strawberries. No treatment (control), *M. anisopliae* premixed (MaPM), *M. anisopliae* applied as drench (MaDR), *M. anisopliae* -bare root treatment (Ma bare), chlorpyrifos applied as a drench at the recommended rate at planting (Chl@P). The trial was conducted in clay loam soil under a polytunnel at Ledbury, Gloucestershire, UK between June and October 2009. Each strawberry plant was infested with 10 black vine weevil eggs and plants were destructively assessed 10 weeks post inoculation. Each treatment was replicated 4 times with 15 plants/treatment. Treatments are listed in Table 2.

**Table 7.** Average number of black vine weevil larvae recovered from each treatment. *Metarhizium anisopliae* applied at different doses for the control of a natural infestation of black vine weevil in field grown strawberries. The trial was conducted in **clay loam** soil under a polytunnel at Ledbury, Gloucestershire, UK between June and October 2009.

| **Treatments** | **Conidia /ha** | **Mean ± SE** |
|---|---|---|
| Control | - | 31.5 ± 11.6 |
| *M. anisopliae* – applied as premixed | $1 \times 10^{12}$ | 30.3 ± 11.6 |
| *M. anisopliae* – applied as premixed | $1 \times 10^{13}$ | 11.3 ± 2.5 |
| *M. anisopliae* – applied as premixed | $1 \times 10^{14}$ | 10.3 ± 4.9 |
| *M. anisopliae* - applied a drench | $1 \times 10^{12}$ | 29.0 ± 11.2 |
| *M. anisopliae* - applied as drench | $1 \times 10^{13}$ | 15.3 ± 2.3 |
| *M. anisopliae* - applied as drench | $1 \times 10^{14}$ | 3.8 ± 1.7 |
| *M. anisopliae* - bare root treatment | $1 \times 10^{12}$ | 29.0 ± 11.4 |
| *M. anisopliae* - bare root treatment | $1 \times 10^{13}$ | 15.0 ± 6.3 |
| *M. anisopliae* - bare root treatment | $1 \times 10^{14}$ | 12.3 ± 12.3 |
| Chlorpyrifos applied as drench at recommended rate at planting | - | 2.3 ± 1.3 |

Each treatment was replicated 4 times with 15 plants/treatment. Within a column, values followed by the same letter are not significantly different ($P > 0.05$; Tukey's test).

### Efficacy of *M. anisopliae* against BVW larvae

BVW larval control differed non-significantly among different application methods ($P > 0.05$), however, larval control differed significantly among different doses ($P < 0.05$) 5 months after treatment (Fig. 5 and Table 8). Interaction between application methods and doses were not significantly different ($P > 0.05$). Irrespective of application methods, higher doses of *M. anisopliae* proved to be the most effective, providing between 73 and 89% larval control, compared to medium dose (40-58%) and low dose (6-10%). Chlorpyrifos provided the same level of larval control (94%) as observed in the high dose of *M. anisopliae* (Fig. 5).

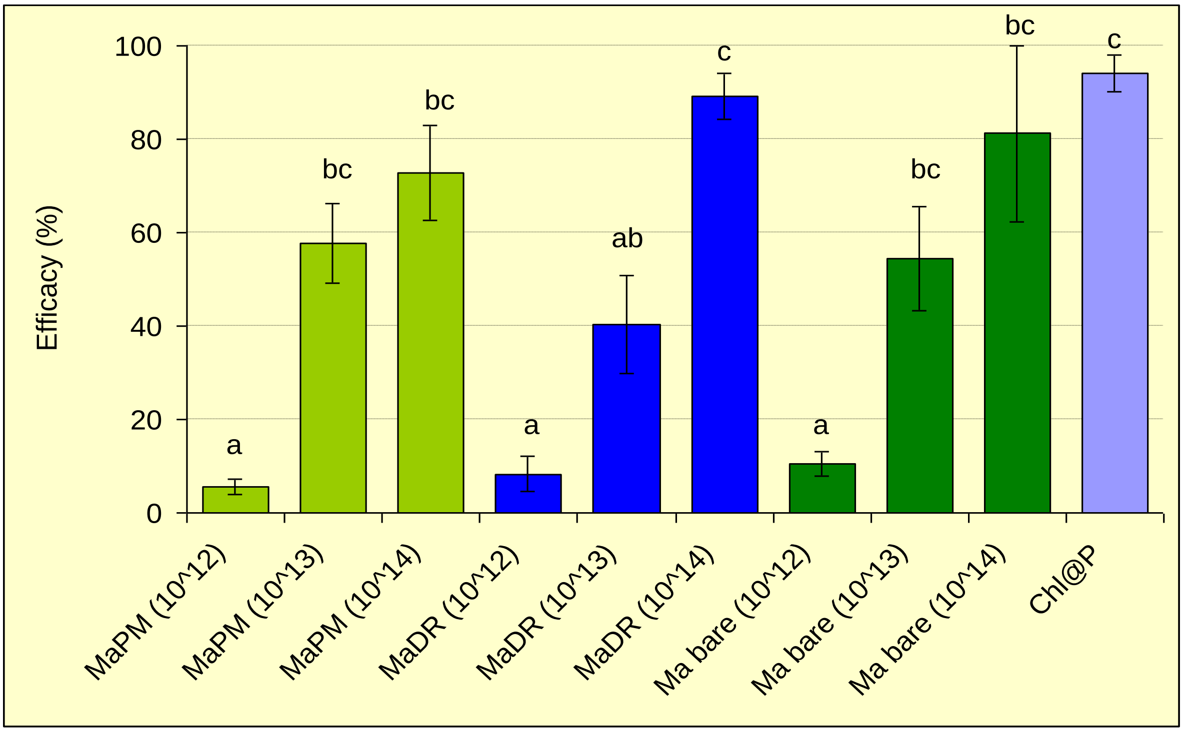



**Figure 5.** Efficacy (%) of *Metarhizium anisopliae*, applied at different doses in soil for the control of a natural infestation of black vine weevil in field grown strawberries. *M. anisopliae* premixed (MaPM), *M. anisopliae* applied as drench (MaDR), *M. anisopliae*-bare root treatment (Ma bare), chlorpyrifos applied as a drench at the recommended rate at planting (Chl@P). The trial was conducted in **clay loam** soil under a polytunnel at Ledbury, Gloucestershire, UK between June 2009 and October 2009. Each strawberry plant was infested with 10 black vine weevil eggs and plants were destructively assessed 10 weeks post inoculation. Each treatment was replicated 4 times with 15 plants/treatment. Bars displaying the same letters are not significantly different ($P > 0.05$; Tukey's test). Treatments are listed in Table 2.

**Table 8.** Efficacy (%) of *Metarhizium anisopliae*, applied at different doses in soil for the control of a natural infestation of black vine weevil in field grown strawberries. The trial was conducted in clay loam soil under a polytunnel at Ledbury, Gloucestershire, UK between June 2009 and October 2009.

| **Treatments** | **Conidia/ha** | **Mean ± SE** |
|---|---|---|
| *M. anisopliae* – applied as premixed | $1 \times 10^{12}$ | $5.5 \pm 1.6^{a}$ |
| *M. anisopliae* – applied as premixed | $1 \times 10^{13}$ | $57.6 \pm 8.6^{bc}$ |
| *M. anisopliae* – applied as premixed | $1 \times 10^{14}$ | $72.9 \pm 10.2^{bc}$ |
| *M. anisopliae* - applied as drench | $1 \times 10^{12}$ | $8.3 \pm 3.7^{a}$ |
| *M. anisopliae* - applied as drench | $1 \times 10^{13}$ | $40.5 \pm 10.5^{ab}$ |
| *M. anisopliae*- applied as drench | $1 \times 10^{14}$ | $89.2 \pm 4.5^{c}$ |
| *M. anisopliae* - bare root treatment | $1 \times 10^{12}$ | $10.5 \pm 2.6^{a}$ |
| *M. anisopliae* - bare root treatment | $1 \times 10^{13}$ | $54.5 \pm 11.2^{bc}$ |
| *M. anisopliae* - bare root treatment | $1 \times 10^{14}$ | $81.2 \pm 18.9^{bc}$ |
| Chlorpyrifos applied as drench at recommended rate at planting | - | $94.1 \pm 3.8^{c}$ |

Each treatment was replicated 4 times with 15 plants/treatment. Within a column, values followed by the same letter are not significantly different ($P > 0.05$; Tukey's test).

## Second year trials-2010

## Trial 1 (2010) Newent Site

### Effect of *M. anisopliae* against larval number

Significantly fewer larvae were recovered from plants treated with *M. anisopliae* compared with the untreated control, 5 months post-treatment (Fig. 6 and Table 9). There were no significant differences among doses but fewer larvae were recovered from plants treated with the highest dose of *M. anisopliae* (1 x $10^{15}$ conidia/ha) and double application of *M. anisopliae* (1 x $10^{14}$ + 1 x $10^{14}$ conidia/ha). At the same dose (1 x or 2 x $10^{14}$ conidia/ha), the premixed gave slightly better control (0.5 larvae/plot) than the drench application (1.5 larvae/plot).

Plants treated with chlorpyrifos at planting had fewer larvae (0.05/plot) than plants treated 8 weeks post planting (4.5/plot).

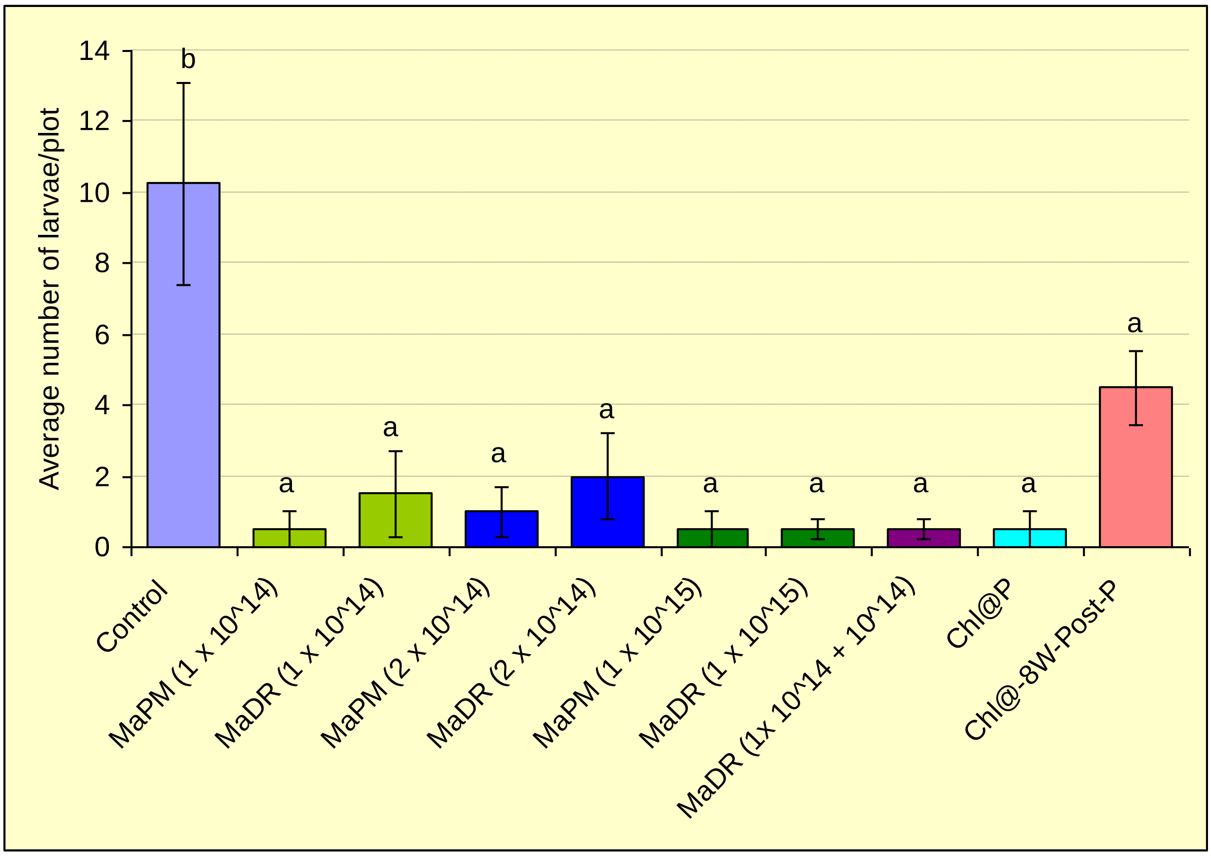



**Figure 6**. Average number of BVW larvae recovered following different treatments in field grown strawberries. The fungus, *M. anisopliae* was tested at different application rates. Treatments consisted of: no treatment (control), *M. anisopliae* premixed (MaPM), *M. anisopliae* applied as drench (MaDR), chlorpyrifos applied as a drench at the recommended rate at planting (Chl@P), chlorpyrifos applied as a drench at the recommended rate 8 weeks post planting (Chl-8w-Post-P). The trial was conducted in **sandy soil** under a polytunnel at Newent, Gloucestershire, UK between May and November of 2010. Each strawberry plant was infested with 10 black vine weevil eggs and plants were destructively assessed 10 weeks post inoculation. Each treatment was replicated 4 times with 15 plants/treatment. Bars displaying the same letters are not significantly different ($P$ > 0.05; Tukey's test). Treatments are listed in Table 3.

**Table 9.** Average number of BVW larvae recovered following different treatments in field grown strawberries. The fungus, *M. anisopliae* was tested at different application rates. The trial was conducted in **sandy soil** under a polytunnel at Newent, Gloucestershire, UK between May and November 2010.

| **Treatments** | **Conidia /ha** | **Average number of larvae/plot** |
|---|---|---|
| Control | - | 10.3 ± 2.9 [b] |
| *M. anisopliae* – applied as premixed | $1 \times 10^{14}$ | 0.5 ± 0.5 [a] |
| *M. anisopliae* - applied as drench | $1 \times 10^{14}$ | 1.5 ± 1.2 [a] |
| *M. anisopliae* – applied as premixed | $2 \times 10^{14}$ | 1.0 ± 0.7 [a] |
| *M. anisopliae* - applied as drench | $2 \times 10^{14}$ | 2.0 ± 1.2 [a] |
| *M. anisopliae* – applied as premixed | $1 \times 10^{15}$ | 0.5 ± 0.5 [a] |
| *M. anisopliae* - applied as drench | $1 \times 10^{15}$ | 0.5 ± 0.3 [a] |
| *M. anisopliae* - premixed + drench* | $1 \times 10^{14} + 1 \times 10^{14}$ | 0.5 ± 0.3 [a] |
| Chlorpyrifos applied as drench at recommended rate at planting | - | 0.5 ± 0.5 [a] |
| Chlorpyrifos applied as drench at recommended rate -8 weeks post planting | - | 4.5 ± 1.0 [a] |

Each treatment was replicated 4 times with 15 plants/treatment. Within a column, values followed by the same letter are not significantly different ($P > 0.05$; Tukey's test).

* = A second application *M. anisopliae* was made 8 weeks after first application.

### Efficacy of *M. anisopliae* against BVW larvae

All treatments provided significantly higher BVW control (87-97%) than chlorpyrifos (53%) that was applied 8 weeks post planting. All three doses ($1 \times 10^{14}$ conidia/ha), ($1 \times 10^{14}$ conidia/ha +$1 \times 10^{14}$ conidia/ha) and ($1 \times 10^{15}$ conidia/ha) provided same level of BVW control. Plants treated with chlorpyrifos at planting provided higher level of larval control (97%) than when applied 8 weeks post planting (Fig. 7 and Table 10).

Plants treated with *M. anisopliae* or insecticides appeared healthy and had extensive root systems while untreated plants were stunted and had poorly developed root systems because control roots had incurred feeding damage by BVW larvae (Figure 8)

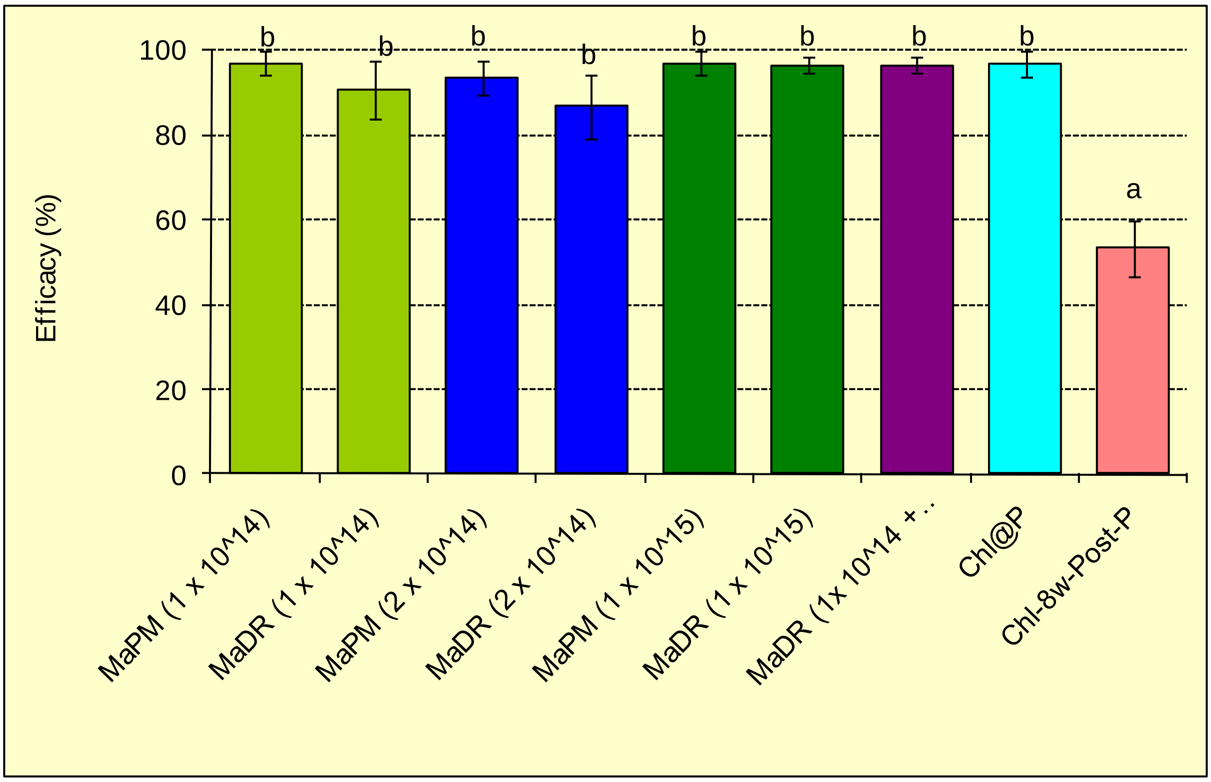



**Figure 7**. Efficacy (%) of *Metarhizium anisopliae*, applied at different doses in soil for the control of BVW in field grown strawberries. *M. anisopliae* applied as premixed (MaPM), *M. anisopliae* applied as drench (MaDR), chlorpyrifos applied as a drench at the recommended rate at planting (Chl@-P) , chlorpyrifos applied as a drench at the recommended rate 8 weeks post planting (Chl-8w-Post-P). The trial was conducted in **sandy soil** under a polytunnel at Newent, Gloucestershire, UK between May and November 2010. Each strawberry plant was infested with 10 black vine weevil eggs and plants were destructively assessed 10 weeks post inoculation. Each treatment was replicated 4 times with 15 plants/treatment. Bars displaying the same letters are not significantly different ($P > 0.05$; Tukey's test). Treatments are listed in Table 3.

**Table 10.** Efficacy (%) of *Metarhizium anisopliae*, applied at different doses in soil for the control of black vine weevil in field grown strawberries. The trial was conducted in **sandy soil** under a polytunnel at Newent, Gloucestershire, UK between May and November 2010.

| Treatments | Conidia/ha | Mean ± SE |
|---|---|---|
| *M. anisopliae* – applied as premixed | $1 \times 10^{14}$ | $97.1 \pm 2.9^{b}$ |
| *M. anisopliae* - applied as drench | $1 \times 10^{14}$ | $90.7 \pm 7.0^{b}$ |
| *M. anisopliae* – applied as premixed | $2 \times 10^{14}$ | $93.7 \pm 4.2^{b}$ |
| *M. anisopliae* - applied as drench | $2 \times 10^{14}$ | $86.9 \pm 7.7^{b}$ |
| *M. anisopliae* – applied as premixed | $1 \times 10^{15}$ | $97.1 \pm 2.9^{b}$ |
| *M. anisopliae* - applied as drench | $1 \times 10^{15}$ | $96.6 \pm 2.0^{b}$ |
| *M. anisopliae* – premixed + drench* | $1 \times 10^{14} + 1 \times 10^{14}$ | $96.6 \pm 2.0^{b}$ |
| Chlorpyrifos applied as drench at recommended rate at planting | - | $97.1 \pm 3.0^{b}$ |
| Chlorpyrifos applied as drench at recommended rate 8 weeks post planting | - | $53.4 \pm 6.7^{a}$ |

Each treatment was replicated 4 times with 15 plants/treatment. Within a column, values followed by the same letter are not significantly different ($P > 0.05$; Tukey's test).

* = A second application *M. anisopliae* was made 8 weeks after first application.

## Trial 2 (2010) Ledbury site

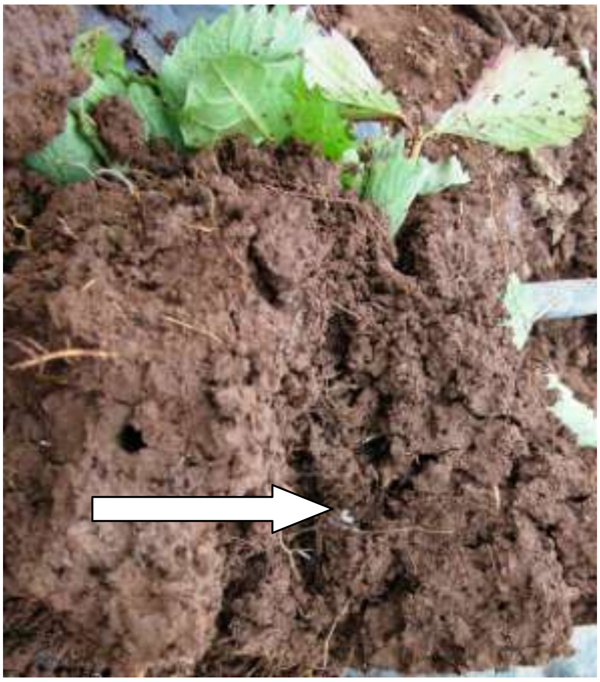
Unfortunately, the whole of the 2010 Ledbury trial site was accidentally ploughed in due to a misunderstanding between the farm supervisor and workers on 19th November 2010 before the planned assessment. Therefore it was not possible to undertake the destructive assessment.

Granular formulation of *Metarhizium*

*Metarhizium* spread on the soil surface before mixing in soil

Searching for black vine weevil larvae

Treated plant



Untreated plant. Arrow points to BVW larva

**Figure 8.** Trial conducted at Haygrove Farms, Newent, Gloucestershire, UK to control black vine weevil between May and November 2010.

**Additional studies- Enhancement of fungal efficacy through the strategic use of reduced rates of entomopathogenic nematodes**

**Effect of combined application of nematodes and fungus against larval number**

Significantly low numbers of larvae were recovered from plants treated with *M. anisopliae* alone or when combined with *H. bacteriophora* or *S. kraussei* than from the untreated control (Fig. 9 and Table 11). No significant difference in larval number was found between the low dose of *H. bacteriophora* and *S. kraussei*. However, significantly low numbers of BVW larvae were recovered in plants treated with the high dose of *H. bacteriophora* (7.0 larvae/plot) than *S. kraussei* (≤ 3.0 larvae/plot). No larvae were found when *S. kraussei* was applied 5 months after plants were treated with *M. anisopliae*.

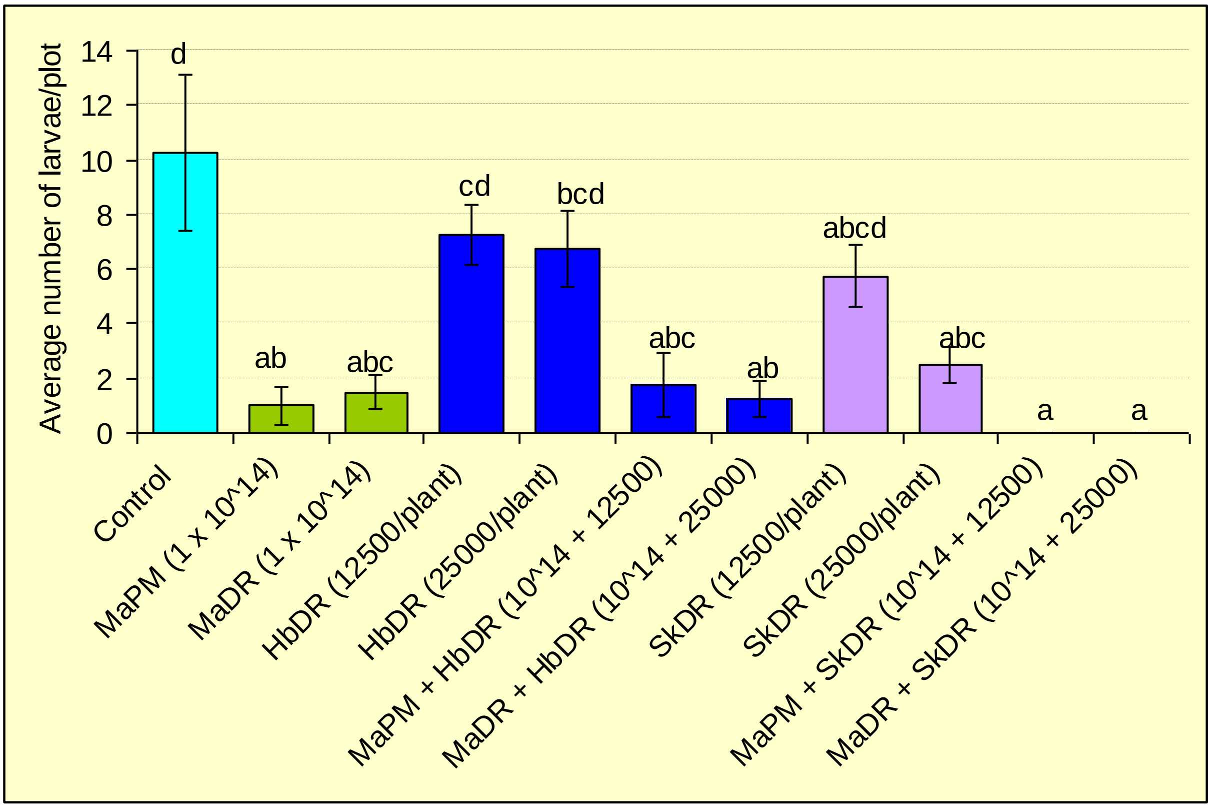



**Figure 9.** Average number of black vine weevil larvae recovered from *Metarhizium anisopliae* and entomopathogenic nematodes, used alone or in combination, for the control of an artificial inoculation of black vine weevil in field grown strawberry. No treatment (control), *M. anisopliae* premixed (MaPM), *M. anisopliae* applied as drench (MaDR), *Heterorhabditis bacteriophora* applied as drench at 12,500 and 25,000 nematode/plant (HbDR) and *Steinernema kraussei* applied as drench at 12,500 and 25,000 nematode/plant (SkDR). Nematodes were applied alone or same doses applied to plants that were previously treated with *M. anisopliae* for 5 months and plants were destructively assessed 6

weeks after nematode application. The trial was conducted in sandy soil under a polytunnel at Newent, Gloucestershire, UK between May 2010 and November 2010. Each treatment was replicated 4 times with 15 plants/treatment. Each strawberry plant was infested with 10 BVW eggs and plants were destructively assessed 10 weeks post inoculation. Bars displaying the same letters are not significantly different ($P$ > 0.05; Tukey's test). Treatments are mentioned in the Table 4.

**Table 11.** Average number of black vine weevil larvae recovered from *Metarhizium anisopliae* and entomopathogenic nematodes, used alone and in combination for the control of an artificial inoculation of black vine weevil in field grown strawberry. The trial was conducted in sandy soil under a polytunnel at Newent, Gloucestershire, UK between May 2010 and November 2010.

| **Treatments** | **Conidia/ha** | **Nematode (x $10^9$/ha)** | **Average number of larvae/plot** |
|---|---|---|---|
| Control | - | - | $10.3 \pm 2.9^{d}$ |
| *M. anisopliae* – applied as premixed | $1 \times 10^{14}$ | - | $1.0 \pm 0.7^{ab}$ |
| *M. anisopliae* - applied as drench | $1 \times 10^{14}$ | - | $1.5 \pm 0.6^{abc}$ |
| *H. bacteriophora* – applied as drench | - | 12,500 | $7.3 \pm 1.1^{cd}$ |
| *H. bacteriophora* – applied as drench | - | 25,000 | $6.8 \pm 1.4^{bcd}$ |
| *M. anisopliae* – premixed + *H. bacteriophora* - drench | $1 \times 10^{14}$ | 12,500 | $1.8 \pm 1.2^{abc}$ |
| *M. anisopliae* – drench + *H. bacteriophora* - drench | $1 \times 10^{14}$ | 25,000 | $1.3 \pm 0.6^{ab}$ |
| *S. kraussei* –applied as drench | - | 12,500 | $5.8 \pm 1.1a^{bcd}$ |
| *S. kraussei* - applied as drench | | 25,000 | $2.5 \pm 0.6^{abc}$ |
| *M. anisopliae* – premixed + *S. kraussei* - drench | $1 \times 10^{14}$ | 12,500 | $0.0 \pm 0.0^{a}$ |
| *M. anisopliae* – drench + *S. kraussei* - drench | $1 \times 10^{14}$ | 25,000 | $0.0 \pm 0.0^{a}$ |

Nematodes were applied alone or the same doses were applied to plants that were previously treated with *M. anisopliae* for 5 months and plants were destructively assessed 6 weeks after nematode application. Each treatment was replicated 4 times with 15 plants/treatment. Within a column, values followed by the same letter are not significantly different ($P$ > 0.05; Tukey's test).

## Efficacy of combined application of nematodes and fungus to control BVW

Significant differences were observed among treatments ($P < 0.05$) against BVW (Fig 10 and Table 12). There were significant differences in BVW control between *M. anisopliae* (88%) and *H. bacteriophora* (20-29%) or low dose of *S. kraussei* (39%) when applied alone. When used together, the low dose of *S. kraussei* plus *M. anisopliae* gave 100% control of the larvae 6 weeks after nematode application. No significant differences were observed between the high dose of *S. kraussei* alone or in combination with *M. anisopliae*.

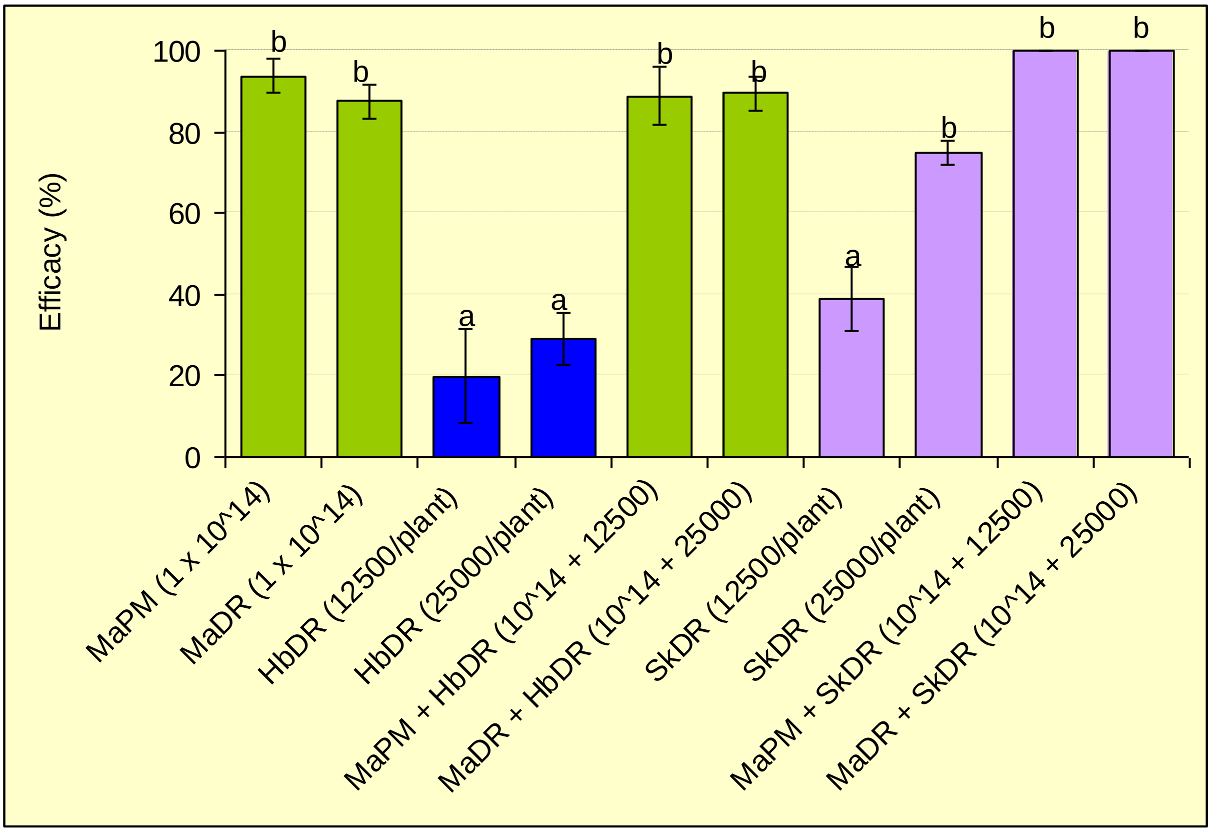



**Figure 10.** Efficacy (%) of *Metarhizium anisopliae* and entomopathogenic nematodes, applied alone or in combination for the control of black vine weevil in field grown strawberry. *M. anisopliae* premixed (MaPM), *M. anisopliae* applied as drench (MaDR), *Heterorhabditis bacteriophora* applied as drench at 12,500 and 25,000 nematodes/plant (HbDR) and *Steinernema kraussei* applied as drench at 12,500 and 25,000 nematodes/plant (SkDR). Nematodes were applied alone or the same doses were applied to plants that were previously treated with *M. anisopliae* for 5 months and plants were destructively assessed 6 weeks after nematode application. The trial was conducted in sandy soil under a polytunnel at Newent, Gloucestershire, UK between May 2010 and November 2010. Each strawberry plant was infested with 10 black vine weevil eggs and plants were destructively assessed

10 weeks post inoculation. Each treatment was replicated 4 times with 15 plants/treatment. Bars displaying the same letters are not significantly different ($P$ > 0.05; Tukey's test). Treatments are mentioned in Table 4.

**Table 11.** Efficacy (%) of *Metarhizium anisopliae* and entomopathogenic nematodes, applied alone or in combination for the control of black vine weevil in field grown strawberry. The trial was conducted in sandy soil under a polytunnel at Newent, Gloucestershire, UK between May 2010 and November 2010.

| **Treatments** | **Conidia/ha** | **Nematode/ plant** | **Mean ± SE** |
|---|---|---|---|
| *M. anisopliae* – applied as premixed | $1 \times 10^{14}$ | - | $93.7 \pm 4.2^{b}$ |
| *M. anisopliae* - applied as drench | $1 \times 10^{14}$ | - | $87.6 \pm 4.2^{b}$ |
| *H. bacteriophora* – applied as drench | - | 12,500 | $19.9 \pm 11.5^{a}$ |
| *H. bacteriophora* – applied as drench | - | 25,000 | $29.1 \pm 6.3^{a}$ |
| *M. anisopliae* – premixed + *H. bacteriophora* -drench | $1 \times 10^{14}$ | 12,500 | $88.8 \pm 7.1^{b}$ |
| *M. anisopliae* – drench + *H. bacteriophora* - drench | $1 \times 10^{14}$ | 25,000 | $89.5 \pm 4.2^{b}$ |
| *S. kraussei* – applied as drench | - | 12,500 | $39.0 \pm 7.9^{a}$ |
| *S. kraussei* – applied as drench | | 25,000 | $75.0 \pm 2.9^{b}$ |
| *M. anisopliae* – premixed + *S. kraussei* - drench | $1 \times 10^{14}$ | 12,500 | $100 \pm 0.0^{b}$ |
| *M. anisopliae* – drench + *S. kraussei* - drench | $1 \times 10^{14}$ | 25,000 | $100 \pm 0.0^{b}$ |

Nematodes were applied alone or the same doses were applied to plants that were previously treated with *M. anisopliae* for 5 months and plants were destructively assessed 6 weeks after nematode application. Each treatment was replicated 4 times with 15 plants/treatment. Within a column, values followed by the same letter are not significantly different ($P$ > 0.05; Tukey's test).

## Discussion

This study showed that Novozymes granular formulation of *M. anisopliae* F52 (Met52, MAPP 15168) can be premixed into soil or alternatively experimental suspension formulations of conidia can be applied as a drench or used as a slurry treatment of the bare roots to control BVW in field grown strawberries. Although *M. anisopliae* efficacy on its own was dose dependant, we showed for the first time that *M. anisopliae* can provide 71-96% control of BVW larvae when applied at $1 \times 10^{14}$ conidia/ha using the above methods in soil. Prior to this study, no detailed studies had been done to evaluate *M. anisopliae* for control of BVW in the soil environment. However, field applications of granular formulations of *M. anisopliae* are typically made to control other soil inhibiting insect pests at doses of $6.6 \times 10^{13}$ conidia/ha (Logan *et al*., 2000). In addition to soil application, *M. anisopliae* has also been successfully used to control BVW in soil-less potting media in glasshouse and outdoor studies (on a range of ornamentals) at the rate of $5.1 \times 10^{13}$/ha to $1 \times 10^{14}$ conidia/ha (Moorhouse *et al*., 1993; Bruck and Donahue, 2007). Similarly, when *M. anisopliae* was premixed thoroughly in the composts or applied as drench at the rate of $1 \times 10^{9}$ conidia/l or $1 \times 10^{10}$ conidia/l compost, controlled 85-100% BVW larvae on a range of container grown hardy nursery stock (Moorhouse *et al*., 1993; Shah *et al*., 2007) and strawberries in grow bags (Ansari *et al*., 2010). Together with our findings, this data show that *M. anisopliae* can be applied at $1 \times 10^{14}$ conidia/ha, the recommended rate, to control BVW in field grown strawberries.

In the current study, the efficacy of *M. anisopliae* was influenced by the method of application. For example, the level of control was consistently 8-16% better using drench application than premixing into soil or bare root treatment. This supports earlier work where we showed that between 15 and 25% higher control was achieved when applying *Metarhizium* as a drench to soil-less potting media (Shah *et al*., 2007).

Laboratory studies revealed that significantly more conidia colonised roots of strawberries when *M. anisopliae* was applied as a drench or as bare root treatment than if the inoculum was premixed into the soil (data not shown). This suggests that the application method that ensures better distribution of conidia in the rhizosphere or the rhizoplane (root surface) gives better pest control. Several studies show that *M. anisopliae* survival is better in the rhizosphere of many plant species (e.g. *Picea abies*, Cabbage) than the surrounding soil (Hu and St. Leger, 2002; Bruck, 2005, 2010). There is no clear explanation at the moment why this is so. Root exudates provide nutrition for many micro-organisms (Butler *et al*., 2003) but there is no evidence that they sustain conidia of *Metarhizium*.

In our opinion, drench applications ensure uniform distribution of conidia around the base of plant and close to where BVW eggs would be laid. Premixing the conidia ensures broader distribution, and since pest mortality is dose-dependent, this may slightly dilute the impact of the fungal BCA. In other words, there may be less inoculum in the root zone where BVW eggs are laid or where larvae normally feed.

Whatever the application method used, of the live larvae recovered from plants treated with the high dose of *M. anisopliae* in 2010, 25% of these died of the fungus within one week of incubation at 25°C in the laboratory. In the 2009 trials all the larvae recovered from *M. anisopliae* treated plots ultimately died of infection with *Metarhizium* (data not shown). The differences in larval mortality may be due to the low soil temperature (11°C) at the time of destructive assessment in 2010 compared to a comparatively higher soil temperature (14°C) in 2009. Temperature does influence the speed of kill. Indeed, Moorhouse *et al*., (1994) reported that *M. anisopliae* provided 98% control of BVW larvae at 25°C, but only 93%, 87% and 49% control at 20°C, 15°C and 10°C, respectively. It is not known if the live larvae recovered would have died in the field either over the winter or in the following spring, but it is clear that a large proportion were infected with the fungus.

In additional studies, *S. kraussei* provided better BVW larvae control at low and high doses (39-75%) whereas the efficacy of *H. bacteriophora* was more variable (20-29%), confirming that the *H. bacteriophora* is more sensitive to temperature fluctuations. Indeed one of the greatest limitations in using *Heterorhabditis* species in field grown strawberries is temperature, with temperatures below 12°C severely impairing their efficacy (Fitters *et al*., 2001). However, *H. bacteriophora* provided 90-100% control of BVW larvae in *Epimedum* x *rubrum* at temperatures ranging between 15-28°C (Gill *et al*., 2001). In contrast, Willmott *et al*., (2002) showed that *S. kraussei* was fairly efficacious in controlling BVW larvae in pots at temperatures ranging from -1.5 to 7.3°C. Long *et al.,* (2000) reported that *S. kraussei* caused 37% mortality of early instar of BVW at 6°C. Recently, Ansari *et al*., (2010) reported 60-69% of BVW control by *S. kraussei* at temperatures ranging between -1.5 and 13.5°C. Although *S. kraussei*, *H. bacteriophora* or *M. anisopliae* alone are unlikely to provide 100% control of BVW, this study shows that control is greatly improved (100%) when *S. kraussei* is applied 5 months after *M. anisopliae*. This preliminary study suggests that growers have a choice to combine *S. kraussei* with *M. anisopliae* when temperatures reach below 10°C and *H. bacteriophora* when temperatures are above 15°C. Additional benefits of using the *S. kraussei-M. anisopliae* combination would be the control of the older, overwintering BVW larvae infesting strawberries. These results support our previous studies where a simultaneous application of *S. kraussei* and *M. anisopliae* provided 100% control of overwintering BVW larvae in strawberry grow bags (Ansari *et al*., 2010).

It should be noted that control from the application of *H. bacteriophora* was marginally lower than *S. kraussei*. The reason for the lack of higher control may be due to low soil temperatures between the period of nematode application (13°C) and destructive assessment (8°C). Previously, a delayed application of *H. bacteriophora* in rooted cuttings of *Euonymus fortunei* treated with *M. anisopliae* has resulted in 100% BVW control in potting media (average media temperature 16.5°C) under glasshouse conditions. These observations suggest that *H. bacteriophora* could significantly reduce BVW populations if used in conjunction with *M. anisopliae* only at temperatures above 15°C.

At present, when protecting plants from BVW using *M. anisopliae*, efforts are focused on applying huge amounts of inoculum to increase the fungal population throughout the soil. This method presents many problems such as: (i) a requirement for large quantities of fungal inoculum, possibly making application uneconomical, (ii) difficulty in getting the fungal conidia applied to the soil surface to penetrate into the soil more than a few centimeters and (iii) a large amount of time, money and effort is spent protecting areas of the field where the pest is absent or is not of concern. As demonstrated in this project, targeted delivery of inoculum would be more cost effective. For example, treating bare roots with *M. anisopliae*, the costs and logistics of doing so would be greatly reduced. However, this may have to be followed by drench application of the fungus or even delivery *via* irrigation lines. There is no doubt that more work needs to be done to establish cost-effective application/delivery systems of *M. anisopliae* for BVW control in field strawberries.

Finally, the granular formulation of *M. anisopliae* shows potential for controlling BVW in field grown strawberries at the recommended dose ($1 \times 10^{14}$ conidia/ha). At the tested rate, growers would benefit from the continued protection given by the fungus against fresh infestations of BVW in the second year after planting. This obviously affects the economic equation for crops kept in the soil for more than a year. Another permutation, revealed in this study, is the application of entomopathogenic nematodes at significantly reduced rates to plots pre-treated with *M. anisopliae* for improved BVW control. However, the efficacy and cost benefits need to be verified through future studies.

## Conclusions

- This study shows that *Metarhizium anisopliae* F52 (=V275 = BIPESCO 5) is highly efficacious for the control of black vine weevil larvae in different soil types.
- The level of control appears to be similar to the recommended insecticide (e.g. chlorpyrifos).
- Premixing, drenching or bare root treatment with *M. anisopliae* all gave similar levels of black vine weevil control.
- The highest dose tested ($1 \times 10^{14}$ conidia/ha) gave significantly better control than the low or intermediate doses.
- These observations suggest that Novozymes granular formulation (Met52, MAPP 15168), can be premixed into the soil or the spores removed and applied as a drench or as a slurry treatment of the bare roots and when used at $1 \times 10^{14}$ conidia/ha is efficacious in the prophylactic control of BVW larvae. ***The removal of spores in this way would not be a permitted use of the product. It as an experimental technique but cannot be recommended at present.***
- This study shows that full rates of *S. kraussei*, *H. bacteriophora* or *M. anisopliae* alone are unlikely to provide 100% control of black vine weevil; however, control in the autumn/winter is greatly improved (100%) when reduced rates of the cold-tolerant nematode *S. kraussei* is applied 5 months after application of *M. anisopliae*.

## Technology transfer

HDC members in the soft fruit sector were informed of progress in the 2009-2011 and 2010-2011 Soft Fruit Review publications produced by HDC in December 2009 and 2010 respectively. The work also featured at the Soft Fruit Agronomists' day in February 2010 and February 2011 and the associated handbook produced each year. The work also contributed to the Met 52 launch at WHRI, Wellesbourne on 1 February 2011.

## References


1. Ansari, M.A., Brownbridge, M., Shah, F.A. and Butt, T.M. (2008a). Efficacy of entomopathogenic fungi against soil-dwelling life stages of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae) in plant growing media. Entomologia Experimentalis et Applicata 127: 80-87.

2. Ansari, M.A., Shah, F.A. and Butt, T.M. (2010). The entomopathogenic nematode *Steinernema kraussei* and *Metarhizium anisopliae* work synergistically in controlling overwintering larvae of the black vine weevil, *Otiorhynchus sulcatus*, in strawberries growbags. Biocontrol Science and Technology 20: 99-105.

3. Ansari, M. A, Shah, F. A., Whittaker, M., Prasad, M. and Butt, T.M. (2007). Control of western flower thrips (*Frankliniella occidentalis*) pupae with *Metarhizium anisopliae* in peat and peat alternative growing media. Biological Control 40: 293-297.

4. Ansari, M.A., Shah, F.A. and Butt, T.M. (2008b). Combined use of entomopathogenic nematodes and *Metarhizium anisopliae* as a new approach for black vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) control. Entomologia Experimentalis et Applicata 129: 340-247.

5. Bruck, D.J. (2005). Ecology of *Metarhizium anisopliae* in soilless potting media and the rhizosphere: implications for pest management. Biological Control 32: 155-163.

6. Bruck, D.J. and Donahue, K.M. (2007). Persistence of *Metarhiziumanisopliae* incorporated into soilless potting media for control of the black vine weevil, *Otiorhynchus sulcatus* in container grown ornamentals. Journal of Invertebrate Pathology 95: 146–150.

7. Bruck, D.J. (2010). Fungal entomopathogens in the rhizosphere. BioControl 55:103-112.

8. Cross, J.V. and Burgess, C.M. (1997). Localised insecticide treatment for the control of vine weevil larvae (*Otiorhynchus sulcatus)* on field-grown strawberry. Crop Protection 16: 565-574.

9. Butler, J.L., Williams, M.A. Bottomley, P.J., and Myrold, D.D. (2003). Microbial community dynamics associated with rhizosphere carbon flow. Applied and Environmental Microbiology 69: 6793-6800.

10. Fitters, P.F.I., Dunne, R., and Griffin, C.T. (2001). Improved Control of *Otiorhynchus sulcatus* at 9°C by Cold-stored *Heterorhabditis megidis* UK211. Biocontrol Science and Technology 11: 483-492.

11. Gill, S., Lutz, J., Shrewsbury, P., and Raupp, M. (2001). Evaluation of biological and chemical control methods for black vine weevil, *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae), in container grown perennials. Journal of Environmental Horticulture 19: 166-170.

12. Hu, G. and Leger, R.J. (2002). Field trials using a recombinant mycoinsecticide (*Metarhizium anisopliae*) reveal that it is rhizosphere competent. Applied and Environental Microbiology 68: 6383-6387.

13. Logan, D.P., Robertson, L.N., and Milner, R.J. (2000). Review of the development of *Metarhizium anisopliae* as a microbial insecticide BioCane™, for control of greyback canegrub *Dermolepida albohirtum*, Waterhouse (Coleoptera: Scarabaeidae) in Queensland sugarcane. IOBC/WPRS Bulletin 23 (8), 131-137.

14. Moorhouse, E R., Charnley, A.K. and Gillespie, A.T. (1992). A review of the biology and control of the vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). Annals of Applied Biology 121: 431-454.

15. Moorhouse, E.R., Gillespie, A.T., and Charnley, A.K. (1993). Application of *Metarhizium anisopliae* (Metsch.) Sor. conidia to control *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae) larvae on glasshouse pot plants. Annals of Applied Biology 122: 623-636.

16. Moorhouse, E.R., Gillespie, A.T., and Charnley, A.K. (1994). The Influence of Temperature on the Susceptibility of Vine Weevil, *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae), Larvae to *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). Annals of Applied Biology 124: 185-193.

17. Scheepmaker, J.W.A. and Butt, T.M. 2010. Natural and released inoculum levels of entomopathogenic fungal biocontrol agents in soil in relation to risk assessment and in accordance with EU regulations. Biocontrol Science and Technology 20: 603-662

18. Shah F. A., Ansari, M.A., Prasad, M., and Butt, T.M. (2007). Evaluation of black vine weevil (*Otiorhynchus sulcatus*) control strategies using *Metarhizium anisopliae* with sublethal doses of insecticides in disparate horticultural growing media. Biological Control 40: 246-252.

19. Shah, F.A., Hutwimmer, S., Greig, C., Dyson P., Strasser, H., and Butt T. M. 2010. Influence of natural microbial populations in horticultural growing media on the efficacy of *Metarhizium anisopliae*. Fungal Ecology 3: 185-194

20. SPSS Inc., 2007. SPSS Statistical Software CD-ROM Version 16.0 for Windows. SPSS Inc., Chicago Illinois, USA.

21. van Tol R.W.H.M., Van Dijk N. and Sabelis M.W (2004). Host plant preference and performance of the vine weevil *Otiorhynchus sulcatus.* Agricultural and Forest Entomology 6: 267-278.

22. Willmott, D.M., Hart, A.J., Long, S.J., Edmondson, R.N., and Richardson, P.N. (2002). Use of cold-active entomopathogenic nematode *Steinernema kraussei* to control overwintering larvae of the black vine weevil *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) in outdoor strawberry plants. Nematology 4: 925-932.