Project title:	Development of the entomogenous fungus, *Metarhizium anisopliae*, for control of vine weevil and thrips in horticultural growing media
Project number	DEFRA Horticulture Link Code HL 0171 HDC Project Code HNS 133
Government sponsor(s)	DEFRA, HortiLINK
Project leader	Dr Tariq M. Butt Reader Department of Biological Sciences University of Wales, Swansea SA2 8PP
Report:	First Annual report, July, 2006
Previous reports:	First six monthly report, November, 2005
Location	University of Wales, Swansea
Project coordinator:	Dr. Paul Sopp Fargro Ltd. Toddington Lane Littlehampton West Sussex BN17 7PP
Date commenced:	1st April 2005
Key words	*Metarhizium*, Black vine weevil, western flower thrips, sublethal doses, reduced chemical insecticides, fipronil, imidacloprid, growth media, peat, coir, bark, composted green waste

Growers Summary

Headline

- The insect pathogenic fungus *Metarhizium anisopliae* V275 was highly efficacious against black vine weevil (BVW) larvae and western flower thrips (WFT) pupae in a range of different plant growing media (peat, bark, coir, peat blends with 10% and 20% composted green waste).
- Good control (over 65%) was achieved whether the fungus applied as a drench or premixed into growing media.
- Excellent (over 85%) control of BVW larvae was achieved when *M. anisopliae* V275 was used with sublethal rates of imidacloprid (1% recommended rate) and fipronil (10% recommended rate). The control achieved was similar to or better than the full dose chemical.
- *M. anisopliae* V275 alone gave significantly better control of WFT pupae than the chemical insecticides (fipronil or imidacloprid) in all the media tested

Background and expected deliverables

BVW is considered the most important pest of hardy nursery stock (HNS) causing annual losses of ca. £30 million. WFT are pests of protected plants causing damage directly through feeding and indirectly through the transmission of plant viruses. Both BVW and WFT spend part of their life cycle in growing media. Control of these subterranean stages would contribute significantly to the overall IPM programme. This project builds on or complements earlier studies funded by HDC and the EU which aim to reduce insecticide inputs especially for BVW and WFT control. The overall aim of this project is to develop the V275 strain of the entomogenous fungus *M. anisopliae* for the control of BVW larvae and WFT pupae in plant growing media. It offers a benign alternative to chemical pesticides that are currently under threat of being phased out (e.g. chlorpyriphos) or where pests have developed resistance (WFT is resistant to many pesticides e.g. chlorpyriphos) or likely to develop resistance soon because of extensive use (e.g. imidacloprid).

The expected project deliverables will include:

- Reduced inputs of chemical insecticides for the control of BVW larvae and WFT pupae.
- Data on the efficacy and robustness of *M. anisopliae* V275 in peat and peat alternative plant growing media.

- Elucidation of the interactions between *M. anisopliae* and sublethal doses of insecticides.
- Determination of whether *M. anisopliae* is compatible with cold tolerant entomophilic nematodes for the control of BVW larvae.
- Grower protocols (advisory leaflet) on the use of *M. anisopliae* for the control of BVW larvae and WFT pupae.
- Standardized protocols for testing the efficacy of microbial pest control agents in plant growing media.

Summary of the project and main conclusion

The main findings of this project are listed below.

- Plant growth studies showed that composted green waste (CGW) cannot be used on its own as a plant growth medium but can be used if blended with peat i.e. CGW : peat, 1:4. The pH, Electrical conductivity (EC) and Potassium (K) levels were very high in CGW whereas studies on the physical properties showed low air space and easily available water for CGW.
- *M. anisopliae* was efficacious against BVW in the different plant growth media tested (peat, coir, bark and peat blended with 10% or 20% CGW).
- *M. anisopliae* used with sublethal doses of imidacloprid/fipronil was consistently efficacious in the control of BVW larvae**.**
- *M. anisopliae* provided significantly higher control of WFT pupae than chemical insecticides (fipronil or imidacloprid) in all the plant growth media tested.
- *M. anisopliae* is compatible with entomopathogenic nematodes (*Steinernema kraussei* and *Steinernema feltiae*). Further studies on the efficacy of *M. anisopliae* with and without cold tolerant entomophilic nematodes will be initiated in autumn 2006.
- Leaching studies showed that significantly more conidia (i.e. the active ingredient or infective unit of *Metarhizium*) were leached from growth media if the fungus was applied as a drench. Since over 90% of total inoculum loss was at the time or within the 1st hour of application it is recommended that excessive volumes of the conidial suspension should not be used since any excess would result in immediate loss of inoculum. Comparatively little inoculum was lost if premixed into the growing media.
- Conidia of *M. anisopliae* were not inhibited by humic-fulvic acid preparations but germination was comparatively slower than those incubated in water soluble leachates

from the different plant growth media. Humic-fulvic substances are ubiquitous, heterogeneous macromolecules derived from biological, chemical and physical degradation of organic matter. They play an important role in plant nutrition, detoxification, and will influence the microbial composition of growing media. Conidia of *M. anisopliae* were marginally less virulent when formulated in concentrated generic humic-fulvic acids. Leachates from the test media had no significant effect on the germination and virulence of *M. anisopliae* conidia. These findings suggest that the water-soluble components of the growth media have no adverse effects on *Metarhizium* efficacy.

- The efficacy of *M. anisopliae* V275 for thrips pupae control was compared with ten potentially competing, commercially viable fungal strains. V275 was found to be as good as or better than these strains in the control of WFT pupae in five different plant growth media.

Financial benefits

Studies are in progress. A report on the financial benefits will be submitted either at the end of 2nd year or with the final project report.

Action points for growers

Studies in progress, recommendation (grower protocol) will be prepared and circulated after demonstration trials in 2007.

Science Section

Objective 1: Characterization of growing media properties

Introduction

Peat, bark, coir and composted green waste (CGW) were characterized to evaluate their performance as horticultural growth media as well as to gain a better understanding of their physical and chemical properties which might influence the efficacy of *Metarhizium anisopliae*.

Materials and Method

pH and EC were determined in water using the standardized methods BS EN 13037:2000 and BS EN 13038:2000, respectively. Nutrient levels were determined in a $CaCl_2$/DTPA extract using the method according to the BS EN 13651:2001 protocol. Nitrogen retention and E4/E6 studies were done as described by Prasad (1997) and Schnitzer (1982), respectively. Biological stability of the media was determined using the SOUR method (Lasaridi & Stentiford 1996) and Oxitop method (Wageningen University & Nutrient Management Institute, 2003). Physical Properties were determined using the ISEN 13041:2000 protocol.

Results

Considerable variation in pH, EC and nutrient levels were observed in the media tested (Table 3). The pH of CGW was very high, while the pH of peat was very low, and bark and coir were intermediate. The EC values were high for CGW, very low for peat whereas coir and bark had moderate values. All media, except bark, had low levels of N and P. Coir and bark had moderate levels of K whereas CGW had extremely high levels of K. All media were stabilized as regards nitrogen retention, with coir and CGW tending to retain a small amount of nitrogen, over ten weeks of incubation (data not presented). The E4/E6 test indicated high levels of humic acid in all media; however CGW and composted bark had relatively higher humic acid levels than peat and coir. Biological stability studies confirmed above findings of nitrogen retention and E4/E6 studies (Table 4).

Studies on the physical properties showed low air space and easily available water for CGW (Table 4). Peat has a good balance of air as well as easily available water. Both coir and bark have high levels of air space but easily available water is low in bark. Bulk density is very high in CGW but low in peat and coir, and intermediate in bark.

Table 3 Chemical Analysis of various growing media

Sample	pH (units)	EC (us/cm)	NH_4N (mg/L)	NO_3N (mg/L)	P (mg/L)	K (mg/L)	E4/E6
Composted green waste	8.23	3290	85	7	20	1073	8.17
Peat	3.90	108	1	2	0	0	12.8
Coir	6.70	398	1	0	7	400	10.8
Bark	5.80	145	0	0	5	82	3.94

Table 4 Biological Stability and physical properties of plant growing media

Sample	Oxitop (mmol O2/kg OS/h)	SOUR (o_2/g dm/ h)	Bulk Density (g/L)	% Air (v:v) *After applying 10cm pressure IS EN 13041:2000*	% Easily Available Water (v:v)	Total pore space
Composted green waste	3.11	0.7	411.77	27	16	63
Peat	0.70	2.37	252.97	34	8	87
Coir	0.90	0.4	80.96	34	20	92
Bark	6.5	0.69	148.63	25	31	93

Discussion

Although the plant growth media differed considerably in their physical and chemical properties all except CGW were suitable for horticultural use. However, good plant growth was observed in peat blends containing 10% or 20% CGW. The exact combination of peat and CGW blends would depend upon the source and degree of green waste composting. The results of this study will help determine the robustness of *M. anisopliae* (i.e. its ability to control pests in different growing media).

Objective 2: Elucidate synergistic interactions between *M. anisopliae* and low doses of insecticide

Task 2.1: Pot bioassay to determine synergistic interactions between *M. anisopliae* and low doses of insecticides for the control of BVW and WFT in different plant growth media

A. Studies against Black Vine Weevil

Introduction

The black vine weevil (BVW), *Otiorynchus sulcatus*, is an important and widespread pest of ornamental nursery stock and soft fruit (Cross and Burgess, 1997; Masaki *et al.,* 1984, Moorehouse et al., 1992; Van Tol et al., 1998; Lola-Luz et al., 2005). Adult weevils feed on leaves causing mostly cosmetic damage whilst the larvae feed on 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) but there is considerable interest to reduce the input of such pesticides because of the risks they pose to humans and the environment and increased resistance among pest populations. Growers are currently trapped between the diminishing number of available chemical insecticides and the availability of safe alternatives. Entomopathogenic nematodes offer a more benign alternative for BVW control in potted plants and glasshouse crops (Kakouli-Duuarte *et al*., 1997; Fitters *et al*., 2000) but they have limited success due to a relatively short shelf life and inconsistent results (Georgis *et al*., 2006; Koppenhofer, 2000; van Tol *et al*., 2004).

The entomogenous fungus, *Metarhizium anisopliae* (Metsch.) Sorokin, shows considerable potential for the control of BVW larvae (Bruck, 2004; Moorhouse *et al.,* 1992, 1993). However, an inherent weakness of *M. anisopliae* is that it is slow acting, particularly at low temperatures. Since the grower often wants rapid protection then a strategy needs to be devised where pesticide inputs can be reduced yet benefit from the control given by *M. anisopliae*. One approach is the exploitation of synergistic interactions between *M. anisopliae* and low doses of chemical insecticides. Of the few studies to date, synergy has been observed in the control of termites, root weevils and aphids (Quintela and McCoy, 1998; Roditakis *et al*., 2000; Inglis *et al*., 2001). Low doses of insecticide usually alter insect behaviour.

Depending on the species, insects may increase in mobility with a corresponding increase in the acquisition of conidia (Roditakis *et al*., 2000), or they may stop grooming (i.e. dislodging conidia from the cuticle) and feeding (Quintela and McCoy, 1998; Boucias *et al*., 1996). Insects that are debilitated due to starvation or other forms of stress may be more susceptible to fungal infection. This study examines, for the first time, if *M. anisopliae* and pesticides work synergistically in the control of BVW larvae. Since plant growing media can influence the efficacy of chemical insecticides and survival of BVW larvae (Ramarkrishnan *et al*., 2000; Buxton, 2003), we investigated such interactions in conventional peat and alternative horticultural growing media (coir, bark, peat blends).

Materials and Methods

Fungal strain, maintenance and mass production

M. anisopliae strainV275 was used in all studies. Details of its maintenance and production are given in Shah *et al*., (2005).

Inoculation of plants with BVW eggs

Rooted cuttings of *Euonymus fortunei* 'Emerald Gold' kindly provided by Johnsons of Whixley (York, UK) were transplanted in 0.5 l pots filled with one of the test media and inoculated 7 days later with 15 melanized BVW eggs which were gently placed around the base of the plant. The media provided by Bord Na Mona (Ireland) included: peat (seed and potting compost), bark (multipurpose peat free), coir and peat blended with 10% (v/v) or 20% (v/v) composted green waste (CGW).

Application of *M. anisopliae* and insecticides

M. anisopliae was applied as a drench or premixed such that the final concentration was 1x 10^{10} conidia/l of compost. The chemical insecticides Provado® (Bayer a.i. 5% w/w imidacloprid) and Vi-Nil® (Certis, a.i. 1% fipronil) were used at the recommended and pre-determined sublethal rates. Imidacloprid and fipronil sublethal rates corresponded to 1% and 10% of the recommended rate. These were used alone or with *Metarhizium* V275. Untreated plants constituted one of the controls.

Trials were conducted between July-October, 2005 when the average day and night temperatures ranged between 15-25ºC and 10-15ºC, respectively. Plants were destructively assessed six weeks post egg infestation to determine the efficacy of the above treatments.

Task 2.2 Pest susceptibility and spore acquisition

To determine if *M. anisopliae* spore acquisition increased in the presence of sublethal doses of insecticides, additional plants were prepared in peat with *M. anisopliae* being applied as a drench with and without a sublethal dose of imidacloprid or fipronil. Both insecticides were also applied alone at the sublethal and recommended rates. All studies included untreated controls.

Each pot was infested with two 2^{nd} instar BVW larvae. Each treatment was replicated 5 times and the whole experiment repeated twice. Larvae were recovered from two pots per treatment 2 days post-infestation. Half the larvae were fixed in 2% formaldehyde and stored at 4ºC until required. The specimens were stained with calcofluor and examined in a Nikon, Eclipse E600 microscope equipped with epifluorescence (Butt, 1997). Spore adhesion to the second larval group was determined as outlined by Moorehouse (1993). Briefly, the larvae were macerated in 0.5ml of 0.03% Aq. Tween and conidial numbers counted using an improved Neubaur haemocytometer (Weber Scientific Ltd., UK). The remaining pots from each treatment were destructively assessed 2 weeks post infestation to determine larval mortality.

Statistical analysis

Percentage efficacy data 6 weeks post-treatment were corrected for control mortality (Abbott's 1925), arcsine square root transformed, and analyzed using ANOVA and Duncan test for means separation (SPSS, 2003). Differences among means were considered significant at $P < 0.05$.

Results

Efficacy experiments

Both *M. anisopliae* and chemical insecticides provided good control of BVW larvae; however, efficacy was dependant on the application method, plant growth medium and insecticide dose (Figs 1-5). For example, *M. anisopliae* appeared to give better control if applied as a drench as opposed to premixing, providing between 85% and 100% control irrespective of the growing medium (Figs 1-5). Premixed applications gave less than 80% control and appeared to be influenced by the medium ($P < 0.05$) (Figs 1-5). In reality, *M. anisopliae* efficacy using the two application methods differed non-significantly ($P > 0.05$) for the all the media tested with the exception of peat blends with 10% and 20% CGW.

Imidacloprid when used at the recommended and sublethal rates gave 70-85% and 30-50% control, respectively in the different media (Figs. 1-5). Its activity appeared to be influenced by compost type. Fipronil usually gave 100% control at the recommended rate but 85-100% control at the sublethal dose in all the media tested (Figs 1-5). At the reduced rate it appeared to be least effective in peat and peat blends with 10% CGW ($P < 0.05$) (Figs. 1-5).

Application of *M. anisopliae* with sublethal doses of insecticides provided excellent control of BVW larvae independent of the chemical used. Usually the control was higher than use of the *M. anisopliae* or chemical insecticides used alone. In combined applications, *M. anisopliae* efficacy was consistently over 75% irrespective of application method and/or plant growing medium.

Compost type significantly influenced BVW infestation with significantly ($P < 0.05$) more larvae being recovered from coir than all other media (Figs. 6-7). Non-significant ($P < 0.05$) differences in larval infestation were observed for the remaining growth media (Fig 6). As stated earlier, compost type influenced the efficacy of the different control agents (Figs.1-5). For example, drench application of *M. anisopliae* was most efficacious in peat blended with 10 and 20% CGW than all other media tested (Figs 1-5). Use of *M. anisopliae* with fipronil or imidacloprid gave good control of BVW larvae in all growing media with 95-100% control being achieved if the fungus was applied as a drench but 78-92% control achieved if it was premixed into the different media (Figs.1-5, 8).

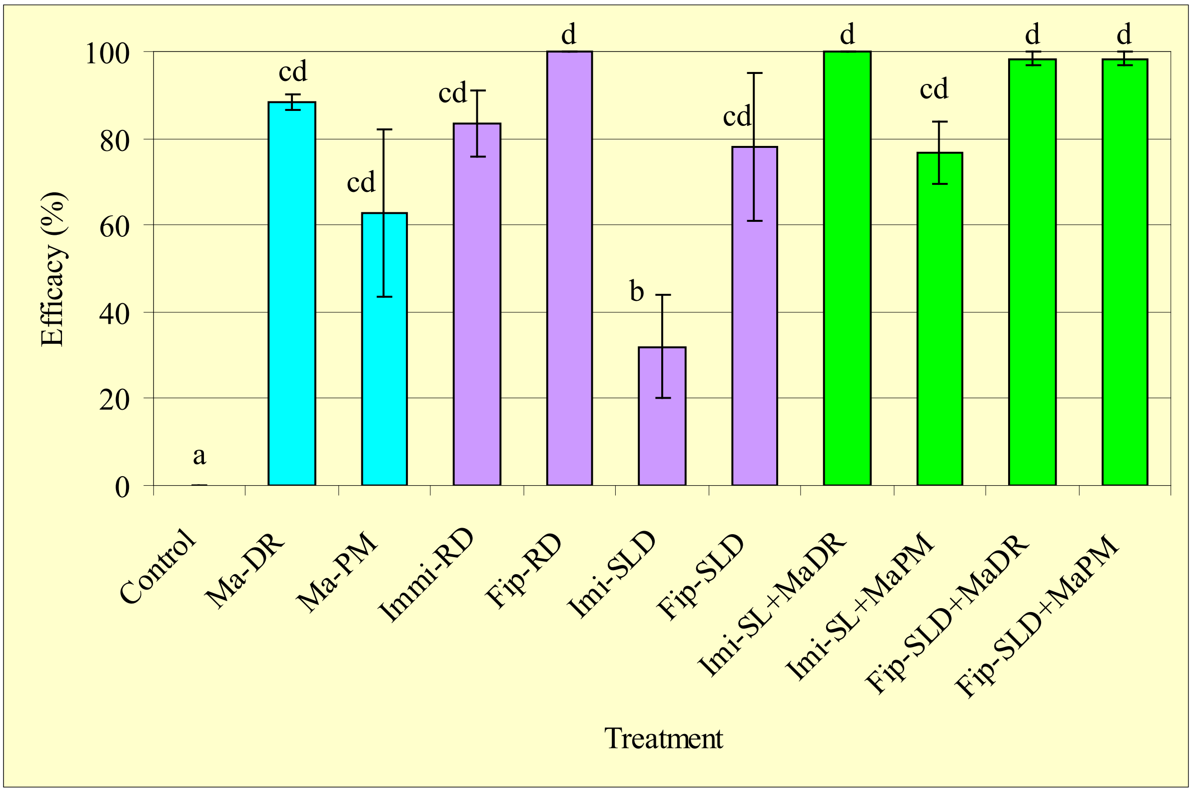

Fig 1. Efficacy (%) of *M. anisopliae* and insecticides against BVW in *Euonymus* potted in peat growth media. The treatments are listed below. No treatment- (control), *Metarhizium* applied as drench (Ma-DR), *Metarhizium* premixed (Ma-PM), Imidacloprid applied as a drench at the recommended rate (Imi-RD), Fipronil premixed at the normal recommended rate (Fip-RD), Imidacloprid applied as a drench at the sublethal rate (Imi-SLD), Fipronil premixed at the sublethal rate (Fip-SLD), Imidacloprid used at the sublethal rate with *Metarhizium* applied as a drench (Imi-SLD+MaDR), Imidacloprid used at the sublethal rate with *Metarhizium* premixed (Imi-SLD+MaPM), Fipronil used at the sublethal rate with *Metarhizium* applied as a drench (Fip-SLD+MaDR), Fipronil used at the sublethal rate with *Metarhizium* premixed (Fip-SLD+MaPM).

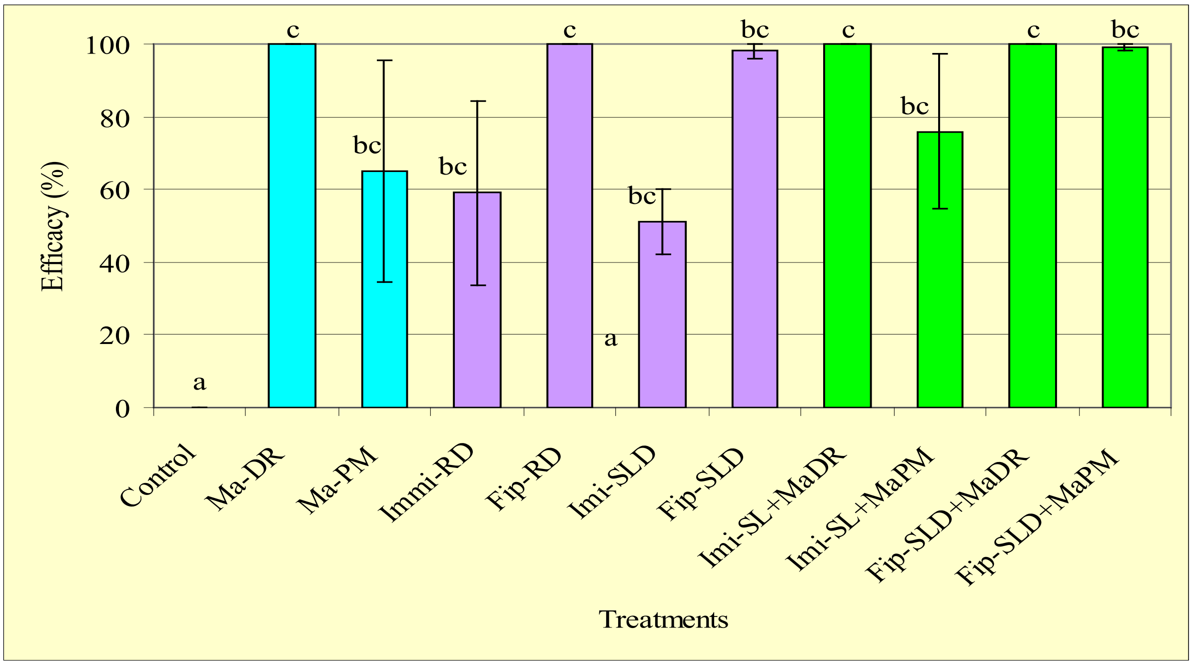

Fig 2. Efficacy (%) of *M. anisopliae* and insecticides against BVW in *Euonymus* potted in bark growth media. Treatments are same as described in Fig.1.

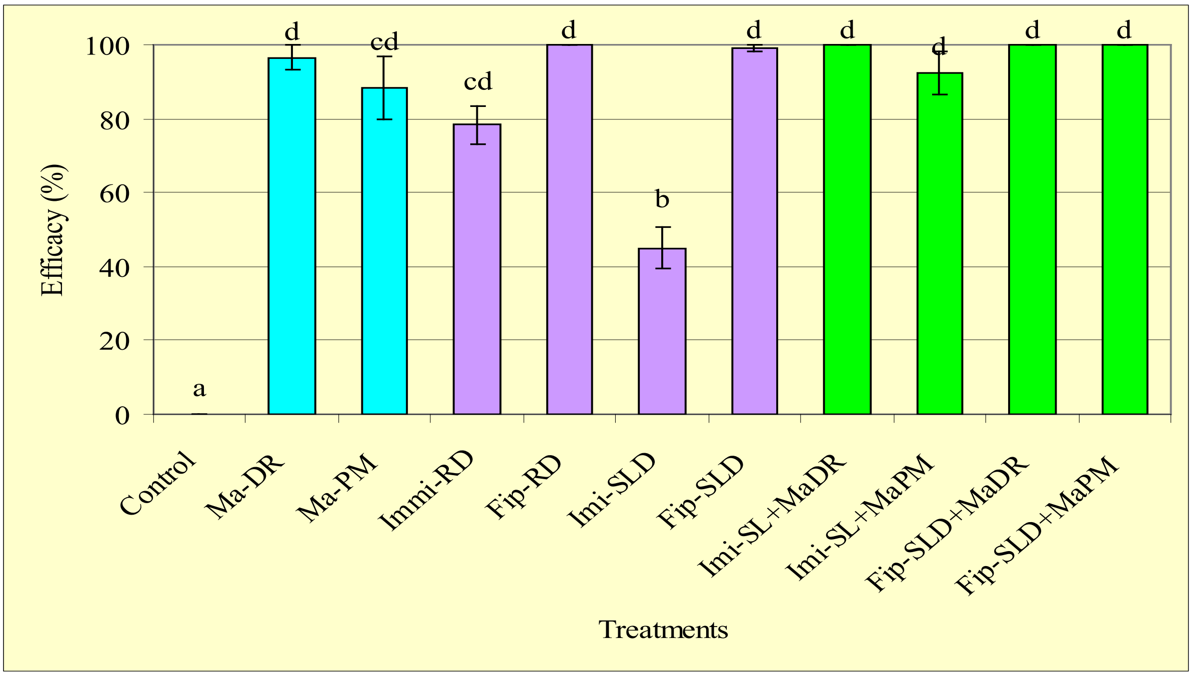

Fig 3 Efficacy (%) of *M. anisopliae* and insecticides against BVW in *Euonymus* potted in coir growth media. Each pot was treated with either of following treatments. Treatments are same as described in Fig.1

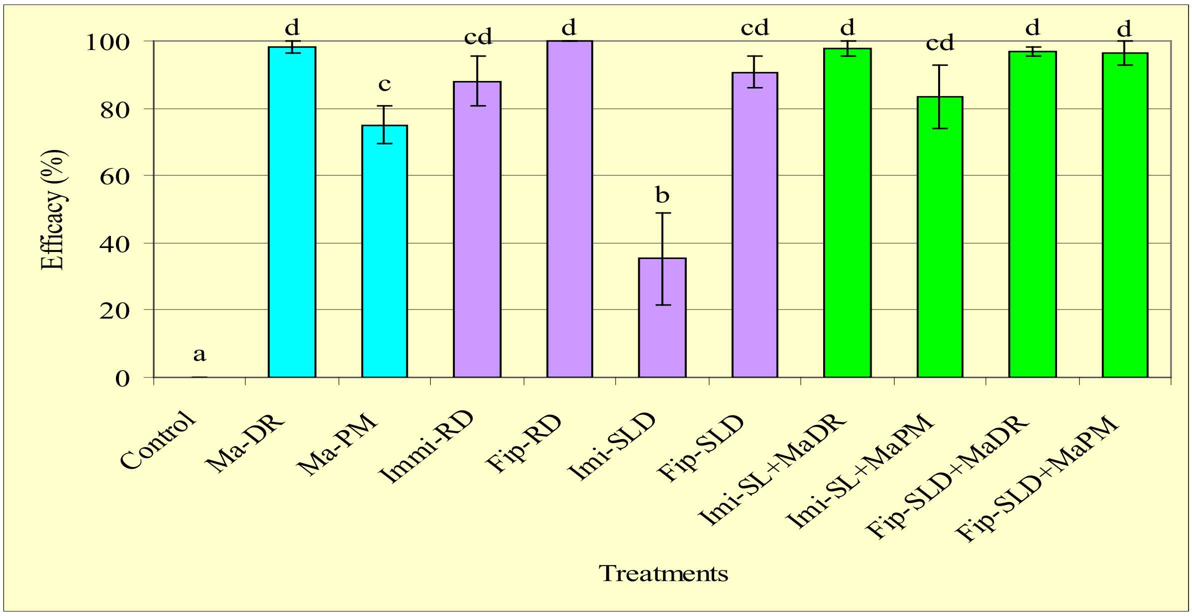

Fig 4. Efficacy (%) of *M. anisopliae* and insecticides against BVW in *Euonymus* potted in 10% green waste blend growth media. Each pot was treated with either of following treatments. Treatments are same as described in Fig.1.

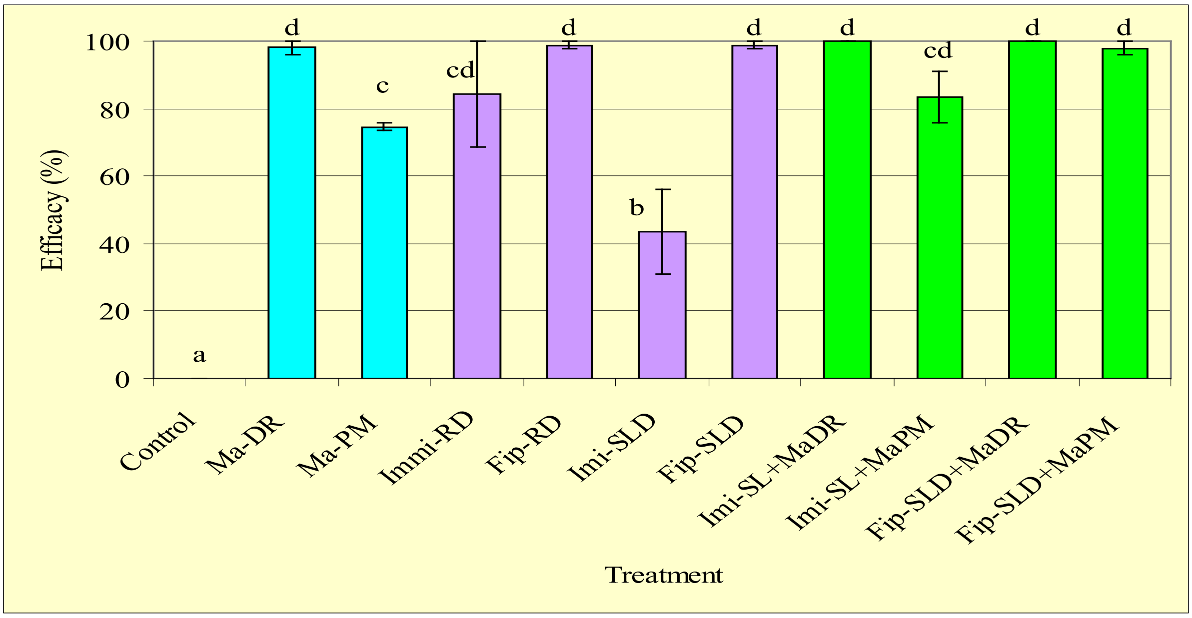

Fig 5. Efficacy (%) of *M. anisopliae* and insecticides against BVW in *Euonymus* potted in 20% green waste blend growth media. Each pot was treated with either of following treatments. Treatments are same as described in Fig.

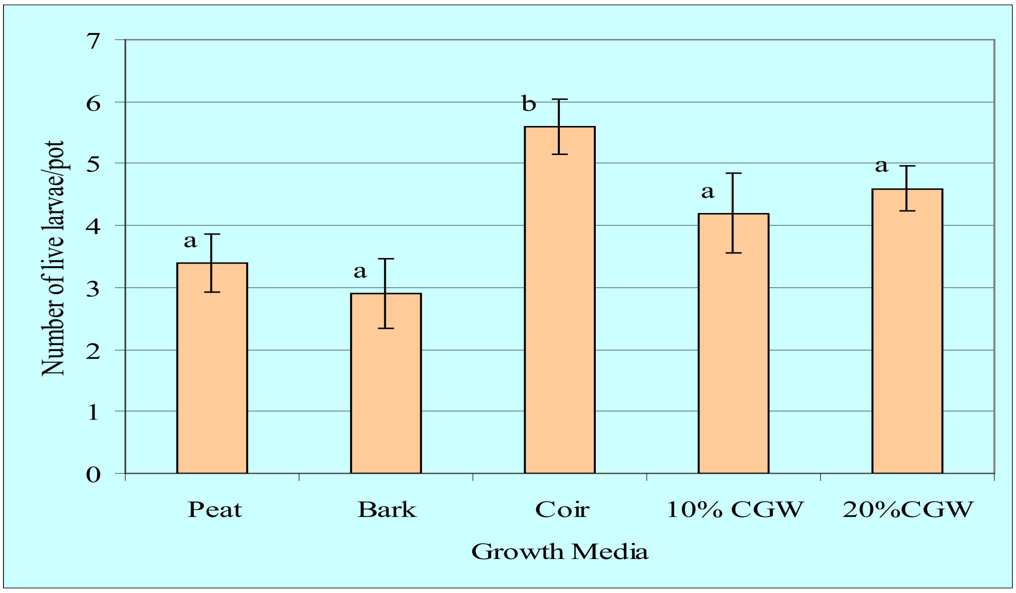

Fig 6. Establishment of BVW larvae in different plant growth media. Each pot was infested with 15 melanized eggs and destructively assessed 6 weeks post infestation

Fig. 7 An untreated *Euonymus* plant with the root system infested with BVW larvae

Fig.8 Comparison between untreated (right) and plant treated with combined application premixed application of *M. anisopliae* and sublethal dose of imidacloprid (left). Note differences in the root system

Conidial adhesion

Significantly more conidia of *M. anisopliae* adhered to BVW larvae when used with sublethal doses of fipronil than imidacloprid or if used alone (10.66 ± 0.84 versus 3.5 ± 0.34 versus 2.17 ± 0.54 conidia per 10µl of macerate). In the fipronil treatment, conidia were usually clustered and had germinated with many producing appressoria (Fig. 9). None of the conidia in the imidacloprid or chemical free controls had germinated during the same timeframe. *In vitro* studies show that neither chemical inhibited *Metarhizium* conidia (Butt & Shah, unpublished observations). In these studies, *M. anisopliae* used with sublethal doses of fipronil resulted in 100% control 2 weeks post inoculation whereas *M. anisopliae* and fipronil when used alone caused 25% and 75% mortality, respectively (data not shown). Use of *M. anisopliae* with sublethal doses of imidacloprid resulted in 50% larval mortality. Imidacloprid alone caused 50 and 25% mortality at the recommended and sublethal doses, respectively.

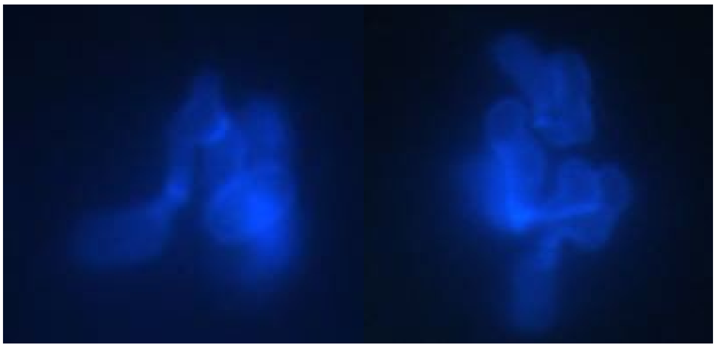

Fig 9. Spore adhesion on larvae recovered from pots treated with sublethal dose of fipronil and *M. anisopliae*. Note spores are attached in clusters and most have germinated and differentiated appressoria (infection structures). Photographed at 60X magnification of fluorescent microscope (Nikon, Eclipse E600) using Cool Snap digital camera.

Discussion

This study shows that *M. anisopliae*V275 can be used for the prophylactic control of BVW larvae in a range of horticultural growing media. The level of control appears to be just as good as imidacloprid but not that of fipronil. Since the objective is to reduce inputs of chemical pesticides, we show for the first time that *M. anisopliae* can give excellent control when used with 1% and 10% recommended rates of imidacloprid and fipronil, respectively. The level of control achieved was similar to that of the recommended rates for these synthetic pesticides. The chemicals give almost immediate crop protection and concomitantly provide *M. anisopliae* more time to kill its host. Presumably the low rate insecticides stress the target or alter their behaviour in such a way as to make it more susceptible to fungal infection.

Maximum recovery of *M. anisopliae* conidia was from larvae exposed to sublethal doses of fipronil. Compared with the latter, almost 67% and 80% fewer conidia were recovered from larvae exposed to sublethal doses of imidacloprid and chemical free treatments, respectively. It is tempting to speculate that fipronil provided an unfavourable environment causing the larvae to move and acquire more inoculum. Indeed, chemical insecticides can induce greater movement and acquisition of inoculum as observed by Roditakis *et al* (2000). The fact that the insects are moving and not feeding can result in starvation stress which is also considered to make insects susceptible to infection (Amiri *et al*., 1999; Thomsen and Eilenberg, 2000). Since the conidia had also germinated and differentiated infection structures suggest that either fipronil stimulated germination or induced changes in the insects which accelerated infection and may explain why more larvae were killed compared with the other treatments.

Almost 38% more conidia were recovered from larvae exposed to sublethal doses of imidacloprid and mortality was higher compared to the insecticide free treatment suggesting that this insecticide is working in an additive or synergistic way with *M. anisopliae*. Sublethal doses of imidacloprid are considered to prevent grooming and subsequent dislodging of inoculum from insect cuticle and since mortality is dose-related it may improve pest control (Quintela and McCoy, 1998).

The physical-chemical properties of the different media may account for some of the variation observed in the control of BVW larvae. The media may exert some inhibitory effect and/or enhance pest establishment. Our studies showed that more BVW larvae survived in untreated coir than bark or peat and peat blends with CGW. These observations corroborate the findings of Buxton (2003) who reported better BVW establishment in coir and fine bark than peat or coarse bark media.

The efficacy of *M. anisopliae* was also influenced by a complex interaction between the method of application and type of growing media. With the exception of coir, the level of control was usually between 15% and 25% better using drench application than premixing into media. Chandler and Davidson (2005) observed a high concentration of *M. anisopliae* inoculum in the top 10 cm of compost following drench application. This would expose newly hatched BVW larvae to a relatively high dose and since mortality is dose related, it would result in better targeted control of the pest. Inoculum would be diluted when premixed into composts but in spite of this it was still a highly efficacious application method. It should be noted that whatever the application method or media used, all the live larvae recovered from *M. anisopliae*-treated pots ultimately died of this pathogen (Fig. 10).This observation suggests that, at the time of destructive assessment, these larvae were at the early stages of infection.

In conclusion, our study shows that *M. anisopliae* V275 can reduce inputs of synthetic insecticides for BVW larval control either when used alone or in combination with low doses of fipronil or imidacloprid. The latter approach not only gives more immediate protection but at a level correspondingly similar to the recommended rate of the chemical pesticide, independent of the application method and/or growing medium used.

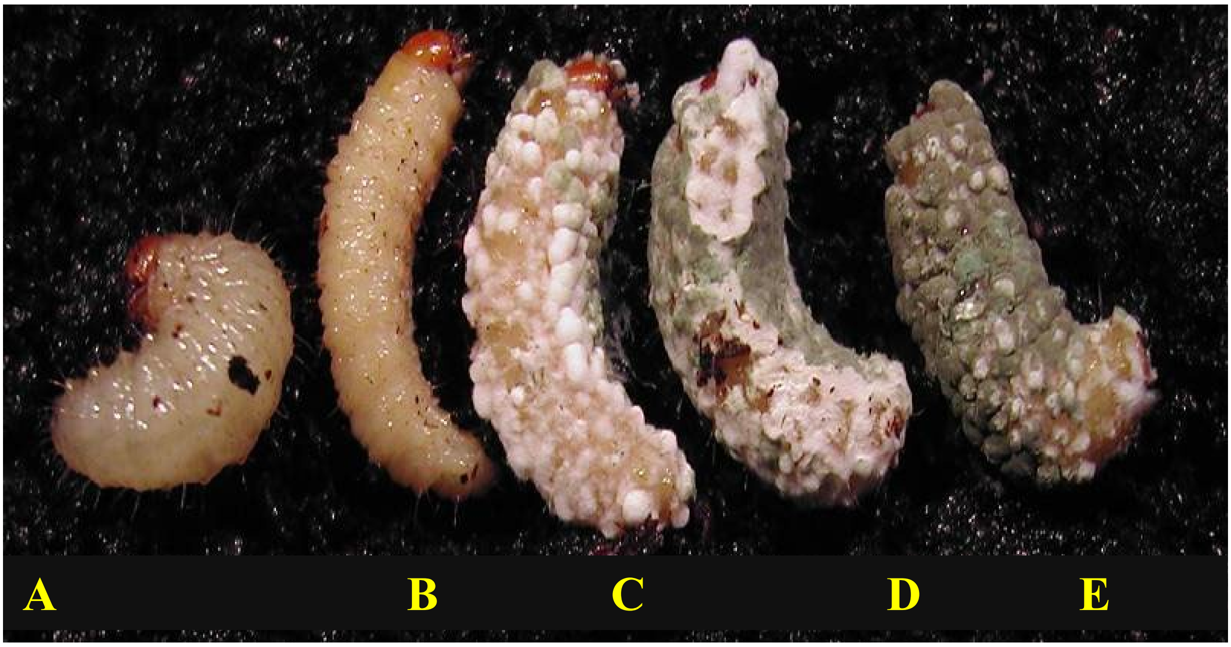

Fig. 10 BVW larvae at different stages of *M. anisopliae* infection. A. Healthy larvae, B. 3-5 days post inoculation, C-E, 2-3 post mortem

B. Studies against Western flower thrips

Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is one of the world's major pests causing damage to a wide range of economically important crops directly through feeding and indirectly through the transmission of harmful plant virus diseases (van Lenteren *et al*., 1992; Kirk & Terry, 2003). Thrips are difficult to control because of their high reproductive rate, cryptic habit (larvae hide in closed buds and pupate in soil) and resistance to many insecticides (van Lenteren and Loomans, 1998; Jensen, 2000; Herron and James, 2005). In addition to chemical insecticides, a range of biological agents are available for thrips control including arthropod predators and parasitoids, and insect pathogenic nematodes and fungi (Jacobson *et al.,* 2001; Blaeser *et al*., 2004; Georgis *et al*., 2006; Xu *et al.,* 2006). Whereas most attention has focused on the control of adults and larvae in the crop canopy little effort has been made to interrupt the life cycle by controlling the pupae.

As stated earlier, *Metarhizium anisopliae* (Metsch) Sorokin has been studied extensively for the control of a wide range of pests, including WFT (Butt *et al.,* 2001; Vestegaard *et al*., 1995; Maniania *et al*., 2002) and shows much promise for the control of subterranean pests (Zimmermann, 1992; Ansari *et al*., 2004). Unlike the canopy layer, the soil environment is less prone to dramatic fluctuations in temperature and humidity which can check fungal development. Indeed, Helyer *et al* (1995) showed that fungal BCAs applied to peat-based composts was effective in killing WFT pupae and helped reduce thrips populations. In light of growing pressure in the UK horticultural industry to reduce dependency on peat as a growing medium, we determined the efficacy of *M. anisopliae* for WFT pupal control in a range of alternative media.

Materials and Methods

WFT were reared in ventilated plastic containers (29 cm ×29 cm ×16 cm) kept at 24 ± 2°C, 50-60% relative humidity (RH), and 16 L: 8D h photoperiod. Between 40-50 adult WFT were introduced into the containers and provided 3-4 pieces of green bean (*Phaseolus vulgaris* L.) and 2-3 yellow chrysanthemum flowers. After three days, the egg infested beans were transferred to fresh ventilated plastic containers (28 cm × 20 cm × 10 cm). First instar larvae usually started to hatch 2 days later. Three days post-eclosion second instar larvae (L2) were collected for experimental use.

Metarhizium anisopliae V275 was produced on broken Basmati rice according to Jenkins et al. (1998) with slight modifications. Conidia used in bioassays had a minimum of 98% viability.

Assays were done using 250 ml white opaque plastic pots (8 cm dia) obtained from Tesco, UK. Ventilation holes were made in the lids and subsequently sealed with thrips-proof nylon gauze. A 5cm × 4cm yellow/blue sticky trap (AgriSense, UK) was attached to the inner part of the lid to trap any emergent adult WFT. The efficacy of *M. anisopliae* was determined in five different types of potting media kindly provided by Bord na Mona, Ireland. These included peat, coir, bark, and peat blended with 10% and 20% composted green waste (CGW). Approximately 125 ml of medium at field capacity was added to each pot.

Conidia of *M. anisopliae* were either applied as a drench or pre-mixed into the compost such that the final concentration corresponded to 1×10^{10} conidia/l compost. Controls consisted of aqueous carrier and two commercial insecticides Provado® (0.125g a.i. imidacloprid/l; Bayer, UK) and Vi-Nil® (1 g a.i. fipronil/Kg; Certis, UK,). Provado® was applied as a drench while ViNil® was premixed into the compost at the recommended rates of 65 ml/l and 1g/l of compost, respectively.

Additional studies were done to see if there was any synergy between *M. anisopliae* and sublethal doses of these insecticides. Conidia of *M. anisopliae* were applied as a drench or premixed as described above but used with ViNil® and Provado® used at 10% and 1% the recommended rates, respectively.

Each pot was inoculated with twenty L2 WFT and a small piece of bean provided as a source of food. The pots were sealed and kept at 24 ± 2°C, 50-60% RH, and 16:8 light and dark photoperiod. Four days after the introduction of the L2, adult WFT started to emerge. These either adhered to the sticky traps or were found on top of the compost. Adults were counted daily for 7 days until no more WFT were observed. Trapped adults on sticky card were incubated in Petri dishes lined with moist filter paper and examined in a binocular microscope to see if they were infected with *M. anisopliae*. Both the number of mycosed cadavers and any surviving emergent adults were recorded. Each treatment consisted of five replicates and the whole experiment repeated twice. Percentage efficacy data 11 days post-treatment were corrected for control mortality (Abbott's 1925), arcsine square root transformed, and analyzed using ANOVA and Duncan test for means separation (SPSS, 2003). Differences among means were considered significant at $P < 0.05$. Percentage efficacy data shown in figure 11-15 are control-corrected.

Results and Discussion

Metarhizium anisopliae V275 was far more efficacious in controlling WFT pupae than either fipronil or imidacloprid (Fig. 11 -15). Pest mortality, whether using fungi or chemicals, is dose-related which suggests that thrips larvae acquired sufficient conidia of V275 to cause infection. Pupal mortality ranged between 72% and 91% with marginally more pupae being killed if the pathogen was premixed into the compost than if applied as a drench. Presumably, more conidia adhere to the larvae as they burrow in the compost to pupate. Our results corroborate the findings of other workers. For example, Heyler *et al* (1995) reported 75% control of WFT pupae in peat-based media treated with *M. anisopliae.* Brownbridge (1995) found that applying *M. anisopliae* as a soil drench reduced the glasshouse population of WFT by approximately 72%. These observations show that it is important to target the pupal as well as larval and adult stages of this pest.

We show that of the few adults (ca. 10%) that emerged over 40% were infected and ultimately killed by V275, and subsequently covered with conidiophores and conidia (Fig. 16). The latter provide a source of fresh inoculum to infect larvae and adults in the crop canopy. In contrast, few pupae (20-51 %) were killed when exposed to fipronil and imidacoprid suggesting that these had low to moderate contact activity (Fig. 11-15).

There was no statistical difference between use of V275 alone or with sublethal doses of insecticide suggesting that there was no additive or synergistic activity between these agents (Fig. 11-15). Other workers have observed synergy or additive effects when *M. anisopliae* is used with sublethal doses of imidacloprid for control of the larval stages of larger subterranean pests (Quintela and McCoy, 1998; Jaramillo *et al.* 2005). Pesticides are likely to have a bigger impact on larval stages since these are still actively moving and feeding. Pesticides would be ingested with the root or adsorbed onto the cuticle as the larvae seek a less toxic environment.

Significant differences were observed between the overall treatments ($F = 118.92$; $df = 9, 50$; $P = < 0.001$) including the different potting media ($F = 5.56$; $df = 4, 50$; $P = < 0.001$), however, interactions between the treatments and potting media were not significant ($F = 1.21$; $df = 36, 50$; $P = > 0.260$). This suggests that *M. anisopliae* V275 is robust and can be used in both the conventional peat and new generation peat alternative and peat blend growing media. The studies also show that the WFT is a robust pest since adult emergence was high in all the untreated media ($F = 1.63$; $df = 4, 45$; $P = < 0.181$). Marginally more adult WFT emerged from bark (90.5 ± 3.9 %) than peat (84.0 ± 4.3%) and coir (83.0 ± 6.6%). Thrips emergence was lowest in peat media blended with either 10% (76.0 ± 6.6 %) or 20% (73.5 ± 4.5%) CGW.

Overall, our results show that premixing *M. anisopliae* into composts can be used as apart of an integrated pest management programme to control thrips populations in glasshouses. Premixing the pathogen into growing media is not only ergonomic for the growers but offers immediate control of thrips pupae.

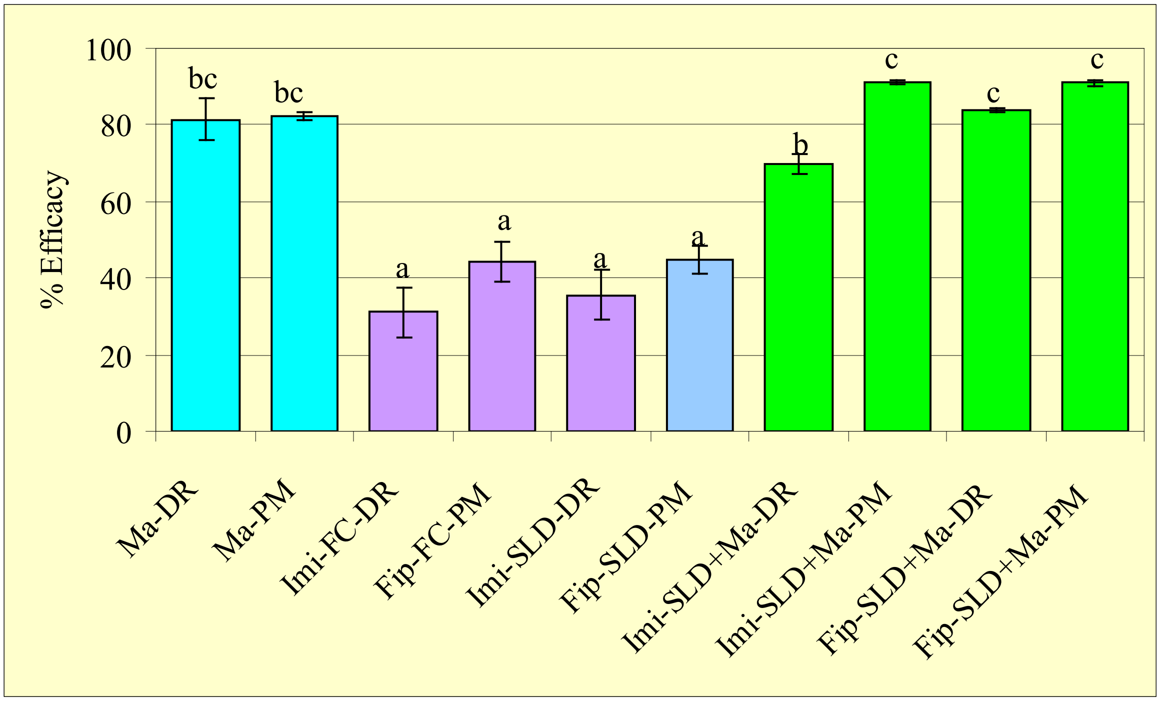

Fig. 11. Mortality (% ± SE) of WFT with *M. anisopliae* (1×10^{10} conidia/l compost) alone, sublethal dose or recommended dose of imidacloprid or fipronil alone, or the combination of *M. anisopliae* and sublethal dose of insecticides in 250-ml cups with different potting media. Ma-DR: *Metarhizium* applied as drench, Ma-PM: *Metarhizium* premixed, Imi-FC-DR: Imidacloprid applied as a drench at the recommended dose, Fip-FC-PM: Fiprinol premixed at the recommended dose, Imi-SLD-DR: Imidacloprid applied as a drench at the sublethal dose, Fip-SLD-PM: Fiprinol premixed at the sublethal dose, Imi-SLD+Ma-DR: Imidacloprid used at the sublethal dose with *Metarhizium* applied as a drench, Imi-SLD+Ma-PM: Imidacloprid used at the sublethal dose with *Metarhizium* premixed, Fip-SLD+Ma-DR: Fiprinol used at the sublethal dose with *Metarhizium* applied as a drench, Fip-SLD+Ma-PM: Fiprinol used at the sublethal dose with *Metarhizium* premixed. Means (± SE) with same letter (11 days after treatment) are not significantly different by Duncan test ($P < 0.05$).

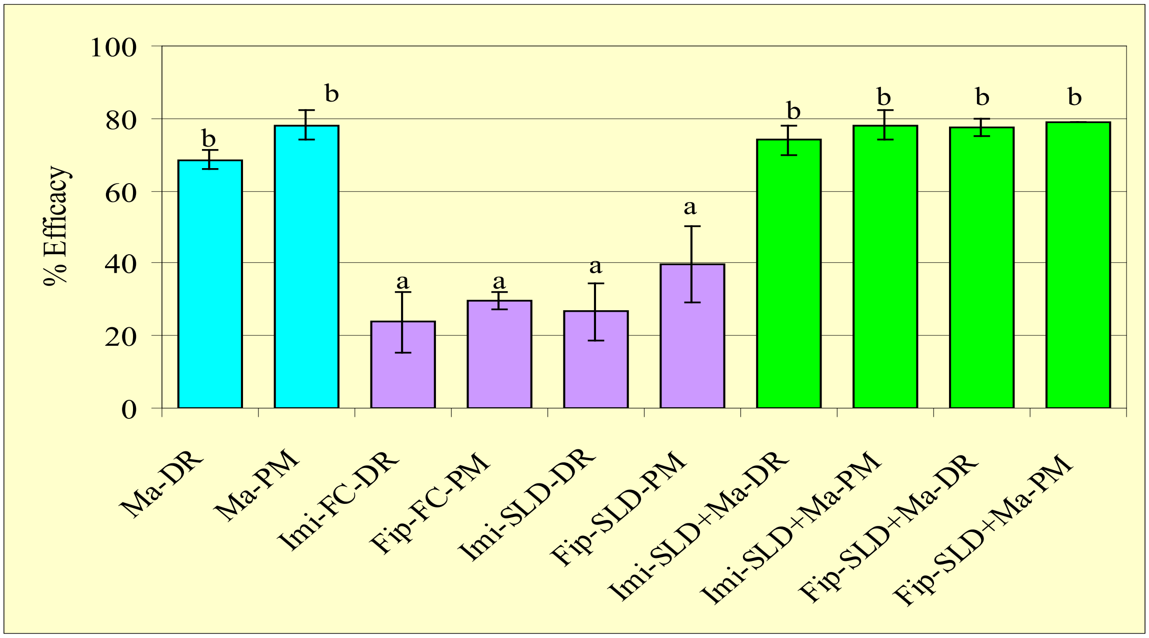

Fig 12. Efficacy (%) of *M. anisopliae* and insecticides against WFT in bark based media Treatments are same as described in Fig.11.

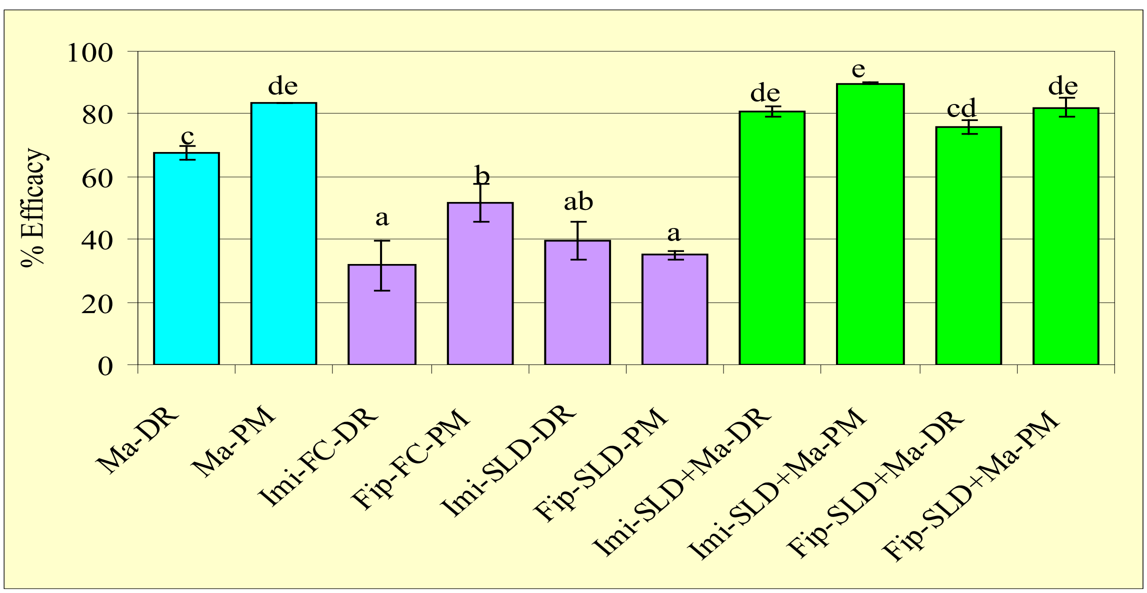

Fig 13. Efficacy (%) of *M. anisopliae* and insecticides against WFT in coir based media Treatments are same as described in Fig.11.

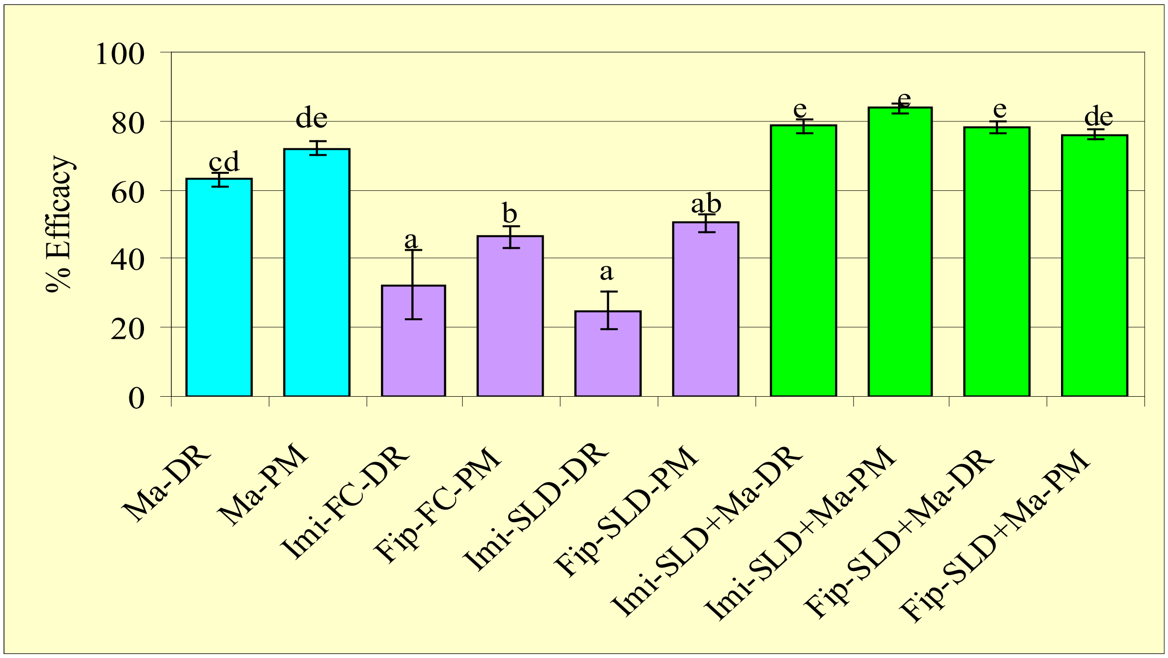

Fig 14. Efficacy (%) of *M. anisopliae* and insecticides against WFT in peat blended with 10% CGW. Treatments are same as described in Fig.11.

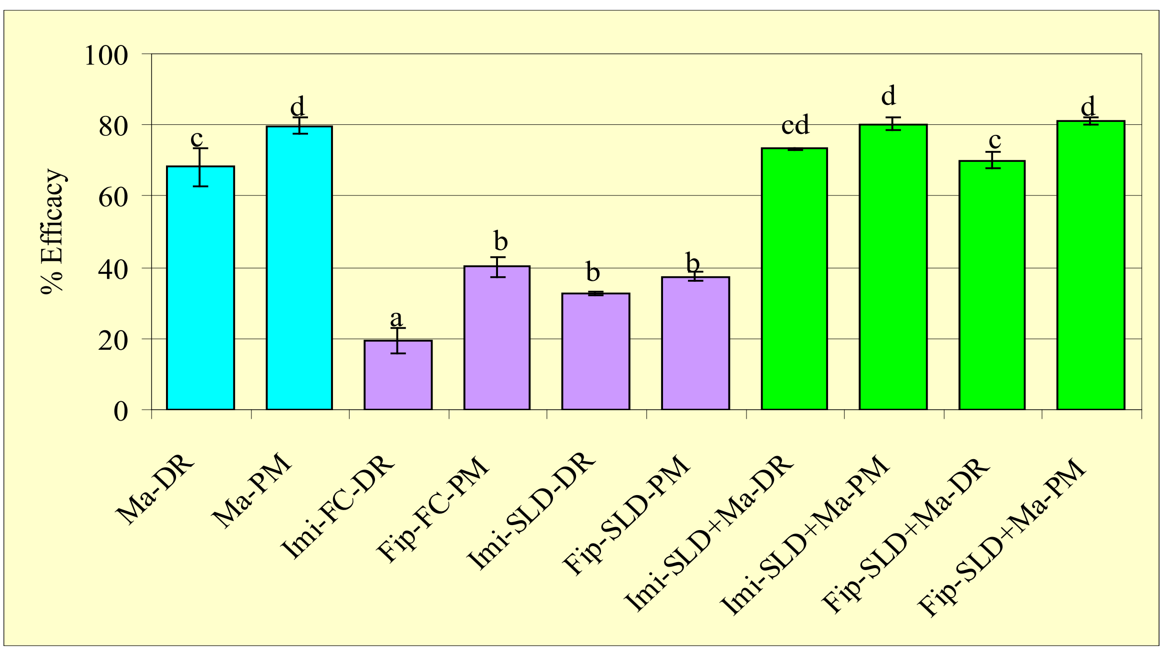

Fig 15. Efficacy (%) of *M. anisopliae* and insecticides against WFT in peat blended with 20% CGW. Treatments are same as described in Fig.11.

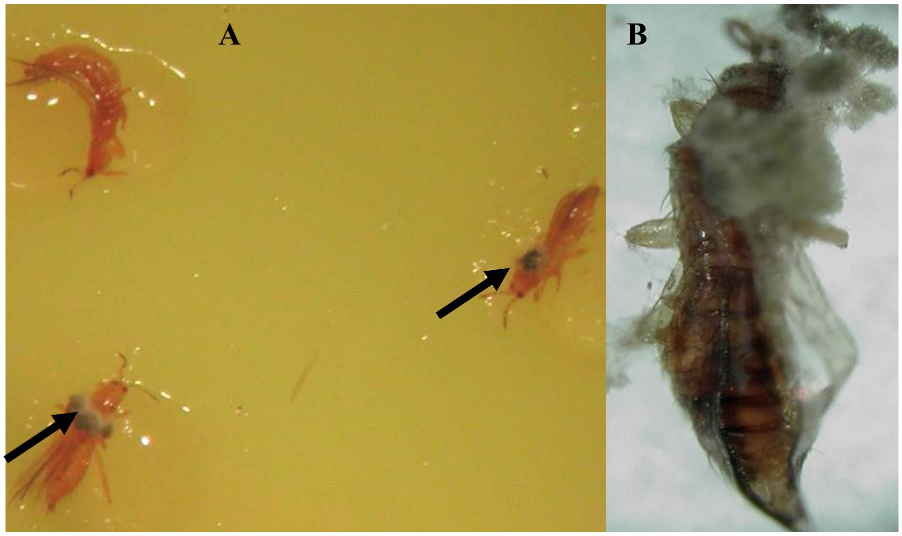

Fig. 16. (A) Adult thrips stuck to yellow sticky card including mycosed individuals (arrow). (B) Adult WFT infected with *Metarhizium anisopliae*.

Objective 3 Determine compatibility of *M. anisopliae* with cold tolerant entomophilic nematodes

Cold tolerant entomophilic nematodes (CTEN) offer an alternative to low dose insecticides for use with *M. anisopliae* to control BVW larvae during the winter when the fungus is less active. Studies on the compatibility of *M. anisopliae* with CTEN would benefit growers in different ways. It gives growers choice – they can choose between the CTEN or the low dose insecticides to use with *M. anisopliae.* For soft fruit (e.g. raspberry, strawberry) growers this strategy would allow for continuous cropping since the absence of chemicals means that there would no safety period. Organic growers would have a fully integrated pest control strategy. In this first of a series of experiments we investigate the compatibility of *M. anisopliae* with the CTEN *Steinernema kraussei* or *Steinernema feltiae* for the control of BVW. Due to the absence of BVW eggs or highly susceptible 1st instar larvae we conducted preliminary studies using the less susceptible 3rd instar larvae.

Materials and Methods

Third instar BVW larvae were transferred to 25-ml ventilated 4.5 cm dia plastic containers containing 20 ml of peat and a carrot slice and inoculated with *M. anisopliae* (1 x 10^{10} conidia/l of compost) and *S. kraussei* or *S. feltiae* (400 infective juveniles/container which is equivalent to 2.5 billion nematodes /ha) applied as a drench in combination or alone. The nematodes *S. kraussei* and *S. feltiae* were kindly provided by Becker Underwood and Koppert, respectively. Control pots were untreated. The containers were kept at 10°C and 20°C in the dark. Each treatment was replicated five times and whole experiment was repeated twice. Larval mortality was assessed at weekly intervals for 4 weeks. Cadavers were examined for signs of nematode or fungal infection.

Results and discussion

At 10°C, *M. anisopliae* caused no larval mortality while *S. kraussei* and *S. feltiae* gave 30 (±10) % and 20% control, respectively (Fig. 17). Control was significantly higher when *M. anisopliae* was used with the CTEN. This observation not only shows that *M. anisopliae* is compatible with these nematodes but suggests that they act synergistically (Figs. 17, 18). The synergy was particularly pronounced using combinations of *M. anisopliae* and *S. kraussei* which resulted in 100% control. This is the first time this synergy has been observed between these organisms for BVW control. At 20°C, *M. anisopliae* gave 60% control of 3rd instar larvae while the nematodes gave 50% (± 10%) control (Fig. 18). When *M. anisopliae* was used with *S. kraussei* and *S. feltiae* then it resulted in 100% control confirming synergy between these organisms (Fig. 18).

Small scale grower trials will be conducted later this year and should confirm if *M. anisopliae* works synergistically with *S. kraussei* and *S. feltiae.* If time permits we will also try and elucidate the underlying mechanisms for this synergy.

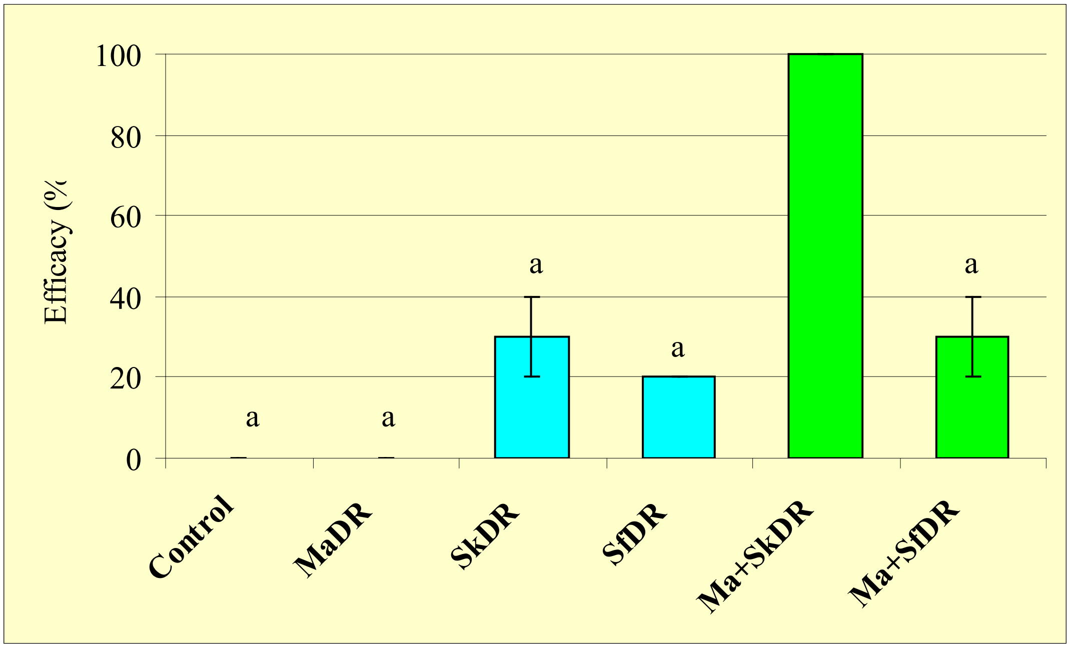

Fig. 17. Efficacy (%) of *M. anisopliae* against BVW larvae (3rd instar) with and without *S. kraussei* or *S. feltiae* at 10° C. Larval mortality was recorded weekly for 4 weeks.

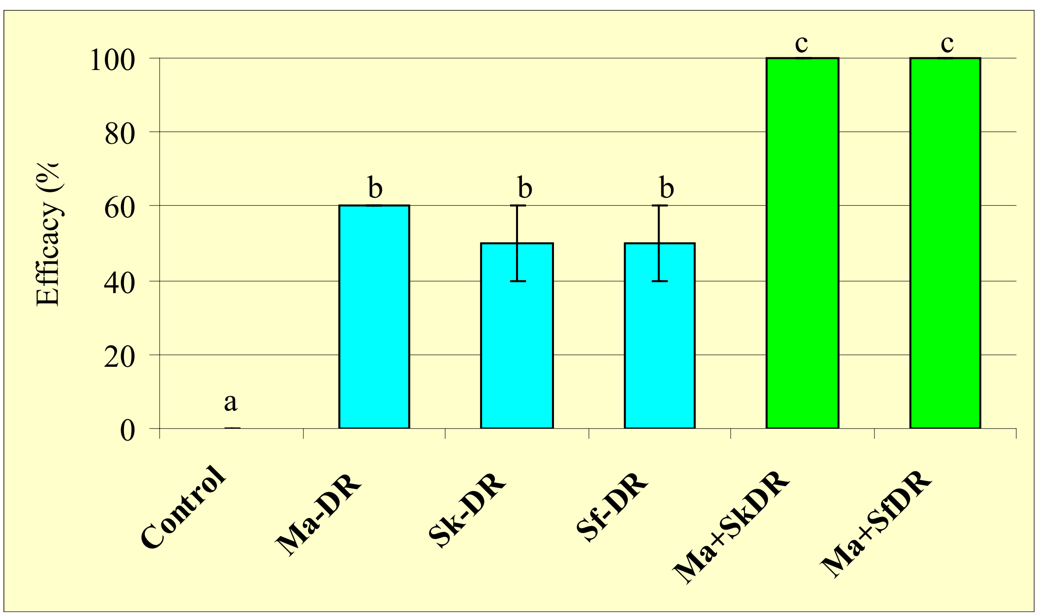

Fig. 18. Efficacy (%) of *M. anisopliae* against BVW larvae (3rd instar) with and without *S. kraussei* or *S. feltiae* at 20° C. Larval mortality was recorded weekly for 4 weeks

Objective 4: Elucidate the impact of physical properties of media on conidial leaching

Introduction

Insect-mortality is dose-related, therefore, inoculum rapidly leached from growing media will result in poor pest control. Conversely, media that retain inoculum will give better pest control. At present the interactions between conidia of *M. anisopliae* and the substrate are poorly understood. Furthermore, extrapolation from field soil studies may not apply to horticultural growing media. By elucidating which physical aspects of growing media affect leaching it may be possible to devise better formulations of the fungus and design media that improve pest control.

Materials and Methods

Percolation of inoculum through disparate growth media was evaluated using leaching columns. Each leaching column was filled with one litre of growth medium, and conidia of *M. anisopliae* applied as a drench or premixed into the compost. The growth medium was subsequently flushed with one litre of water at 0 hr, 1 hr, 24 hrs and then daily up to 7 days post inoculation. The columns were flushed weekly for another 4 weeks. After each flushing, leachates from media were collected in a beaker and samples were taken to determine the number of conidia using a haemocytometer. Each sample was fixed with 2% formaldehyde and stored at 4ºC until evaluated. Each treatment was replicated and the whole experiment repeated twice.

Results & Discussion

Both the application method and growth medium influenced conidial leaching. Drench applications resulted in greater (95%) leaching from all media (Fig. 19). Relatively more conidia were leached from bark and coir media as compared to peat and peat blend media. Leaching of conidia if premixed was less than 15% irrespective of growth medium but was more pronounced in peat than the other media (Fig. 19). Over 90% of the conidia, following drench application had been leached from the medium at 0 and 1 hr after flushing (Fig. 20). Premixed inoculum leaching varied non significantly till 2 days post application and accounted for up to 80-90% of total loss (Fig. 21). These observations suggest that immediately after potting that channels exist for rapid loss of conidia applied as a drench. Subsequent watering/flushing results in compaction of the medium and less conidia being lost from the medium. In contrast, premixing appears to facilitate better entrapment or adhesion of spores to the substrate since very little is lost irrespective of the media type.

None of the conidia collected had germinated suggesting that plant growing media do not stimulate germination of *M. anisopliae* conidia or conversely the media contain factors that inhibit germination.

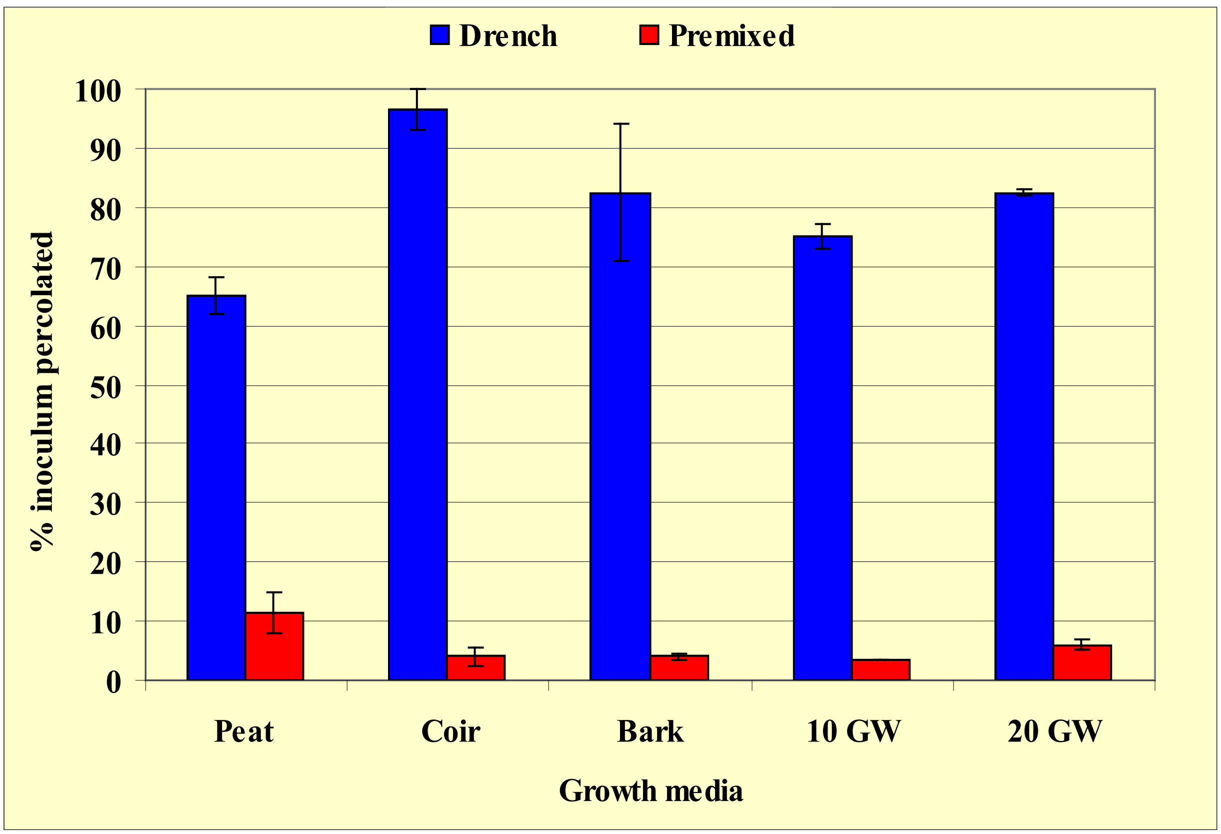

Fig. 19 Influence of application method on the percolation (total) of *M. anisopliae* inoculum from different plant growth media

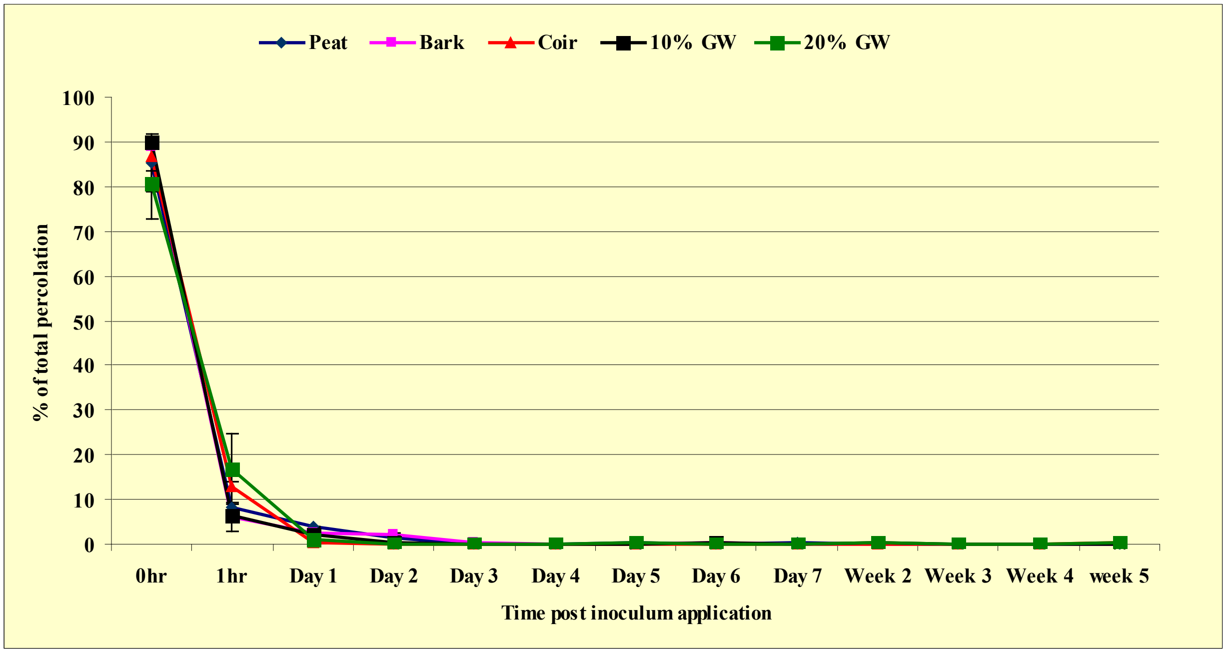

Fig. 20 Time course analysis of the inoculum percolation applied as drench. Note more than 90% of the total inoculum loss occurs at the time of application.

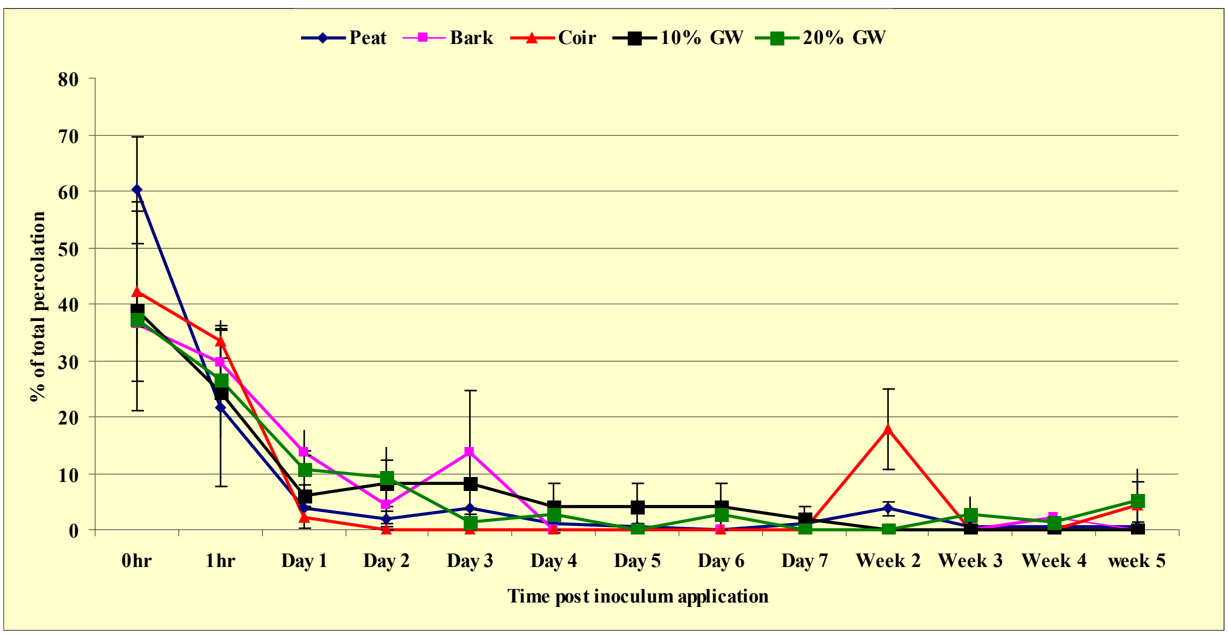

Fig. 21 Time course analysis of the inoculum percolation when applied as premixed. Note up to 90% of the total inoculum loss occurs during the first 2-3 days.

Objective 5: Elucidate the impact of chemical properties of media on the infectivity of *M. anisopliae* conidia

Introduction

Humic-fulvic substances are ubiquitous, heterogeneous macromolecules derived from biological, chemical and physical degradation of organic matter. They play an important role in plant nutrition, detoxification, and will influence the microbial composition of growing media (Hoitink and Boehm, 1999; Ashley, 2002). The quantity of fulvic and humic substances vary with the different media. At present, there is no information on their influence on the efficacy of insect-pathogenic fungi. Many other compounds are present in the media such as tannins (oligomeric and polymeric phenolics) and terpenoids but detailed evaluation of these substances would be outside the scope of this project. In addition to humic-fulvic substances, additional studies were conducted to see whether leachates from different plant growth media influence *M. anisopliae* germination and virulence.

Task 5.1. Effect of humic/fulvic substances on *M. anisopliae* germination

Materials and Methods

Preparation of growth media lactates

Humic-fulvic substances were provided by BNM. Leachates were prepared by suspending 400ml of plant growth medium in 800 ml of distilled water for 1 hour at room temperature. The suspension was blended in a laboratory shaker for 30 sec. and filtered through a double layer of cheese cloth. The filtrate was then divided into two parts with one part being filtered through a 0.2µm Millipore filter to exclude microorganisms.

M. anisopliae incubation in leachates and humic/fulvic acids

M. anisopliae conidia were suspended in leachates from the different plant growth media or generic humic-fulvic substances at a final concentration of $1x10^7$ conidia/ml. Conidia were also suspended in 2, 4 and 20 fold dilutions of humic-fulvic substances. Conidia suspended in 0.03% aq. Tween 80 (Fisher Scientific) were used as a control. Samples were incubated at 25ºC (LEEC incubator) for 24 hrs. Each treatment was replicated three times and the whole experiment was repeated twice.

Germination assays

Influence of humic-fulvic substance on *M. anisopliae* germination was assessed in sterile 24 well microtire plates (Nunc^{TM}). Each well contained 500 µl of Sabouaraud dextrose broth and was inoculated with 500 µl of the conidial suspension from the above treatments. Plates were incubated in a Gallen-Kamp orbital incubator (Sanyo) at 25ºC and 70 rpm. At 8 and 24 hpi, samples were fixed with 2% formaldehyde and germination assessed at 40X magnification of light microscope (Leitz WETZLAR, Germany)). For each replicate sample, three fields of 100 conidia/field were randomly observed. Conidia exhibiting germ tube equal or greater than conidial width was regarded as germinated. Each treatment was replicated three times and whole experiment was repeated twice.

Task 5.2 Bioassays - influence of humic-fulvic substances on virulence

Influence of humic-fulvic substances and leachates on *M. anisoplaie* virulence was determined in peat based media. *M. anisopliae* was applied with the solutions of humic-fulvic substances or leachates at 1 X 10^7 conidia/ml of peat. Controls consisted of water with and without the conidia. The treated and control peat was added to 250 ml pots and five mealworm (*Tenebrio molitor*) larvae were transferred to each vial. Each treatment was replicated three times. Mortality was recorded daily over a period of seven days.

Results and discussion

Germination varied significantly at 8 hpi in Sabouraud broth, however, at 24 hpi no significant difference was observed. At 8 hpi germination were less than 5% in conidia incubated with different dilutions of generic humic-fulvic substances (Table 5). Conidia incubated in leachates (un-filtered) from peat and bark also germinated relatively slow (35-40%). Conidial germination varied non-significantly among conidia incubated in the remaining treatments (Table 5).

M. anisopliae was significantly less virulent when applied with full, ½ and ¼ dilutions of humic-fulvic substances (Table 5). No significant difference in virulence was observed in the rest of the treatments. These findings suggest that only very high concentrations of humic-fulvic substances can influence *M. anisopliae* efficacy but such levels are unlikely to be encountered in growing media or field conditions.

Table 5 Effect of humic/fulvic substances and plant growth media leachates on *M. anisopliae* germination and virulence.

Treatments	Germination (%)	LT_{50} (Days post inoculation)
Untreated – Conidia incubated in water	65.5 ± 5[def]	2.7[a]
Conidia incubated in leachate (filtered through 0.2 µm filter) from peat	64.7 ± 2.5[def]	-
Conidia incubated in leachate (filtered through 0.2 µm filter) from bark	64.0 ± 1.0[def]	-
Conidia incubated in leachate (filtered through 0.2 µm filter) from coir	74.2 ± 3.0[f]	-
Conidia incubated in leachate (filtered through 0.2 µm filter) from 10% green waste blend	68.0 ± 4.0[ef]	-
Conidia incubated in leachate (filtered through 0.2 µm filter) from 20% green waste blend	73.5 ± 4.8[f]	-
Conidia incubated in leachate from peat	41.7 ± 4.2[bc]	2.4[a]
Conidia incubated in leachate from bark	34.0 ± 3.3[b]	2.6[a]
Conidia incubated in leachate from coir	70.5 ± 6.7[f]	2.6[a]
Conidia incubated in leachate from 10% green waste blend	53.0 ± 3.9[cde]	3.2[a]
Conidia incubated in leachate from 20% green waste blend	49.7 ± 2.5[bcd]	2.2[a]
Generic humic/fulvic substances-full concentration	0 ± 0[a]	18.2[d]
Generic humic/fulvic substances-1/2 dilution of full concentration	0 ± 0[a]	9.0[c]
Generic humic/fulvic substances- ¼ dilution of full concentration	0 ± 0[a]	4.5[b]
Generic humic/fulvic substances-1/20 dilution of full concentration	4.2 ± 1.4[a]	3.1[a]

Objective 6: Elucidate the impact of microorganisms in growing media on the efficacy of *M anisopliae*

Studies were initiated in May 2006 with the sample from the first batch (0 month) being currently analysed to determine the microbial community profile using physiological (Biolog) and molecular approaches (ARISA, TRFLP). Microbial community profiling and linked studies will be repeated at 1, 6 and 12 months to determine the shift in microbial population.

Objective 7: Determine shelf life of *M. anisopliae* in different media

This work is in progress with conidia of *M. anisopliae* being recovered and tested for viability and efficacy every six months over a 24 month period.

Objective 8: Grower trials to demonstrate the efficacy and robustness of *M. anisopliae* in selected media

Some of the partners have been consulted to discuss the logistics and planning for the trials and workshops.

References

Abbott, W.S., (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265-267

Amiri B., Ibrahim L.and Butt T.M. (1999). Antifeedant properties of destruxins and their potential use with the entomogenous fungus *Metarhizium anisopliae* for improved control of crucifer pests. Biocontrol Sci. Tech. 9:487-498.

Ansari, M.A., Tirry, L., Vestergaard, S. and Moens, M. (2004). Selection of a highly virulent fungal isolate, *Metarhizium anisopliae* CLO 53, for controlling *Hoplia philanthus*. J. Invertebr. Patholo. 85:89-96

Ashley, M. W. (2002). Understanding the carbon-fertiliser link. Paper presented at the Anniversary Symposium, Research Station of Crop Culture, 16th October 2002. http://www.lifeforcetm.com/carbon_fertiliser_link.pdf

Blaeser, P., Sengonca, C. and Zegula, T. (2004). The potential use of different predatory bug species in the biological control of *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). J. Pest Sci 77:211-219.

Boucias, D., Stokes, G., Storey, C., G. and Pendland, J.C. (1996). The effect of imidacloprid on the termite *Reticultermers flavipes* and its interaction with the mycopathogen *Beauveria bassaiana*. Pflanzenschutz Nachr. Bayer 49:103-144.

Brownbridge, M., 1995. Prospect for mycopathogens in thrips management. In: Parker, M. Skinner, M., Lewis, T. (eds), thrips biology and management. New York Plenum Press, USA. 281-295.

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

Butt, T.M., 1997. Complementary techniques: Fluorescence Microscopy. In: Lacey, L.A (Ed), Manual of techniques in insect pathology. Academic Press, San Diego, USA.355-365.

Butt, T. M., 2002. Use of entomogenous fungi for the control of insect pests, In: Esser, K, Bennett, J. W., (Eds), Mycota, Springer-Verlag, Berlin.111- 134.

Butt, T.M., Jackson, C. and Magan, N., (2001). Introduction-fungal biocontrol agents: progress, problems and potential. In: Butt, T.M., Jackson, C., Magan, N. (eds.), Fungi as biocontrol agents: Progress, Problems and Potential. CABI International, UK. 1-8.

Buxton, J., 2003. Vine weevil control in hardy nursery stock. Horticultural Development Council Factsheet 02/03.

Certis press release, 6 June 2002, http://www.certiseurope.com/_Attachments/News/DC6AD961-3915-4F6C-83FF-B434916B447A.DOC

Chandler, D. and Davidson, G. (2005). Evaluation of entomopathogenic fungus *Metarhizium anisopliae* against soil-dwelling stages of cabbage maggot (Diptera: Anthomyiidae) in glasshouse and field experiments and effect of fungicides on fungal activity. J. Eco.Entomolo. 98;1856-1862.

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.

Fitters, P. F. L., Dunne, R. and Griffin, C. T. (2000). Vine weevil control in Ireland with entomopathogenic nematodes: optimal time and frequency of application. Irish J. Agr. Food Res. 40:199-213

Georgis, R., Koppenhöfer, A. M., Lacey, L. A., Bélair, G., Duncan, L. W., Grewal, P. S., Samish, M., Tan, L., Torr, P. and van Tol, R.W.H.M. (2006). Successes and failures in the use of parasitic nematodes for pest control. Biological Control 38: 103-123.

Herron, G. A. and James, T. M. (2005). Monitoring insecticide in Australian *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) detects fipronil and spinosad resistance. Australian J. Entomol. 44:299-303.

Helyer, N. L., Brobyn, P. N. and Edmondson, R. N., (1995). Control of western flower thrips (*Frankliniella occidentalis* Pergande) pupa in compost. Ann. Appl. Biol. 127:405-412.

Hoitink, H. A. J. and Boehm, M. J. (1999). Biocontrol within the context of soil microbial communities:a substrate-dependent phenomenon. Annual Review of Phytopathology 37:427–46

Inglis, D. G., Goettel, M. S., Butt T. M. and Strasser, H. (2001). Use of hyphomycetous fungi for managing insect pests. In: Butt T.M., Jackson, C.W., Magan, N., (Eds), Fungi as biocontrol agents: Progress, Problems and Potential. CABI, Wallingford, UK. 23-69.

Jacobson, R.J., Chandler, D., Fenlon, J. and Russell, K.M. (2001). Compatibility of *Beauveria bassiana* (Balsaamo) Vuillemin with *Amblyseius cucumeris* Oudemans (Acarina: Phytoseiidae) to control *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) on cucumber plants. Biocontrol Sci. Technol. 11;391-400.

Jaramillo, J., Borgemeister, C., Ebssa, L., Gaigl, A., Tobón, R. and Zimmermann, G. (2005). Effect of combined applications of *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) strain CIAT 224 and different dosage of imidacloprid on the subterranean burrower bug *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae). Biological Contr. 34;12-20.

Kakouli-Duuarte, T., Labuschagne, L., Hague, N. G. M. (1997). Biological control of the black vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) with entomopathogenic nematodes (Nematoda : Rhabditida). Ann. Appl. Biol. 131;11-27.

Kirk, W. D.J. and Terry, L. I. (2003). The spread of the western flower thrips *Frankliniella occidentalis* Pergande. Agric. Forest Entomol. 5;301-310.

Koppenhöfer, A.M., (2000). Nematodes. In: Lacey, L., Kaya, H.K., (Eds.), Field Manual of Technique in Invertebrate Pathology. Kluwer Academic Publishers, Dordrecht, Netherlands.. 283-301.

Lasaridi K. E. and E. I. Stentiford (1996) Respirometric Techniques in the Context of Compost Stability Assessment: Principles and Practice, The Science of Composting part 1, Ed. Marco be Bertoldi, Paolo Sequi, Bert Lemmess, Tiziano Pape. Blackie Academic and professional London. 1-659

Lola-Luz T., Downes, M. and Dunne, R. (2005). Control of Black vine weevil *Otiorhynchus sulcatus* (Fabricus) (Coleoptera: Cruculionidae) in grow bags outdoors with nematodes. Agr. forest Entomol. 7;121-126.

Maniania, N.K., Ekesi, S., Löhr, B. and Mwangi, F. (2002). Prospects for biological control of the western flower thrips, *Frankliniella occidentalis*, with the entomopathogenic fungus, *Metarhizium anisopliae*, on chrysanthemum. Mycopathologia, 155;229-235.

Masaki, M., Ohmura, K. and Ichinohe, F. (1984(. Host range studies of the black vine weevil *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae). Appl. Entomol. Zoolo. 19;95-106.

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). Ann. Appl. Biol. 121;431–454.

Moorhouse, E. R., Easterbrook, M. A., Gillespie, A. T. and Charnley, A. K. (1993). Control of *Otiorhynchus sulcatus* (*Fabricius*) (Coleoptera: Curculiondae) larvae on a range of hardy ornamental nursery stock species using the Entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Sci. Technol. 3;63-72.

Quintela, E.D. and McCoy, C.W. (1998). Conidial attachment of *Metarhizium anisopliae* and *Beauveria bassiana* to the larval cuticle of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) treated with imidacloprid. J. Invertebr. Pathol.72;220-230.

Ramakrishnan, R., Suiter, D. R., Nakatsu, C.H.and Bennet, G. W. (2000). Feeding inhibition and mortality in reticulitermes flavipes (Isoptera: Rhinotermitide) after exposure to imidacloprid-treated soils. J. Econ. Entomol. 93;422-428.

Roditakis, E., Couzin, I.D., Barlow, K., Franks, N. R. and Charnley, A. K. (2000). Improving secondary pick up of insect fungal pathogen conidia by manipulating host behaviour. Ann.,Appl. Biol. 137;329-335.

Schnitzer, M. (1982). Organic Matter Characterization 581-594 Methods of soil analysis part 2. Chemical and Microbial Properties Ed. A.L. Page. American Society of Agronomy Madison WI USA . 1-1159

Shah, F. A., Wang, C-S. and Butt, T. M. (2005). Nutrition influences growth and virulence of the insect-pathogenic fungus *Metarhizium anisopliae* FEMS Microbiology Letters 251;259-266.

SPSS (2003) "Statistical product and service solution, system user's guide" Version 13. Psychology Press Ltd, Publishers, East Sussex, UK.

Thomsen L. and Eilenberg J. (2000).Time-concentration mortality of *Pieris brassicae* (lepidoptera :Pieridae) and *Agrotis segetum* (lepidoptera : noctuidae) larvae from different destruxins. Environmental Entomology 29;1041-1047.

van Lentere, J.C., Benuzzi, M., Nicoli, G. and Maini, S. (1992). Biological control in protected crops in Europe. In: van Lenteren, J.C. Minks, A.K., Ponti, O.M.B. (eds), Biological Control and Integrated Crop Protection: Towards Environmentally Safer Agriculture. Pudoc Scientific Publishers, Wageningen, the Netherlands, 77-89.

van Tol, R. W. H. M., Bezooijen, J. V. and Ketelaars, T. A. C. M. (1998). Searching behaviour of entomopathogenic nematodes: roots and soil temperature determine success of black vine weevil (*Otiorhynchus sulcatus*) control. Insect Pathogens and Insect Parasitic Nematodes, IOBC Bulletin 21:187 –191.

van Tol, R.W.H.M., van Dijk, N. and Sabelis, M.W. (2004). Host plant preferences and performance of the vine weevil *Otiorhynchus sulcatus*. Agr. Forest Entomol. 6:267-278.

Veeken, A.H. M., Wilde V de and Hamelers, H. V. M. Wageningen University, Dr SW Moolenaar and R Postma Nutrient Management Institute, Wageningen, The Netherlands, Oxitop measuring system for standardized determination of the respiration rate and N mineralization rate of organic matter in waste material, compost and soil. Unpublished observations

Zimmermann, G., (1981). Gewachshausversuche zur Bekampfung des Gefurchten Dickmaulrublers, *Otiorhynchus Sulcatus* F., mit dem Pliz *Metarhizium aniso*pliae (Metsch.) Sorok.) Nachrichtenblartt des Deutschen Pflanzenschutzdienste, 33:103- 108.