Efficacy of *Metarhizium anisopliae* as a Biological Control of Vine Weevil Larvae in Growing Media©

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We tested the efficacy of the insect-pathogenic fungus, *Metarhizium anisopliae* strain V275, for the control of black vine weevil on a range of plant species and three sorts of peat growing media. Two different application methods (drench or pre-mix) and two different doses (1 × 108 conidia or 1 × 1010 conidia per litre of growing medium) were assessed. Efficacy was compared with imidacloprid, a widely used synthetic insecticide. The higher dose rate was as effective as imidacloprid for a wide range of plant species including ornamentals, strawberry, and hardy nursery stock. It was also effective in all three growing media and was effective whether applied as a drench (70%-95% control) or pre-mixed (60%-90% control) into growing media. The lower rate was not effective.

INTRODUCTION

Black vine weevil, *Otiorhynchus sulcatus*, is a serious, worldwide pest, which attacks a wide range of protected and field-grown ornamental and fruit crops (Zimmermann and Simons, 1986; Westerman and Werf, 1998; Van Tol et al., 1998). Typical damage includes notching of leaves by adults and root feeding by larvae. The latter can cause serious damage, often requiring destruction of the plants (Van Tol. et al, 1998; Cross and Burgess, 1997).

Damage to commercial nursery stock crops is estimated at around €45 million per annum in the U.K. alone. Current control is still heavily dependent on the use of chemical insecticides (e.g., fiprinol, imidacloprid, chlorpyriphos) but there is much interest in developing alternatives. Entomophilic nematodes (*Heterorhabditis megidis*, *Steinernema carposcapsae*) can be used but growers find these are expensive and have a short shelf life.

Biological control of vine weevil larvae using the insect-pathogenic fungus *Metarhizium anisopliae* has previously been shown to be very effective (Moorhouse et al., 1992; 1993a; b). Previously this agent was not exploited commercially because of its high cost (Cross and Burgess, 1997) but recent developments in its production now make it commercially viable. In this study, we explored the potential of a commercially important strain of *M. anisopliae* for the control of vine weevil in a range of plants and peat-based composts.

MATERIALS AND METHODS

Maintenance of Fungal and Insect Cultures. We used air-dried conidia of *M. anisopliae* strainV275 in all the assays (Shah et al., 2004). We produced the conidia on parboiled rice, or on Sabouraud dextrose agar. We bred the adult vine weevils in small, ventilated boxes and fed them on *Euonymus* leaves. The insects were incubated at 22 to 24°C and 14-h light/10-h dark photoperiod. We collected their eggs twice weekly and could store these at 4 °C for several weeks. To encourage larval development, we transferred the eggs to young potted polyanthus plants as described in Shah et al. (2004). When the plants showed signs of wilting, we destructively checked pots for larvae, which were subsequently transferred to young potted polyanthus or impatiens (i.e., plants with soft fibrous roots). We transferred second instar larvae to chambers containing seed and potting compost and fed them on pieces of carrot.

Plants. In the study we used a range of plants including *Primula* hybrids (polyanthus), *Cyclamen*, *Heuchera*, *Fuchsia*, *Choisya ternata* (choisya), and *Fragaria ×ananassa* (strawberry), all as rooted cuttings, runners or as seedlings at 3-4 true-leaf stage. Except for nursery stock species we grew them all in an unheated glasshouse where the temperature ranged from 12 to 30 °C during the experimental period in a 14-h light/10-h dark photoperiod. We conducted the trials involving the nursery stock species in the open during the summer and early autumn.

Application of *Metarhizium anisopliae* and Assessment of Efficacy. We assessed two different application methods (drench or pre-mix) and two different doses (1×10^8 conidia or 1×10^{10} conidia per litre of growing medium) as described by Shah et al. (2004). We compared the efficacy of *M. anisopliae* with a chemical insecticide, imidacloprid, used at 0.28 $g{\cdot}L^{-1}$ of compost. We gave the untreated control plants a dose of 0.03% aqueous Tween 80 only.

We infested each plant with 15 melanized black-vine-weevil eggs, a higher number than is usually laid around the base of a plant by an adult. We assessed cyclamen and polyanthus destructively for surviving larvae after 4 weeks, and strawberry and hardy nursery plants after 10 weeks.

We also checked for any influence of growing medium type on *M. anisoplia's* performance by replicating the trials in in three commercial peat-based media: Shamrock Irish peat moss (P), Shamrock Seed and Modular Compost (SM), and Shamrock Seed and Potting compost (SP). The peat used in P and SP is H_4 on the von Post scale, while SM contains 20% H_8 and 80% H_4 peat.

RESULTS

Effect of Different Inoculum Doses and Application Methods. The efficacy of *M. anisopliae*, both in preliminary and glasshouse studies, was dose-related (Fig. 1). Larval mortality was 20% at the lower dose and 100% at the higher (Fig. 1). We saw no adverse effect on plant growth at the higher dose. Plants were stunted or wilted due to larval damage to the roots at the lower dose. In the untreated control, bedding plants were completely damaged within 4 weeks while nursery stock and strawberry plants were stunted at the time of assessment.

Applying *M. anisopliae* as a drench appeared to give slightly better control than pre-mix applications but in some of the trials there was no statistical difference between the two application methods (Figs. 1–3). Mean larval mortality using drench and pre-mix applications usually ranged between 70%–95% and 60%–90%, respectively (Figs. 1–4).

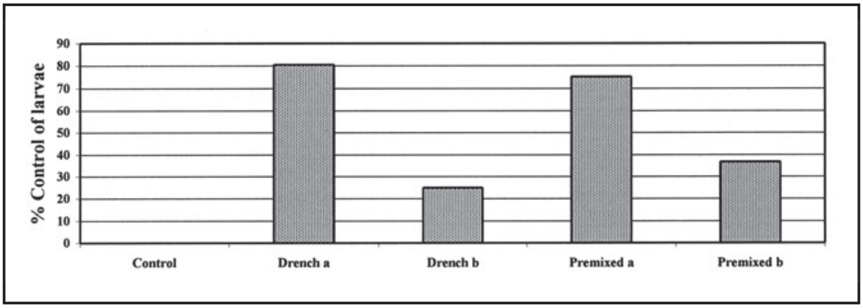

Figure 1. Efficacy of *Metarhizium anisopliae* applied as a drench or premixed into growing media for BVW control using two different doses (a) 10^{10} conidia/L and (b) 10^{8} conidia/L of compost.

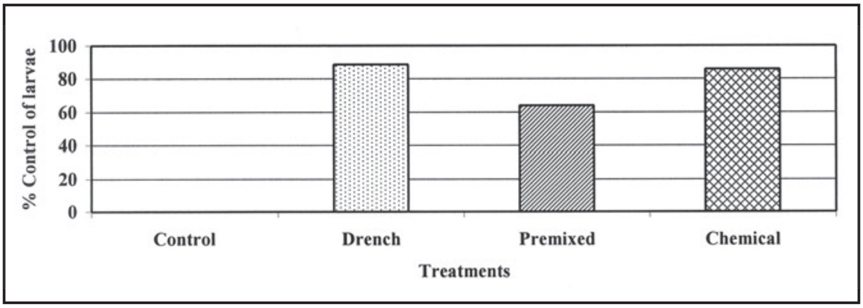

Figure 2. Comparison of the efficacy of *Metarhizium anisopliae,* applied as a drench or premixed into growing media, with drench application of chemical insecticide imidacloprid. Experiments were conducted in potted polyanthus plants.

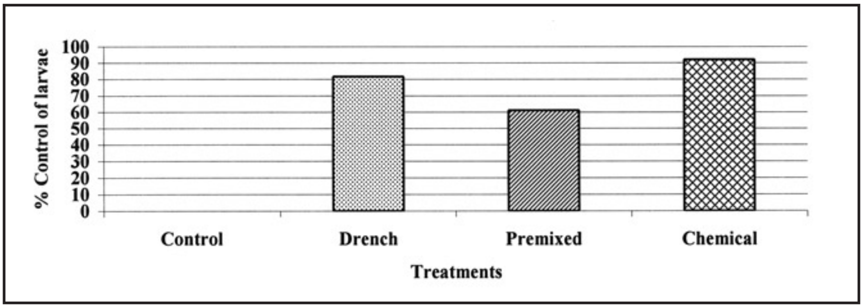

Figure 3. Comparison of the efficacy of *Metarhizium anisopliae,* applied as a drench or premixed into growing media, with drench application of chemical insecticide imidacloprid. Experiments were conducted in potted cyclamen plants.

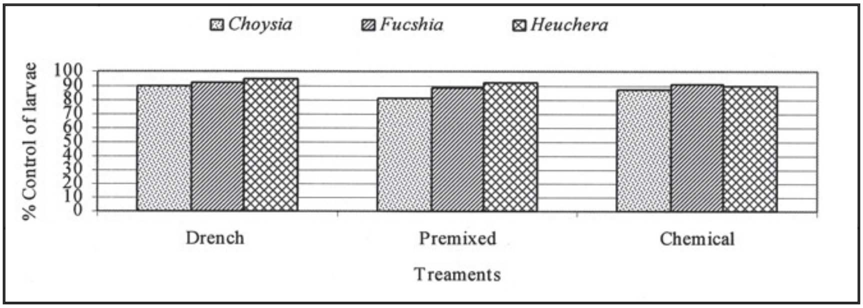

Figure 4. Effect of different application methods on the efficacy of *Metarhizium anisopliae* using drench and pre-mix application methods for control of BVW larvae in two HNS and one herbaceous perennial. Control is similar to that achieved using imidacloprid (synthetic chemical insecticide). Adapted from Shah et al., 2004.

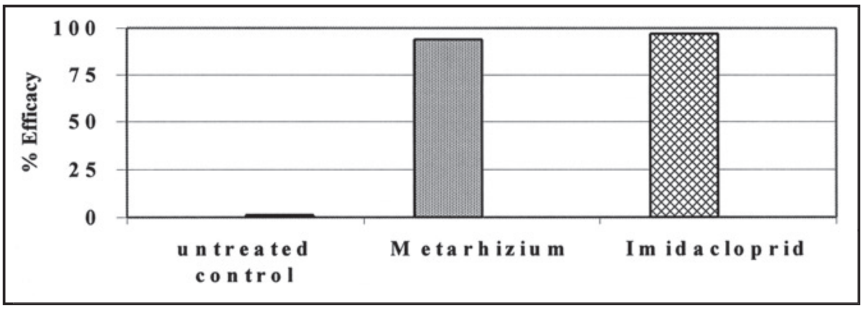

Figure 5. Comparison of the efficacy of *Metarhizium anisopliae* and imidacloprid in the control of vine weevil larvae. Both *Metarhizium* and imidacloprid were applied as a drench to strawberries in grow bags.

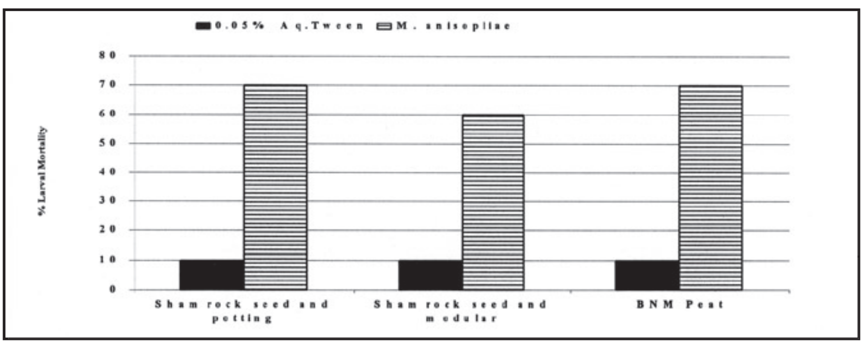

Figure 6. Efficacy of *Metarhizium anisopliae* against vine weevil in different peat growing media. Seed and modular peat is diluted with 20% H_8 peat.

Effect of Plant Species and Peat Type. A similar degree of control was achieved in all plants tested despite the fact that the vine weevil larvae seemed to establish more readily in certain plants, notably fuchsia. At the high dose the degree of control ranged between 80%-95%, which was similar to, and in some cases better than, that achieved with imidacloprid (Figs. 2-5). When surviving larvae recovered from *M. anisopliae*-treated plants at assessment time (4 weeks or 10 weeks after inoculation and treatment depending on plant species) were incubated in fresh growing media — they all died as a result of infection by *M. anisopliae* (data not shown).

Metarhizium anisopliae was effective in all three peat media tested although it appeared to perform slightly better in the media containing only H_4 peat than in the seed and modular compost, which contains 20% H_8 peat (Fig. 6).

DISCUSSION

In this study *M. anisopliae* V275 gave highly effective control of vine weevil larvae in a wide range of plant species. Larval mortality is dose related (Butt et al., 1994; 2001) and commercially significant control was achieved only at the higher dose we used, although we should point out that in our trials we presented the control agent with a worst case scenario — rarely would plants be infested with such large numbers of larvae under normal commercial conditions. Even so, *M. anisopliae* V275 achieved control comparable with, if not better than, imidacloprid.

By our results *M. anisopliae* V275 can achieve total control if used as a prophylactic (i.e., to prevent pest establishment). Early instar larvae of vine weevil and other pests are usually highly susceptible to fungal infection (Butt et al., 2001; Moorhouse, 1993; Shah et al., 2004). Furthermore, if used in this way, control is achieved before the pest can do significant damage to the host plant.

Metarhizium anisopliae achieved good control whether applied as a drench or premixed into growing media. It achieved marginally better control used in a drench application, possibly because more inoculum comes into contact with the early instar larvae, which are mostly found feeding close to the surface. Deeper located larvae would probably be better controlled by the pre-mix application method, but we still have to verify this. Growers may prefer *M. anisopliae* pre-mixed into media because it is more ergonomical, so we may need to investigate the use of higher dose rates to improve control using this application method. At present we know very little about the movement of inoculum through the growing media. Such information is important as it helps in the formulation of the fungus to prevent leaching but also informs growers about when to recharge the media with fresh inoculum.

The fact that slight differences were noted in performance between the different sorts of peat media suggests that the physical-chemical and possibly microbial properties of these media may slightly influence *M. anisopliae's* efficacy. Again, we'll need to do more studies to determine the influence of these parameters on the efficacy and shelf life of the agent and to see how efficacious it is in peat blends or peat-alternative growing media (e.g., coir, bark, or composted materials). Virulent inoculum has been recovered from peat 18 months after application suggesting that this growing medium offers a favourable environment for survival of the agent (Shah et al., 2004). Furthermore, the shelf life of this fungus is significantly better than that of entomophilic nematode biological controls, which usually survive only a few weeks outside their insect host.

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