

**Biological Control
of Vine Weevil**

Final Report

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RELEVANCE TO GROWERS AND PRACTICAL APPLICATION

Application

The fungus *Metarhizium anisopliae* has potential as a biological control agent for larvae of vine weevil. Control is likely to be best on glasshouse-grown plants with the exception of *Cyclamen*. However, good control can also be achieved with the fungus on hardy ornamentals if it is incorporated into the potting compost, and if temperatures are not too low. The fungus has potential for use on soft fruit crops, but more research is required on application methods and dose rates. *Metarhizium* is not registered for use in the U.K., and would probably be subject to the full registration procedure.

Summary

Objectives of the Project

The objective of the project was to investigate the potential of the fungus *Metarhizium anisopliae* to control the larvae of vine weevil, with the emphasis on hardy ornamental nursery stock, but also on protected ornamentals and soft fruit.

Results

Control of vine weevil larvae on hardy ornamentals with *Metarhizium* was variable. The best results were achieved by incorporating the fungus into the compost at potting. Trials with different dose rates have shown the most appropriate rate of incorporation. At this optimum treatment, the most likely

cause of variability in levels of control was temperature. For glasshouse-grown plants, temperature will not usually be a limiting factor, but it can be outdoors.

When *Metarhizium* was incorporated into the compost at potting at a rate of 5×10^9 conidia/litre of compost or higher, good control of vine weevil larvae on various HNS subjects was achieved in most cases. The poor control that occurred in some experiments was probably due to low temperatures after the weevil eggs were applied, thus reducing fungal activity. Where eggs were applied earlier in the summer, so that the fungus had the opportunity to act at more favourable temperatures, it gave successful control, and showed the ability to persist in the compost in the absence of the host larvae. This is an important and necessary attribute, as potting would usually be carried out several months before the natural egg-laying period of the weevil (mainly July to September in unprotected situations).

Drench treatments of *Metarhizium* were less effective, possibly because the fungal spores were filtered out in the upper layers of the compost, leaving untreated areas lower down in the pot, where weevil larvae could survive. However, this method of application could still be useful when it is too late to carry out a compost-incorporation treatment, or to treat a previously untreated plant which is about to be re-potted into a larger size pot with treated compost.

On glasshouse plants, temperature will not usually be a limiting factor and our trials confirmed the results of earlier work by Moorhouse at HRI Littlehampton that good control of weevil larvae could be achieved with *Metarhizium*. An exception is on *Cyclamen*, which appears to be a particularly

difficult subject for control of weevil larvae. It is possible that the *Cyclamen* corm exudes a fungicidal substance, thus reducing the activity of the fungus. However, it may be that the larvae find areas in and around the large corm where the amount of fungus present is too low to kill them.

On soft fruit crops, application of *Metarhizium* presents more of a problem. It cannot be applied to the soil before fumigation, as it will be killed. One trial gave promising results where the fungus was incorporated into the top few inches of soil before planting of strawberries, without any fumigation. Otherwise, plants growing through polythene mulch on fumigated beds would have to be drenched with the fungus during the summer. Trials to investigate this technique were inconclusive, due to poor survival of larvae on untreated plants.

Anticipated Benefits

The availability of *Metarhizium* for use against vine weevil depends on it being produced by a commercial company, and on the company backing UK registration. HDC is engaged in discussions to attempt to achieve this, but the commercial decision has not yet been made.

The recent registration of SuSCon Green for use on hardy ornamental nursery stock has reduced the urgency of the search for alternative vine weevil control techniques in this commodity area. Although an examination of this chemical was not an aim of this project, it was included as a standard treatment in most of the trials on ornamentals reported here. With the exception of one trial on *Cyclamen*, SuSCon Green was very effective against vine weevil, thus confirming the findings of the associated part of this project,

on chemical control of the pest.

The problem remains acute, however, in those commodities for which SuSCon Green is not approved, and even where it is approved it is undesirable to be dependent on a single material. There is thus still an important potential role for *Metarhizium*. The fungus has been shown to be effective provided that soil/compost temperatures are not too low. An important advantage of the *Metarhizium* formulation is that it can be applied by watering onto the surface, so it could be used as a top-up or where no treatment has been incorporated into the original compost mixture. As a biological control agent, it also avoids any possible future constraints on the use of insecticidal materials where runoff into water courses is a possibility.

Background Information on Vine Weevil and *Metarhizium*

Life-cycle of vine weevil

The following is a short summary of the biology of the vine weevil. Growers requiring more details should consult the HDC Report 'A review of the biology and control of the vine weevil' by Moorhouse, Charnley and Gillespie.

Under outdoor conditions there is one generation of weevils per year, with new adults starting to emerge in late May or early June. These adults are all females, and each is capable of laying over 500 eggs, so numbers can build up very rapidly from year to year. They do not start laying eggs as soon as they mature to adults, but feed for 4-6 weeks first, so the main egg-laying period outdoors is July to September. The eggs are laid on or slightly below the surface of the compost or soil, and the larvae spend all of their life

underground during the autumn and winter, feeding on roots, before pupating in the spring.

This basic life-cycle can be altered if plants are kept for some or all of the time at higher temperatures. At high temperatures under glass larvae will develop faster, and there may be more than one generation per year.

Overwintering protection of plants will bring forward the emergence of adults in late spring/early summer and subsequent egg laying. A wide range of different temperature regimes are provided by gauze houses, plastic tunnels, etc., so the timing of weevil development can vary considerably.

Some adult weevils probably overwinter successfully and are able to lay eggs in their second year. They would lay eggs earlier than the adults developing from overwintered larvae, and so advance the life cycle. The type of protective structure under which plants are grown may affect overwintering survival of adult weevils.

The fungus *Metarhizium anisopliae*

This fungus occurs naturally in the soil and attacks the larvae of various insects. Different strains of the fungus have been isolated, and these vary in their response to temperature and which insects they attack. Previous work at HRI Littlehampton, which had HDC funding, compared some of these strains for efficacy against vine weevil at different temperatures.

The fungus can attack adult weevils, but is most effective against the larvae in compost or soil, where the high humidity provides ideal conditions for growth and production of spores. Temperature can be a limiting factor,

however. When a spore of the fungus lands on the cuticle ('skin') of the larva, it is able to send out a growth which penetrates into the body. The fungus grows within the body of the larva, eventually killing it. Large numbers of spores are then produced on the body of the dead larva, ready to infect other larvae.

The fungus itself does not move through the compost or soil, so good distribution is vital.

Research on the fungus at HRI Littlehampton by Moorhouse and Gillespie also examined the effects of some fungicide drenches on *Metarhizium*. Drenches of twelve different fungicides, including Aaterra, Fongarid and Filex, applied 7 days after *Metarhizium*, did not reduce control of weevil larvae by the fungus in their tests on potted plants. Therefore, biological control of weevil larvae with *Metarhizium* should be compatible with disease control.

EXPERIMENTAL SECTION

INTRODUCTION

Vine weevil has become an important pest on hardy and protected ornamentals, and on soft fruit. Since the withdrawal of aldrin, control has been variable and alternatives are needed. Previous HDC-funded research carried out at HRI by E. Moorhouse and colleagues had indicated the potential of a fungus that attacks vine weevil larvae. Various strains of this fungus, *Metarhizium anisopliae*, were assessed, and the most promising strain was used throughout this project. The most effective method of applying the fungus was also investigated.

Wherever possible, the efficacy of the fungus was compared with that of nematode products, a fungal product being developed by Bayer (BIO 1020), and a slow-release formulation of chlorpyrifos (SuSCon Green). The complementary part of the overall project on control of vine weevil concentrated on chemical control with SuSCon Green and has been reported separately by John Buxton of ADAS.

MATERIALS AND METHODS

Strain 275 of the fungus *Metarhizium anisopliae* was used throughout these experiments. In 1990 it was cultured at HRI, but in 1991 and 1992 it was produced by a commercial company, under an arrangement with HRI and HDC.

Hardy and Protected Ornamentals

The fungus was applied by incorporating it into the compost at potting, or drenching the plant with a suspension of the fungal spores. SuSCon Green and BIO 1020 (the Bayer fungus product) were also mixed into the compost before potting. In the case of BIO 1020 a pre-mix of product and compost was made at 1 g product per litre of compost, as recommended by the manufacturers, and incubated under warm damp conditions for 7 days before potting. The rate of SuSCon Green was 750 g product per m³ of compost in all experiments. Mixing of products and compost was done in a cement mixer, except in 1990 when a compost mixer was used.

Drenches of suspensions of spores of *Metarhizium anisopliae* were made at a rate of 100 mls liquid per litre of compost. The wetter Triton was added to the suspensions at 0.05% in 1990, but water only was used in 1991 and 1992. Nematodes were applied as drenches, according to the recommendations of the distributors.

Eggs of vine weevil were taken from cultures maintained in controlled environment rooms at HRI East Malling. They were applied at intervals after treatment by scratching a groove c. 2 cm deep in the compost, dropping the eggs in and covering them with compost. In most trials two applications of 20 eggs per plant were made. Samples of eggs were kept to assess their viability.

Previous experience has indicated a considerable variation in survival of vine weevil larvae in pots, so that even when the same number of eggs is placed in each pot, and no treatments are applied, numbers of mature larvae

recovered later can vary greatly between pots. For this reason we used a minimum of 10 replicate plants for each treatment. Having so many replicates increased the size of each experiment and, in particular, increased the time spent on the hand-sorting of the compost for assessment of larvae. This restricted the number of plant species that could be included in each set of experiments, but this replication was necessary to increase the chances of achieving statistically significant results.

Soft Fruit

In the 1990 trial *Metarhizium* was applied to an area of 22.5 x 22.5 cm of the soil surface on the grain on which it had been cultured, and was lightly forked in to the top few inches. A strawberry plant was planted in the centre of this area. Drenches were applied at 100 mls per plant. No polythene mulch was used in this trial.

In the 1991 trial on a second year strawberry field with black polythene mulch, all treatments were applied as drenches of 400 mls per plant.

For the trial on blackcurrant in 1992, incorporation treatments of *Metarhizium* and SuSCon Green were made by applying them with a 'pepperpot' shaker to a 30 cm wide band on 8th April before the black polythene was laid. Blackcurrant cuttings were then pushed in at the centre of this band, through 5 cm diameter holes in the mulch. Drenches were applied later (30th July) at 500 mls per plant through these holes in the mulch.

In all these trials plants were artificially infested with weevil eggs buried c. 2 cm deep in the soil near the plant.

Assessment of efficacy

Assessment of the efficacy of the treatments was made by counting the surviving larvae in the spring in outdoor trials, or after 10-12 weeks for glasshouse trials. The root ball and compost from potted plants was tipped into a 0.6 m x 0.3 m plastic tray and carefully teased apart by hand to find weevil larvae or pupae. In soft fruit trials a volume of soil surrounding and including the root system was dug up and bagged, then larval numbers assessed by a combination of visual sorting and washing the soil through sieves, followed by flotation of organic material in a saturated solution of magnesium sulphate.

RESULTS

Hardy and Protected Ornamentals

In 1990, trials were carried out on a range of hardy ornamentals and a semi-protected ornamental (*Camellia*) to examine two rates of fungal incorporation into the potting compost, and application as a drench of spores. The results showed that where the fungus was incorporated into the compost on cereal grain at the higher rate, control of weevil larvae was very good (Table 1). Control with the lower incorporation rate was less effective and much more variable. Where a drench application was compared, this was always inferior to incorporation. Several other HNS species such as *Ilex*, *Buddleia* and *Philadelphus* were also included in the trials, but recovery of larvae was too low to give any valid data.

Table 1. Control of vine weevil on HNS with *Metarhizium*, 1990-91

Plant species	Mean number of weevil larvae found per plant				
	Untreated	Fungus incorporated		Fungus applied as drench ³	Least significant difference
		Low rate ¹	High rate ²		
<i>Potentilla</i>	10.8	6.6	0.6	--	2.23
<i>Viburnum plicatum</i>	5.4	0	--	1.2	1.54
<i>Hydrangea</i>	3.2	1.7	0.1	--	1.11
<i>Camellia</i> , trial 1	2.7	0.6	0	--	1.13
<i>Camellia</i> , trial 2	0.8	0.7	0.1	1.0	0.82
<i>Weigela variegata</i>	0.3	1.9	0	0.7	0.84
<i>Erica</i> 'Winter Chocolate'	3.0	0.2	0	1.1	1.15
<i>Erica</i> 'J.H.Hamilton'	3.2	0.3	--	--	2.00
<i>Pittosporium</i>	0.9	1.1	--	--	
<i>Forsythia</i>	3.6	0.5	--	--	2.42

¹ *Metarhizium* applied on cereal grain at 10⁹ spores/litre compost

² *Metarhizium* applied on cereal grain at 10¹⁰ spores/litre compost

³ *Metarhizium* applied as spore suspension at 10⁹ spores/litre compost
25 weevil eggs per plant, applied 5-6 weeks after treatment

-- not tested

In 1991-92 further comparisons were made between incorporation of the fungus into the compost at potting and application as a drench shortly after potting, and the benefit of adding a later drench to either of these treatments was also investigated. The second drench was applied in the interval between the two applications of eggs, to simulate a treatment during the long egg-laying period of the weevil. Each of these treatments was tested at three different dose rates of the fungus.

The incorporation treatments of *Metarhizium* gave better results than

the drenches at the same dose rate (Table 2). There was a clear dose response, with the highest rate of the fungus giving the best control (98% reduction on *Weigela*, 88% on *Potentilla*, 69% on *Euonymus*). A later drench usually improved control where the first treatment was also a drench, but had little effect on the efficacy of the incorporation treatments. BIO 1020, the fungal product from Bayer, and SuSCon Green, the slow-release formulation of chlorpyrifos, both used as compost-incorporation treatments, gave similar control to the highest incorporated rate of *Metarhizium*.

Table 2. Control of vine weevil larvae on HNS species, 1991-92

Treatment	First application	Second application	Vine weevil larvae per plant		
			<i>Euonymus</i>	<i>Potentilla</i>	<i>Weigela</i>
Untreated			2.9	7.3	4.4
<i>Metarhizium</i> at low rate	Inc.	-	2.4	2.6	2.2
	Inc.	Drench	1.4	4.1	2.1
	Drench	-	2.2	4.9	4.7
	Drench	Drench	2.0	3.7	0.1
<i>Metarhizium</i> at medium rate	Inc.	-	1.2	2.3	1.0
	Inc.	Drench	1.6	2.3	0.6
	Drench	-	2.2	6.5	1.9
	Drench	Drench	1.2	3.0	0.7
<i>Metarhizium</i> at high rate	Inc.	-	0.9	0.9	0.1
	Inc.	Drench	0.6	1.0	0.2
	Drench	-	1.0	4.5	2.1
	Drench	Drench	1.4	2.2	0.2
BIO 1020			0.5		
SuSCon Green			0.3		
Least Significant Difference (5% Probability)			1.44	2.4	1.2

The low rate of *Metarhizium* was 10⁹ conidia/litre of compost; the medium rate was 2½ x the low rate, and the high rate was 5 x the low rate.

A trial was also done on a protected ornamental, *Cyclamen*, in 1991, with comparison of application of *Metarhizium* by incorporation or drench at different dose rates. Again, incorporation treatments gave better control than drenches at the equivalent dose rate (Table 3). However, in this trial the highest level of control achieved was 67%. This was inferior to SuSCon Green, which gave very good control. BIO 1020 and the nematode product Nemasys gave only moderate control in this trial.

Table 3. Control of vine weevil on *Cyclamen*, 1991

Treatment	Number of vine weevil larvae per plant
Untreated	12.1
<i>Metarhizium</i> incorporated:	
at 5×10^8 conidia/litre compost	8.6
at 10^9 conidia/litre compost	5.4
at 2×10^9 conidia/litre compost	6.1
at 4×10^9 conidia/litre compost	4.0
<i>Metarhizium</i> drench:	
5×10^8 conidia/litre compost	11.2
10^9 conidia/litre compost	8.9
2×10^9 conidia/litre compost	9.5
4×10^9 conidia/litre compost	9.3
BIO 1020 1 g/litre compost	6.9
suSCon Green 750 g/m ³	1.0
Nemasys	6.5
Least Significant Difference (5% Probability)	3.5

Further trials were carried out on a range of hardy ornamentals in 1992-3, concentrating on the high rate of *Metarhizium*, which had given very good control in 1991-92. Glasshouse plants experience a higher temperature

regime, which is likely to improve the efficacy of *Metarhizium*. Consequently, a lower dose rate was included in the 1992-93 trials on protected ornamentals. Interpretation of these trials is complicated by the poor survival of weevil larvae in the untreated plants in several cases. However, it is clear that control was not as effective on the hardy ornamentals as with the same rate of *Metarhizium* used in the previous year, even from incorporation, which had previously been the most effective application method (Table 4). The addition of a second drench conferred no advantage over a single drench in these trials. Control from BIO 1020 was similar to these *Metarhizium* treatments. However, SuSCon Green gave excellent control.

Table 4. Control of vine weevil larvae on hardy ornamentals, 1992-93

Treatment	Numbers of weevil larvae per plant			
	<i>Euonymus</i>	<i>Thuja</i>	<i>Mahonia</i>	<i>Cupressocyparis</i>
Untreated	1.8	3.4	4.3	1.0
<i>Metarhizium</i> * incorporated	1.0	2.8	1.2	0.5
<i>Metarhizium</i> * single drench	1.0	3.9	2.2	0.8
<i>Metarhizium</i> * two drenches	1.1	4.4	1.5	0.3
BIO 1020	2.0	2.5	1.3	0.8
SuSCon Green	0.1	0	0	0
Least Significant Difference	0.7	1.6	0.56	0.9

* at 5×10^9 conidia per litre of compost

In the glasshouse trials both rates of *Metarhizium* gave good control of low populations of weevil larvae, though there was some indication that the higher rate performed better (Table 5). There were no differences between incorporation and drenching in these trials. BIO 1020, Nemasys and SuSCon Green were all very effective.

Table 5. Control of vine weevil larvae on protected ornamentals, 1992

Treatment	Numbers of weevil larvae per plant		
	<i>Begonia</i>	<i>Primula</i>	<i>Impatiens</i>
Untreated	3.1	1.1	0.3
<i>Metarhizium</i>			
incorporated low rate ¹	0.1	0.5	0
incorporated high rate ²	0	0.2	0
drench low rate ¹	0.2	0.6	0
drench high rate ²	0	0.3	0.1
BIO 1020	0	--	0
Nemasys	0	0	0
SuSCon Green	0.4	0.1	0
Least Significant Difference	1.83	0.66	

¹ 1 x 10⁹ conidia per litre of compost

² 5 x 10⁹ conidia per litre of compost

In a trial on *Cyclamen* in a glasshouse we attempted to improve control from *Metarhizium* by including an additive. It has been suggested that *Cyclamen* corms may produce an exudate which is fungicidal, and the aim was to neutralise any such effect. However, the use of this additive failed to improve control (Table 6). There were only small differences between any of

Table 6. Control of vine weevil larvae on *Cyclamen*, 1992

Treatment	Numbers of weevil larvae per plant
Untreated	9.8
Additive only	10.6
<i>Metarhizium</i> *	
single drench	6.9
drench (+ additive)	7.8
two drenches	5.6
two drenches (+ additive)	8.3
incorporation	5.6
incorporation + drench	6.3
incorporation + drench (+ additive)	6.9
BIO 1020	10.2
SuSCon Green	4.3
Nemasys	7.6
Nemasys H	6.8
Least Significant Difference	2.61

* used at 5×10^9 conidia per litre of compost in all treatments

the *Metarhizium* treatments, and the best ones (incorporation or two drenches) gave only a 43% reduction in numbers of larvae compared with the untreated. Control from the two nematode products was also poor (22-30% reduction), and SuSCon Green reduced infestation by only 56%.

Persistence of the fungus in compost

The company producing the fungus in 1991 and 1992 was able to make supplies available in July in both years, so the potting and incorporation of the fungus had to be done in July/August; the plants were then inoculated with eggs 4-8 weeks later. When potting is carried out in the autumn to spring period in commercial practice, there would be an interval of several months before weevil eggs are laid on the plants. Therefore three trials were carried

out to test the persistence of *Metarhizium*. In the first trial *Potentilla* were potted in May 1991 using HRI-produced fungus and inoculated with 25 weevil eggs/plant five months later. Excellent control was achieved with both rates of fungus used (Table 7). In another trial *Sedum* were potted and treated in late July 1992, kept outdoors, and infested with weevil eggs (20/plant) in early June 1993, ten months later. The *Metarhizium* incorporation treatment at potting gave excellent control, equal to that from SuSCon Green (Table 7). BIO 1020 incorporation and the *Metarhizium* drench were less effective. In a trial on *Fuchsia* in a glasshouse, inoculation with weevil eggs was made five months after treatments and again the *Metarhizium* incorporation treatment was very effective, as were BIO 1020 and SuSCon Green (Table 7). The *Metarhizium* drench allowed some survival.

Table 7. Persistence of *Metarhizium* in compost

Trial 1 on <i>Potentilla</i>		Trials 2/3 on <i>Sedum/Fuchsia</i>		
Treatment	Larvae/plant	Treatment	Larvae/plant	
			<i>Sedum</i>	<i>Fuchsia</i>
Untreated	7.6	Untreated	13.7	1.7
<i>Metarhizium</i> low rate ¹	0.1	<i>Metarhizium</i> drench ³	3.7	0.6
<i>Metarhizium</i> high rate ²	0	<i>Metarhizium</i> incorporated ³	0.4	0
		BIO 1020	2.8	0
		SuSCon Green	0.3	0
LSD			2.15	1.14

¹ 2×10^9 conidia per litre of compost

² 2×10^{10} conidia per litre of compost

³ 5×10^9 conidia per litre of compost

Treatment of liners

In the trials described so far, most of the old compost was removed from the root system of the plant when re-potting into treated compost. In practice, a core of old compost would often be left around the root ball when potting into new compost. A trial was set up to test the importance of treating plants at every potting stage, so that there is no block of untreated compost in the centre of the pot at re-potting. Liners of *Cornus alba* were either drenched with a spore suspension of *Metarhizium* or left untreated. Half of each group of treated or untreated liners were then potted into untreated compost, and the other half into compost in which *Metarhizium* had been incorporated. Drenching the liner apparently had little effect in this trial but the incorporated *Metarhizium* gave good control of weevil larvae, regardless of whether the liner had been treated or not (Table 8).

Table 8. Effect of liner treatment on control of vine weevil larvae with *Metarhizium*

Treatment	Larvae per plant
Untreated liner into untreated compost	10.3
Untreated liner into fungus-incorporated* compost	1.1
Drenched* liner into untreated compost	9.7
Drenched* liner into fungus-incorporated* compost	0.5
Least Significant Difference	2.6

* Drenches and incorporation treatments at 5×10^9 conidia/litre compost

Soft Fruit

A trial was carried out in 1990 on newly-planted strawberries, grown

without polythene mulch. Some plants were planted in early June into a 22.5 cm wide band onto which *Metarhizium* growing on cereal grain had been sprinkled and forked into the top few centimetres. Other plants were treated in July with a drench of a suspension of spores of the fungus in a solution containing 0.05% Triton wetter, with equal numbers of plants receiving 0.05% Triton solution only, as a comparison. Plants that had been drenched then had weevil eggs (25/plant) applied at intervals after the treatment. On the second egg-inoculation date the plants in the fungus-incorporation treatments also received eggs.

Table 9. Effect of *Metarhizium* on vine weevil larvae on field-grown strawberries

Treatment	Application method	Interval before eggs applied	Larvae per plant
<i>Metarhizium</i> , Low Rate ¹	Incorp.	56 days	0#
<i>Metarhizium</i> , High Rate ²	Incorp.	56 days	0#
<i>Metarhizium</i> ³	Drench	3 days	0
Triton	Drench	3 days	0.1
<i>Metarhizium</i> ³	Drench	18 days	0.1
Triton	Drench	18 days	1.5
<i>Metarhizium</i> ³	Drench	33 days	0.9
Triton	Drench	33 days	3.2
<i>Metarhizium</i> ³	Drench	47 days	2.1
Triton	Drench	47 days	2.5

¹ at 2.2×10^{11} conidia/m² soil surface

² at 4.3×10^{11} conidia/m² soil surface

³ 10^9 conidia/plant in 100 mls suspension per plant

'untreated' comparison is Triton treatment at 18 days

When larval numbers were assessed in the following spring, none were found in either of the fungus-incorporation treatments, though the Triton-

treated comparison only had 1.5/plant (Table 9). No conclusion can be drawn for the level of control at 3 days after drench treatment because of extremely low numbers in the Triton-treated comparison. However, there were reductions in larval numbers from the *Metarhizium* drench treatment when eggs were applied at 18 and 33 days after the treatment, whereas by 47 days after treatment no reduction was apparent.

In 1991 another trial was carried out on strawberries, in this case on second year plants growing through black polythene mulch. *Metarhizium* was applied as suspensions of spores in water (no wetter) at four different rates, with the suspensions drenched on the plants through the holes in the polythene at 400 mls per plant. Comparisons were made with drenches of chlorpyrifos (2 mls Spannit/litre) and Nemasys H. Drenches of *Metarhizium* and Spannit were applied on 21st August and Nemasys H on 4th September. The plants were infested with weevil eggs on 30th August (20/plant) and again on 2nd October (20/plant).

Table 10. Effect of treatments against vine weevil larvae on mature strawberry plants, 1991

Treatment	Weevil larvae per plant
Untreated	0.63
<i>Metarhizium</i> drenches	
5 x 10 ⁸ conidia/plant	0.25
1 x 10 ⁹ conidia/plant	0
2 x 10 ⁹ conidia/plant	0.13
4 x 10 ⁹ conidia/plant	0
Spannit	0
Nemasys H	0

Because of poor recovery of larvae from untreated plants in the samples of root systems and surrounding soil taken in spring 1992, it is difficult to interpret the results of this trial (Table 10). However, there are indications that the higher rates of *Metarhizium* drench and the drenches of Dursban and Nemasys H had controlled the larvae.

Recovery of larvae from around the roots of untreated plants in the trial on newly-planted blackcurrants in 1992-3 was also poor (Table 11), despite an application of 20 eggs per plant in mid-August, followed by another in early September. However, a few larvae were found in all treatments, suggesting that none of them had been very successful.

Table 11. Effect of *Metarhizium* and SuSCon Green on survival of vine weevil larvae on newly-planted blackcurrants, 1992

Treatment	Weevil larvae per plant
Untreated	0.6
<i>Metarhizium</i>	
Incorporated Low Rate ¹	0.3
Incorporated High Rate ²	0.2
Drench Low Rate ³	0.3
Drench High Rate ⁴	0.2
Incorporation ¹ + Drench ³ (both low rate)	0.3
Incorporation ² + Drench ⁴ (both high rate)	0.2
SuSCon Green Low Rate ⁵	0.1
SuSCon Green High Rate ⁶	0.8

¹ 5 x 10⁹ conidia/m²

² 10¹⁰ conidia/m² ³ 10⁹ conidia/plant

⁴ 5 x 10⁹ conidia/plant

⁵ 40 kg Product/treated ha

⁶ 80 kg Product/treated ha

CONCLUSIONS

Metarhizium can provide a biological control method for controlling larvae of vine weevil, particularly on glasshouse-grown plants. Good results have also been obtained on outdoor-grown ornamentals, but control has been rather variable. The main reason for this is probably the temperatures experienced during the trials. Although the strain of *Metarhizium* used in these trials remains effective at lower temperatures than most other strains, it nevertheless performs best at temperatures above c. 14°C. Thus, when plants are infested with weevil eggs (either artificially or naturally) in late summer, the fungus has only a short time at these temperatures before temperatures drop and it is less effective. This may explain the poorer control achieved in the 1992 trials compared to 1991. Plants were potted in summer in both sets of trials, but temperatures in September/October were higher in 1991 than 1992; soil temperatures recorded at 10 cm depth at East Malling were on average 2°C higher in 1991 for both months.

The results indicated that if *Metarhizium* was incorporated into the compost at potting in the autumn-spring period, it was able to persist in the compost in the absence of weevil larvae, and control larvae hatching from eggs during the summer. In this situation the fungus would have been in the compost for several months during the summer, at temperatures optimal for its growth. For most of the trials reported here the fungus was produced by a commercial company, and was not available until July/August; applications were thus made towards the end of the period in which unprotected plants were experiencing temperatures optimal for fungal growth.

Incorporation of the fungus into the compost usually gave better control than a drench of spores, probably because of better distribution throughout the compost. When the spores are drenched on they may be filtered out in the upper layers of the compost, leaving areas of compost lower in the pot where weevil larvae can survive. However, drenching may still be a useful method for treatment during the summer when it is too late to carry out compost-incorporation, or for treating plants that are about to be re-potted into a larger pot with treated compost. Although the experiment on *Cornus* liners did not indicate an advantage from drenching the liner, it is likely to be an important factor when re-potting from larger pots and may vary between plant species, depending on the extent of the root system.

In the trials on *Cyclamen*, none of the dose rates or application methods of *Metarhizium* gave commercially-acceptable levels of control. There are several possible explanations for this. There may be a fungicidal exudate from the *Cyclamen* corm which inhibits growth of the fungus. Also, the *Cyclamen* were kept at lower temperatures than in the other glasshouse trials, so the fungus may not have grown as well. In the 1992 trial, even the SuSCon Green treatment did not prove very effective, so the weevil larvae may be avoiding treated compost by burrowing into the corm, or remaining close to its surface.

The field trials on soft fruit were rather inconclusive, due to poor recovery of larvae from untreated plants. The poor survival may have been due to soil conditions affecting eggs or young larvae, or to predation by natural enemies. A project to study predation of different life stages of vine weevil in

the field, funded by HDC, has recently started at HRI East Malling. Incorporation of *Metarhizium* into the top few inches of soil before planting did give a promising result in one trial, but it would only be possible to do this where the soil was not about to be fumigated. Otherwise, the fungus would have to be applied into the planting hole at the time of planting, or drenched on in the summer, and these methods merit further investigation.