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Application of *Metarhizium anisopliae* (Metsch.) Sor. conidia to control *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae) larvae on glasshouse pot plants

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Summary

The efficacy of the entomogenous fungus *Metarhizium anisopliae* was assessed against vine weevil (*Otiorhynchus sulcatus*) larvae in the glasshouse. Prophylactic application of *M. anisopliae* conidia to begonia resulted in total larval control, but curative applications were less effective with only 65% control when conidial application was delayed until 8 weeks after egg infestation. Prophylactic applications also provided effective larval control on begonia plants which received multiple egg applications over a six week period. Larval mortality was monitored on cyclamen plants which had received a prophylactic drench of *M. anisopliae* conidia. The population was reduced by 78% within 5 weeks of egg application and control rose to 90% after 17 weeks, although the increase was not significant. Prophylactic conidial drenches were compared with a similar number of compost incorporated conidia on cyclamen, but there was no significant difference between the two spore application strategies. Application of *M. anisopliae* conidia to impatiens modules before potting-on resulted in over 89% larval control compared to over 97% control when a similar number of conidia were applied to the plants after potting. Larval control was further reduced to 79% when the module drenches were reduced to one quarter of the highest dose (5×10^7 compared to 2×10^8 conidia per module). The persistence of three *M. anisopliae* strains was examined over a 20 week period on impatiens. There was no overall decline in efficacy over this period, although there was variability in the performance of the different strains and it was suggested that this was linked to temperature. The results of these experiments suggest that *M. anisopliae* has considerable potential as a microbial control agent for *O. sulcatus* on glasshouse ornamentals.

Key words: Entomogenous fungus, *Metarhizium anisopliae*, vine weevil, *Otiorhynchus sulcatus*, horticultural pest, glasshouse pot plants, biological control

Introduction

The vine weevil, *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae) was first recognised as pest of horticultural crops in the 1830s (Smith, 1932). Reports of damage by *O.*

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sulcatus larvae, and to a lesser extent adults, have been increasing over recent years as a result of the withdrawal of the effective and resistant cyclodiene insecticide, aldrin, and changes in horticultural practices (Moorhouse, Charnley & Gillespie, 1992*a*). A number of alternative chemical control agents, such as fonofos and carbofuran have been examined, but reduced efficacy (compared with aldrin) and phytotoxicity problems have limited their application. Insect parasitic nematodes have been developed for vine weevil control and a number of products are now on the market. However, nematodes have their limitations, such as limited persistence and there is a need for additional environmentally acceptable control agents for *O. sulcatus* (Moorhouse *et al.*, 1992*a*).

Vine weevil larvae are known to be susceptible to a number of fungal species, including *Metarhizium anisopliae* (Metsch.) Sor. and *Beauveria bassiana* (Bals.) Vuill. (Zimmermann, 1981), under favourable conditions. The glasshouse environment is particularly suited to the use of entomogenous fungi as microbial control agents because of the relatively high temperatures (without large fluctuations) compared to field situations. In addition, larval infection will not normally be restricted by lack of moisture. Good control of *O. sulcatus* larvae under glasshouse conditions has already been demonstrated by Prado (1979) who found that *M. anisopliae* was over four times more pathogenic to weevil larvae than *B. bassiana*. More recently, Gillespie (1989) also reported good control with a number of *M. anisopliae* strains following trials with 21 isolates from the genera *Metarhizium, Beauveria* and *Paecilomyces*. This preliminary work was expanded by Moorhouse, Gillespie & Charnley (1993*a*) who demonstrated significant differences between *M. anisopliae* isolates in a glasshouse trial on begonia.

Most of the published work on entomogenous fungi for control of *O. sulcatus* larvae has been based on simple experimental protocols with one or two plant species. The object of the present work was to carry out a more detailed examination of the performance of *M. anisopliae* on glasshouse ornamentals using a range of spore application strategies so that the potential of *M. anisopliae* as a mycoinsecticide could be determined.

Materials and Methods

All the experimental plants were grown from seed in peat compost. The seeds were germinated at approximately 30°C and maintained under high humidity conditions for about 3 wk. The seedlings were then transplanted directly into pots or into polystyrene cellular trays prior to final potting 7–8 wk later. All the pots and cellular trays were transferred to an aluminium glasshouse (10 m × 6.5 m × 4 m) and placed on capillary matting on raised benches. The glasshouse thermostat was set for a minimum of 15°C and over-heating during the summer months was reduced by shading and venting. The plants were fed twice weekly using a standard pot plant fertiliser mix (Moorhouse, 1990) which was applied in the irrigation water. The only pesticide applied during the trial periods was Pirimor (a.i. pirimicarb; 0.2 g in 400 ml water sprayed to run-off). This product was selected because it had been shown to have no effect on either *M. anisopliae* or *O. sulcatus* (Moorhouse, Gillespie, Sellers & Charnley, 1992*d*).

Adult vine weevils were maintained at 20–22°C in ventilated plastic propagation boxes and were provided with strawberry leaves (Moorhouse, Fenlon, Gillespie & Charnley, 1992*c*). Eggs were collected weekly and placed on moist filter paper for a further two to three days at 20°C so that any fresh, white eggs could complete the melanisation process. Only "tanned" eggs were used in the experiments because the white and partially melanised eggs were non-viable (Moorhouse, 1990). The eggs were buried 5–10 mm deep in the compost around the base of the plant stem using a camel hair brush (size 00). The hatch

rate of three batches of 100 eggs was assessed on moist filter paper in 9 cm Petri dishes. The eggs were incubated under glasshouse conditions and viability was determined by counting the number of empty egg shells. Egg viability in experiments was greater than 91.7%, unless otherwise stated.

Four *M. anisopliae* isolates were used in these experiments: strain 37–80 was the only strain which had been isolated from *O. sulcatus* (kindly supplied by L. R. Wardlow, ADAS–Wye, England); strains 100–82 and 101–82 were isolated from *Melolontha melolontha* (L.) (both kindly supplied by B. Papierok, Pasteur Institute, France); and strain 275–86 originated from *Cydia pomonella* (L.) (kindly supplied by G. Zimmermann, Darmstat, Germany). The isolates were maintained in liquid nitrogen and subcultured onto Sabouraud's dextrose agar (SDA) and incubated at 23°C for 10 days. These stock culture plates were then placed at 5°C and conidia from these plates were used to inoculate fresh SDA plates to produce spores for the experiments. Conidia were harvested after 10 days incubation at 23°C by flooding the SDA plates with 0.05% Triton X-100. The spores were removed by agitation with a metal rod and the conidial suspensions were filtered through four layers of sterile, coarse-mesh cheesecloth. The suspensions were centrifuged for 10 min at 3000 rpm and resuspended in fresh 0.05% Triton X-100. Spore concentrations were determined using an improved Neubauer haemocytometer adjusted to the desired concentration by dilution with 0.05% Triton X-100. The germination of each spore suspension was determined on SDA at 25°C using the method developed by Hall (1977) and exceeded 95% in all experiments.

Effect of spore application timing

Seventy two begonia plants (*Begonia semperflorens*: cv. Happy Choice) in 0.5 litre pots were infested with 10 weevil eggs per pot and then arranged in a six treatment, 12 replicate randomised complete block design. Two groups of 12 pots were treated 24 h later with either a 25 ml per pot drench of a 10^7 conidia/ml suspension of strain 275–86 or a similar volume of 0.05% Triton X-100 (earlier work by Moorhouse (1990) had shown that this wetter had no insecticidal activity). Further spore applications were made (using fresh suspensions) to groups of 12 untreated plants at 2, 4, 6 and 8 wk after egg application. The pots were destructively sampled 12 wk after egg application and the numbers of live weevil larvae recorded.

Time of larval death on cyclamen after treatment with M. anisopliae

Seventy cyclamen plants (*Cyclamen persicum*: cv. Cinderella) growing in 1 litre pots were each drenched with 50 ml of a spore suspension containing 10^7 conidia/ml of strain 275–86. A second group of 70 plants was treated with a similar volume of 0.05% Triton X-100 solution. The treated plants were then arranged in a randomised complete block design and 15 weevil eggs were applied to each pot. Ten Triton X-100 and 10 *M. anisopliae* treated plants (one from each block) were destructively sampled 5 wk after egg application; this assessment was repeated fortnightly for the following 12 wk and the number of live larvae were recorded on each occasion. The larvae from the pots treated with Triton X-100 were also weighed at each assessment time.

Control of larvae originating from multiple egg applications

Fifty begonia plants (*Begonia semperflorens*: cv. Olympic Red) in 0.4 litre pots were each treated with a 20 ml drench of 0.05% Triton X-100. A further group of 50 plants was treated with a similar volume of a 10^7 conidia/ml suspension of strain 275–86. The plants were arranged in a randomised complete block design and 24 weevil eggs were applied to each

Table 1. *Timing of weevil egg treatments applied to begonia plants previously treated with 0.05% Triton X-100 or* M. anisopliae *strain 275–86*

Drench treatment	Number of eggs applied Week 0[a]	Week 2	Week 3	Week 4	Week 6
Triton X-100	24	—	—	—	—
275–86	24	—	—	—	—
Triton X-100	12	—	—	—	12
275–86	12	—	—	—	12
Triton X-100	8	—	8	—	8
275–86	8	—	8	—	8
Triton X-100	6	6	—	6	6
275–86	6	6	—	6	6
Triton X-100	—	—	—	—	24
275–86	—	—	—	—	24
Egg hatch	93.7%	96.3%	94%	95.3%	92%

[a] The week 0 eggs were applied immediately after the drench treatments.

pot in one or a number of applications over a 6 wk period (Table 1). The plants were destructively assessed after 14–18 wk and numbers of live weevil larvae recorded.

Comparison of drench application to whole pots or modules

One hundred and ninety five impatiens seedlings (*Impatiens wallerana*: cv. Super Elfin Blush) were planted into peat compost in polystyrene cells (4 cm × 4 cm × 4 cm) and grown-on for 7–8 wk. Sixty impatiens modules (cells) were each drenched with 5 ml of 0.05% Triton X-100 and further batches of 15 modules were treated with similar suspensions containing 1×10^7, 2×10^7 or 4×10^7 conidia/ml of *M. anisopliae* strain 100–82 or 275–86. The impatiens modules were potted into peat compost in 0.4 litre square polythene pots after the cell drenches had been applied. The three remaining batches of 15 untreated plants received a 20 ml drench of 0.05% Triton X-100 or a spore suspension containing 10^7 conidia/ml of strain 100–82 or 275–86. Ten weevil eggs were applied to each pot once the plants had become established (approximately 3 wk after potting) and the larvae were allowed to develop for 10 wk before the pots were destructively assessed.

Comparison of a drench application with spore incorporation

Spore suspensions containing 10^7 conidia/ml of four *M. anisopliae* strains (37–80, 100–82, 101–82 and 275–86) were mixed into separate 5 litre batches of peat compost at a rate of 5 ml/litre compost. The control batch of compost was prepared by mixing 1.5 litre of 0.05% Triton X-100 into 30 litre of compost. Cyclamen seedlings (*C. persicum*: cv. Tucana) were potted into the treated compost in 0.4 litre square polythene pots and the plants were maintained on capillary matting. The drench treatments containing 10^7 conidia/ml of the four strains were applied after 7 wk to groups of 10 plants growing in the spore-free compost at a rate of 20 ml per pot. The remaining Triton X-100 and fungal incorporated pots each received a similar drench of 0.05% Triton X-100. Twenty weevil eggs were applied to each pot 7 days after the drench treatments and the plants were maintained for a further 11 wk prior to destructive assessment.

Persistence on impatiens plants

Groups of 60 impatiens plants (*I. wallerana*: cv. Sequins Mixed) were treated with either a 20 ml per pot drench of 0.05% Triton X-100 or a similar volume of a fungal suspension

containing 10^7 conidia/ml of strain 37–80, 101–82 or 275–86. The plants were arranged in a randomised complete block design (six pots of each treatment per block) on capillary matting and 20 eggs were applied immediately after the suspensions to one pot of each treatment per block. A second group of 40 plants was infested with 20 weevil eggs after four weeks and the remaining batches of plants were infested with similar numbers of eggs after 8, 12, 16 or 20 weeks. Each group of infested pots was destructively assessed approximately 10–12 wk after egg application and the numbers of live weevil larvae were recorded.

Results

Effect of spore application timing

Weevil control on begonia was clearly influenced by spore application timing (Table 2). Total control was recorded in the pots treated with a drench of *M. anisopliae* conidia prior to egg application. Delaying spore treatment until 2, 4, 6 and 8 wk after egg application resulted in reduced levels of control. The lowest reduction in larval numbers (65%) occurred in the pots treated 8 wk after egg application. The data fitted a probit model very well and were analysed as a classical 'Wadley' problem (Finney, 1971; Table 2). The model suggested that a delay in spore application by 2.5, 4 and 7 wk would result in a decrease in control of 5%, 10% and 25% respectively, although the predicted reduction in effectiveness after 2.5 wk was not significant.

Time of larval death on cyclamen

The numbers of live larvae per pot were subjected to a square root transformation and the results were assessed by analysis of variance. The weevil population in the pots drenched with *M. anisopliae* conidia was consistently lower ($P < 0.001$) than the pots drenched with Triton X-100 (Table 3). The level of control ranged from 78% at week 5 to 90% at week 17, however this increase was not significant ($P < 0.05$). The fungus was still active at the

Table 2. *Effect of spore application timing on* O. sulcatus *control on begonia*

Treatment	Application time (weeks after eggs)	Larval population[a]	Predicted population[b]	Percentage control[c]
0.05% Triton X-100	0*	4.33	4.34	—
M. anisopliae (strain 275–86)	0*	0.00	0.06	100%
M. anisopliae (strain 275–86)	2	0.33	0.17	92%
M. anisopliae (strain 275–86)	4	0.33	0.40	92%
M. anisopliae (strain 275–86)	6	0.66	0.80	85%
M. anisopliae (strain 275–86)	8	1.50	1.40	65%

* Treatments applied 24 h after the *O. sulcatus* eggs.

[a] Mean number of larvae recovered per pot 12 wk after egg application.

[b] Predictions of larval populations based on a probit transformation of the application timing data.

Analysis of heterogeneity:

	chi^2	df
Lack of fit	3.472	3
Residual	57.821	66
Heterogeneity	61.293	69

[c] Percentage control compared to weevil population on plants treated with 0.05% Triton X-100.

Table 3. *Mortality of weevil larvae on cyclamen over 17 wk following treatment with* M. anisopliae *strain 275–86*

Assessment time (weeks)[a]	Mean number of larvae pot[b] Triton X-100	*M. anisopliae*	Percentage control[c]
5	6.7 (2.53)	1.5 (1.08)	78%
7	5.8 (2.37)	0.8 (0.62)	86%
9	4.5 (2.05)	0.8 (0.68)	82%
11	6.1 (2.42)	1.3 (0.94)	79%
13	6.5 (2.50)	0.9 (0.50)	86%
15	7.2 (2.65)	1.3 (0.92)	82%
17	5.2 (2.19)	0.5 (0.44)	90%

[a] Assessment time—weeks after egg application.
[b] Figures in brackets are the means of the square root transformed data: SED (117 df) = 0.278.
[c] Percentage control compared to the mean population on the pots treated with Triton X-100 at each assessment time.

end of the experiment and infected larvae were recovered from the pots on the last two assessment times. The mean percentage control over the 12 wk of the experiment was 83.3%.

The weevil population in the pots treated with Triton X-100 fell by approximately 55% between egg application and the first observation time (Table 3). The larval population remained relatively stable over the following 12 wk, although there was a significant difference between the populations recovered after 9 and 15 wk. Larval weights increased progressively throughout the experiment up to a maximum weight of 71.1 mg after 17 wk (Fig. 1). The largest weight increase between two successive observation times occurred between 9 and 11 wk. There was only a very small increase in weight between 15 and 17 wk and this was probably the result of cessation of feeding prior to pupation. The first pupae were observed after 15 wk and a number of other larvae had formed prepupal cells in the compost at the same time.

Control of larval populations originating from multiple egg applications

The level of control recorded with the different egg applications was highly significant ($P < 0.001$) and very consistent ranging from 83% to 90% (Table 4). Larval survival from

Table 4. *Effect of different* O. sulcatus *egg application timings on larval control on begonia*

Egg application[a]	Mean number of larvae pot[b] Triton X-100	*M. anisopliae*	Percentage control[c]
24.0.0.0.0	7.8 (2.736)	0.8 (0.541)	90%
12.0.0.0.12	6.5 (2.498)	0.9 (0.656)	86%
8.0.8.0.8	6.9 (2.563)	1.0 (0.697)	86%
6.6.0.6.6	7.3 (2.671)	1.3 (1.007)	83%
0.0.0.0.24	7.2 (2.598)	1.0 (0.756)	86%

[a] Number of eggs applied at 0, 2, 3, 4 and 6 wk (see Table 1 for full experimental design).
[b] Figures in brackets are the means of the square root transformed data: SED (81 df) = 0.2178.
[c] Percentage control compared to the mean population on the pots treated with Triton X-100 at each application time.

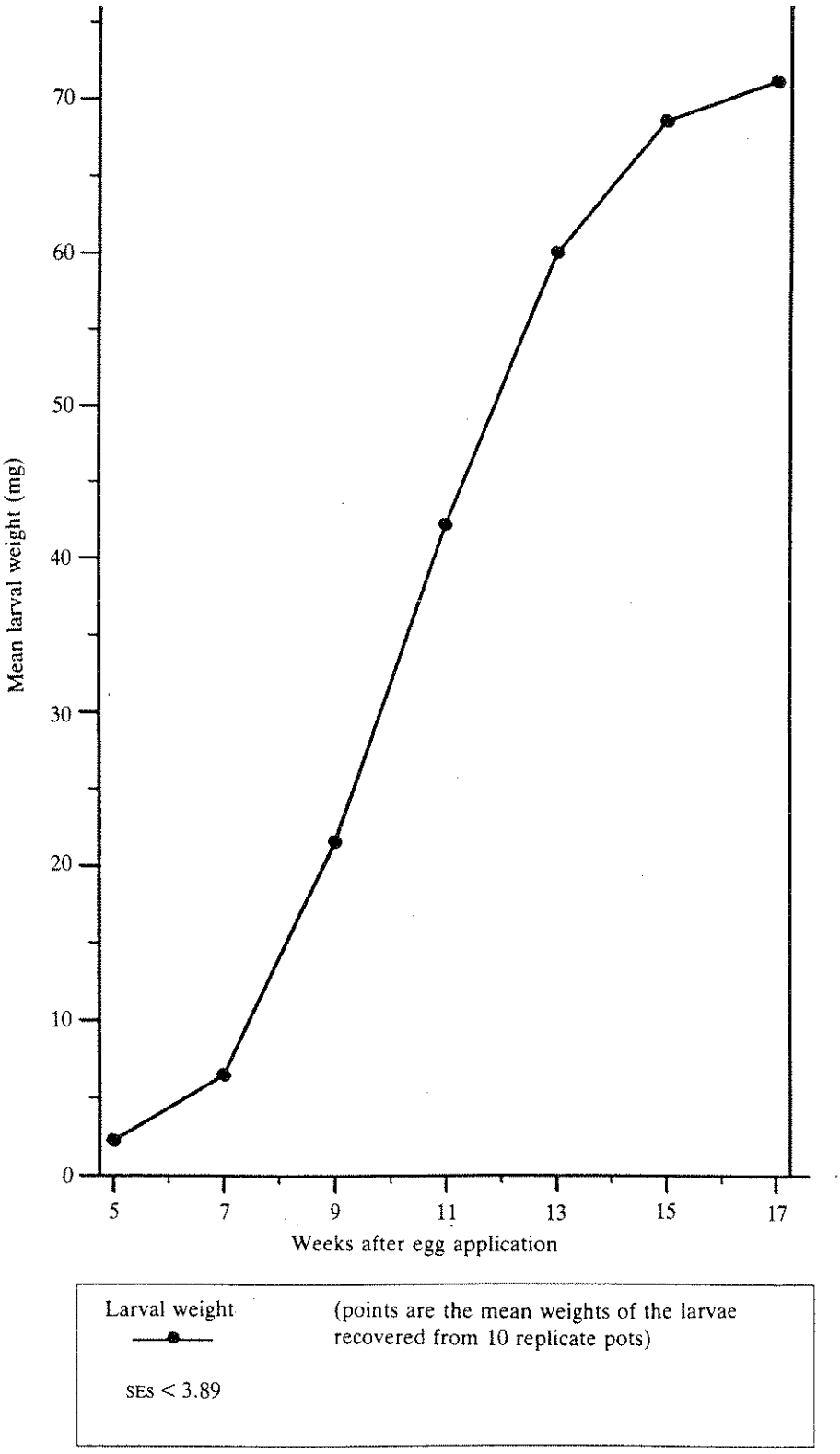

Fig. 1. The development of vine weevil larvae on Cyclamen plants.

Table 5. *Control of* O. sulcatus *larvae on impatients following application of* M. anisopliae *conidia to either pots or modules*

Treatment (conidia/pot or module)		Mean number of larvae/pot[a]	Mean square root transformation[b]	Percentage control[c]
Triton X-100	Pot	5.07	2.203	—
100–82	Pot 2×10^8	0.5	0.378	89.9%
275–86	Pot 2×10^8	0.27	0.267	94.6%
Triton X-100	Module	4.88	2.143	—
100–82	Module 5×10^7	1.33	1.002	73.3%
100–82	Module 1×10^8	1.07	0.855	78.5%
100–82	Module 2×10^8	0.87	0.710	82.5%
275–86	Module 5×10^7	0.93	0.771	81.3%
275–86	Module 1×10^8	0.40	0.400	92.0%
275–86	Module 2×10^8	0.67	0.589	86.5%

[a] Mean of 15 replicate pots (60 in the case of the modules treated with Triton X-100).
[b] SEDs (with 170 df)
Between Triton X-100 pot and fungal means = 0.2098.
Between Triton X-100 module and all the other means = 0.1659.
[c] Percentage control is the reduction in larval numbers compared to the mean Triton X-100 populations.

eggs in the pots treated with Triton X-100 was also very stable and ranged from 27.1% to 32.5%. Strain 275–86 was clearly able to control larvae from multiple egg applications as effectively as single applications and there were no significant differences in the square root transformation means of any of the treated pots.

Comparison between drench applications to pots and modules

Both module and pot applications of *M. anisopliae* conidia reduced weevil numbers ($P < 0.001$) on impatiens plants by at least 73% (Table 5). Analysis of the square root transformed data revealed no significant differences between the pot applications of strains 100–82 and 275–86 (89.9% and 94.6% control, respectively). There were differences (although not always significant) between the module applications and in all cases strain 275–86 reduced larval numbers more than a similar spore concentration of strain 100–82. The control resulting from module treatment with strain 275–86 was less than the pot drench, but this was only significant ($P < 0.05$) with the lowest concentration (5×10^7 conidia per module). The treatment of module or potted plants with 2×10^8 conidia per pot of strain 100–82 resulted in a similar level of control. The lower concentrations of spores applied to modules were significantly ($P < 0.05$) less effective at reducing larval numbers, however even the lowest application rate (5×10^7 conidia per module) reduced the larval population by 73.3%.

Comparison of a drench application with spore incorporation

The highest reduction in larval numbers (48.5%) occurred in pots containing the incorporated spores of strain 100–82. A drench application was less effective giving only 33% control, although this still represented a significant ($P < 0.05$) reduction in larval numbers (Table 6). Drench application of the other three strains was superior to compost incoporation, but the difference was not significant. Larval populations on pots drenched with the different strains were similar with reductions ranging from 27.4% to 35.8%. There were significant strain differences in larval control resulting from the incorporation of spores into compost and two strains (37–80 and 101–82) failed to reduce larval numbers significantly ($P > 0.05$).

Table 6. *Control of weevil larvae on cyclamen plants by compost incorporation and drench applications of conidia*

Strain	Application method	Mean number of larvae/pot[a]	Mean sq. root transformation	Percentage control
Triton X-100	Incorporation	10.6	3.227	—
	Drench	10.9	3.279	
37–80	Incorporation	7.5	2.703	30.2%
	Drench	6.9	2.593	35.8%
100–82	Incorporation	5.5	2.281	48.8%
	Drench	7.2	2.538	33.0%
101–82	Incorporation	10.5	3.184	2.3%
	Drench	7.8	2.722	27.4%
275–86	Incorporation	7.2	2.607	33.0%
	Drench	6.9	2.526	35.8%
			SED = 0.2858 (81 df)	

[a] Mean of 10 replicate pots.

Conidial persistence on impatiens plants

There were significant differences in the larval populations in the pots treated with Triton X-100 and infested with eggs at different times. The highest larval population (73% survival from eggs) was recorded with the batch of eggs applied 12 wk after the spores (Table 7). This was significantly larger ($P < 0.01$) than the population in the pots infested at both 8 and 20 wk. The percentage reduction in larval numbers by the *M. anisopliae* treatments at the different egg application times ranged from 64% for strain 101–82 at 12 wk to 100% for strain 37–80 at 20 wk. There was no significant difference in the level of control between

Table 7. *Mean populations of* O. sulcatus *larvae (square root transformed value) developing from monthly egg applications to impatiens plants treated with* M. anisopliae *conidia*

Egg application time (weeks after the fungal and Triton X-100 treatments)	Mean larval number/pot (transformed value) *M. anisopliae* strain				Application time means[a]
	Triton X-100	37–80	101–82	275–86	
0	10.6	0.8	1.9	0.1	
	(3.147)	(0.524)	(1.174)	(0.100)	(0.559)
4	11.2	0.7	2.5	0.8	
	(3.313)	(0.446)	(1.415)	(0.673)	(0.845)
8	8.9	0.4	1.3	0.3	
	(2.872)	(0.273)	(0.929)	(0.300)	(0.501)
12	14.6	1.2	5.2	2.4	
	(3.805)	(0.924)	(2.148)	(1.197)	(1.423)
16	12.9	0.8	2.8	0.6	
	(3.578)	(0.541)	(1.416)	(0.541)	(0.833)
20	8.3	0	0.3	0.3	
	(2.809)	(0)	(0.300)	(0.241)	(0.180)
Treatment means	(3.254)	(0.451)	(1.230)	(0.509)	

[a] Application time means are the average of the fungal treatments only

SEDs (207 df)

Between treatment means = 0.1175.

Between application time means = 0.1662.

Between Triton X-100 and application time mean = 0.2350.

Between treatments = 0.2878.

37–80 and 275–86 ($P > 0.05$), but both these strains were consistently better ($P < 0.001$) than strain 101–82.

The difference between the populations treated with Triton X-100 and *M. anisopliae* was highly significant ($P < 0.001$) at all six egg application dates (Table 7). There were large variations ($P < 0.05$) in the larval populations on the pots treated with the three fungal strains. The range in population size was particularly large in the pots treated with 101–82 (5.2 – 0.3 larvae per pot). Interestingly, the highest level of control (96%) was recorded with the oldest spores. The level of control recorded with the 20 wk old spores of the other two strains was also very high. All three strains were clearly able to remain active for at least 20 wk and there was no sign of declining spore viability.

Discussion

The natural survival of *O. sulcatus* larvae from eggs in the different experiments was variable ranging from 33% to 73%. This variation is consistent with observations from other experiments on the development of *O. sulcatus* from eggs (Moorhouse, 1990). The reasons for this variation are not fully understood, although it is recognised that factors, such as varietal resistance (Moorhouse, Gillespie & Charnley, 1993*b*) and potting medium (Moorhouse, Gillespie & Charnley, 1992*b*) have an influence on larval survival.

The economic damage threshold for *O. sulcatus* larvae has not been accurately quantified, although it is known that a single mature larva can kill a cyclamen plant (Moorhouse, 1990) and three larvae can be lethal to rhododendron plants (La Lone & Clarke, 1981). A number of the plants in the current experiments which had only been treated with Triton X-100 were showing severe signs of stress at the time of assessment. In some cases, the larvae had eaten all the plant roots and they were beginning to burrow into the stems and corms. This was particularly evident in the cyclamen experiment at the later assessment times and it is likely that the plant mortality in the other experiments would have increased with delayed assessments. The sensitivity of ornamental species to low larval populations increases the need to identify highly effective control agents.

Soil inhabiting larvae are a difficult target for both chemical and microbial insecticides because of the difficulties in getting good contact between the pest and control agent and other factors, such as microbial competition. An understanding of larval biology is an important consideration in the development of effective control strategies for *O. sulcatus*. Moorhouse (1990) demonstrated rapid downward migration of the neonate larvae following egg hatch. Over 50% of the larvae had migrated down more than 30 mm after 4 wk and nearly 20% had reached the lowest layer of the pot (60–75 mm) after a similar period. Larval distribution at the time of spore application might explain the reduced levels of control that were observed on the begonia plants where spore application was delayed. It is likely that some of the larvae hatching from eggs applied to pots before drench application are able to migrate down through the compost without being exposed to lethal spore concentrations. Larvae feeding near the surface when the pots are subsequently drenched with conidia will probably become infected, however those in the lower layers may survive because conidial penetration through peat compost is limited (Moorhouse *et al.*, 1992*b*).

Larvae hatching after the prophylactic application of conidia will have to migrate down through a concentrated spore layer and it is likely that infection will result from the increased exposure to conidia. It is also possible that the natural body movements of the larvae and the action of the compost may have resulted in localised conidia concentrations on sensitive parts of the cuticle such as the intersegmental membranes. The significance of infection

during this initial migratory period is demonstrated in the cyclamen experiment when mortality had risen to 78% within 5 wk of egg application (Table 3). Infection of insect species, such as *Curculio caryae* (Horn) and *Chalcodermus aeneus*, has also been demonstrated after the larvae burrowed into the soil through contaminated surface layers prior to pupation (Bell & Hamalle, 1970; Champlin *et al.*, 1981).

Movement would be minimal after the initial migratory period, therefore the chances of contact between larvae and infective conidia would be greatly reduced. Forced migration resulting from food shortages may occur during the late instars and this would explain the recovery of a number of infected larvae from the cyclamen plants at the last two assessment times (Table 3). Infection at this time may have limited importance from a practical point of view because the amount of damage done by mature larvae would probably be unacceptable (La Lone & Clarke, 1981). Increased infection rates in older larvae may also be associated with migration prior to pupation. Inclusion of an irritant or locomotion-inducing chemical in the conidial formulation could enhance spore pick-up and improve larval control.

Reduced levels of vine weevil control with delayed spore treatments have also been observed by other workers. Zimmermann (1981) found that control on azalea by *M. anisopliae* was reduced from 94% to 78% by delaying spore treatment until four weeks after egg infestation. Similar results were obtained by Tillemans & Coremans-Pelseneer (1987) using another fungal pathogen, *B. brongniartii* (Sacc.) Petch, with a reduction in control from 84% to 72% following a 14 day delay in conidial application. It is interesting to note that the conidial doses used by Zimmermann (1981) were significantly higher than those used in the present experiment (1.9×10^{10} compared to 2.5×10^{8} conidia per pot) and yet control was similar. Direct comparison between dose rates is not ideal because of differences in pot size and plant species. Moorhouse (1990) demonstrated a 100 fold difference in LC_{95} on impatiens compared with cyclamen using the same *M. anisopliae* isolate.

The results from the multiple egg application experiment (Table 4) are particularly encouraging because they demonstrate that a single application of *M. anisopliae* can control a larval population originating from a number of egg applications. All the other experiments reported here, and most of the published work on *O. sulcatus* control, involves the control of weevil larvae from one, or at most two, egg applications. In contrast, natural populations of parthenogenetic *O. sulcatus* adults oviposit over a prolonged period and individual adults may produce over 2000 eggs (Moorhouse *et al.*, 1992*c*). Adults may lay eggs on one or a group of plants and it is likely that there would be significant natural variation in the age of the larvae on infested plants.

High levels of control were recorded in nearly all the experiments reported here, however total control was only observed in one experiment and this suggests that the surviving larvae must have resisted or avoided infection. It is possible that, in addition to natural variation in susceptibility, rapid ecdysis following migration and removal of conidia from the cuticle by the sloughing action of compost may have increased the chances of survival. It is also possible that uneven spore distribution may have led to sub-lethal exposure to infective conidia.

It is possible to overcome uneven spore distribution by incorporating conidia into compost prior to potting. Similar levels of control were recorded on cyclamen plants that received a drench of *M. anisopliae* conidia compared with those growing in compost with incorporated spores (Table 6). These results contrast with the observations of Zimmermann (1981) who reported 81% control with a prophylactic soil drench compared with 16% control with an equivalent number of incorporated conidia. The conflicting results may reflect the failure of Zimmermann (1981) to achieve an even distribution of conidia within his pots. It is also

possible that the root geometry of the different plant species may have had an effect. The distribution of conidia on plants, such as cyclamen, where the roots originate from a corm is likely to be quite different from that on plants with an adventitious root system. The dynamics of spore movement within artificial potting media and the interaction with root systems are very poorly understood.

Movement of conidia within the compost profile is limited, therefore infection is dependent on larvae coming in contact with spores. Incorporation of conidial suspensions into compost maximises spore distribution, although the concentration around individual larvae would be low. Mixing granular mycelial formulations of *M. anisopliae*, such as BIO 1020 (Bayer AG), into compost would lead to the formation of dense, localised areas of infective inoculum as a result of sporulation on the granule surface. The spore concentration in the surrounding compost would be low or non-existent. In this situation, the level of control will depend more on the number and distribution of conidial foci rather than on total spore numbers. This is perhaps analogous to an epizootic in field soil where healthy larvae become infected by picking up conidia from sporulating cadavers. Incorporation of BIO 1020 granules into compost at 0.1 and 1.0 g/litre resulted in a reduction in larval numbers by 25.6% and 75.8%, respectively (Andersch, Hartwig, Reinecke & Stenzel, 1990). It is likely that the efficacy of granule formulations would be improved by reducing granule size because the increase in the number of granules/g would give rise to a more even distribution of conidial foci.

Targeting spore applications to a precise area has some potential for achieving high levels of control with low application volumes (Table 5). Conidia applied to modules of species such as cyclamen would protect the corm and main roots (the most vulnerable parts of the plant). In addition, some conidia may be transferred from the central area to the surrounding compost by the growing roots. This application technique would probably be unsuitable for species with large root systems because larvae could feed around the edge of the pot and would not be exposed to lethal spore doses. Plants can also be treated by dipping in conidial suspensions, although the results using this treatment technique were poor (Zimmermann, 1984).

Persistence is a necessity for prophylactic control and it is also a desirable characteristic for curative products. The results of the persistence experiment on impatiens plants (Table 7) demonstrated that conidia of all *M. anisopliae* isolates remained active for at least 20 wk in peat compost. This contrasts with the observations of Fargues & Robert (1985) who reported significantly reduced virulence in one strain of *M. anisopliae* after 4 months in soil compared to another strain which was still highly active after 21 months. The control of larvae at the different egg application times is probably a reflection of temperature conditions shortly after the time of application. This would explain the reduction in larval control when eggs were applied 12 wk after the conidia (early January). The most pronounced decline in efficacy was observed with strain 101–82 and this is probably due to the particular sensitivity of this strain to low temperatures (Moorhouse, 1990).

The results of these experiments demonstrate that *M. anisopliae* has potential as a microbial agent for *O. sulcatus* larvae on a range of glasshouse ornamentals and it is possible that a single application of a product based on *M. anisopliae* could provide effective protection for the whole season.

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