

## The development of *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae) larvae on a range of ornamental pot-plant species and the potential for control using *Metarhizium anisopliae*

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### SUMMARY

Aspects of the biology and control of vine weevil (*Otiorhynchus sulcatus*) on glasshouse pot plant species were investigated. In a natural situation, *O. sulcatus* preferred *Primula* and *Cyclamen* to *Begonia*, *Impatiens* and *Campanula*. The mean population size ranged from 0.4 larvae per pot for *Campanula* to 36.6 larvae per pot for *Primula*. The larval populations on all species were variable and differences were observed in species sensitivity to larval damage. All the *Cyclamen* plants showed significant signs of damage, whereas only 20% of the *Primula* plants were similarly affected, even though the mean larval population was 42% higher. Larval populations failed to develop on *Campanula*, *Euphorbia*, *Hypoestes* and *Solanum* following artificial egg infestation, while nearly 70% of the eggs applied to *Cyclamen* were recovered as larvae. Larval development on *Impatiens* and *Cyclamen* was compared, but no significant differences in larval number or weight were recorded. Significant differences in larval numbers and weight were observed on six *Impatiens* cultivars with survival from eggs and mean larval weight ranging from 84% to 58% and 38.4 to 27.4 mg respectively. Larval control using a prophylactic conidial drench of the entomogenous fungus, *Metarhizium anisopliae*, was very effective when conidia were applied at the higher rate of  $1 \times 10^9$  conidia  $l^{-1}$  compost. Total control was recorded on five of the species examined and control on the other species exceeded 85%. The results achieved using the higher rate of *M. anisopliae* were consistently better than those achieved using the lower rate ( $5 \times 10^8$  conidia  $l^{-1}$  compost), but the difference in most cases was not significant. The results of these experiments demonstrate the potential of *M. anisopliae* as a microbial control agent for *O. sulcatus* on glasshouse ornamentals.

VINE WEEVIL (*Otiorhynchus sulcatus*) (Coleoptera: Curculionidae) infestations can result in substantial crop damage and significant economic loss for the grower. Serious damage has been reported on a wide range of protected and outdoor horticultural species, including *Cyclamen*, *Taxus*, *Rhododendron*, *Ribes* and *Fragaria* (Smith, 1932; Moorhouse *et al.*, 1992a). The

damage reported on most species results from the feeding activity of larvae on the root system. Adult feeding damage is rarely significant in terms of plant mortality, though plant consignments may be rejected if characteristic leaf notches are observed on the leaf margins. The damage threshold in certain situations is very low. Moorhouse (1990) reported that a single larva could kill a *Cyclamen* plant and La Lone and Clarke (1981) found that *Rhododendron* plants could be killed by as few as three larvae. Established crops are more resistant to weevil damage than young plants and newly transplanted cuttings, because their larger root

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system can withstand some feeding without adverse effects on growth (Neiswander, 1953).

Changes in control strategies and modern husbandry practices have contributed to the increase in the pest status of *O. sulcatus* over recent years. The routine use of aldrin, a highly effective and persistent organochlorine insecticide, helped to prevent severe infestations of *O. sulcatus*, but this product is now unavailable. The modern alternatives to aldrin are less effective and more expensive and consequently growers have adopted curative rather than preventative control strategies. The widespread use of peat based potting mixes and the expansion of horticultural production are also thought to have influenced the increasing frequency of weevil infestations (Nielsen and Roth, 1985; Stimmann *et al.*, 1985). It has also been suggested that the extensive use of insecticides has disrupted the balance between *O. sulcatus* and its natural enemies (Evenhuis, 1983). In addition to these factors, various aspects of weevil biology, such as the ability to reproduce parthenogenetically, make it a particularly successful pest species.

The lack of suitable chemical replacements for aldrin and increasing environmental concern over the use of insecticides has encouraged growers to consider other pest control strategies. Insect parasitic nematodes have shown considerable potential as a biological control agent for *O. sulcatus* and they have out-performed chemical treatments in experiments on protected crops (Stimmann *et al.*, 1985). Nematodes are now available commercially in a number of countries including the UK.

Encouraging results have also been achieved using the entomogenous fungus *Metarhizium anisopliae* in glasshouse trails using a small number of pot plant species (Moorhouse, 1990). The results obtained by other workers, such as Zimmermann (1984), suggest that the performance of *M. anisopliae* is influenced by the host plant and there is therefore a need to evaluate the fungus on a number of plant species. Weevil biology on different host plants with respect to resistance is also poorly understood. Partial resistance to *O. sulcatus* adults has already been reported in a limited number of genera, including *Rhododendron* (Nielsen and Dunlap, 1981) and *Fragaria* (Shanks and Doss, 1986). Resistance to *O. sulcatus* larvae

has not been studied, although highly variable larval populations have been observed in experiments with mixed cultivars (Moorhouse, 1990).

These experiments were conducted to study weevil establishment and development on a variety of common ornamental species and to examine the efficacy of *M. anisopliae* as a potential control agent for *O. sulcatus*.

#### MATERIALS AND METHODS

##### *Weevil production*

A vine weevil population was maintained on a range of plant species including blackcurrant, strawberry and *Rhododendron* in a large outdoor 'Tygan' mesh cage. Small numbers of adults were removed from the cage during the early summer months and placed in plastic plant propagators together with laboratory-reared adults and adults collected from field populations. The weevils were maintained at 20–22°C under a 16:8 h light:dark photoperiod and fed solely on strawberry (cv. Elsanta) leaves that were removed from mature strawberry plants (Moorhouse *et al.*, 1992b). The adults were fed every 3–4 days with six fresh leaves per box. To maintain leaf freshness, the leaf petioles were pushed through a piece of plastic and into a 60 ml bottle containing water. Many adults survived for over one year under these conditions and thus populations were comprised of first and second season adults. Adults were transferred to a clean propagating box every week and then the eggs were separated from the larger pieces of frass and plant debris using a small sieve. Egg viability was determined under glasshouse conditions by incubating three groups of 100 eggs on moist filter paper in 9 cm Petri dishes.

##### *Plant production*

The experimental plants were either purchased as modules or grown from seed (Asmer seeds Ltd., Leicester, UK) in peat compost with added nutrients. The seedlings were pricked out at varying periods of time after emergence (depending on species) into peat based compost in 0.4, 0.5 or 1.0 l polythene pots. The modules were similarly re-potted into peat compost. All the glasshouse trials, with the exception of the *M. anisopliae* experiment, were conducted in an aluminium glasshouse (10

× 6.5 × 4.0 m) with a minimum temperature of 15°C. Overheating was reduced during the summer months by venting and glasshouse shading. The *M. anisopliae* experiment was conducted in a similar glasshouse (14.0 × 6.5 × 3.8 m) which had automatic shade screens. The plants in both glasshouses were maintained on capillary matting and were fed twice weekly with a standard pot plant fertilizer solution (Moorhouse, 1990).

#### *Natural preference for host plant*

Ten pots (0.4 l) of five ornamental species (Table I) were arranged in a randomized complete block design inside the 'Tygan' mesh cage (containing the free-living population of *O. sulcatus*) on 8 July. Each pot was temporarily sealed inside a plastic bag at 1100 hours on 27 August and the number of adults on each plant was determined in the laboratory. The plants were carefully searched and any adults hiding in the foliage were dislodged by vigorous shaking. The plants were returned to the 'Tygan' cage after assessment and were left for a further five weeks before larval numbers were determined by destructive assessment. The results were assessed by analysis of variance (ANOVA) using a square root transformation of the larval numbers.

#### *Influence of plant species on larval development*

Weevil eggs were collected over a two day period and maintained at 20°C on moist filter paper until hatching occurred (approximately 12 d after collection). Groups of 30 neonate larvae were placed on the roughened compost surface of eight *Cyclamen* (cv. Tucana) and eight *Impatiens* (cv. Super Elfin Blush) pots (0.4 l) using a moist camel hair brush (size 00). The plants were maintained in the glasshouse for seven weeks prior to destructive assessment. Both the number and weight of the larvae recovered per pot were recorded and analysed as above using a square root transformation of the larval numbers and the actual weights.

#### *Assessment of cultivar resistance to weevil larvae*

Two seed trays were sown with *Impatiens* seeds (cv. Sequins mixed) and the resultant seedlings were potted into peat compost in

0.5 l square plastic pots. The plants were allowed to develop until flowering and then ten replicate plants of six cultivars (Sequins Orange, Sequins White, Sequins Orchid, Sequins Red and White, Sequins Scarlet and Sequins Rose) were arranged in a randomized complete block design on capillary matting on a glasshouse bench. Weevil eggs were collected over a 24 hour period and 10 tanned eggs were applied to each pot seven days later. The plants were destructively assessed after 10 weeks and the number and weights of weevil larvae were analysed as above.

#### *Weevil control by *M. anisopliae* on pot plants*

The *M. anisopliae* strain (275-86) used in this study had originally been isolated from a codling moth (*Cydia pomonella*) larva in Western Germany by G. Zimmermann. This isolate was selected for further study as a result of the promising activity against *O. sulcatus* larvae obtained in laboratory and glasshouse assessments (Moorhouse 1990). A sample of conidia was removed from the liquid nitrogen vivostat and used to inoculate a Sabouraud's dextrose agar (SDA) Petri plate (9 cm diameter). The plate was incubated for 10 d at 23°C and then conidia were subcultured onto 10 SDA plates. These plates were incubated at the same temperature for a further 10 d and then a conidial suspension was prepared by flooding the plates with 0.05% Triton X-100 (BDH Chemicals Ltd., Poole, UK). The conidia were removed by agitation using a metal rod and the resulting suspension was filtered through four layers of sterile coarse-mesh cheesecloth to remove the hyphal debris. The suspension was centrifuged (10 min, 3000 rpm) and resuspended in fresh 0.05% Triton X-100. The spore concentration was determined using an improved Neubauer haemocytometer and adjusted to  $10^7$  conidia  $\text{ml}^{-1}$  by dilution with 0.05% Triton X-100. Conidial viability was determined on SDA using the technique developed by Hall (1977). The germination after 24 h at 25°C was 98.3%.

A range of ornamental pot plant species (Table I) was treated with a spore suspension containing  $10^7$  conidia  $\text{ml}^{-1}$  of strain 275-86 or with a solution of 0.05% Triton X-100. The *M. anisopliae* spores were applied as a drench at two rates; 50 and 100  $\text{ml l}^{-1}$  compost, whereas all the control treatments were applied

TABLE I  
List of ornamental species and cultivars used in the experiments where the natural preference (np) of *O. sulcatus* was examined and the efficacy of *M. anisopliae* (Ma) was evaluated

Species	Cultivar	Pot size (ℓ)	Experiment	
			np	Ma
<i>Begonia semperflorens</i>	Olympic Red	0.4	*	*
<i>Campanula isophylla</i>	Stella Blue	0.4	*	*
<i>Coleus blumei</i>	Wizard Mixed	0.4		*
<i>Cyclamen persicum</i>	Sierra Scarlet	0.4		*
		1.0		*
<i>Dianthus</i>	Magic Charm	0.4	*	*
<i>Euphorbia pulcherrima</i>	Annette Hegg Diva	1.0		*
<i>Gazania splendens</i>	Chan Sonnet Mix	0.4		*
<i>Hypoestes sanguinolenta</i>	Pink Splash	0.4		*
<i>Impatiens wallerana</i>	Super Elfin Blush	0.4	*	*
<i>Kalanchoe blossfeldiana</i>	Rode Sinapore	0.4		*
<i>Pelargonium</i> F1 hybrid	Century Rose	1.0		*
<i>Primula veris</i>	Giant Bouquet Mixed	0.4	*	*
<i>Sinningia speciosa</i>	Gala Blue	0.4		*
<i>Solanum capsicastrum</i>	Ballard	0.4		*

\*Species included in the experiment.

at 100 ml ℓ<sup>-1</sup> compost. The actual volume of drench applied to each plant was adjusted to take account of the two different pot sizes (Table I). Each treatment was applied to 10 replicate pots of each species and the groups of 30 treated plants were arranged in a randomized complete block design. Only 10 *Primula* plants were available and consequently these plants were treated only with 0.05% Triton X-100. Two additional treatments (20 ml pot<sup>-1</sup> of 0.05% Triton X-100 and 60 ml pot<sup>-1</sup> of the spore suspension) were each applied to ten more *Begonia* and *Impatiens* plants. An extra group of ten *Cyclamen* plants growing in the 0.4 ℓ pots was also treated with 60 ml pot<sup>-1</sup> of the 10<sup>7</sup> conidia ml<sup>-1</sup> suspension.

Twenty weevil eggs per pot were applied to the *Begonia*, *Coleus*, *Cyclamen*, *Gazania*, *Impatiens*, *Kalanchoe* and *Sinningia* immediately after the treatments. The same number of eggs were applied to the remaining seven species one week later. The viabilities of the two egg batches was 93.7 and 95.7%, respectively.

An infestation of cabbage moth and silver Y moth midway through the experiment was controlled using *Bacillus thuringiensis* ('Bactospeine', Duphar, BV. Weesp, Holland; 300 g per 10 ℓ water). All the plants were destructively assessed between ten and twelve weeks after egg application and the number of live larvae per pot recorded and analysed as above.

## RESULTS

### Natural preference for host plant

A total of only 13 adults were found on the experimental plants in the 'Tygan' cage which was surprising considering the large number of adults in the cage. There was an indication that the adults had a preference for the *Primula* plants because six of them were found on this species (Table II). There were no adults on the *Begonia* plants, however it was not possible to be certain whether or not this was significant. There was a weak correlation between the number of adults and larvae recovered on the different species in that larval and adult popula-

TABLE II  
Weevil population on five ornamental species exposed to a caged population of *O. sulcatus* adults

Species	Mean number of adults recovered pot <sup>-1</sup>	Mean number of larvae recovered pot <sup>-1</sup>	Mean square root transformation of larval numbers
<i>Begonia</i>	0	8.9	2.80
<i>Campanula</i>	0.1	0.4	0.28
<i>Cyclamen</i>	0.3	26.4	5.04
<i>Impatiens</i>	0.3	16.3	3.83
<i>Primula</i>	0.6	36.6	5.83
			SED = 0.548 (36 d.f.)

tions were highest on *Primula* plants, while *Campanula* plants had the lowest larval numbers and the second lowest adult population.

There were significant differences in the numbers of larvae recovered from each plant species (Table II). The *Primula* plants had significantly ( $P < 0.001$ ) higher larval populations than the other species (with the exception of *Cyclamen*). The number of larvae in each *Primula* pot ranged from 10 to 74, but in only two of the pots did plants show signs of stress from larval feeding. By contrast, all the *Cyclamen* plants were severely stressed even though the larval populations were lower. The other three species showed no outward signs of larval damage. Larval development on *Campanula* was poor and only four larvae were recovered from the ten pots and this population was significantly lower ( $P < 0.001$ ) than on the other species.

#### *Influence of plant species on larval development*

There was no significant difference ( $P > 0.05$ ) between the mean number of larvae recovered from the *Cyclamen* pots ( $9.75 \text{ pot}^{-1}$ ) and *Impatiens* pots ( $11.38 \text{ pot}^{-1}$ ). The larval populations were highly variable with standard deviations (SD) of 3.95 and 5.37 for *Cyclamen* and *Impatiens* respectively. Variation in the larval weights was reduced compared with larval numbers with SD values of 5.48 and 6.75 for *Cyclamen* and *Impatiens* respectively. The mean weight of the larvae recovered from *Cyclamen* pots was 29.4 mg, while the larvae on *Impatiens* plants had a mean weight of 23.6 mg, but the difference was not significant ( $P > 0.05$ ).

#### *Assessment of cultivar resistance to weevil larvae*

There were significant differences ( $P < 0.01$ ) in the number of weevil larvae recovered from the *Impatiens* cultivars (Table III). The most susceptible cultivar was Sequins Red and White on which 84% of the maximum possible total number of larvae were recovered. This survival rate was reduced to 58% on the most resistant cultivar of the six, Sequins Scarlet. There were significant differences ( $P < 0.001$ ) between the mean larval weights of the groups of larvae from the different *Impatiens* cultivars (Table

III). Larval development and survival on the different cultivars showed weak correlation ( $r = 0.693$ ; 4 d.f.). The lightest larvae were recovered from the two most resistant cultivars and the heaviest larvae were recovered from the most sensitive cultivar, Sequins Red and White. Regression analysis demonstrated that there was no significant intra-cultivar interaction between larval number per pot and larval weight (Table III).

#### *Weevil control by M. anisopliae on a range of ornamental pot plant species*

Weevil larvae were recovered from all the plant species examined with the exception of *Campanula*, *Euphorbia*, *Hypoestes* and *Solanum* (Table IV). The population in the Triton X-100 treated pots of the other species ranged from 0.14 to  $12.95 \text{ larvae pot}^{-1}$  (*Dianthus* and *Cyclamen*, respectively). Both application rates of strain 275-86 significantly reduced larval numbers on the different species ( $P < 0.05$  and  $< 0.01$  for the lower and higher rates, respectively). In all cases the highest rate of *M. anisopliae* increased larval mortality compared with the lower rate, although the difference between the two was significant only ( $P < 0.01$ ) on the *Cyclamen* plants in 1  $\ell$  pots. Total larval control was recorded on five of the species examined and the maximum control on the remaining species was greater than 85%.

#### DISCUSSION

The host plant clearly influenced larval numbers and it is likely that this was due to the combined effects of the plant on the adult and the developing larvae. The host plant has been shown by many workers to have a major effect on oviposition. Maier (1981) reported that adults feeding on *Taxus cuspidata* produced a mean total of 507 eggs, while those on *T. canadensis*, *Kalmia latifolia* and *Cornus florida* produced 324, 143 and 15 eggs, respectively. Hanula (1988) found that adults chose to oviposit on *T. cuspidata* in preference to alternative species and it was suggested that *Taxus* foliage contained an oviposition stimulant. The larval population sizes in the 'Tygan' cage experiment suggest that *O. sulcatus* adults had a preference for *Primula* and *Cyclamen*. However, it is possible that the numbers of larvae recovered from the test plants do not accurately

TABLE III  
Mean number and weights of larvae recovered from several *Impatiens* cultivars

Sequins cultivar	Mean number of larvae/pot	Mean square root transformation	Mean larval weight (mg)	Correlation coefficient <sup>a</sup>
S. Red and white	8.4	2.89	38.38	-0.324
S. Orange	8.3	2.88	32.07	-0.410
S. Rose	8.1	2.84	29.88	-0.046
S. Orchid	7.2	2.64	32.98	-0.521
S. White	6.9	2.61	27.06	0.520
S. Scarlet	5.8	2.37	27.42	0.344
SED (with 45 d.f.)		0.161	2.216	

<sup>a</sup>The correlation coefficient between larval number and weight ( $P=0.05$ ) with 10 d.f. = 0.576.

reflect adult preference and the low larval populations on *Campanula* plants in both experiments may be a reflection of poor larval survival rather than reduced oviposition.

Mortality from eggs on the *Cyclamen* plants

treated with Triton X-100 in the experiment evaluating the efficacy of *M. anisopliae* was only 32.5% compared with over 75% on *Begonia*, *Primula* and *Impatiens* suggesting that *Cyclamen* is a more acceptable host species for

TABLE IV  
Larval control on glasshouse ornamentals using *M. anisopliae* (strain 275-86)

Species <sup>a</sup>	Drench treatment (volume/pot)	Mean number of larvae/pot	Mean square root transformation	SED (d.f.)	Percentage control <sup>b</sup>
<i>Begonia</i>	20 ml Triton	4.8	2.01		
	40 ml Triton	5.4	2.29		
	20 ml <i>M. anisopliae</i>	0.1	0.10	0.221	98%
	40 ml <i>M. anisopliae</i>	0	0	(36)	100%
<i>Coleus</i>	60 ml <i>M. anisopliae</i>	0	0		100%
	40 ml Triton	4.6	2.07		
	20 ml <i>M. anisopliae</i>	1.5	0.79	0.338	67%
	40 ml <i>M. anisopliae</i>	0.2	0.2	(18)	96%
<i>Cyclamen</i>	40 ml Triton	13.5	3.48		
	20 ml <i>M. anisopliae</i>	5.0	1.93		63%
0.4 l pots	40 ml <i>M. anisopliae</i>	3.3	1.63		76%
	60 ml <i>M. anisopliae</i>	2.0	1.23		85%
	100 ml Triton	12.4	3.47	0.405	
1.0 l pots	50 ml <i>M. anisopliae</i>	5.4	2.17	(54)	56%
	100 ml <i>M. anisopliae</i>	1.6	1.04		87%
	40 ml Triton	0.14	—		
<i>Dianthus</i>	20 ml <i>M. anisopliae</i>	0	—		
	40 ml <i>M. anisopliae</i>	0	—		
	40 ml Triton	2.3	1.14		
<i>Gazania</i>	20 ml <i>M. anisopliae</i>	0.6	0.34	0.339	74%
	40 ml <i>M. anisopliae</i>	0	0	(18)	100%
	20 ml Triton	4.25	2.02		
<i>Impatiens</i>	40 ml Triton	6.10	2.41		
	20 ml <i>M. anisopliae</i>	0.10	0.10	0.175	98%
	40 ml <i>M. anisopliae</i>	0	0	(34)	100%
	60 ml <i>M. anisopliae</i>	0	0		100%
<i>Kalanchoe</i>	40 ml Triton	8.0	2.49		
	20 ml <i>M. anisopliae</i>	0.2	0.30	0.469	98%
	40 ml <i>M. anisopliae</i>	0	0	(11)	100%
<i>Pelargonium</i>	100 ml Triton	3.4	1.58		
	50 ml <i>M. anisopliae</i>	0.9	0.78	0.341	74%
	100 ml <i>M. anisopliae</i>	0.56	0.36	(17)	84%
<i>Primula</i>	40 ml Triton	4.25			
	40 ml Triton	2.9	1.37		
	20 ml <i>M. anisopliae</i>	0.1	0.10	0.304	97%
<i>Sinningia</i>	40 ml <i>M. anisopliae</i>	0	0	(18)	100%

<sup>a</sup>Larvae were not recovered from any plants of *Campanula*, *Euphorbia*, *Hypoestes* or *Solanum*.

<sup>b</sup>Reduction in larval numbers as a percentage of the population on the pots treated with Triton X-100.

*O. sulcatus* larvae. These results are not consistent with the larval populations in the 'Tygan' cage experiment where the highest population was recorded on *Primula* plants. Assuming similar levels of mortality from eggs, these results suggest that populations of *O. sulcatus* larvae on the *Cyclamen* pots would have originated from 39 eggs compared with 172 eggs on *Primula*. Similarly, the larval population on *Begonia* and *Impatiens* would have originated from 37 and 77 eggs respectively. These simple comparisons illustrate that complex interactions between *O. sulcatus* and its host plant and demonstrate the limited understanding of the processes involved.

It has been suggested that adults are more polyphagous than larvae (Nielsen and Dunlap, 1981), but there have been few detailed reports on the influence of the host plant on larvae. A number of factors could have been responsible for the increased resistance of some of the *Impatiens* cultivars to *O. sulcatus* larvae. Leaf physiology has been implicated in resistance to adult feeding (Doss *et al.*, 1987; Nielsen and Dunlap, 1981) and it is also possible that physiological differences between the roots may have similar effects on larvae. In addition, roots may possess chemical defence mechanisms similar to those observed in leaves (Doss, 1984).

Resistance to adult feeding does not necessarily mean that the plant will be resistant to larvae. Cram (1970a) examined adult activity on a range of cranberry cultivars and found that adults did not oviposit or survive when fed exclusively on cvs Weymouth and Cabot leaves. Larval development was later examined on three other cultivars which were acceptable to adult weevils and one (cv. Weymouth) which was resistant (Cram, 1970b). There was no difference in larval survival or size on the cv. Weymouth plants compared with the other cultivars. These incongruous results may have been due to differential sensitivity of the adults and larvae or the non-expression of resistance factors in the roots.

The absence of a significant difference between larval development on *Impatiens* and *Cyclamen* was atypical in that retarded development on *Impatiens* had been observed in a number of previous experiments (Moorhouse, 1990). In one experiment, pupae were

first observed on *Cyclamen* plants nine weeks before they were recovered from *Impatiens*. It was noted that only a small number of larvae pupated on *Impatiens* plants and it is possible that the pupation process was inhibited by secondary chemicals from the *Impatiens* plants. The reasons for the inconsistency between the results of different experiments are uncertain, however it is possible that differences between cultivars and methods of weevil infestation (eggs rather than neonate larvae were used in the other experiments of Moorhouse, 1990) may have been important.

There was considerable variability in the natural distribution of larvae in the experimental pots of the different species. Parrella and Keil (1982) also noted this when they observed that the numbers of pupae on *Taxus* plants in an infested nursery ranged from 0 to 70 per pot. The variability of larval populations in experimental pots was large, even when the pots were artificially infested with defined numbers of eggs. To reduce the impact of variable weevil populations, Anon. (1987) recommended that pot experiments on *O. sulcatus* should be carried with at least 15 replicates per treatment. In addition, it is also suggested that the pots should be infested with 30 eggs followed 10–14 d later by a further 20 eggs.

The host plant had a significant influence on the efficacy of *M. anisopliae* and similar effects have also been reported with the control of adult *O. sulcatus* using pyrethroids (Shanks and Chamberlain, 1988). Moorhouse (1990) quantified the influence of host plant on *M. anisopliae* and reported that the  $LC_{95}$  of strain 275–86 on *Cyclamen* plants was much greater than that on both *Begonia* (ca. 10x) and *Impatiens* (ca. 100x) plants. Zimmermann (1984) also reported that larval control on *Cyclamen* plants (30–45%) was much lower than on *Azalea*, *Fragaria*, and *Kalanchoe* plants (80–100%) from similar spore applications. It is possible that fungistatic root exudates from the host plant may inhibit infection of *O. sulcatus* larvae by *M. anisopliae*. Zimmermann (1984) suggested that the saponin, 'cyclamin', might be the casual agent of the reduced infection on *Cyclamen* plants and this was supported by the work of Gillespie and Sellers (unpublished) who demonstrated *in vitro* inhibition of germination and growth of *M. anisopliae* by aqueous

extracts from *Cyclamen* corms. Inhibition of the infection processes has not been demonstrated *in vivo* and the presence of sufficiently large quantities of secondary plant chemicals in compost has yet to be determined. The influence of the glycoalkaloid content of several *Solanum* species on the infection of *Leptinotarsa decemlineata* by *Beauveria bassiana* was examined by Costa and Gaugler (1989), but no effects were reported.

Secondary plant chemicals may also influence infection indirectly by reducing insect fitness. Hare and Andreadis (1983) found that *L. decemlineata* larvae were more sensitive to infection by *B. bassiana* on unfavourable plant species. Retarded development and physiological stress of *O. sulcatus* larvae on some host plants may similarly reduce their resistance to infection. The distribution and nutritive value of the roots will also influence larval movement and this might be associated with increased exposure to infective conidia.

The results from this study suggest that *M. anisopliae* has considerable potential as a microbial control agent for *O. sulcatus* on a number of pot plant species. Larval control on some species might be reduced and in this situation it may be necessary to develop an integrated control system using *M. anisopliae* and resistant cultivars.

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