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Control of *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae) Larvae on a Range of Hardy Ornamental Nursery Stock Species Using the Entomogenous Fungus *Metarhizium anisopliae*

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The potential of the entomogenous fungus Metarhizium anisopliae *as a microbial control agent for vine weevil (*Otiorhynchus sulcatus*) larvae was examined on a range of outdoor hardy nursery stock species. A curative application of* M. anisopliae *conidia (5×10^8 conidia l^{-1} compost) reduced larval numbers by 62% on* Skimmia japonica *'Rubella' and by up to 43% on* Viburnum plicatum *'Mariesii'. Four* M. anisopliae *isolates were examined and all reduced the larval populations on both species. However, the reductions were only significant with strains 159–83 and 100–82 on* S. japonica *'Rubella' and 100-82 on* V. plicatum *'Mariesii'. Larval development on two other species (*Hydrangea macrophylla *'Blue Wave' and* Thuja plicata *'Zebrina') which had been treated with 0.05% Triton X-100 (the control treatment) was very poor and therefore it was not possible to determine whether or not the fungal drench had any effect. The experiment was repeated in the following year at two different sites, East Malling and Littlehampton, using a prophylactic drench of two* M. anisopliae *isolates on a greater number of plant species. Strain 275–86 was more effective than 159–83 on all species at East Malling, with the exception of* V. davidii. *The difference was less pronounced at Littlehampton and the results from the two isolates were similar. Larval control was highly variable and species dependent with a reduction in larval numbers ranging from zero to 96% and zero to 90% at East Malling and Littlehampton respectively. The larval populations in pots treated with Triton X-100 were also highly variable, ranging from zero (*Chaemaecyparis lawsoniana *'Stardust',* Dianthus *'Maria',* Escallonia *'Crimson Spire' and* Pittosporum tenuifolium *'Garnettii') to 17.8 larvae per pot (*Ribes nigrum *'Baldwin'). The results indicate the potential of* M. anisopliae *and demonstrate the complexity of plant–weevil–fungus interactions.*

Keywords: *entomogenous fungus,* Metarhizium anisopliae, *vine weevil,* Otiorhynchus sulcatus, *biological control, hardy ornamental nursery stock*

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INTRODUCTION

Vine weevil (*Otiorhynchus sulcatus*) is currently rated as one of the most serious pests of hardy ornamental nursery stock (HONS) and soft fruit in the UK (Moorhouse *et al.*, 1992a). Losses of certain species resulting from the feeding activity of *O. sulcatus* larvae on the root systems can be severe. Damage to plant foliage resulting from the feeding activity of the adult weevils is less important, although plant quality and marketability can be reduced if leaf notching is significant. The nocturnal, parthenogenetic adults are difficult to control using insecticides and growers have traditionally relied on compost incorporation of aldrin (a persistent organochlorine insecticide) to control the *O. sulcatus* larvae. Aldrin is no longer available and environmentally acceptable alternatives, such as entomogenous fungi, are now being examined.

Several species of entomogenous fungi have been isolated from field populations of *O. sulcatus*; these include *Metarhizium anisopliae*, *M. flavoviride*, *Beauveria bassiana*, *B. brongniartii*, *Paecilomyces farinosus*, *P. fumosoroseus* and *Verticillium lecanii* (Zimmermann, 1981). Isolates from two of these genera, *Metarhizium* and *Beauveria*, have been studied extensively over recent years as potential biological control agents for *O. sulcatus* on protected ornamental species (Prado, 1979; Zimmermann, 1981, 1984; Tillemans & Coremans-Pelseneer, 1987). However, data on the control of *O. sulcatus* by entomogenous fungi on outdoor crops is surprisingly limited considering the insect's importance on outdoor species. Soares *et al.* (1983) reported the failure of isolates from three entomogenous species (*B. bassiana*, *B. brongniartii* and *P. fumosoroseus*) to reduce significantly the population of *O. sulcatus* larvae on strawberry plants in a small-scale field trial. These authors did however observe a natural reduction in larval numbers of up to 72% in the control plots as a result of infection by *M. anisopliae*.

Temperature is likely to be one of the most important factors limiting the exploitation of entomogenous species on outdoor crops. A maximum mean soil temperature (10 cm) of 20.4°C was recorded during July 1988 at Littlehampton and this was well below the optimum for *M. anisopliae* (Soares *et al.*, 1983). Bioassay data demonstrate that *O. sulcatus* larvae can still be infected by *M. anisopliae* at 10°C, but the mortality rate is greatly reduced (Moorhouse *et al.*, 1990). The present study was therefore initiated to determine the potential of *M. anisopliae* to control vine weevil under sub-optimal temperature conditions which are normally experienced during British summers. Other factors, such as the effect of fluctuating moisture conditions, the increased persistence requirement and the greater diversity of potting media may also be important on outdoor crops and there will be a need for these factors to be addressed in subsequent experiments before the full potential of *M. anisopliae* can be determined.

The successful application of *M. anisopliae* to control *O. sulcatus* on outdoor container-grown nursery stock and field crops would greatly enhance its commercial attractiveness because these crops cover a much larger area than glasshouse ornamentals. The experiments described in this study aim to provide an initial evaluation of the potential of *M. anisopliae* as a microbial control agent for *O. sulcatus* on a range of nursery stock species.

MATERIALS AND METHODS

Four different plant species, *Thuja plicata* 'Zebrina', *Hydrangea macrophylla* 'Blue Wave', *Skimmia japonica* 'Rubella' and *Viburnum plicatum* 'Mariesii', were selected for a preliminary trial to evaluate the potential of *M. anisopliae* isolates. The plants were produced from rooted cuttings and grown on in a standard peat and grit container compost. The *T. plicata* 'Zebrina' plants were grown in 3 l pots, whilst the other three species were grown in 2 l pots. Fifty plants of each species were arranged on a concrete pad in the nursery area at Horticulture Research International (HRI), East Malling, Kent in ten blocks of five plants. A treatment plan was then

drawn up by randomly allocating one plot in each block to each treatment. The plants were irrigated using an overhead spray line and they were protected from the wind by other, larger, ornamental species and artificial windbreaks. Pesticides were not applied to any of the experimental plants during the trial period.

Vine weevil eggs were collected over a 7-day period from a population of adult weevils maintained on strawberry leaves at 20–22°C under a 16 : 8 hour light : dark photoperiod (Moorhouse *et al.*, 1992b). The eggs were placed on moist filter paper in a 9 cm Petri dish and maintained at 20°C for a further 3 days. Twenty eggs were buried approximately 1 cm below the compost surface of each pot on 23 June 1988. Egg viability was determined by placing three batches of 100 eggs on moist filter paper in Petri dishes. The Petri dishes were maintained under ambient conditions and egg hatch was observed. Seventy-seven per cent of the eggs hatched within 6 weeks and the remaining eggs were considered non-viable.

Four virulent *M. anisopliae* strains were selected for further evaluation following promising results in bioassay and glasshouse experiments against *O. sulcatus* larvae (Gillespie, 1989; Moorhouse *et al.*, 1993a; Moorhouse *et al.* 1993b). Strain 35-79 was isolated from *O. sulcatus* in the UK, strain 100-82 was isolated from *Melolontha melolontha* in France, strain 159–83 was isolated from *Wiseana* sp. in New Zealand and strain 275–86 was isolated from *Cydia pomonella* in Germany. All four isolates were maintained in polypropylene drinking straws in liquid nitrogen using the technique developed by Challen and Elliott (1986).

Conidial samples of each strain were removed from liquid nitrogen 8 days after egg application and were subcultured on to 9 cm Petri dishes containing Sabouraud's dextrose agar (SDA). The cultures were maintained at 23°C for 10 days and then conidia were subcultured on to fresh SDA plates. These cultures were incubated under the same conditions for a further 10 days. The plates were then flooded with 0.05% Triton X-100 and the conidia were released by agitation with a metal rod. The resulting conidial suspensions were filtered through four layers of sterile coarse-mesh cheesecloth, centrifuged and resuspended in fresh 0.05% Triton X-100. The concentrations of the four suspensions were determined using an improved Neubauer haemocytometer and adjusted to 10^7 conidia ml^{-1} using fresh 0.05% Triton X-100. The four *M. anisopliae* treatments and the control treatment (0.05% Triton X-100) were applied to the four nursery stock species 4 weeks after the eggs. One *T. plicata* 'Zebrina' plant in each block received a 150 ml drench of one of the four spore suspensions or 0.05% Triton X-100. The treatments were similarly applied to the other three species using a lower application rate (100 ml per pot) to allow for the reduced pot size. Spore viability was determined on SDA using the technique developed by Hall (1977) and germination exceeded 95% in all cases. The pots were destructively assessed after 16 weeks and larval populations were recorded.

The trial was expanded in the following year to include a greater range of host species at two sites, HRI East Malling and HRI Littlehampton (Table 1). Two host species were common to both sites, *Fuchsia* 'Display' and *Pernettya mucronata* 'Crimsonia'. Different cultivars of the same species were also included at both sites to determine any host species–weevil–fungus interactions. The plants at East Malling were grown in the nursery area described earlier, whereas those at Littlehampton were grown on a sheet of 'Mypex' (Fyffes-Monro, Goodwood, UK) with watering from an overhead spray line. The Littlehampton plants were protected on three sides: by glasshouses on the east and western sides, and by an artificial windbreak on the southern side.

Only two strains, 159-83 and 275-86, were examined in the 1989 trials. Fresh conidial samples were removed from liquid nitrogen and subcultured on to SDA as before. Conidial suspensions (10^6 conidia ml^{-1}) were prepared after the first 10-day incubation period and each suspension was then used to inoculate four sterile Erlenmeyer flasks (1 ml/flask) containing 25 g millet and 25 ml deionized water (Moorhouse, 1990). The flasks were incubated at 25°C for 14 days and then the *M. anisopliae* suspensions were prepared as before.

The pots at East Malling were treated on 14 July 1989 with a drench of 0.05% Triton X-100 or a spore suspension containing 10^7 conidia ml^{-1} of strain 159–83 or 275–86 (95% and 98% germination respectively). Each treatment was applied to ten replicate pots at a rate of 50 ml l^{-1} compost. Twenty-five 'tanned' weevil eggs per pot were applied to the *Buddleia davidii* 'Royal

TABLE 1. Hardy ornamental species examined at East Malling (EM) and Littlehampton (L)

Species	Cultivar	Pot volume (l)	Site[a]
Buddleia davidii	'Royal Red'	2	EM
Camellia × *williamsii*	'Debbie'	2	L(5)
Chamaecyparis lawsoniana	'Stardust'	2	L(5)
Cryptomeria japonica	'Elegans'	1	EM
Cupressocyparis leylandii	'Clone 122'	3	L(5)
Cupressocyparis leylandii	'Castlewellan'	1	L(4)
Dianthus	'Maria'	1	L(4)
Elaeagnus pungens	'Maculata'	3	L(4)
Escallonia	'Crimson Spire'	1	L(4)
Euonymus fortunei	'Emerald & Gold	3	L(5)
Fuchsia	'Display'	2	EM L(4)
Hedera helix	'Buttercup'	2	EM
Hydrangea macrophylla	'Blue Wave'	2	EM
Mahonia × *media*	'Charity'	3	L(5)
Myrtus communis	'Microphylla'	2	L(4)
Pernettya mucronata	'Crimsonia'	1	EM L(4)
Pittosporum tenuifolium	'Garnettii'	2	L(4)
Ribes nigrum	'Baldwin'	2	EM
Rhododendron	'Lady Clementine Mitford'	1	L(4)
Skimmia japonica	'Rubella'	3	L(4)
Taxus baccata		0.9	EM
Thuja plicata	'Zebrina'	2	EM
Viburnum davidii		2	EM
Viburnum plicatum	'Grandiflorum'	2	EM
Viburnum plicatum	'Mariesii'	2	EM
Vitis coignetiae		2	EM
Weigela	'Bristol Ruby'	2	EM

[a] Parentheses shows date of second egg application in weeks after spore application.

Red', *Hedera helix* 'Buttercup', *H. macrophylla* 'Blue Wave', *Taxus baccata*, *T. plicata* 'Zebrina' and *V. plicatum* 'Grandiflorum' pots 3 weeks later. Eggs were applied to the remaining plant species after a further week (4 weeks after spore application). The viability of the first and second egg batches was 84% and 88% respectively. The plants were maintained for 17–22 weeks and then they were destructively assessed and larval populations recorded.

The 1989 East Malling experiment was repeated at Littlehampton. The two *M. anisopliae* and the Triton X-100 treatments were applied 1 week after the East Malling treatments at the same rates. The fungal treatments were prepared from freshly harvested spores and the initial *in vitro* germination percentages of 159–83 and 275–86 were 98% and 99% respectively. Twenty-five 'tanned' weevil eggs were applied to each pot immediately after the treatments. However, egg viability was only 14% and, consequently, a second egg application was necessary. The repeat applications were made to 10 plant species after 4 weeks (96% hatch) and to the remaining 5 plant species one week later (96% hatch). The plants were destructively assessed 20–28 weeks after treatment and larval numbers were recorded.

RESULTS

There were large differences in the larval populations on the four ornamental species following treatment with 0.05% Triton X-100 (control) with percentage survival from eggs ranging from 3.5% to 46.5% on *H. macrophylla* 'Blue Wave' and *V. plicatum* 'Mariesii', respectively (Table 2). The larval populations on the *H. macrophylla* 'Blue Wave' and *T. plicata* 'Zebrina' plants which had been treated with 0.05% Triton X-100 were low and variable; consequently, the pots which had been treated with *M. anisopliae* were not assessed. The larval numbers were analyzed by analysis of variance on the square root transformed data and the transformed means were

compared using *t* tests with a significance threshold of $P = 0.05$. The most effective fungal treatment on *S. japonica* 'Rubella' was strain 159–83 which reduced larval numbers by 62% ($P < 0.05$). Larval populations on *S. japonica* 'Rubella' were also significantly reduced by strain 100–82. This strain also reduced larval numbers on *V. plicatum* 'Mariesii' by 43% ($P < 0.05$). The other three strains reduced the larval population on this species by up to 38.7%, but this was not statistically significant.

TABLE 2. Preliminary trial to examine control of *O. sulcatus* larvae by four strains of *M. anisopliae* on four HONS species

Species and cultivar	Treatment	Larvae recovered per pot	Mean square root transformation	Percentage control
H. macrophylla 'Blue Wave'	Triton X-100	0.7		
	M. anisopliae	not assessed		
S. japonica 'Rubella'	Triton X-100	4.7	2.03	0
	35–79	2.8	1.48	40.4
	100–82	2.3	1.17	51.1
	159–83	1.8	1.08	61.7
	275–86	2.1	1.31	55.3
			SED = 0.358 (36 df)	
T. plicata 'Zebrina'	Triton X-100	1.1		
	M. anisopliae	not assessed		
V. plicatum 'Mariesii'	Triton X-100	9.3	2.938	0
	35–79	6.1	2.418	34.4
	100–82	5.3	2.157	43.0
	159–83	7.5	2.695	19.4
	275–86	5.7	2.333	38.7
			SED = 0.303 (34 df)	

In contrast to the initial experiment described above, where the fungus was applied after the insects. in the 1989 expeiments at East Malling and Littlehampton the fungus was applied prophylactically. The development of vine weevil in the 1989 experiment at East Malling was highly variable with populations in the pots treated with Triton X-100 ranging from 0.3 to 17.8 larvae per pot (Table 3). The populations in similarly treated *Bu. davidii* 'Royal Red' and *Ta. baccata* were low and therefore the *M. anisopliae*-treated plants were not assessed. Larval populations in pots treated with either strain 159–83 or 275–86 were reduced by up to 96%. Strain 275–86 was more effective than 159–83 on all the species examined, except *V. davidii*. Larval control was highly dependent on the host plant species. Both strains reduced larval numbers by over 50% on *H. macrophylla* 'Blue Wave', whereas the maximum control on *T. plicata* 'Zebrina' was only 30% and was not significant. Strain 275–86 significantly reduced the larval populations ($P < 0.05$) on four species: *Cryptomeria japonica* 'Elegans', *H. macrophylla* 'Blue Wave', *Ribes nigrum* 'Baldwin' and *Weigela* 'Bristol Ruby'. This strain also reduced the larval numbers on seven of the remaining eight species on which larvae developed. The reduction in larval numbers by strain 159–83 was only significant ($P < 0.01$) on one of the species (*V. davidii*). The lower populations recorded on seven of the remaining eleven plant species drenched with this strain were not significant.

The larval populations on the Triton X-100-treated nursery stock species at Littlehampton were also highly variable. Larvae failed to develop on *Chamaecyparis lawsoniana* 'Stardust', *Dianthus* 'Maria', *Escallonia* 'Crimson Spire' and *Pittosporum tenuifolium* 'Garnettii', and the population on three other species was below one larva per pot. In contrast, the population on four other species was about ten larvae per pot (Table 4). Weevil control varied between zero and 90% and was species dependent. The maximum control recorded on *Cr. japonica* 'Elegans' was 22% (not significant at the 5% level), whereas larval numbers were reduced by 90% on the *Fuchsia* 'Display' plants treated with strain 275–86 ($P < 0.01$). Both strains reduced the larval populations

TABLE 3. Control of *O. sulcatus* on a range of hardy ornamental species at East Malling

Species and cultivar	Treatment	Larvae/pot	Mean square root transformation	SED[a]	Percentage control[b]
Bu. davidii 'Royal Red'	Triton X-100	0.3			
	159–83	—			
	275–86	—			
Ca. × *williamsii* 'Debbie'	Triton X-100	12.3	3.49		
	159–83	10.7	3.13	0.459	13
	275–86	4.3	1.71		65
Fuchsia 'Display'	Triton X-100	3.7	1.23		
	159–83	1.2	0.74	0.622	68
	275–86	0.3	0.24		91
He. helix 'Buttercup'	Triton X-100	7.4	2.40		
	159–83	9.2	2.84	0.536	− 24
	275–86	4.5	1.82		39
H. macrophylla 'Blue Wave'	Triton X-100	5.4	2.13		
	159–83	2.5	1.43	0.347	54
	275–86	0.4	0.27		93
Pe. mucronata 'Crimsonia'	Triton X-100	4.0	1.33		
	159–83	1.3	0.75	0.519	68
	275–86	0.6	0.41		85
Ri. nigrum 'Baldwin'	Triton X-100	17.8	4.16		
	159–83	13.2	3.29	0.652	26
	275–86	8.6	2.39		52
Ta. baccata	Triton X-100	2.0			
	159–83	—			
	275–86	—			
T. plicata 'Zebrina'	Triton X-100	8.9	2.88		
	159–83	9.0	2.70	0.521	− 1
	275–86	6.2	2.35		30
V. davidii	Triton X-100	6.9	2.24		
	159–83	0.3	0.30	0.517	96
	275–86	9.8	2.92		− 42
V. plicatum 'Grandiflorum'	Triton X-100	7.6	2.73		
	159–83	9.5	2.83	0.653	− 25
	275–86	4.6	1.44		39
V. plicatum 'Mariesii'	Triton X-100	3.0	1.50		
	159–83	5.0	2.10	0.454	− 66
	275–86	2.0	1.12		33
Vi. coignetiae	Triton X-100	6.0	2.19		
	159–83	3.6	1.36	0.450	40
	275–86	3.5	1.71		42
Weigela 'Bristol Ruby'	Triton X-100	12.3	3.42		
	159–83	8.8	2.66	0.438	28
	275–86	6.1	2.23		50

[a] The SEDs of the transformed means with 18 df (11 and 15 for *Fuchsia* 'Display' and *V. plicatum* 'Grandiflorum' respectively).

[b] The percentage control is the reduction in the mean larval population of the treated pots compared to the control pots of each species.

on all the species examined (with the exception of 159–83 on *Myrtus communis* 'Microphylla'). The reduction by both strains was only significant ($P < 0.05$) on two species: *Cupressocyparis leylandii* 'Castlewellan' and *Mahonia* × *media* 'Charity'; 275–86 also significantly reduced larval numbers on *Fuchsia* 'Display' and *My. communis* 'Microphylla'. The difference between the two strains was not as pronounced as it was in the East Malling experiment and the results were similar in most cases.

The results from the two *C. leylandii* cultivars were interesting because they indicate varietal differences. The weevil population on the 'Clone 122' plants treated with Triton X-100 was over 2.5 times larger than the population on similarly treated 'Castlewellan' plants. The fungal treatments were also influenced by the different varieties. Larval numbers were reduced by a maximum of 70% and 31% on the 'Castlewellan' and 'Clone 122' plants respectively, as a result of fungal treatments. Control by both strains of *M. anisopliae* was significant ($P < 0.01$) on the 'Castlewellan' plants; however, the reduction in larval numbers on the 'Clone 122' plants was not significant ($P > 0.05$).

DISCUSSION

Control of *O. sulcatus* on several HONS species was highly variable, ranging from zero to a 96% reduction in larval numbers. The relatively poor results obtained in the first East Malling experiment could be explained partially by the curative rather than prophylactic application of conidial drenches. Total control has been recorded on glasshouse *Begonia semperflorens* plants which had been prophylactically treated with strain 275–86, whereas control was reduced to 92% and 65% when conidia were applied 2 and 8 weeks after egg infestation respectively (Moorhouse *et al.*, 1990). Similar differences between the levels of control achieved using prophylactic and curative spore applications have also been reported on glasshouse *Azalea* (Zimmermann, 1981; Tillemans & Coremans-Pelseneer, 1987).

The conidial treatments in the 1989 experiments were applied prophylactically in an attempt to improve larval control, but control was not improved on either the *S. japonica* 'Rubella' or the *V. plicatum* 'Mariesii' plants. Zimmermann and Simons (1986) also reported small reductions (30–50%) in the population of *O. sulcatus* larvae on outdoor strawberry and ornamental plants following prophylactic application of *M. anisopliae* conidia and they suggested that the poor control was probably the result of low soil temperature. This hypothesis is supported by bioassay observations which demonstrated that LT_{50} was inversely related to temperature over the range 10–25°C (Moorhouse *et al.*, 1990). Larval control on a number of species in the 1989 experiments was high and therefore low temperature is unlikely to have been a major limiting factor.

The variable results on the different HONS species may be the result of other factors, such as the physical and chemical interaction of the host plant with *M. anisopliae*. The root system of *Fuchsia* 'Display' was very open; therefore, spores would have been evenly distributed resulting in good contact between larvae and infective conidia. A number of other species, including *T. plicata* 'Zebrina', had very dense, fibrous root systems and this may have inhibited conidial penetration. It is possible that larval control of these species could have been improved by increasing the drench rate or incorporating conidia into compost before potting to improve distribution (Moorhouse, 1990). Fungistatic root exudates from plant species, such as *Cyclamen persicum* (Zimmermann, 1984), have been implicated in reduced levels of larval control and it is possible that exudates from species such as *T. plicata* 'Zebrina' may have similarly reduced control.

Larval numbers on the ornamental species treated with Triton X-100 were also very variable. The population on *H. macrophylla* 'Blue Wave' in the first experiment was 0.7 larvae per pot compared with 5.4 in the second. Similarly, there was a large difference between the larval population on the two *C. leylandii* cultivars at Littlehampton. There were also differences between our observations and those reported by other workers. Infestations of *O. sulcutus* larvae on *Rhododendron* and *Taxus* plants have frequently been reported on nurseries in the USA (La

TABLE 4. Control of *O. sulcatus* on a range of hardy ornamental species at Littlehampton

Species and cultivar	Treatment	Larvae/pot	Mean square root transformation	SED[a]	Percentage control[b]
Cr. japonica 'Elegans'	Triton X-100	6.3	2.47		
	159–83	4.9	1.90	0.364	22
	275–86	5.4	2.27		14
Ch. lawsoniana 'Stardust'	Triton X-100	0			
	159–83	—			
	275–86	—			
C. leylandii 'Clone 122'	Triton X-100	11.3	3.26		
	159–83	7.8	2.58	0.387	31
	275–86	8.0	2.78		29
C. leylandii 'Castlewellan'	Triton X-100	4.4	1.97		
	159–83	1.3	0.92	0.317	70
	275–86	1.5	0.89		66
Dianthus 'Maria'	Triton X-100	0			
	159–83	—			
	275–86	—			–
E. pungens 'Maculata'	Triton X-100	0.7	0.42		
	159–83	0.3	0.24	0.28	57
	275–86	0.2	0.20		71
Escallonia 'Crimson Spire'	Triton X-100	0			
	159–83	—			
	275–86	—			
Eu. fortunei 'Emerald & Gold'	Triton X-100	11.7	3.38		
	159–83	6.7	2.43	0.551	43
	275–86	8.6	2.38		25
Fuchsia 'Display'	Triton X-100	10.2	2.81		
	159–83	4.0	1.90	0.509	61
	275–86	1.0	0.79		90
Ma. × media 'Charity'	Triton X-100	13.3	3.55		
	159–83	3.7	1.66	0.389	72
	275–86	4.4	1.85		67
My. communis 'Microphylla'	Triton X-100	7.4	2.64		
	159–83	7.5	2.66	0.332	– 1
	275–86	3.5	1.71		53
Pe. mucronata 'Crimsonia'	Triton X-100	1.1	0.58		
	159–83	0.9	0.63	0.373	18
	275–86	0.6	0.54		45
Pi. tenuifolium 'Garnettii'	Triton X-100	0			
	159–83	—			
	275–86	—			
Rhododendron 'Lady C. Mitford'	Triton X-100	0.1			
	159–83	0			
	275–86	0			
S. japonica 'Rubella'	Triton X-100	0.6	0.48		
	159–83	0.3	0.30	0.345	50
	275–86	0.33	0.22		45

[a] The SEDs of the transformed means with 18 df for all species except *Fuchsia* 'Display' (17).
[b] The percentage control is the reduction in the mean larval population of the treated pots compared to the control pots of each species.

Lone & Clarke, 1981; Parrella & Keil, 1984). However, poor survival on these two species was recorded in the current experiments. The differences in survival rates may have been caused by a number of factors, such as varietal resistance to *O. sulcatus* larvae. Antonelli and Campbell (1981) demonstrated resistance within the genus *Rhododendron* to *O. sulcatus* adults and they reported that one hybrid and four species were completely resistant to adult feeding. They also noted that *Rhododendron* 'Lady Clementine Mitford' was moderately susceptible to adult feeding. This contrasts with the result obtained in the present experiment with *Rhododendron* 'Lady Clementine Mitford' and it suggests that there may be differences between the bases of resistance to *O. sulcatus* larvae and adults.

Larval survival from eggs in the outdoor trials was generally much lower than that observed in similar glasshouse experiments and variability between replicates was greater (Evenhuis, 1978; Moorhouse, 1990). Parrella and Keil (1984) reported large differences between the natural populations of *O. sulcatus* larvae and pupae on potted *Taxus* and they concluded that this was linked to limited adult movement between pots. The results obtained in the current experiments suggest that the conditions within each pot, such as compaction of the potting medium (Shanks & Finnigan, 1973), strongly influence larval survival. It is also possible that localized predation may be an important factor causing the variability in larval populations between the replicate pots of the same species. Carabid species and the common earwig are known to be predators of *O. sulcatus* eggs and larvae (Garth & Shanks, 1978), although the significance of natural predation in pot plants has not been quantified.

The minimum level of acceptable larval control will depend on host plant species and age. Larval infestations on recently established strawberry and blackcurrant crops can be devastating, with total destruction in severe situations. This contrasts with established crops and robust nursery stock species where low levels of feeding can be tolerated without economic damage. Damage on HONS is normally measured in terms of plant mortality rather than reduction in yield and therefore higher larval populations can often be tolerated. However, the long-term effect of poor weevil control on HONS would result in a significant increase in the weevil populations on nurseries and an increase in consumer dissatisfaction. Plant health standards are also increasing and plants must be guaranteed free from *O. sulcatus* larval infestation for sale in certain situations. The performance of *M. anisopliae* will obviously need to be improved to meet the increasing health standards and it is possible that *M. anisopliae* will need to be combined with other control agents as part of an integrated crop protection programme for *O. sulcatus* on outdoor nursery stock

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