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Effect of Potting Media on the Control of *Otiorhynchus sulcatus* Larvae on Outdoor Strawberry Plants Using the Entomogenous Fungus *Metarhizium anisopliae*

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The potential of entomogenous fungi to control black vine weevil (*Otiorhynchus sulcatus* (Fabricius)) larvae has been demonstrated on a range of protected crops species, but very little attention has been paid to weevil control on outdoor crops, such as strawberry. Curative applications of *Metarhizium anisopliae* (Metschnikoff) Sorokin conidia to strawberry plants potted in field soil (5×10^8 conidia/pot) reduced the population of *O. sulcatus* larvae by up to 94%, whereas similar treatments reduced larval numbers in peat compost by a maximum of 53%. Significant differences in relative performance were observed among the four *M. anisopliae* strains (37-80, 101-82, 189-83, and 275-86) in the two media. Strain 189-83 was the most virulent isolate in both media, whereas strain 101-82 reduced the *O. sulcatus* population in field soil by only 15% and had no effect in peat compost. Larval establishment in the pots treated with 0.05% Triton X-100 (control treatment) was greater in peat compost than in field soil with nearly twice as many larvae in the former medium. The larval population was evenly distributed in most of the pots with the exception of the pots containing peat compost treated with strain 189-83 which showed significant heterogeneity. Spore density declined with increasing depth in pots containing peat compost. The situation was reversed in the pots of field soil and the highest spore concentrations were found at the base of the pots. More conidia were recovered from the pots containing peat compost than from the pots of field soil. There were also significant differences between strains in the number of colony forming units per sample. In both media, mean counts for strains 37-80 and 275-86 were significantly greater than those for strain 101-82; strain 189-83, however, had a low count in peat relative to 37-80 and 275-86, but had a higher count in field soil.

KEY WORDS: ***Metarhizium anisopliae; Otiorhynchus sulcatus;* black vine weevil; strawberry pest; biological control; conidial movement.**

INTRODUCTION

The black vine weevil, *Otiorhynchus sulcatus* (Fabricius), is an increasingly serious pest on a range of protected and outdoor horticultural species including cyclamen, fuchsia, strawberry, and blackcurrant (Moorhouse *et al.*, 1992a). Damage to plants normally results from the feeding activity of larvae on the root system which may result in plant death. *O. sulcatus* adults feed mainly on the leaf margins and seldom cause serious damage, although leaf notching may cause rejection of nursery stock consignments.

The organochlorine insecticide, aldrin, was widely used until recently for control of *O. sulcatus* larvae; however, this chemical has been withdrawn and there are no equally effective replacements. Reports of severe damage by *O. sulcatus* larvae are becoming more frequent and this is possibly the result of natural population increases in the absence of aldrin. Controlled release insecticide formulations have been developed for use on hardy nursery stock species, but their use on soft fruit is restricted because of concern over insecticide residue levels. A number of potential biological control agents for *O. sulcatus* larvae are currently being developed, such as insect-parasitic nematodes (Klein, 1990) and entomogenous fungi (Andersch *et al.*, 1990). These biological control agents would seem to be the obvious answer for this pest species, and our approach has been to evaluate the potential of the entomogenous fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin, as a biological control agent for black vine weevil larvae on soft fruit.

There have been a number of reports on the potential of entomogenous fungi as microbial control agents for

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O. sulcatus larvae on protected crops (Zimmermann, 1981; Moorhouse *et al.*, 1990). However, data on outdoor crops are surprisingly limited considering the potential threat to hardy ornamental nursery stock and field grown soft fruit. Early results from field experiments with entomogenous fungi fully justified the initial concentration on protected crops. For example, Soares *et al.* (1983) reported the failure of isolates from three entomogenous species (*Beauveria bassiana* (Balsamo) Vuillemin, *B. brongniartii* (Saccardo) Petch, and *Paecilomyces fumosoroseus* (Wize) Brown & Smith) to reduce the population of *O. sulcatus* larvae significantly 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 natural infection by *M. anisopliae*.

The commercial attractiveness of *M. anisopliae* would be greatly enhanced if good control of *O. sulcatus* on outdoor hardy nursery stock and field crops could be clearly demonstrated. Expansion of the potential market for *M. anisopliae* would greatly increase its viability as a commercial product and would help to offset the high registration costs. The sensitivity of *M. anisopliae* to low temperatures (Moorhouse *et al.*, 1990) is likely to be one of the major factors that would restrict commercial application in a field environment. Other factors, such as the influence of soil microflora, the interaction with different potting media or soil types, application timing, persistence under field conditions, and lack of available moisture during dry summers may also be important and need to be considered. The influence of some of these factors on the effectiveness of four strains of *M. anisopliae* against *O. sulcatus* larvae and the distribution of conidia in two potting media is reported below.

MATERIALS AND METHODS

Strawberry runners (cv. Pandora) were potted in either peat compost or field soil (Hamble series; classed as silty brick earth) taken directly from a cultivated field at Littlehampton. Fifty plants were potted into each medium (1 runner/pot) in 13-cm-diameter polyethylene pots (approximately 1 liter) and the pots were placed on gravel beds in an uncovered cold frame at Horticulture Research International (HRI)-Littlehampton. The cold frame was sheltered from the prevailing wind by glasshouses on either side, but it was fully exposed to the sun and rain. Additional water was applied to the pots as required to ensure that the potting medium was never excessively dry. The mean monthly soil temperatures at 10 cm at HRI-Littlehampton during the experimental period ranged from 16.9°C (August) to 11.1°C (October).

Vine weevil eggs used during this experiment were obtained from cultures of adult weevils maintained on strawberry leaves at 20–22°C with a 16:8 h light:dark regime (Moorhouse *et al.*, 1992b). Eggs were collected every 7 days and incubated on moist filter paper for another 3 days at 20°C. Twenty "tanned" eggs were buried approximately 1 cm deep in each pot on July 19th. Five batches of 100 eggs were also placed on moist filter paper in petri dishes and maintained under the same conditions as the pots so that egg hatch could be monitored (mean egg hatch, 93.4%). The pots were then randomized in the cold frame and left for 24 days before spore application.

Four strains of *M. anisopliae* were selected for this experiment. Two of these strains (37-80 and 189-83) were originally isolated from *O. sulcatus* in England, the third strain (101-82) was isolated from European cockchafer (*Melolontha melolontha* (L.)) in France, and the fourth strain (275-86) was isolated from codling moth (*Cydia pomonella* (L.)) in Germany. The virulence of all four strains against *O. sulcatus* larvae had previously been demonstrated in laboratory bioassays (Moorhouse, 1990), but their performance in the field has not been quantified.

Conidia were produced on Sabouraud's dextrose agar (SDA) in 9-cm-diameter petri plates inoculated with conidia from stock culture plates and incubated for 10 days at 23°C. Conidial suspensions of each strain were prepared by flooding 10-day-old cultures 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 (10 min, 3000 rpm) and resuspended in fresh 0.05% Triton X-100. The conidial concentrations were determined using an improved Neubauer hemocytometer and adjusted to 10^7 conidia/ml by diluting with 0.05% Triton X-100. One milliliter of each suspension was removed, adjusted to 10^6 conidia/ml, and sonicated (amplitude of 10 μm) for approximately 10 s to break up the conidial clumps. Aliquots (0.005 ml) of each suspension were removed and spread onto three SDA pools on a glass microscope slide so that germination could be assessed (Hall, 1977). Germination was determined after 24 h incubation at 25°C, by observing 100 conidia on each agar pool using phase-contrast microscopy. Mean germination was 92, 94, 94, and 98% for 37-80, 101-82, 189-83, and 275-86, respectively.

Ten replicate pots of each growing medium were drenched with 0.05% Triton X-100 at a rate of 50 ml/pot on August 12th. Further batches of 10 soil and 10 peat pots were each drenched with a 50-ml suspension containing 10^7 conidia/ml of strain 37-80. The remaining 60 pots received a similar volume drench containing 10^7 conidia/ml of one of the other *M. anisopliae* isolates on the same date. The plants were then returned to the cold frame and laid out in a randomized block design (each pot was treated as a single plot for statistical analysis). The pots were maintained in the cold frame for 11 weeks post-treatment before assessment.

Each pot (minus the strawberry foliage) was initially

TABLE 1

Distribution of Live Weevil Larvae in Strawberry Pots Treated with *Metarhizium anisopliae* or 0.05% Triton X-100

Medium	*M. anisopliae* strain	Larvae recovered per layer (mm) 0–25	25–50	50–75	75–100	Total	χ^2 (3 *df*) uniformity	Larvae per pot[a]	Percentage control[b]
Peat compost	Control (Triton X-100)	15	15	18	11	59	1.712	5.9	—
	37-80	11	15	12	10	48	1.167	4.8	19
	101-82	7	21	14	14	56	7.000 ($P < 0.1$)	6.2	−5
	189-83	1	14	4	9	28	14.000 ($P < 0.01$)	2.8 ($P < 0.001$)	53
	275-86	7	9	8	6	30	0.666	3.0 ($P < 0.001$)	49
Field soil	Control (Triton X-100)	6	11	8	8	33	1.545	3.3	—
	37-80	1	1	2	1	5	0.600	0.5 ($P < 0.001$)	85
	101-82	6	2	12	8	28	7.428 ($P < 0.1$)	2.8	15
	189-82	0	1	1	0	2	2.000	0.2 ($P < 0.001$)	94
	275-86	1	2	0	0	3	3.667	0.3 ($P < 0.001$)	91

[a] Larval population based on the mean of 10 pots in all cases, except strain 101-82 peat (9 pots).
[b] Reduction in mean larval population compared to the pots of the same medium which had been treated with Triton X-100.

covered during winter months due to the unfavorable temperatures for *M. anisopliae*. Larval activity also declines over this period, although feeding still continues at a reduced rate (Smith, 1932). It is possible that temperatures in the following spring would be suitable for infection, but there will also be an increased risk of plant damage from mature larvae.

Significant numbers of *M. anisopliae* conidia were isolated from the pots 11 weeks after application. Some of these conidia may have originated from sporulating insect cadavers; however, the majority would have been the original spores because sporulation was restricted to the cadaver surface. Larvae that became infected and died would have formed very small dense foci of conidia within the pot and earlier work demonstrated that sixth instar larvae killed by strain 189-83 produced a mean of 2.09×10^8 conidia after 10 days under laboratory conditions at 20°C (Moorhouse, 1990). It is likely that *in vivo* sporulation in the current experiment would have been greatly reduced as a result of the nonsterile media and smaller larvae. The sporulation behavior of *M. anisopliae* on *O. sulcatus* cadavers contrasts with the observations of hyphal elongation from *Curculio caryae* (Horn) cadavers infected with *B. bassiana* (Gottwald and Ted-

TABLE 2

Distribution of *Metarhizium anisopliae* Conidia in Strawberry Pots Containing Peat Compost or Field Soil

Media	*M. anisopliae* strain	Log_{10} of CFU[a]/g peat or field soil at each depth (mm) 0–25	25–50	50–75	75+	Means
Peat compost						
	37-80	6.024	5.798	5.551	5.163	5.634
	101-82	5.664	5.380	5.241	4.805	5.272
	189-83	5.527	5.392	5.206	5.266	5.348
	275-86	5.962	5.945	5.685	5.686	5.820
	Means	5.794	5.629	5.421	5.230	5.518
Field soil						
	37-80	5.012	5.042	5.031	5.564	5.162
	101-82	4.716	4.739	4.701	4.781	4.735
	189-83	5.073	5.094	5.186	5.481	5.208
	275-86	4.899	4.992	5.128	5.199	5.055
	Means	4.925	4.967	5.011	5.256	5.040

[a] *M. anisopliae* conidia were assessed as colony forming units (CFU) on the assumption that conidial number and CFU were proportional.

SEDS of the $\log_{10}$ (CFU)		Peat compost	Field soil
Between strains	(12 *df*)	0.1361	0.1064
Between depths	(48 *df*)	0.0845	0.0639
Between strain × depth	(48 *df*)	0.1998	0.1535
(comparing depths only)		0.1689	0.1278

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 where low levels of feeding can be tolerated without economic damage. None of the plants was killed by weevil larvae in this experiment, but a number of the Triton X-100-treated plants were severely stressed by larval feeding activity around the stem base. Penman and Scott (1976) estimated that economic loss on strawberry plants would occur with between two and eight larvae per plant. The control in field soil with the leading *M. anisopliae* strains would have reduced larval numbers below this threshold and thus the potential of *M. anisopliae* as a microbial control agent for black vine weevil larvae on potted plants is clearly demonstrated.

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