Comparison of Efficacy of a Pathogenic Fungus, a Parasitic Nematode, and Neem Seed Extract in Control of Black Vine Weevil©

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INTRODUCTION

The larva of the black vine weevil, *Otiorhynchus sulcatus,* is one of the most serious pests of hardy ornamental nursery stock and soft fruit production. Larvae feeding on plant root systems can cause severe reduction in plant quality and in some cases kill the plants. Damage caused by the adults feeding on foliage is usually less important, except in cases of cosmetic injury to ornamentals. The nocturnal adults are difficult to kill using insecticides and control measures are focused on the soil-living larvae. Although effective, many organochlorine, organophosphorus, and carbamate pesticides are prohibited in the U.K. and Ireland for environmental and safety reasons. As a consequence, the search for new soil pest control measures has focused on biological control agents such as entomopathogenic fungi and nematodes, together with less broad-spectrum insecticidal compounds, including insect growth regulators targeted against soil-inhabiting insect larvae.

The entomopathogenic fungus *Metarhizium anisopliae* has previously been tested for use against vine weevil with varying degrees of success (Moorhouse et al, 1993, Van Tol, 1993). Low temperatures appear to be a major limiting factor in the use of such fungi on outdoor crops (Moorhouse et al, 1993). Use of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) against soil insect pests is also generally temperature limited (Fitters et al, 2001) but a strain of *Steinernema kraussei* (Nemasys L) is now marketed with claims for efficacy at temperatures down to 5 °C (Becker Underwood technical leaflet).

Extracts from neem tree (*Azadirachta indica*) seed kernels have been shown to have insecticidal properties against many members of the coleoptera (Warthen, 1989). The main active ingredient is azadirachtin, which is directly insecticidal, inhibiting the moulting process and disrupting normal development. Other compounds in neem seeds, including salannin and meliantriol, have potent antifeedant effects on insects and may also inhibit oviposition (Mordue and Blackwell, 1993).

MATERIALS AND METHODS

The efficacy of four experimental treatments (*Metarhizium*, *Steinernema*, neem kernels, and an untreated control) was compared against three controlled vine weevil egg infestation times, in heated glasshouse and outdoor conditions in randomised experimental plots. All treatments were initially established in the first week of June 2003 by potting a total of 480 pots (750 ml) with miniplugs of a *Begonia* Semperflorens Cultorum Group.

The *Metarhizium* and the neem kernel treatments were incorporated into the growing medium at potting: the fungus by incorporation of 1×10^{10} conidia (strain V275, original conidial concentration 1.5×10^{11} per gram); and the neem kernel by using 5 g of crushed and sieved (500 µm) neem seed kernel, both per litre of growing medium. Half the pots for each treatment were randomly assigned to capillary bed staging in a heated glasshouse maintained at 10 °C minimum, while the other half were placed outside on a capillary matting bed at ambient temperatures. In both cases, the crop was isolated to prevent naturally occurring weevil infestation. In the glasshouse this was achieved using water baths around the staging legs. We protected the capillary bed using Fluon GP1 strips (Whitford Plastics Ltd. Runcorn, Cheshire, England). Additional trap pots were used to monitor potential natural infestation.

Fresh vine weevil eggs were obtained from adults cultured in plastic boxes containing moist sand and fed on *Euonymus* leaves. The boxes were kept at room temperature (18 to 25 °C) and exposed to natural daylight. The sand in the boxes was sieved weekly to collect the eggs. On each of three separate occasions in July, August, and September (Table 1), 10 melanised eggs were placed just under the surface of the growing medium at the base of the test plants in a randomly selected third of the pots for each treatment (creating 20-pot replicates of each control agent × temperature × egg application date combination). The viability of the eggs used was assessed in a random subsample of 100 eggs from each infestation batch, which were incubated at 25 °C on moist filter paper. The average hatch rate across all three batches was 93% after 4-weeks incubation.

A commercial strain of *S. kraussei* (Nemasys L) was applied as a drench on the 7 Oct. 2003 to the nematode treatment pots for both indoor and outdoor crops. This application was made using a single commercial packet of Nemasys L at the commercially recommended rate, equivalent to approximately 5600 infective nematode larvae in 45 ml water per test pot.

Table 1. Treatments, infestation dates and damage assessment times.

Treatment and application date	Weevil infestation date	Damage assessment dates	
		Greenhouse	Outside
Untreated	1 Jul. 2003	11 Nov. 2003	15 Mar. 2004
	4 Aug. 2003	16 Feb. 2004	23 Mar. 2004
	8 Sept. 2003	1 Mar. 2004	22 Apr. 2004
Metarhizium (3 Jun. 2002)	1 Jul. 2003	11 Nov. 2003	15 Mar. 2004
	4 Aug. 2003	16 Feb. 2004	23 Mar. 2004
	8 Sept. 2003	1 Mar. 2004	22 Apr. 2004
Neem (3 Jun. 2002)	1 Jul. 2003	11 Nov. 2003	15 Mar. 2004
	4 Aug. 2003	16 Feb. 2004	23 Mar. 2004
	8 Sept. 2003	1 Mar. 2004	22 Apr. 2004
Steinernema (7 Oct. 2003)	1 Jul. 2003	11 Nov. 2003	15 Mar. 2004
	4 Aug. 2003	16 Feb. 2004	23 Mar. 2004
	8 Sept. 2003	1 Mar. 2004	22 Apr. 2004

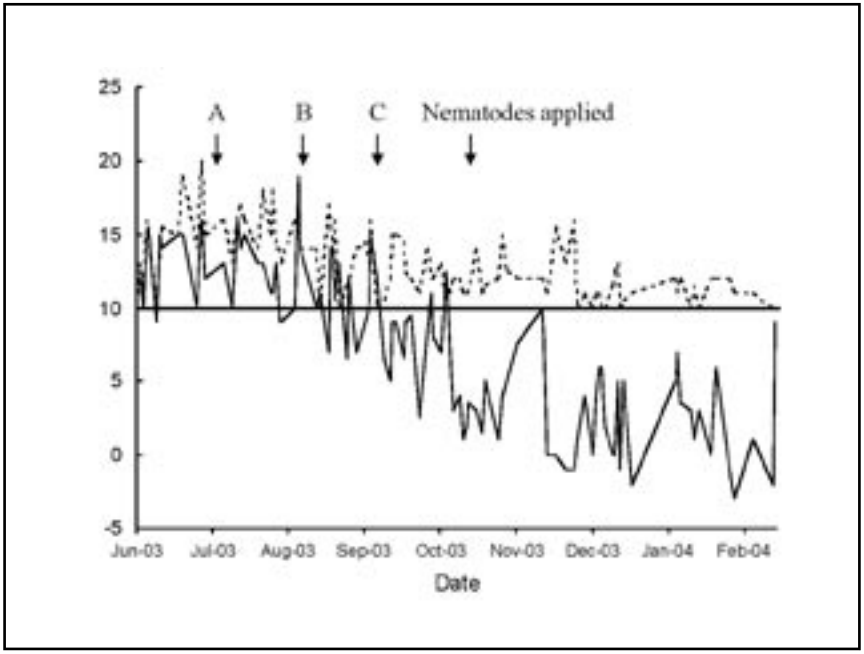

Figure 1. Minimum daily air temperature (°C) recorded in the heated glasshouse and ambient outdoor conditions throughout the study period. Arrows indicate times of manipulated egg infestations (A-July, B-August, and C-September) and the time of application of *S. kraussei* (nematodes applied).

When the untreated plants started to die all plots were destructively assessed for surviving weevil larvae numbers and root damage. Because eggs were added to pots on three separate occasions, this led to three discrete assessment dates (Table 1). At each assessment, we counted the numbers of surviving weevil larvae per pot and scored the plant roots on a qualitative scale of 1 (very severely damaged) to 10 (undamaged) to reflect the relative amount of root system remaining. Many weevil larval counts were zero per pot, so mean treatment counts within egg application dates were compared using the nonparametric Wilcoxon Matched Pairs Test. Root quality scores conformed to normality and were analysed using ANOVA. Treatment means within egg application dates were compared using Duncan's Multiple Range Test.

RESULTS

No natural vine weevil infestations were detected by trap pot monitoring.

Figure 1 shows the minimum daily air temperatures recorded in the glasshouse and outdoors. In the glasshouse, temperatures were maintained at or above 10 °C and the mean minimum temperature recorded throughout the study was 13 °C. Outdoors, from October onwards, minimum temperatures regularly fell below 5 °C and the mean minimum temperature recorded throughout the study was 7.7 °C.

Table 2. Effect of control treatments on weevil infestations (10 eggs per pot applied in either July, August, or September) in the heated glasshouse.

Treatments	Mean no. of live larvae per pot[1] (n = 20) (% control c.f. untreated) July	August	Sept	Overall mean % control
Untreated	5.3[a] (0)	2.5[a] (0)	2.6[a] (0)	0
Metarhizium	0.9[b] (83)	0.3[b] (84)	0.8[b] (70)	79
Neem	5.7[a] (-8)	2.7[a] (-7)	2.4[a] (8)	-2
Steinernema	1.8[c] (66)	0.05[b] (98)	0 [b] (100)	88

[1]Larval numbers within individual columns with a similar superscript are not significantly different ($p<0.05$, Wilcoxon paired test).

The numbers of surviving larvae in the glasshouse crop are presented in Table 2. In untreated pots, establishment and survival of the earliest (July) infestation was substantially greater compared with later infestation dates. *Metarhizium* gave significant control over all three infestations, although the level of control against the last infestation made in September, was less than for earlier infestations. The nematode treatment (*Steinernema*) gave significant, but relatively poor (66%) control of larval numbers resulting from the earliest infestation. In contrast, the nematode treatment was highly effective against the later (August and September) infestations. Neem kernel gave no measurable reduction of larval numbers in the glasshouse crop.

Table 3. Effect of control treatments on weevil infestations (10 eggs per pot applied in either July, August, or September) in outdoor pots.

Treatments	Mean no. of live larvae per pot[1] (n = 20) (% control c.f. untreated) July	August	Sept	Overall mean % control
Untreated	3.8[a] (0)	3.6[a] (0)	2.2[a] (0)	0
Metarhizium	0.15[b] (96)	0.7[b] (81)	0.85[b] (61)	79
Neem	2.8[a] (26)	1.6[b] (56)	0.85[b] (61)	48
Steinernema	1.9[c] (50)	1.2[b] (66)	0.3[b] (86)	67

[1]Larval numbers within individual columns with a similar superscript are not significantly different ($p<0.05$, Wilcoxon paired test).

In the outdoor crop, weevil establishment in the untreated pots was similar following the July and August infestations, but somewhat less following the September infestation (Table 3). The *Metarhizium* formulation performed very well in controlling larval numbers following the July and August infestations but the level of control achieved against the September infestation, although significant, was 61% in contrast to 96% and 81% control of the July and August infestations, respectively. The nematode treatment gave significant, but relatively poor control against the July infestation compared with the level of control of the later infestations. The control performance of the nematode was significantly lower outdoors than in the

glasshouse (98% and 100% indoors, 66% and 86% outdoors). The average minimum air temperature recorded outdoors was 9.2 °C for the 8 days following nematode application, compared with 12.1 °C in the glasshouse.

Neem kernel gave a nonsignificant ($p>0.05$) 26% reduction of weevil larvae numbers from the July infestation. However the treatment gave significant reductions ($p<0.05$) of numbers from the August and September infestations (56% and 61%, respectively). The degree of control of the later infestations was similar to that achieved by the fungus and the nematode.

Table 4. Effect of treatments on root quality.

	Mean Root Quality Scores[1]					
	Glasshouse crop			Outdoor crop		
Treatments	July	Aug.	Sept.	July	Aug.	Sept.
Untreated	3.5[a]	3.9[a]	3.3[a]	1.8[a]	4.2[a]	3.4[a]
Metarhizium	7.8[b]	7.5[b]	7.4[b]	7.5[b]	7.6[b]	7.1[b]
Neem	6.7[b]	5.9[c]	4.6[c]	4.4[b]	7[b]	6.4[b]
Steinernema	4.5[a]	7.1[bc]	7.6[b]	2.7[a]	7.5[b]	6.8[b]

[1]Root scores within individual columns with a similar superscript are not significantly different ($p>0.05$, Duncan's multiple range test), (scale 1 = very severely damaged to 10 = undamaged)

Mean root scores (Table 4) for the untreated glasshouse crop were broadly similar for different infestation dates (mean score range: 3.3 to 3.9). In contrast, damage to the root system of untreated outdoor plants was substantially greater following the July infestation (mean score: 1.8), compared with the later infestations (mean scores: 4.2 and 3.4, respectively). *Metarhizium* treatment resulted in significantly higher root quality compared with untreated pots across all combinations of crop conditions and infestation dates (mean score range: 7.1-7.8). The nematode treatment resulted in significantly improved root quality scores in both the glasshouse and outdoor crops exposed to the August and September infestations but not to the July infestation. Neem kernel treatment significantly improved the root quality of plants in all combinations of crop conditions and infestation dates. However, the scores tended to be lower (sometimes significantly lower, $p<0.05$) than the root quality scores achieved using the best performing biological agent.

DISCUSSION

The *Metarhizium* fungus gave good control of both larval numbers and root damage symptoms across all combinations of temperature conditions and infestation dates. Its effectiveness against even the latest infestation suggested that conidia of the V275 strain of *M. anisopliae* are sufficiently persistent when admixed into a peat-based compost to provide protection of potted crops for at least 3 months.

Steinernema kraussei (Nemasys L) applied as a pot drench after all weevil eggs had hatched also gave good control of both weevil larvae numbers and root damage in both heated and unheated conditions, with the exception of the earliest infestation. In the case of root damage, this might have been expected since a considerable amount of larval feeding following the July infestation would have taken place be-

fore the nematodes were applied. However, the relative lack of effectiveness against the early infestation suggests that at the recommended application rate, it may be less pathogenic to established (larger) larvae compared with younger (smaller) ones. Despite this, *S. kraussei* (Nemasys L) appears to be an effective weevil control in both heated and unheated conditions, but may need to be applied at least twice in the growing season (mid-August and early October) for effective crop protection.

The results obtained using neem kernels are intriguing and warrant further research. In the warmer conditions of the glasshouse, this treatment did not reduce larvae numbers but did significantly reduce root damage. In the lower temperatures on the outdoor crop, neem gave a significant reduction of the mid and late larval infestations, but not the earliest infestation. Taken together, these data suggest that its directly insecticidal effect may be strongly temperature dependant, being more effective at lower temperatures. There are two plausible explanations why this may be so. Firstly, the active insecticidal ingredients in neem (primarily azadirachtin) are susceptible to faster rates of degradation at high temperatures, and therefore may have broken down more rapidly in the heated glasshouse. Secondly, because larvae in the outdoor pots developed more slowly compared with those in the heated glasshouse, they were exposed to the active ingredients for a longer period of time (on average 239 days between infestation and destructive pot assessment compared with only 168 days for indoor pots). This latter effect could also explain why neem failed to significantly reduce the numbers of larvae from the earliest infestation in the outdoor crop, since this group of larvae would have experienced warmer temperatures and developed more rapidly compared with the later infestations.

Despite the somewhat variable and usually lower level of larval kill compared with the tested biological control agents, neem provided a very useful reduction in the amount of root damage inflicted on test plants in both heated and unheated conditions. This result is very likely a reflection of neem's complex chemistry, which in addition to containing insecticidal compounds also contains known anti-feedants, particularly Salannin and Meliantriol. Our data suggest that these components may provide a very useful level of season-long plant protection against vine weevil larvae in both heated and unheated conditions.

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