Horticultural Fellowship Awards

Individual Trial Report - Efficacy of entomopathogenic nematodes against vine weevil

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Industry Representative:	-
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Efficacy of entomopathogenic nematodes against vine weevil

Vine weevil (*Otiorhynchus sulcatus*) remains one of the most serious problems in both soft fruit and nursery stock industries. In order to reduce damage caused by this pest, controls can be targeted against both the larvae in the soil and the adult weevils within the crop. Biological control of vine weevil is preferable to the use of insecticides in Integrated Pest Management (IPM) programmes. Current options for biological control of vine weevil larvae are entomopathogenic nematodes (various species and products) and the entomopathogenic fungus *Metarhizium anisopliae* (Met52).

The aim of this project was to assess the efficacies of four commercially available nematode products Nemasys L® (*Steinernema kraussei*), Nemasys H®, Nematop® and Larvanem® (all *Heterorhabditis bacteriophora)* and the entomopathogenic fungus, Met52® (*Metarhizium anisopliae),* for the control of vine weevil larvae. Efficacy of Met52 combined with each of the nematode products was also determined.

Methods

The experiment consisted of fourteen treatments (Table 1). There were two untreated coir treatments (treatments 1 and 2) and two Met52 coir treatments (treatments 8 and 9).

Table 1 Treatments, rates and methods of application

Trt. num	Product name	Active substance	Supplier	Substrate	Label recommended rate	Equivalent nematodes per litre of compost	Application method
1	Untreated	-	-	Coir	-	-	-
2	Untreated	-	-	Coir	-	-	-
3	Untreated	-	-	Peat	-	-	-
4	Nemasys L	*Steinernema kraussei*	BASF	Coir	25,000 per plant	10,000 per l	Drench
5	Nemasys H	*Heterorhabditis bacteriophora*	BASF	Coir	25,000 per plant	10,000 per l	Drench
6	Nematop	*H. bacteriophora*	e-Nema	Coir	25,000 per plant	10,000 per l	Drench
7	Larvanem	*H. bacteriophora*	Koppert	Coir	1 million per m²	10,000 per l	Drench
8	Met52	*Metarhizium anisopliae* var. *anisopliae*	Novozymes	Coir	500 g/m²	-	Substrate incorporation
9	Met52	*M. anisopliae* var. *anisopliae* F52	Novozymes	Coir	500 g/m²	-	Substrate incorporation
10	Met52	*M. anisopliae* var. *anisopliae* F52	Novozymes	Peat	500 g/m²	-	Substrate incorporation
11	Met52 + Nemasys L	*M. anisopliae* var. *anisopliae* F52 + *S. kraussei*	Novozymes + BASF	Coir	500 g/m² + 25,000 per plant	10,000 per l	Substrate incorporation + Drench
12	Met52 + Nemasys H	*M. anisopliae* var. *anisopliae* F52	Novozymes + BASF	Coir	500 g/m² + 25,000 per plant	10,000 per l	Substrate incorporation + Drench
13	Met52 + Nematop	*M. anisopliae* var. *anisopliae* F52 + *H. bacteriophora*	Novozymes + e-nema	Coir	500 g/m² + 25,000 per plant	10,000 per l	Substrate incorporation + Drench
14	Met52 + Larvanem	*M. anisopliae* var. *anisopliae* F52	Novozymes + Koppert	Coir	500 g/m² + 1,000,000 per m2	10,000 per l	Substrate incorporation + Drench

N.B. All nematode products were recommended on the product leaflet to be applied at 100 ml of water per plant except for Nematop which was recommended at 200ml per plant. Due to concern about the risk of water logging and potential nematode run-off, all treatments were applied at 100ml of water per plant.

Experimental plants and substrate:

Standard one metre-long grow-bags, each containing 25 litres of substrate were obtained from Bulrush Horticulture Ltd. Four grow-bags contained 80% peat and 20% wood fibre, 24 contained coir, four contained Met52 incorporated into 80% peat and 20% wood fibre and 24 contained Met52 incorporated into coir.

Bare-rooted everbearer strawberry plants (cv. Calypso) were purchased from Hargreaves Plants Ltd.

Mealworm test for Met52:

Grow-bags were tested for the presence of Met52 by carrying out a mealworm test (Figure 2).

Figure 2 Mealworm test

On 4 July 2013, substrate samples were taken from each grow-bag and were placed in Petri dishes to which ten mealworms were added. The Petri dish was sealed and kept in an incubator at 25°C 16L:8D. After 7-10 days the presence of mealworms infected with *M. anisopliae* were recorded.

Experiment design

Ten strawberry plants were planted per grow-bag on 20 June. This was later reduced to six plants per bag as explained below. Each grow-bag represented a treatment plot, and there were four replicates per treatment except for treatment 1 and 13 which had eight replicates each.

Treatments were arranged in a randomised block design in a polytunnel at ADAS Boxworth, Cambridgeshire (Figure 3).

Figure 3 Strawberry experiment in grow-bags in a polytunnel at ADAS Boxworth

Irrigation, temperatures and reduction in numbers of plants per bag

Overhead irrigation was used to establish the plants between 20 June and 27 June; those plants which did not establish were replaced. Automatic drip irrigation was used thereafter. Due to a malfunction with the Dosatron between 27 June and 16 July, feed was not delivered correctly. Although this was rectified four plants furthest away from the drippers in each coir bag failed to recover (Figure 4). In order to standardize the number of healthy plants per grow-bag, on 23 August the four plants which failed to establish, and an equivalent four plants in each peat bag, were removed by cutting them just above the crown leaving six plants per bag.

Temperature of the substrate at root depth was measured throughout the experiment using four identical data loggers.

Figure 4 Four of the six strawberry plants failed to establish

Vine weevil egg infestation

On 23 August, 15 vine weevil eggs were washed onto the soil around the stem of each of the six plants (Figure 5). An additional 60 eggs were kept on a damp filter paper in the laboratory and their viability was assessed by recording egg hatch. Fifty-six of the eggs hatched (93%) and the larvae were recovered.

Figure 5 Infesting strawberry plants with vine weevil eggs

Nematode applications

On 5 September, curative applications of each nematode product were applied as per supplier's recommendations to all ten planting holes (Table 1). All ten planting holes were treated as the roots of the removed four plants remained in the substrate and could potentially be attacked by vine weevil larvae. Nematodes were applied with a syringe rather than a sprayer or through the irrigation lines, to ensure dose accuracy to each plant (Figure 6).

Figure 6 Nematode application using syringe

For each product, counts of active nematodes in six sub-samples of the nematode suspension were completed before application. Nematode suspensions were diluted where necessary to make sure all nematodes products were applied at 250 nematodes per ml of water (in the experiment Nemasys L and Nemasys H were diluted slightly to standardize the dose rates, see Table 14). The following method was used:

1) Packs of 50 million nematodes were examined for microbial spoilage. Packs were emptied into a 1L beaker then mixed thoroughly with 500ml of water. The beaker contents were then diluted to 2L in a measuring cylinder and aerated for five minutes.

3) The air supply was turned off and after a few second 80ml (representing the 2 million nematodes needed for the experiment) was transferred into a bucket containing 7,920ml of water.

4) The solution was aerated again and a 5ml pipette was used to take a sample which filled a single counting chamber of a haemocytometer. Using a binocular microscope, counts of live infective juvenile nematodes were then made under each 1ml grid which was repeated six times (a count of 250 nematodes per ml was expected). The numbers of infective juveniles in each

pack were determined by calculating the mean of the six counts multiplied by the dilution factor (200,000).

Assessment of vine weevil larvae and plant vigour

Between 11-14 November, the grow-bags were destructively sampled and the numbers of live vine weevil larvae were recorded by carefully searching through the roots, substrate and breaking open the crown of the plant (Figure 7). Vine weevil larvae were collected from each grow-bag and kept in the laboratory in a Petri dish on damp filter paper to see if further infection developed.

Figure 7 Vine weevil larvae in the crown of the plant and substrate surrounded by red frass produced by the larvae feeding on the roots.

Visual assessments were also made of plant vigour (plant size and foliage health) before destructive sampling using a scale of 1-5 (Figure 8) as follows:

5 - large and healthy

4 - small and healthy

3 - discolored leaves

2 - wilted

1 - dead

5)  4) 

3)  2)  1) 

Figure 8 Vigour score: 5) Large & healthy; 4) small & healthy; 3) discoloured leaves; 2) wilted; 1) dead

Control of other pests and diseases

Regular applications of biological control agents were applied to control other pests e.g. aphids, spider mites and thrips. The biological control agents used included the predatory mite *Neoseiulus (Amblyseius) cucumeris* for thrips control, a mix of six aphid parasitoid species for aphid control and the predatory mite *Phytoseiulus persimilis* for spider mite control.

Statistical analysis

Data on the numbers of live larvae and plant vigour for each treatment were subjected to analysis of variance (ANOVA).

Results and Discussion

Effects of treatment on the number of live larvae

- Analysis of the mean number of live larvae per grow-bag showed a highly significant effect ($F= 15.67$, $p <0.001$) of treatment (Figure 9). As expected the highest numbers of live larvae were recorded in the untreated grow-bags containing either peat (44.3 larvae per bag equivalent to a mean of 7.4 per plant) or coir substrate (39.5 (untreated coir 1) and 44

(untreated coir 2) larvae per bag equivalent to 6.6 and 7.3 larvae per plant respectively). High numbers of larvae (statistically similar numbers to the untreated bags) were also observed in grow-bags containing Met52 in a peat substrate, indicating that this treatment was not effective.

- When considering the effect of nematode treatments alone in coir (without Met52), Nemasys L and Larvanem were the most effective nematode products, reducing the mean number of larvae per bag to 1.5 and 7.5 respectively (equivalent to means of 0.3 and 1.3 per plant respectively). These two treatments were not significantly different.
- Nematop and Nemasys H reduced the numbers of live larvae per bag to 16 and 20.5 respectively (equivalent to means of 2.7 and 3.4 per plant respectively) and were not significantly different from Larvanem. However, these two treatments did not reduce vine weevil larvae as well as Nemasys L.
- When used alone, Met52 in a coir substrate significantly reduced the mean number of larvae per bag when compared with numbers in untreated bags, to 23.5 (Met52 coir 1) and 19.5 (Met52 coir 2) (equivalent to means of 3.9 and 3.3 per plant respectively). However, Met52 in a coir substrate was not as effective as Nemasys L in coir but was not significantly different than Larvanem, Nematop and Nemasys H in coir.
- The difference in the performance between Met52 in peat and coir substrates could possibly have been due to a combination of differences in substrate moisture and nutrition. Between 27 June and 16 July the Dosatron was not delivering feed correctly. As the peat substrate had more nutrients naturally available the plants established better than the plants in the coir substrate. This led to a discrepancy in watering where providing sufficient water for the coir plants resulted in the peat substrates being drier as the larger established plants took up more water. These differences in substrate moisture could have affected the performance of Met52. However, this irrigation discrepancy was rectified quickly and evidence of some control by Met52 in peat would have been expected.
- When each nematode product was combined with Met52 the numbers of live larvae per grow-bag were not significantly lower compared with treatments where the nematode products were used alone. Therefore combining Met52 with nematodes did not result in improved control.

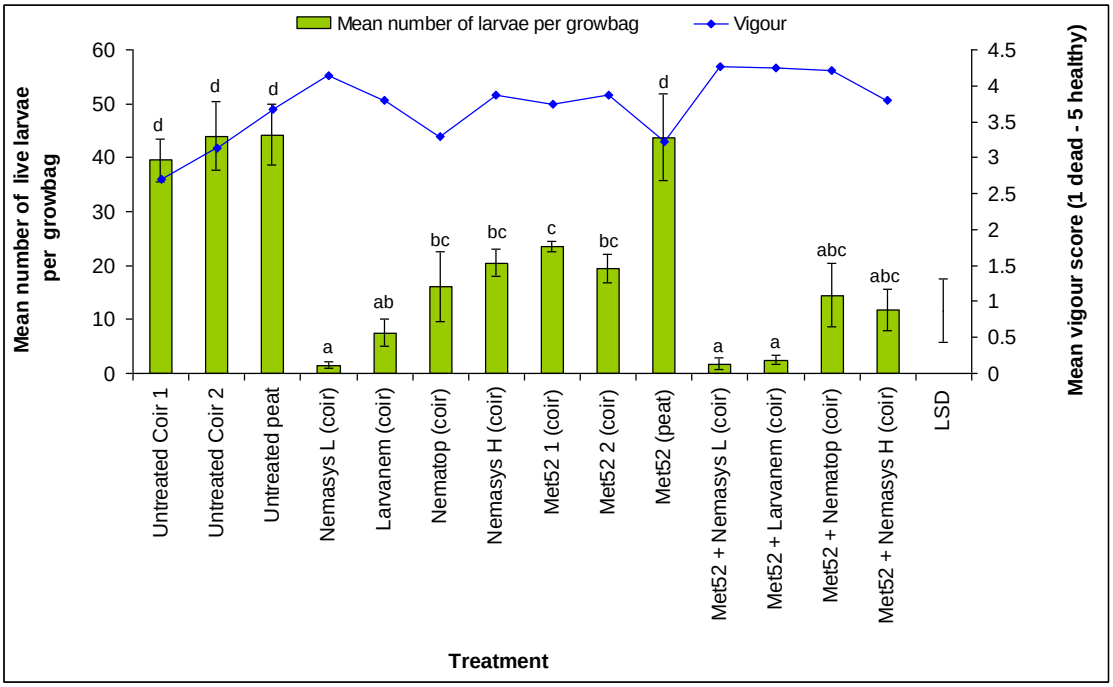

Figure 9 Mean numbers of live vine weevil larvae per grow-bag with standard error of the mean. The least significant difference (LSD) was used to determine any significant differences. Different letters above bars indicate a significant difference. The mean vigour score per grow-bag is also shown.

Effects of treatment on plant vigour

Analysis of the average plant vigour score per plot showed that despite some visual difference in plant vigour (Figure 8) there was no significant effect of treatment on plant vigour observed during the experiment (Figure 10).

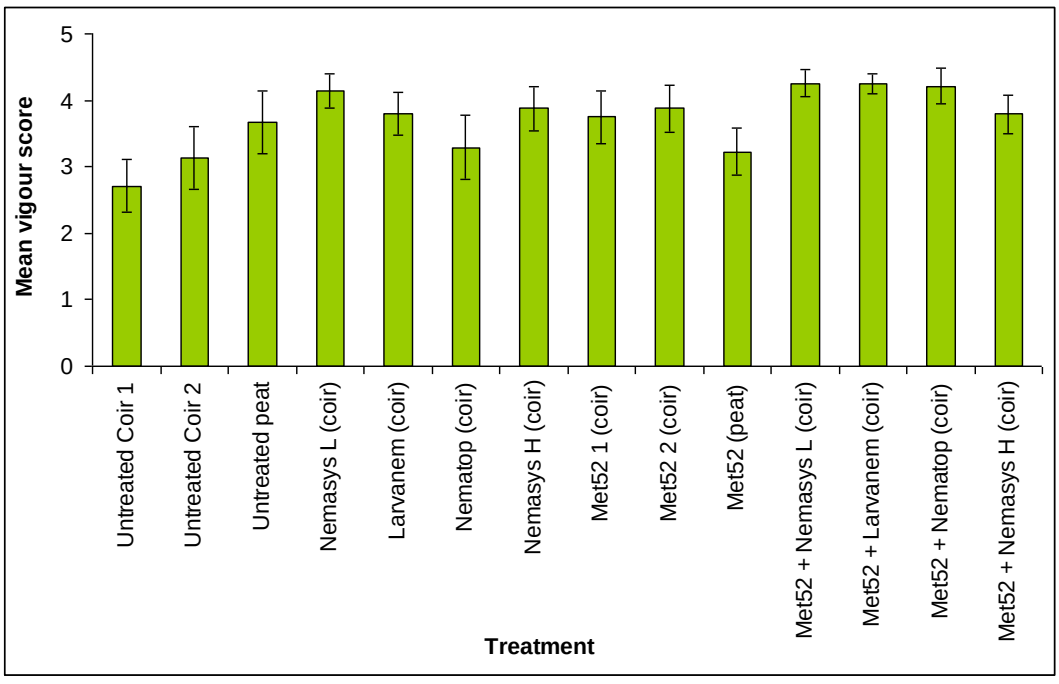

Figure 10 Mean foliage vigour score per plot for all treatments (5 very healthy, 1 dead).

When removing the peat treatments from the analysis to compare the treatments in a coir substrate only, a significant effect (F= 2.11, p= 0.048) of treatment on the vigour scores was observed (Figure 11). Only the combined Met52 and Nemasys L treatment had significantly better vigour than the untreated coir controls. This suggests that the plant vigour scores were similar across all other treatments.

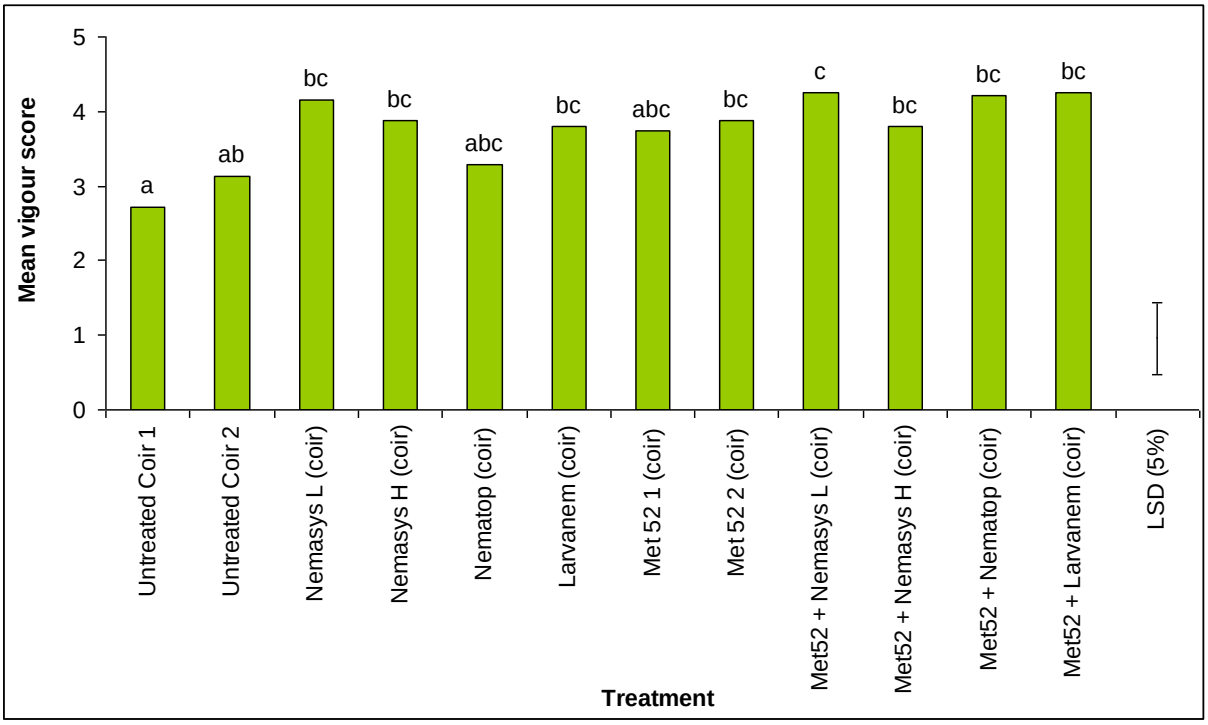

Figure 11 Mean foliage vigour score per plot for coir only treatments (5 very healthy, 1 dead). The least significant difference (LSD) was used to determine any significant differences. Different letters above bars indicate a significant difference.

In the 2012 experiment, no effect on vigour was observed between treatments and untreated controls, indicating that more than a mean of 60 live larvae per grow-bag (equivalent to six larvae per untreated control plant) were required before immediate visible crop damage occurs. It is thought that the damage observed during the 2013 experiment was due to the later planting date meaning the plants were not as well established prior to being infested with vine weevil eggs. Furthermore, Dosatron problems with delivering feed and irrigation early on in the experiment is likely to have also effected establishment. These factors may have made these plants more susceptible to vine weevil feeding damage.

Substrate temperatures

The critical period for substrate temperatures for nematode activity was between the date of nematode application (5 September) and the date assessments were done on surviving vine weevil larvae (11-14 November). During this period, temperatures remained within the activity range of Nemasys L (5-30°C). Minimum substrate temperatures were dropping below 14°C (lower limit of Larvanem) prior to the start of the experiment and average temperatures began to drop below 14°C by 9 September (Figure 12 and Table 13). However, substrate temperature did not appear to adversely affect the level of control provided by Larvanem which gave as effective control as Nemasys L. Nematop and Nemasys H are reported to have a lower minimum temperature (>12°C) than Larvanem (14°C), however these two treatments were less effective than Nemasys L.

The critical period for substrate temperatures for Met52 was between vine weevil egg hatching and the date assessments were done on surviving vine weevil larvae (11-14 November). The activity range of Met52 is reported to be between 15-30°C. During the experimental period average temperatures remained above 15°C until 8 September. Therefore, following egg infestation on 23 August, the larvae (after taking a few days to hatch) would only be exposed to Met52 for up to two weeks before temperatures fell and the activity of Met52 was reduced. Furthermore, minimum temperatures were already falling below 15°C at the beginning of the experiment on 22 June meaning the quality of newly formed spores may have been reduced (see Met52 product leaflet). Lower than optimum temperatures could explain the performance of Met52 in the experiment. As previously discussed, Dosatron problems may also have affected the performance of Met52 in the peat substrate as it was drier than the coir substrate.

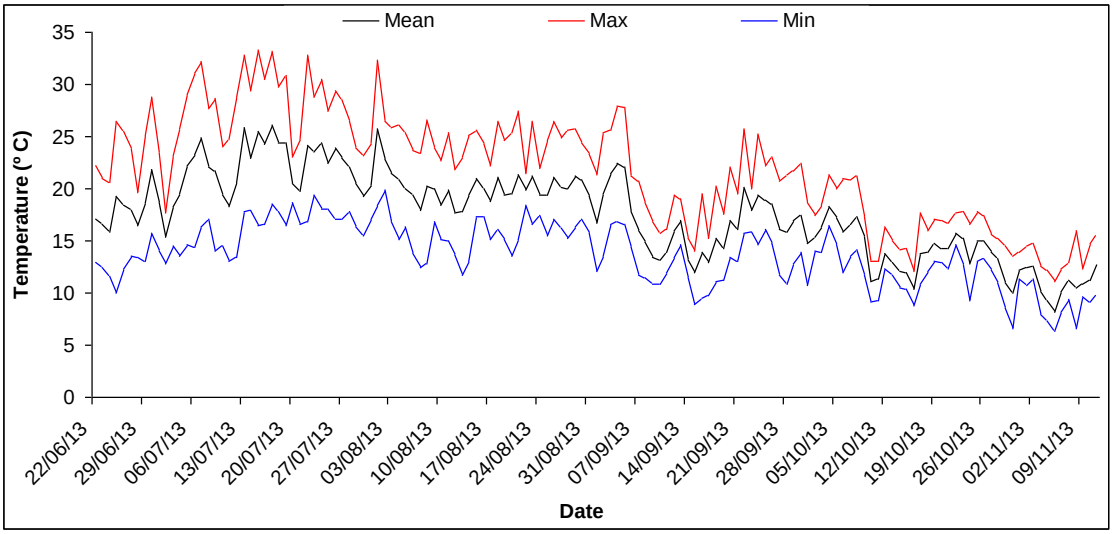

Figure 12 Mean average, maximum and minimum substrate temperatures recorded by four data loggers at root level throughout the experimental period.

Table 13 The optimum temperature range for the nematode products used as per the manufacturer's instructions supplied with the products.

Product	Effective temperature range (°C)
Nemasys L	5-30
Larvanem	14-33
Nematop	>12
Nemasys H	12-30
Met52	15-25

Substrate mealworm test

All the samples taken from the Met52-treated grow-bags had almost 100% of the mealworms infected by *M. anisopliae* after seven days (Table 14). However, of the 28 untreated grow-bags which should not have contained Met52, seven of the grow-bags contained a few infected mealworms after seven days. By day 11, samples from a further five untreated grow-bags contained low numbers of infected mealworms. This result indicated that the 'untreated' grow-bags contained a low level of Met52, however this did not seem to affect the experimental results. It is thought that mealworms are more susceptible to infection by Met52 than vine weevil larvae.

Table 14 Results from the substrate mealworm test to determine the presence or absence of Met52 in each grow-bag used in the experiment

Treatment	Infection after 7 days	Infection after 11 days	Treatment	Infection after 7 days	Infection after 11 days
Met52 + Larvanem (coir)	Yes		Nemasys H (coir)	No	
Met52 + Nematop (coir)	Yes		Untreated (coir)	No	Yes
Met52 + Nemasys H (coir)	Yes		Met52 (Peat)	Yes	
Nemasys H (coir)	No	Yes	Nemasys L (coir)	No	
Untreated (coir)	No		Larvanem (coir)	Yes	
Met52 (coir)	Yes		Met52 (coir)	Yes	
Met52 (peat)	Yes		Met52 + Larvanem (coir)	Yes	
Nemasys L (coir)	No	Yes	Nematop (coir)	No	
Untreated (coir)	No		Met52 (coir)	Yes	
Met52 (coir)	Yes		Untreated (coir)	No	
Met52 + Nemasys L (coir)	Yes		Met52 + Nemasys L (coir)	Yes	
Nematop (coir)	No		Met52 + Nemasys H (coir)	Yes	
Untreated (peat)	No		Untreated (peat)	No	
Larvanem (coir)	Yes		Met52+ Nematop (coir)	Yes	
Untreated (peat)	Yes		Met52 + Nemasys L (coir)	Yes	
Met52 + Nemasys L (coir)	Yes		Met52 + Nemasys H (coir)	Yes	
Nematop (coir)	Yes		Met52 (peat)	Yes	
Met52 + Nematop (coir)	Yes		Nemasys H (coir)	Yes	
Met52 + Larvanem (coir)	Yes		Met52 + Larvanem (coir)	Yes	
Met52 (peat)	Yes		Met52 + Nematop (coir)	Yes	
Met52 +Nemasys H (coir)	Yes		Met52 (coir)	Yes	
Nemasys H (coir)	No		Met52 (coir)	Yes	
Met52 (coir)	Yes		Nemasys L (coir)	No	
Nemasys L (coir)	No		Nematop (coir)	No	Yes
Untreated (coir)	Yes		Larvanem (coir)	No	Yes
Untreated (coir)	No		Untreated (peat)	Yes	
Larvanem (coir)	No		Untreated (coir)	No	
Met52 (coir)	Yes		Untreated (coir)	No	

Counts of nematodes

Active counts of the nematodes within each product were estimated (Table 15). All packs of nematodes claimed to contain 50 million active juveniles per pack. Nemasys L contained the most nematodes per pack, followed by Nemasys H, with both above the claimed 50 million content, while Nematop and Larvanem contained slightly less. Means calculated were based on 6x 1 ml sub-samples.

An analysis of variance based on the six sub-samples showed that Nemasys L and Nemasys H had similar numbers of nematodes per ml of water but had significantly more than Larvanem and Nematop. Larvanem and Nematop had similar numbers of nematodes per ml of water. Nemasys L and Nemasys H numbers were adjusted to standardise numbers of nematodes delivered per plant to 250 per ml (see methods). Despite having slightly lower numbers of nematodes per ml than Nemasys L (Table 15), Larvanem was not significantly different compared with Nemasys L in reducing the number of vine weevil larvae.

Table 15 Mean nematodes counts per 1ml sub-sample for each product. The least significant difference (LSD) was used to determine any significant differences. Different letters next to the mean indicate a significant difference.

Products	Mean numbers per 1ml sub-sample	Estimated numbers per pack
Nemasys L	304.8 a	60,960,000
Nemasys H	286.3 a	57,260,000
Larvanem	235.7 b	47,140,000
Nematop	234 b	46,800,000

Conclusions

- All the nematode products and Met52 in a coir substrate significantly reduced the numbers of live vine weevil larvae in substrate-grown strawberry when compared with untreated controls.
- Met52 in coir was as effective as Larvanem, Nematop and Nemasys H but less effective than Nemasys L.
- Met52 in a peat substrate was ineffective.
- Nemasys L (*Steinernema kraussei)* and Larvanem (*Heterorhabditis bacteriophora)* were the best performing products and were not significantly different in their reduction of mean numbers of live vine weevil larvae. Nematop and Nemasys H (both *Heterorhabditis bacteriophora)* were not significantly different than Larvanem but did not reduce the mean number of vine weevil larvae as well as Nemasys L.

- Combining nematodes with Met52 did not significantly improve the control of vine weevil larvae compared to when using nematodes alone.
- Vine weevil larvae feeding damage and plant vigour was similar across all treatments when analysing peat and coir substrates together. When analysing coir treatments alone the combined Met52 and Nemasys L treatment had significantly better vigour than the untreated coir controls.

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