Project title:	Managing ornamental plants sustainably (MOPS)
Project number:	CP 124
Work package title:	Ornamentals: Novel pesticides and biopesticides for control of vine weevil (*Otiorhynchus sulcatus*) larvae on *Fuchsia erecta*
Work package leader:	Dr Gemma Hough, ADAS
Report:	Annual report, December 2015
Previous report:	None
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Location of work:	ADAS
Date work commenced:	01 May 2014
Date work completed (or expected completion date):	11 November 2014

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Gemma Hough
Entomologist
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Signature Date 30 January 2015

Report authorised by:

John Atwood
Project Leader
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Signature Date 30 January, 2015

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GROWER SUMMARY

Headline

Currently available biological and conventional chemical control measures for controlling vine weevil larvae on ornamentals were shown to be effective. New biopesticides currently in development have also been found to be effective.

Background and expected deliverables

Vine weevil (*Otiorhynchus sulcatus*) is one of the most serious and persistent pest problems in UK hardy nursery stock and it can also damage some ornamental pot plants. Favoured ornamental crop hosts include *Bergenia*, *Cyclamen*, *Euonymus*, *Primula* and *Taxus*. Damage is caused both by the adults, which feed on foliage (resulting in characteristic leaf notching), and the larvae, which feed on plant roots, stem bases and tubers. The adult leaf notching does not severely affect the health of the plant but can make ornamental plants unmarketable or significantly reduce crop value. Damage caused by larvae is serious on both ornamental and soft fruit crops and may result in reduced yields, plant growth and, if damage is severe, may kill the plant.

Conventional chemical insecticides available to growers of container-grown ornamentals include the use of the neonicotinoid insecticides imidacloprid (e.g. Imidasect 5GR) or thiacloprid (Exemptor) in the growing media and the use of foliar sprays for the control of adults. The current restrictions on the use of neonicotinoid insecticides limit the use of imidacloprid to glasshouse crops and non-flowering ornamentals.

Current biological control options against larvae include various species of entomopathogenic nematodes (epns) applied as drenches to the substrate or through drip irrigation systems and the entomopathogenic fungus *Metarhizium anisopliae* (Met52 granular), supplied ready-mixed in substrate or as a product for mixing with the substrate or substrate by the grower. Although epns can give very effective control of vine weevil larvae many growers are unsure of which epn product to use and how best to apply it in their own crop and situation. Similarly, Met52 granular has given variable control of vine weevil in both HNS and soft fruit crops and growers need reliable, impartial information on efficacy and best-practice use in different production systems and environmental conditions.

This trial aimed to evaluate the efficacy of selected pesticides, biopesticides and biological control agents for control of vine weevil larvae on *Fuchsia erecta* in a polytunnel and to evaluate crop safety.

Summary of the work and main conclusions

Materials and methods

The trial was carried out at ADAS, Boxworth between April (timed to coincide with when *F. erecta* cuttings are being taken commercially) and November 2014 (when vine weevil eggs have hatched and larvae are developing) in a 20m long polytunnel.

There were 10 treatments. Each treatment was replicated six times and each treatment consisted of ten plants in a plot (60 plants per treatment).

Treatments were either substrate-incorporated or applied as drenches (Table 1). Substrate-incorporated products were included throughout the plant propagation process (i.e. plugs and final pots) while drenches were applied either preventatively (drench applied 24 hours prior to egg infestation) or curatively (drench applied in September when larvae developing).

Table 1 Treatments used in trial including active ingredients, application timing, rate and drench volume per pot

Product name or MOPS code number	Active ingredient	Application timing
1. Water (negative control)	-	Curative drench in September
2. Exemptor [standard] (positive control)	thiacloprid (conventional)	Substrate incorporation
3. Calypso	thiacloprid (conventional)	Curative drench in September
4. Nemasys L	*Steinernema kraussei* (biological)	Curative drench in September
5. Larvanem	*Heterorhabditis bacteriophora* (biological)	Curative drench in September

6. SuperNemos	*Steinernema feltiae, Steinernema carpocapsae* & *Heterorhabditis* spp. (biological)	Curative drench in September
7. 205	(biopesticide)	Preventative treatment - Drench applied 24 hours prior to infestation with eggs.
8. Met52 granular	*Metarhizium anisopliae* var. *anisopliae* strain F52 (biopesticide)	Substrate incorporation
9. 179	(biopesticide)	Preventative treatment - Drench applied 24 hours prior to infestation with eggs
10. 130	(biopesticide)	Preventative treatment - Drench applied 24 hours prior to infestation with eggs

Cuttings of *Fuchsia erecta* were taken on 1 May at Darby Nursery Stock, Thetford. Cuttings were planted in plugs (77 plug holes per tray) in a propagation mix containing 55% coir, 15% fine grade bark, 30 % Perlite, 1.5kg/cu. m of Osmocote mini (5-6 m) and 200g/cu. m MicroMax Premium TE. For treatment 8 and 2, Met52 granular and Exemptor respectively were incorporated into the propagation mix used for the plugs.

Plugs were transported to ADAS Boxworth on 3 July and potted up into 2 L pots on 4 July using a herbaceous mix (70% peat, 30% bark). Cuttings planted in plugs treated with either Met52 granular or Exemptor were potted up into the same treated substrate.

Following potting up plants were arranged in a randomised design (Figure 1). The trial was surrounded by a border of duct tape coated with Eco Tack® glue to stop any resident naturally-occurring vine weevils on site from infesting the trial.

Figure 1 **Trial on *Fuchsia erecta* which took place in a polytunnel at ADAS, Boxworth.**

All treatments were applied once. Treatments 2 and 8 are substrate-incorporated and were applied preventatively in the plugs for the *F. erecta* cuttings and at potting on.

On 31 July the preventative drench treatments were applied 24 hours prior to egg infestation. On 16 September curative drench treatments were applied (see Table 1 for application timings). Drenches were applied using a small watering can (without the rosette) trying to cover as much of the growing media surface as possible. Drench applications were made to already moist soil to ensure the drench was absorbed.

On 1 August all plant were artificially infested with 15 brown (embryonated) vine weevil eggs per plant. Plants were infested with eggs by removing a small area of the topmost substrate layer next to each plant close to the roots. The eggs were then washed off with water from a piece of filter paper onto the substrate and re-covered lightly with moderately moist substrate. Egg viability was 85% (20 extra eggs were monitored in the laboratory for hatching).

The trial was assessed between 3 and 11 November. In each of the plots five out of 10 plants were assessed (30 pots per treatment). The number of live vine weevil larvae per plant were recorded along with vine weevil weight, plant vigour and signs of phytotoxicity.

Results

All the products tested, except for Code 179, were effective and significantly reduced the number of live vine weevil larvae compared to the water control (Figure 1). The best performing products were Exemptor, Calypso, Code 205 and the three nematode products (Nemasys L, Larvanem, and SuperNemos).

Exemptor, Calypso and Code 205 reduced the mean number of vine weevil larvae per pot to 0, 0.033 and 0 respectively compared to the control which had a mean of 5.1 vine weevil larvae per

pot. The three entomopathogenic nematodes, Nemasys L, Larvanem and SuperNemos, reduced the mean number of vine weevil larvae to 0.67, 0.87 and 1.13 respectively per pot and were equally effective as each other.

Met52 granular and Code 130 reduced the number of vine weevil larvae to 2.23 and 1.7 larvae respectively per plant.

Figure 1 Mean number of live vine weevil larvae per pot (treatments with the same letters are not significantly different).

No effects of treatment were observed on plant vigour or larval weight. There was an effect of treatment observed on root weight and root vigour with a trend for the plants treated with the best performing products to have a higher root vigour and root weight compared to the control. Root weight was significantly higher than the control (mean root weight of 9.9g) when treated with Exemptor (13.0g), Calypso (15.6g) and Larvanem (13.1g). Mean root vigour scores were significantly higher than the control (mean score of 2.9) when plants were treated with Exemptor (3.4), Calypso (3.7), Nemasys L (3.5), SuperNemos (3.4) and Code 205 (3.6). Code 179 had significantly lower root weight and root vigour score compared to the control plants.

Conclusions

The trial confirmed that there are effective control measures currently available to growers for controlling vine weevil larvae. While the trial has shown that growers have the option of using conventional pesticides either preventatively (Exemptor) or curatively (Calypso), it has also confirmed that, when applied correctly, entomopathogenic nematodes can achieve a similar level of control while being safer to the operator, the environment and other beneficial insects. The

temperatures during this trial did not appear to negatively affect the activity of the nematodes (full details given in science section and Appendix B).

Met52 granular was also observed to reduce the number of vine weevil larvae and is currently the only approved biopesticide which can be used preventatively in ornamental plant production. Met52 granular requires temperatures between 15°C - 30°C to infect its host and the temperature data collected during this trial suggests that substrate temperatures were suitable for Met52 granular activity for 504 hours (21 days) during August. Air temperatures indicated that average daily temperatures were suitable until the end of September. It is likely that higher temperatures would have increased the level of control recorded for Met52 Granular.

The trial also identified two additional biopesticides currently in development which were effective in controlling vine weevil larvae, particularly Code 205 which gave 100% control. Different timings of application should be investigated for Code 130 and 179 as this could improve their efficacy. Root weight and root vigour scores were generally higher than the control for the most effective treatments due to the reduction of vine weevil larvae feeding on the roots.

There was no evidence that any of the products tested in the study had a repellent or feeding deterrent effect as there were no observed differences in the average weight of the larvae recovered between treatments. If this was the case it would be expected that larvae from the treatments would weigh less than those from the controls.

While the products used in this trial were effective using our drenching methods on *F. erecta*, growers may see differences in efficacy using different crops (particularly those with dense roots or fleshy crowns) and application methods

Action Points

- If wishing to use a preventative treatment in the substrate of the plugs and substrate used for potting up, use Exemptor or the biopesticide Met52 granular. Exemptor was more effective than Met52 granular in this trial, giving 100% control of live vine weevil larvae.
- Use either entomopathogenic nematodes or Calypso curatively (EAMU (2014/2153) for the use of Calypso as a drench on protected ornamentals).
- When using biopesticides read the label carefully as the application method and timing of application requires more consideration compared to conventional pesticides.
- Biopesticides and biological control agents do not always provide 100% control and need to be used within an Integrated Pest Management programme.

Science Section

Introduction

Vine weevil (*Otiorhynchus sulcatus*) is one of the most serious and persistent pest problems in UK hardy nursery stock and it can also damage some ornamental pot plants. Favoured ornamental crop hosts include *Bergenia*, *Cyclamen*, *Euonymus*, *Primula* and *Taxus*. Damage is caused both by the adults, which feed on foliage (resulting in characteristic leaf notching), and the larvae, which feed on plant roots, stem bases and tubers. The adult leaf notching does not severely affect the health of the plant but can make ornamental plants unmarketable or significantly reduce crop value. Damage caused by larvae is serious on both ornamental and soft fruit crops and may result in reduced yields, plant growth and, if damage is severe, may kill the plant.

Conventional chemical insecticides available to growers of container-grown ornamentals include the use of the neonicotinoid insecticides imidacloprid (e.g. Imidasect 5GR) or thiacloprid (Exemptor) in the growing media and the use of foliar sprays for the control of adults. The current restrictions on the use of neonicotinoid insecticides limit the use of imidacloprid to glasshouse crops and non-flowering ornamentals.

Current biological control options against larvae include various species of entomopathogenic nematodes (epns) applied as drenches to the substrate or through drip irrigation systems and the entomopathogenic fungus *Metarhizium anisopliae* (Met52 granular), supplied ready-mixed in substrate or as a product for mixing with the substrate or substrate by the grower. Although epns can give very effective control of vine weevil larvae many growers are unsure of which epn product to use and how best to apply it in their own crop and situation. Similarly, Met52 granular has given variable control of vine weevil in both HNS and soft fruit crops and growers need reliable, impartial information on efficacy and best-practice use in different production systems and environmental conditions.

Materials and methods

The trial consisted of 10 treatments and each treatment had six replicates. Each replicate was made up of 10 plants (60 plants per treatment) (Table 1).

Cuttings of *Fuchsia erecta* were taken on 1 May at Darby Nursery Stock, Thetford. Cuttings were planted in plugs (77 plug holes per tray) in a propagation mix containing 55% coir, 15% fine grade bark, 30 % Perlite, 1.5kg/cu. m of Osmocote mini (5-6 m) and 200g/cu. m MicroMax Premium TE (Appendix F, Figure 2 and 3). For treatment 8 and 2, Met52 and Exemptor respectively were incorporated into the propagation mix used for the plugs.

Plugs were transported to ADAS Boxworth on 3 July and potted up into 2 L pots on 4 July using a herbaceous mix (70% peat, 30% bark). Substrate-incorporated treatments (treatments 2 and 8) were mixed at Darby Nursery Stock into the herbaceous mix and transported to ADAS, Boxworth in labelled 80L bags for use in the final potting.

Cuttings planted in plugs treated with either Met52 granular or Exemptor were potted up into the same treated substrate. The best cuttings with even root and foliage vigour were selected for potting up. Potting up took place the day after the cuttings were collected.

Following potting up plants were arranged in a randomised design. The trial (Appendix F, Figure 1) was surrounded by a border of duct tape coated with Eco Tack® glue to stop any resident naturally-occurring vine weevils on site from infesting the trial.

Site and crop details

Table 1. Test site and plot design information

Test location:	
County	Cambridgeshire
Postcode	CB234NN
Soil type/growing medium	Plugs: Propagation mix containing 55% coir, 15% fine grade bark, 30 % Perlite, 1.5kg/cu. m of Osmocote mini (5-6 m) and 200g/cu. m MicroMax Premium TE. Potting up: herbaceous mix (70% peat, 30% bark).
Nutrition	As above
Crop	*Fuchsia*
Cultivar	*Fuchsia erecta*
Glasshouse* or Field	Polytunnel
Date of planting/potting	Plug plants potted up on 4 July 2014
Pot size	2 L
Number of plants per plot	10
Trial design (layout in Appendix C)	Randomised block
Number of replicates	6
Plot size w (m), l (m), total area (m²)	10x 2L pots
Method of statistical analysis	ANOVA

*Temperature and relative humidity settings are given in Appendix B

Treatment details

Table 2. Detail of products tested

MOPS code number	Active ingredient(s)	Manufacturer	Batch number	a. i concentration	Formulation type
1. Water (negative control)	-	-	-	-	-
2. Exemptor [standard) (positive control)	thiacloprid	Everris	140414A	10% w/w	GR
3. Calypso	thiacloprid	Bayer	EM4LO11179	480g/l	Flowable insecticide
4. Nemasys L	*Steinernema kraussei*	BASF	SK26.4A (expiry 29/9/14)	50 million	n/a
5. Larvanem	*Heterorhabditis bacteriophora*	Koppert	14 HB3334 (expiry 7/10/14)	50 million	n/a
6. SuperNemos	*Steinernema feltiae, Steinernema carpocapsae* & *Heterorhabditis* spp.	Flowering Plants Ltd	S729K0-0608 (expiry 1/10/14)	50 million	n/a
7. 205	*Metarhizium anisopliae* var. *anisopliae* strain F52	Novozymes	1420NFEC16	11% w/w	EC
8. Met52 granular	*Metarhizium anisopliae* var. *anisopliae* strain F52	Novozymes	-	2% w/w	GR
9. 179	Orange Oil	Oro Agri	7579	60g/L	SL
10. 130	Azadirachtin A	Trifolio-M	140414A	1%	EC

Table 3. Treatments

Product name or MOPS code number	Application timing	Product rate	Drench volume per pot (ml)
1. Water (negative control)	Curative drench in September - A1	-	200ml
2. Exemptor [standard] (positive control)	Substrate incorporation - A2	400g/m^3 of growing media	n/a
3. Calypso	Curative drench in September - A1	83ml product per m^3 (40 g active substance per m^3). 100L per 1000L compost.	200ml
4. Nemasys L	Curative drench in September - A1	500,000 nematodes per m^2 at 4 L/m^2 application volume 10,121 nematodes per pot in 81 ml of water	81ml
5. Larvanem	Curative drench in September - A1	500,000 nematodes per m^2 10,000 nematodes per 2L pot in 50ml of water	50ml

6. SuperNemos	Curative drench in September - A1	500,000 nematodes per m^2 at 5 L/m^2 application volume 10,000 nematodes per 2L pot in 50ml of water	50ml
7. 205	Preventative treatment A3 - Drench applied 24 hours prior to infestation with eggs.	1.78 litres (62.5 fluid oz). / 379 litres	200ml
8. Met52 granular	Substrate incorporation - A2	0.5 kg/m^3 of growing media	N/A
9. 179	Preventative treatment A3 - Drench applied 24 hours prior to infestation with eggs	0.8% (800 ml in 100L)	200ml
10. 130	Preventative treatment A3 - Drench applied 24 hours prior to infestation with eggs	0.5% - (500 ml in 100L)	200ml

Application timing	
A1	Curative treatment – 16 September 2014
A2	Substrate incorporation – 1 May 2014 in plugs and 4 July for potting up
A3	Preventative treatment – 31 July 2014

Table 4. Application details

Application No.	**A1**	**A2**	**A3**
Application date	16 September 2014	1 May 2014 and 4 July 2014	31 July 2014
Time of day	Afternoon	Morning	Afternoon 13:15 - 15:30

Application method	Drench	Substrate incorporation	Drench
Temperature of air – max/min (°C)	27.5°C /13°C (polytunnel data logger)	1 May- unavailable offsite 4 July- 25.05°C /15.97°C (Boxworth weather station)	34.5°C /15.5°C (polytunnel data logger)
Relative humidity (%) –	73.2% day average (polytunnel data logger)	1 May- unavailable offsite 4 July- 75.03% (day average) (Boxworth weather station)	35.5% (13:15) 43.5% (15:30) (polytunnel data logger)
Cloud cover (%)	n/a in polytunnel	n/a in polytunnel	n/a in polytunnel
Crop growth stage	Flowering	Cuttings	Flowering
Crop comments	-	-	-
Other*:	-	-	-

*Includes soil temperature and moisture details where relevant

All treatments were applied once. Treatments 2 and 8 are substrate-incorporated and were applied preventatively in the plugs for the *F. erecta* cuttings and at potting on.

Products were used at the recommended rates (Table 2 and 3). For treatment, 1, 3, 7, 9 and 10, drenches were applied at 200ml (within the recommended water volume of each product) per pot using a small watering can (without the rosette) trying to cover as much of the growing media surface as possible. This volume was decided as drench treatments are usually be applied at 10% pot volume (10% of 2L = 200ml). Drench applications were made to already moist soil to ensure the drench was absorbed. Table 4 shows the weather conditions during each application.

Nematode products (treatments 4, 5 and 6) were applied by weighing a 50 million pack and taking a subsample weight representing the required amount of nematodes. The nematodes were then added to the required amount of tap water and mixed. To confirm the solution contained the right

number of nematodes, three 1ml samples were taken from the nematode solution for each treatment and the number of nematodes were counted using a 1 ml counting chamber (Table 5). Drenches were applied using a small watering can (without the rosette) trying to cover as much of the growing media surface as possible. Applications were made to already moist soil to ensure the drench was absorbed. All other manufacturers' recommendations regarding application were followed.

Table 5. Predicted and actual numbers of nematodes recorded per 1ml sample

Treatment	Rate (as per label)	Expected number of nematode per ml based on rate	Average number of nematodes recorded per 1ml
Nemasys L	10,121 nematodes per pot in 81ml of water per 2L pot	125	129
Larvanem	10,000 nematodes per 2L pot in 50ml of water per 2L pot	200	198.3
SuperNemos	10,000 nematodes per 2L pot in 50ml of water per 2L pot	200	208

Target pest(s)

Table 6. Target pest(s)

Common name	Scientific Name	Infestation level pre-application
Vine weevil	*Otiorhynchus sulcatus*	15 vine weevil eggs per plant (9000 eggs in total)

Vine weevil adults (Table 6) were collected from commercial HNS/ strawberry crops in May-July and maintained in plastic boxes on damp tissue in a controlled environment room (21°C). The weevils were fed with strawberry or yew leaves.

Once a week, eggs were collected from the culture and transferred (using a fine paintbrush) onto damp filter paper in a petri dish labelled with the date and stored in the fridge until needed for plant infestation (eggs would start to hatch after 10 days in the fridge).

All plants were artificially infested with a total of 15 brown (embryonated) vine weevil eggs per plant on 1 August 2014. One week prior to infestation, eggs were collected from the ADAS vine weevil culture and transferred to pieces of damp filter paper (600 pieces of damp filter paper with 15 eggs

on each). A small area of the topmost substrate layer next to each plant was removed and 15 eggs were washed onto the substrate. The eggs were then recovered lightly with moderately moist substrate. Eggs were applied to all the control treatments first.

Percentage egg viability was determined by collecting additional eggs from the culture and determining how many hatched in the laboratory. Egg viability was 85% (20 eggs were monitored for hatching).

Assessments

The trial was assessed between 3 and 11 November. In each of the plots, five out of 10 plants were assessed (300 pots in total - 30 pots per treatment) (Appendix F, Figure 4). As a significant result was obtained from assessing five out of the ten plants per plot the remaining five plants per plot were not assessed as assessments were very time-consuming. The number of live vine weevil larvae per plant were recorded along with vine weevil weight, plant vigour, root vigour, root weight and signs of phytotoxicity (Table 7).

Table 7. Assessments

Assessment No.	Date	Growth stage	Timing of assessment relative to last application	Assessment type(s) (e.g. no./% LAI/crop safety)
1	3-11 November 2014	Flowering	95 days after preventative treatments 48 days after curative treatments	Number of live larvae per pot
2	3-11 November 2014	Flowering	95 days after preventative treatments 48 days after curative treatments	Mean larval weight per pot
3	3-11 November 2014	Flowering	95 days after preventative treatments 48 days after curative treatments	Plant vigour
4	3-11 November 2014	Flowering	95 days after preventative treatments 48 days after curative treatments	Root vigour
5	3-11 November 2014	Flowering	95 days after preventative treatments 48 days after curative treatments	Root weight
6	3-11 November	Flowering	95 days after preventative treatments	Phytotoxicity

	2014		48 days after curative treatments	

Statistical analysis

Data were analysed using analysis of variance (ANOVA) to calculate means, variance, LSDs ($p<0.05$) using Genstat 14th Edition. The statistics used were approved by the ADAS statistician Chris Dyer.

Results

Control of vine weevil larvae

All the products tested, except for Code 179, were effective and significantly reduced the number of live vine weevil larvae compared to the water control (Table 8, Figure 1). The best performing products were Exemptor, Calypso, Code 205 and the three nematode products (Nemasys L, Larvanem, and SuperNemos).

Exemptor, Calypso and Code 205 reduced the mean number of vine weevil larvae per pot to 0, 0.033 and 0 respectively compared to the control which had a mean of 5.1 vine weevil larvae per pot. The three entomopathogenic nematodes, Nemasys L, Larvanem and SuperNemos, reduced the mean number of vine weevil larvae to 0.67, 0.87 and 1.13 respectively per pot and were equally effective as each other.

Met52 granular and Code 130 reduced the number of vine weevil larvae to 2.23 and 1.7 larvae respectively per plant.

Table 8. Effect of treatments on vine weevil larvae. Data presented as live vine weevil larvae per plant. Numbers in a column followed by the same letter are not significantly different at P <0.05 based on Duncan multiple comparisons (which can differ from the Fisher's LSD test).

Product name or MOPS code	**Mean number of live vine weevil larvae per pot**
1. Water (negative control)	5.10 [d]
2. Exemptor [standard] (positive control)	0 [a]
3. Calypso	0.03 [a]
4. Nemasys L	0.67 [ab]

5. Larvanem	0.87 [ab]
6. SuperNemos	1.13 [abc]
7. 205	0 [a]
8. Met52 Granular	2.23 [c]
9. 179	6.27 [e]
10. 130	1.70 [bc]
F value (df)	30.24 (45) (P = <.001)
LSD	1.135

Figure 1 Mean number of live vine weevil larvae per pot (treatments with the same letters are not significantly different).

Larval weight

Larvae from each pot were weighed together and the average weight per larvae was calculated. For the analysis, treatments 2 and 7 were removed from the analysis as there were no surviving larvae to provide a mean weight. Due to the number of missing values present when analysing the mean larval weight per pot (due to some pots having no larvae to provide a weight), a mean weight

per plot was calculated and analysed. No effect of the treatment was observed on mean larval weight (Table 9, Figure 2). The mean larval weight for the untreated larvae was 0.04 grams.

Table 9. Effect of treatments on larval weight. Data presented as mean larval weight per plot.

Product name or MOPS code	**Mean larval weight per plot (g)**
1. Water (negative control)	0.04
2. Exemptor [standard] (positive control)	(excluded from analysis)
3. Calypso	0.04
4. Nemasys L	0.04
5. Larvanem	0.05
6. SuperNemos	0.04
7. 205	(excluded from analysis)
8. Met52 Granular	0.04
9. 179	0.04
10. 130	0.03
F value (df)	0.90 (28) (P = 0.518 n.s.)

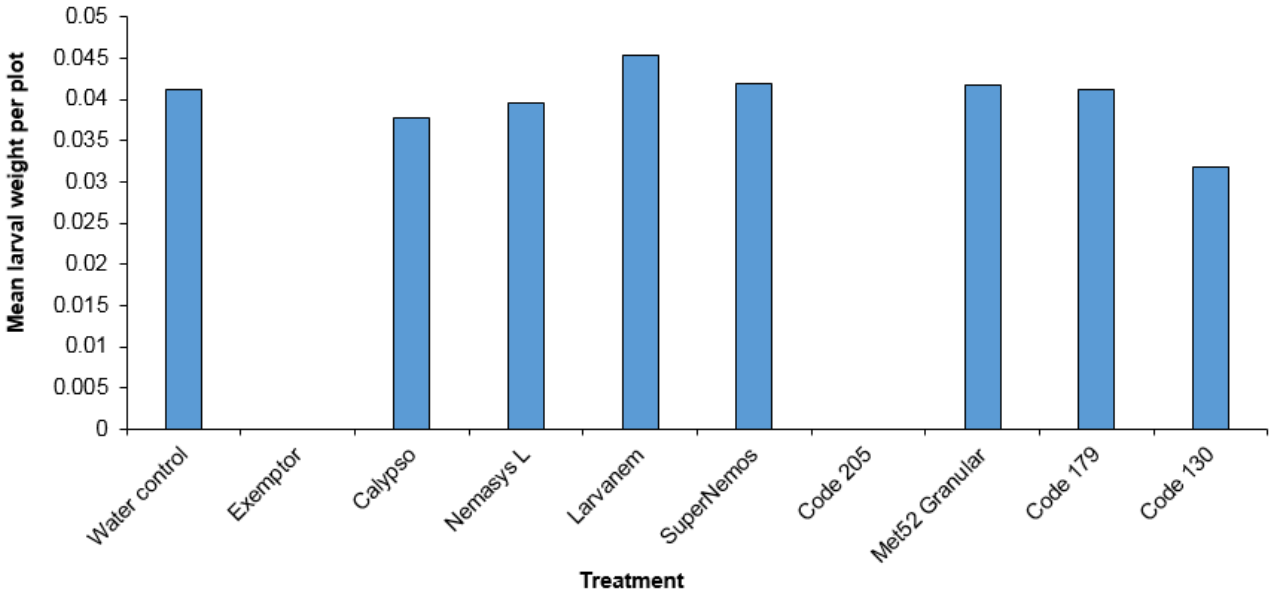


Figure 2 Mean larval weight per plant per plot.

Root vigour

Mean root vigour per plant was scored on a scale of 5 (healthy) to 1 (dead). There was a significant effect of treatment on root vigour. Exemptor, Calypso, Nemasys L, SuperNemos and Code 205 (biopesticide) had mean root vigour scores of 3.4, 3.7, 3.5, 3.4 and 3.6 respectively which was significantly higher than the control which had a mean root vigour score of 2.9. (Table 10, Figure 3). Code 179 had a mean root vigour score of 2.3 which was significantly lower than the control.

Please note that analysis of means of a score should be used with caution as data is not normally distributed.

Table 10. Effect of treatments on root vigour. Data presented as mean root vigour score per plant. Numbers in a column followed by the same letter are not significantly different at P <0.05 based on Duncan multiple comparisons (which can differ from the Fisher's LSD test).

Product name or MOPS code	Mean root vigour score
1. Water (negative control)	2.85 [bc]
2. Exemptor [standard] (positive control)	3.40[d]
3. Calypso	3.73 [d]

4. Nemasys L	3.47 [d]
5. Larvanem	3.40 [bcd]
6. SuperNemos	3.43 [d]
7. 205	3.60 [d]
8. Met52 Granular	2.83 [b]
9. 179	2.27 [a]
10. 130	3.37 [bd]
F value (df)	6.15 (45) (P = <.001)
LSD	0.513

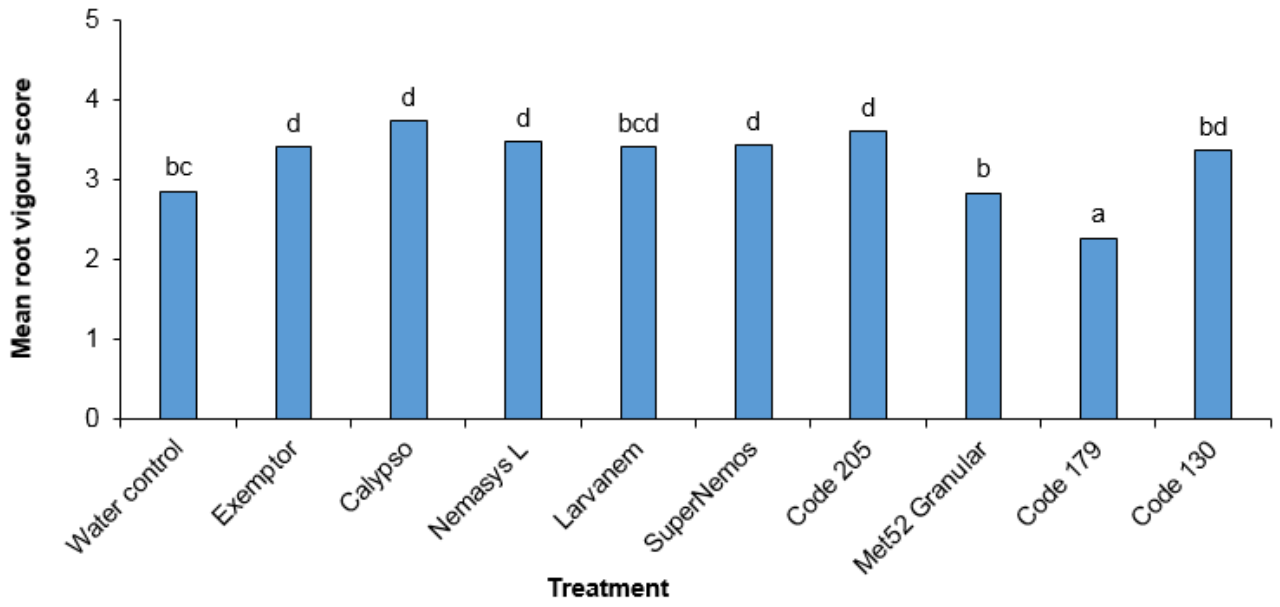


Figure 3 Mean root vigour score (5 healthy, 1 dead) per plant. Treatments with the same letters are not significantly different.

Root weight

Exemptor, Calypso, Larvanem had a mean root weight (g) of 13.0, 15.6 and 13.1 respectively which was significantly higher than the untreated control which had a mean root weight of 9.9

(Table 11, Figure 4). All other treatments except Code 179 performed the same as the control. Code 179 had significantly lower mean root weight (6.6g) compared to the control.

Table 11. Effect of treatments on root weight (g). Numbers in a column followed by the same letter are not significantly different at P <0.05 based on Duncan multiple comparisons (which can differ from the Fisher's LSD test).

Product name or MOPS code	**Mean root weight (g)**
1. Water (negative control)	9.91 ^{bc}
2. Exemptor [standard] (positive control)	13.01 ^{de}
3. Calypso	15.56 ^{e}
4. Nemasys L	10.95 ^{cd}
5. Larvanem	13.12 ^{de}
6. SuperNemos	11.26 ^{cd}
7. 205	11.75 ^{cd}
8. Met52 Granular	7.18 ^{ab}
9. 179	6.62 ^{a}
10. 130	9.72 ^{bc}
F value (df)	7.90 (45) (P = <.001)
LSD	2.756

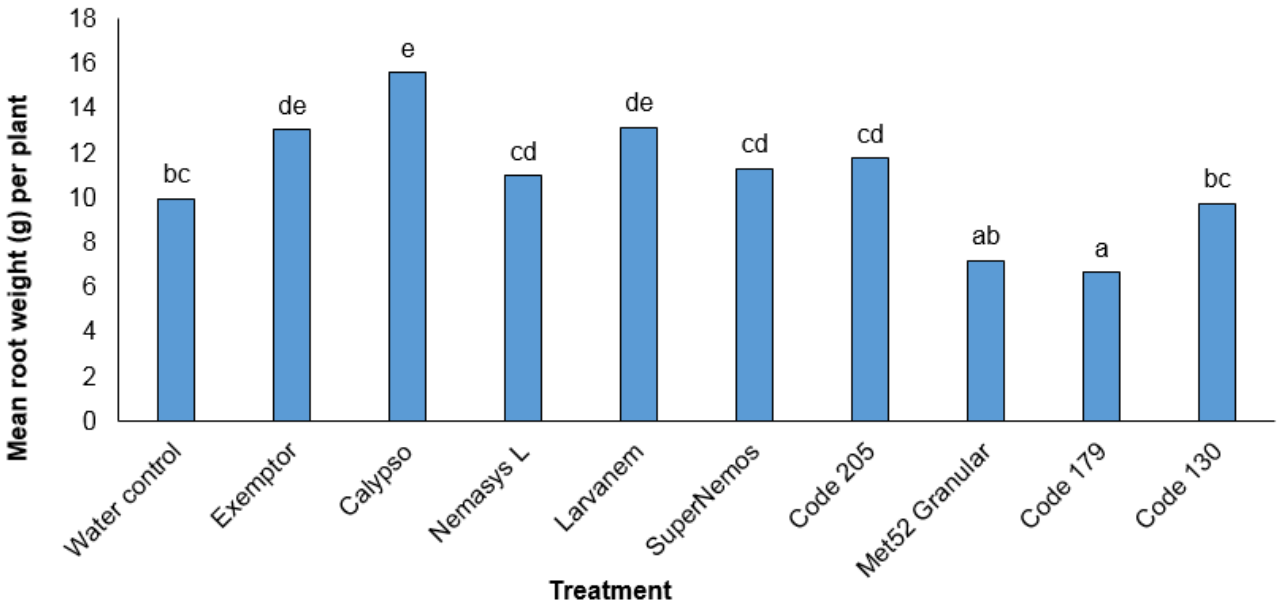


Figure 4 Mean root weight (g) per plant. Treatments with the same letters are not significantly different.

Plant vigour and phytotoxicity

Mean plant vigour per plant was scored on a scale of (5 healthy, 1 dead). There was no effect of the treatments on plant vigour (Table 12, Figure 5). None of the treatments achieved the highest plant vigour score of five because of leaf drop which can occur naturally (deciduous shrub) or in response to stress. Mean plant vigour scores ranged between 2.6 and 3.3. No phytotoxic effects were observed following any treatment.

Please note that analysis of a score should be used with caution as data is not normally distributed.

Table 12. Effect of treatments on plant vigour. Data presented as mean plant vigour per plant.

Product name or MOPS code	**Mean plant vigour score**
1. Water (negative control)	2.8
2. Exemptor [standard] (positive control)	3.1
3. Calypso	3.3
4. Nemasys L	3.0

5. Larvanem	3.2
6. SuperNemos	3.1
7. 205	3.0
8. Met52 Granular	2.8
9. 179	2.6
10. 130	3.2
F value (df)	45 (P = 0.07 n.s.)

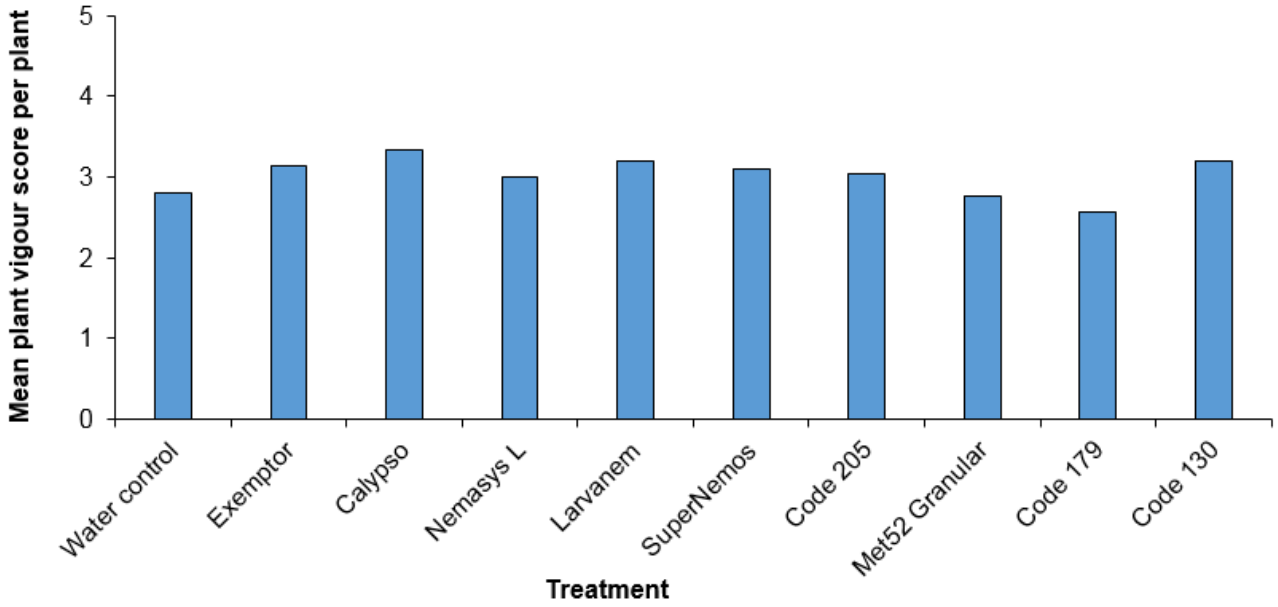


Figure 5 Mean plant vigour score per plant (5 healthy, 1 dead).

Formulations

No problems were encountered during mixing or application of any of the product formulations under test.

Effect on non-target pest

The grower who propagated the cuttings commented that cuttings grown in Exemptor treated substrate generally took better and had improved development compared to the untreated cuttings. It was hypothesised that as Exemptor controls aphids, sciarid fly and other soil-dwelling pests it allowed stronger root development and therefore better nutrient uptake. However, as this was based on a small number of cuttings further work would need to be carried out to confirm this

observation.

Discussion

The trial confirmed that there are effective control measures currently available to growers for controlling vine weevil larvae. While the trial has shown that growers have the option of using conventional pesticides either preventatively (Exemptor) or curatively (Calypso) (EAMU 2014/2153 for the use of Calypso as a drench on protected ornamentals), it has also confirmed that, when applied correctly, entomopathogenic nematodes can achieve a similar level of control while being safer to the operator, the environment and other beneficial insects. The temperatures during this trial (Appendix B) did not appear to negatively affect the activity of the nematodes.

Met52 Granular was also observed to reduce the number of vine weevil larvae and is currently the only approved biopesticide which can be used preventatively in ornamental plant production. Met52 granular was not as effective as Exemptor, Calypso or one of the nematode products. Met52 granular requires temperatures between 15°C - 30°C to infect its host and the temperature data collected during this trial suggests that substrate temperatures were suitable for Met52 activity for 504 hours (21 days) during August (Appendix B). Air temperatures indicated that average temperatures were suitable until the end of September. It is likely that higher temperatures would have increased the level of control recorded for Met52 Granular.

The trial also identified two additional biopesticides currently in development which were effective in controlling vine weevil larvae, particularly Code 205 which gave 100% control. Different timings of application should be investigated for Code 130 and 179 as this could improve their efficacy.

Root weight and root vigour scores were generally higher than the control for the most effective treatments due to the reduction of vine weevil larvae feeding on the roots. There was no evidence that any of the products tested in the study had a repellent or feeding deterrent effect as there were no observed differences in the average weight of the larvae recovered between treatments. If this was the case it would be expected that larvae from the treatments would weigh less than those from the controls.

While the products used in this trial were effective using our drenching methods on *F. erecta*, growers may see differences in efficacy using different crops (particularly those with dense roots or fleshy crowns) and application methods.

Conclusions

- All the products tested, except for Code 179 significantly reduced the number of live vine weevil larvae compared to the water control

- The best performing products were Exemptor, Calypso, Code 205 and the three nematode products (Nemasys L, Larvanem, and SuperNemos).
- No phytotoxicity of the treatments was observed.
- Plants treated with the most effective treatments generally had improved root vigour and higher root weight compared to the control.
- None of the treatments had a repellent or feeding deterrent effect.
- The timing of application of some of the coded biopesticides should be investigated further to determine if efficacy could be improved.

References

J., Bennison, G., Prince, T., Pope, D., Chandler & G., Hough (2014) A review of vine weevil knowledge in order to design best-practice IPM protocols suitable for implementation in UK horticulture (CP 111). HDC Final Report.

Fargro (2014) A practical IPM guide to controlling vine weevil on ornamental nurseries.

Appendix A – Study conduct

ADAS is officially recognised by United Kingdom Chemical Regulations Directorate as competent to carry out efficacy testing. The experiments reported were carried out according the internal ADAS operating procedures

GLP compliance will not be claimed in respect of this study.

Relevant EPPO/CEB guideline(s)		**Variation from EPPO**
PP 1/152(3)	Design and analysis of efficacy evaluation trials	None
PP 1/135(3)	Phytotoxicity assessment	None
PP 1/181(3)	Conduct and reporting of efficacy evaluation trials including GEP	None
PP 1/111 (3)	*Otiorhynchus* spp. Larvae on ornamentals and strawberry	From our experience of working on vine weevil on strawberry we know that four replicates per treatment and 10 plants per plot (40 plants per treatment) has given a significant results. Therefore, six replicates will be sufficient. From our experience artificially infesting strawberry plants individually with 15 eggs gives a significant result.

Appendix B – Meteorological data

N.B. Air temperatures can only be used as an indication of the effect of temperature on temperature sensitive treatments as air temperature and substrate temperature can differ.

Throughout the trial the air temperature was recorded (Figure 6). The mean temperature ranged between 9.7 and 22.6°C. The maximum temperature recorded was 38°C on 1 August and the minimum temperature was 4.5°C on 4 October. Temperature in this trial had a particular influence on Met52 Granular activity and nematode activity. While Met52 Granular was within the substrate from potting, the critical period for its activity against vine weevil larvae in this trial was between the date of egg infestation (1 August) and the date assessments were done on surviving vine weevil larvae (3-11 November). Met52 requires temperatures above 15°C for infection to occur and mean daily air temperatures first dropped below 15°C on 26 August and 20 September before they regularly remained below 15°C. Daily minimum temperatures were already dropping below 15°C at the beginning of the trial. Substrate temperature was also recorded during August (Figure 7) and temperatures were above 15°C for 504 hours (21 days). The data logger should have recorded substrate temperature throughout the entire trial but was mistakenly only set to run for one month.

The critical period for nematode activity was between the date that the nematodes were applied (16 September) and the date the trial was assessed (3-11 November). During this period mean daily air temperatures remained above the lower minimum temperature of Nemasys L (5°C). Daily mean temperatures only dropped below 10 °C, the lower minimum temperature of SuperNemos, on 4, 6 and 22 October. Larvanem had the highest minimum temperature of 14°C and temperatures first dropped below this on 22 and 24 September before regularly remaining below this threshold after the beginning of October. Despite temperatures dropping to below optimums for SuperNemos and Larvanem towards the end of the trial period, all nematode products were equally effective

Location of the weather station		On site (ADAS Boxworth		
Distance to the trial site		0 m		
Origin of the weather data		Weather station for long term average Data logger for average conditions during the trial		
Long-term averages from *location* Boxworth 30 year mean				
Month/period	**Av temp (°C)**	**Min temp (°C)**	**Max temp (°C)**	**Rainfall (mm)**
May	11.8	6.9	16.8	43.5
June	14.8	9.6	19.9	50.8

July	17.3	11.8	22.8	45.8
August	17.5	12.2	22.6	51.9
September	14.6	10.1	19.0	54.5
October	11.1	7.4	14.8	57.1
November	7.0	4.1	9.9	54.2

Average conditions during the trial: August – October using weather station

Month/period	Av temp (ºC)	Min temp (ºC)	Max temp (ºC)	Av RH (%)*	Rainfall (mm)
July	18.1	8.6	30.3	78.5	n/a
August	15.5	6.9	24.6	75.9	n/a
September	15.2	6.9	24.2	83.8	n/a
October	12.7	5.1	20.7	87.5	n/a

***protected crops only**

Average conditions during the trial: August – October using datalogger in polytunnel

Month/period	Av temp (ºC)	Min temp (ºC)	Max temp (ºC)	Av RH (%)*	Rainfall (mm)
August	18.1	11.7	29.8	71.9	n/a
September	16.6	11.5	25.1	82.2	n/a
October	13.7	9.7	20.8	86.2	n/a

***protected crops only**

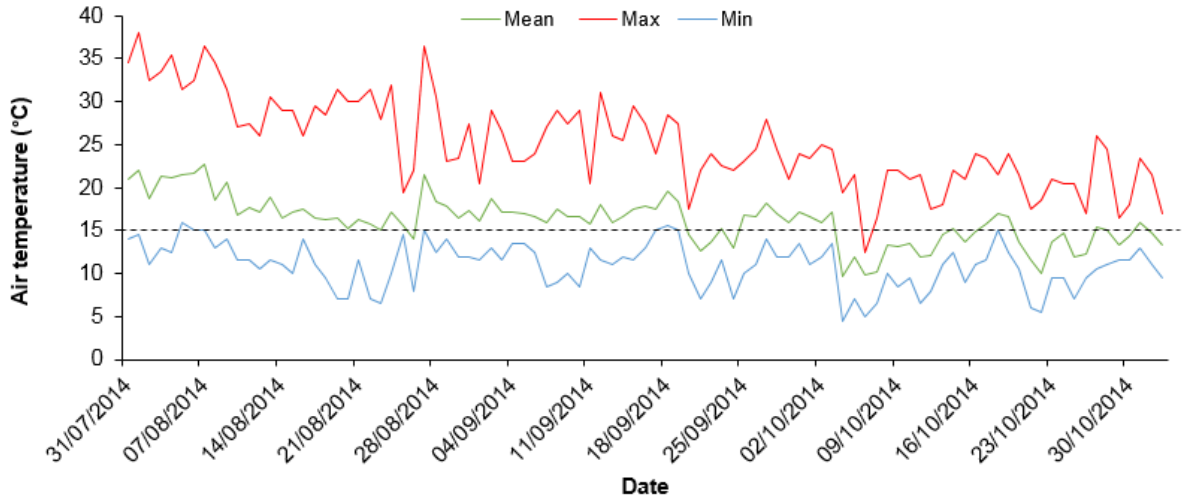


Figure 6 Mean, maximum and minimum air temperatures in polytunnel during trial (using datalogger)

Average substrate conditions during the trial: August using datalogger in substrate

Month/period	Av temp (ºC)	Min temp (ºC)	Max temp (ºC)
August	19.9	8.8	28.3

***protected crops only**

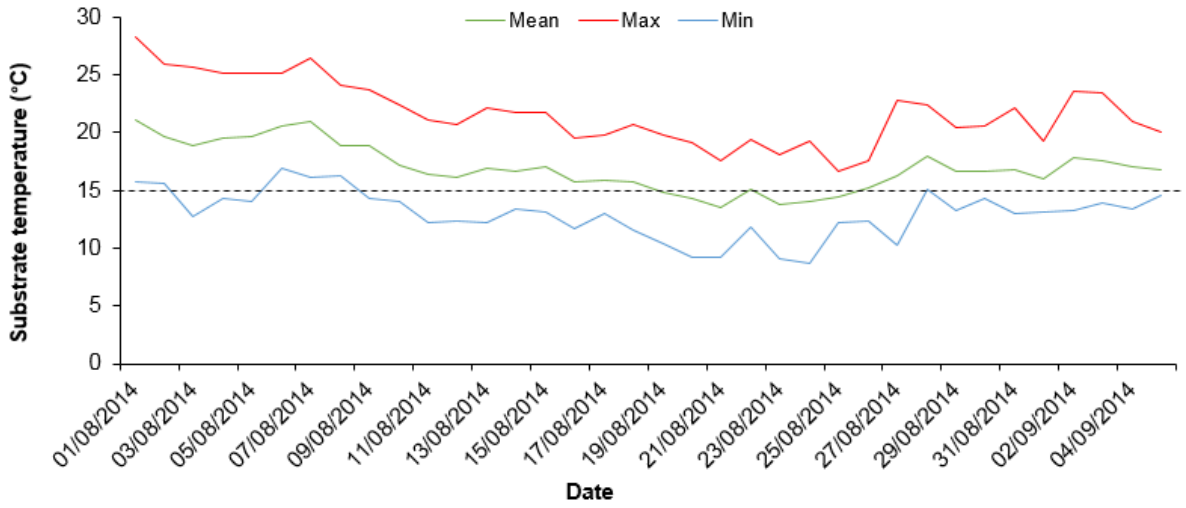


Figure 7 Mean, maximum and minimum substrate temperatures 1 August – 4 September 2014 (using datalogger in substrate of pot)

Weather at treatment application: Air temperature using datalogger in polytunnel

Month/period	Av temp (ºC)	Min temp (ºC)	Max temp (ºC)
Preventative treatment: 31 July 2014 8.30am – 12pm	23.69	15.5	34.5
Curative treatment: 16 September	17.9	13.0	27.5

Appendix C – Agronomic details

Growing system

Crop	Cultivar	Planting/sowing date	Row width (m) or pot spacing
Fuchsia	*erecta*	1 May	77 plug holes trays

Other pesticides - active ingredient(s) / fertiliser(s) applied to the trial area

Date	Product	Rate	Unit
n/a			

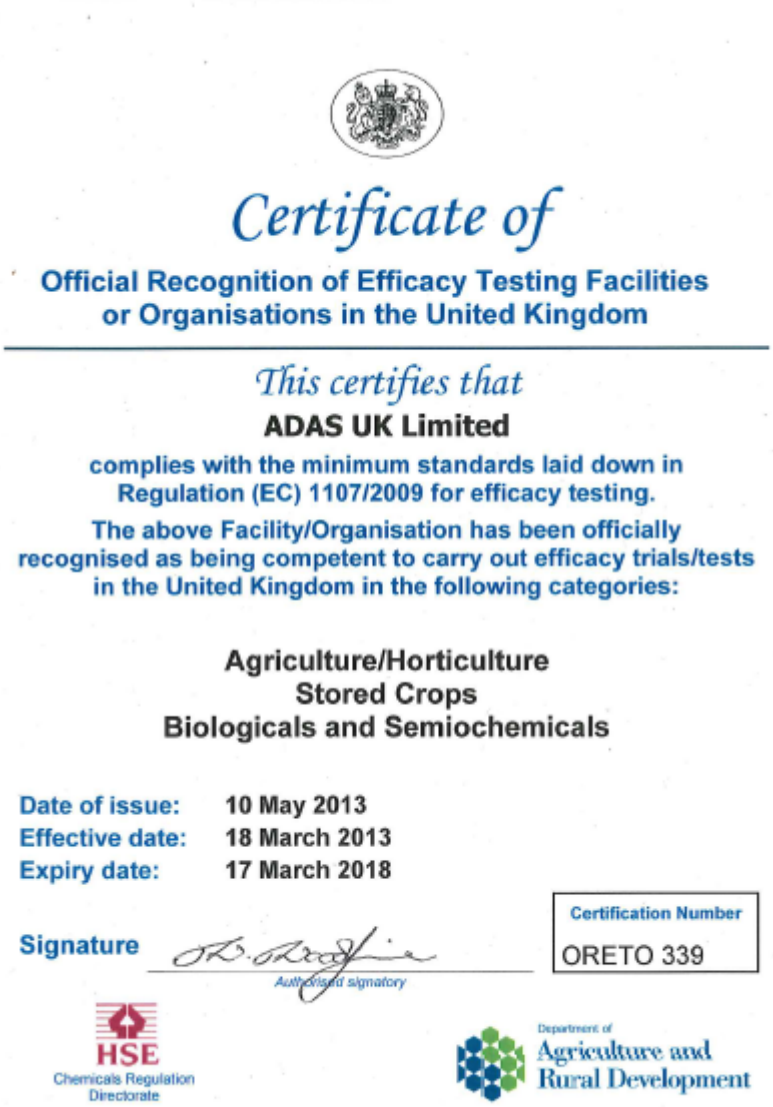
Details of irrigation regime (pot-grown crops)

Type of irrigation system employed (e.g. overhead sprinkler, hand watering, drip, ebb and flow, capillary sandbed or capillary matting)
Overhead sprinklers

Appendix D – Trial layout

PLOT	1	11	21	31	41	51
BLOCK	1	2	3	4	5	6
TREATMENT	5	10	4	9	8	6
PLOT	2	12	22	32	42	52
BLOCK	1	2	3	4	5	6
TREATMENT	8	5	2	1	3	7
PLOT	3	13	23	33	43	53
BLOCK	1	2	3	4	5	6
TREATMENT	6	4	6	3	1	2
PLOT	4	14	24	34	44	54
BLOCK	1	2	3	4	5	6
TREATMENT	9	7	1	6	10	4
PLOT	5	15	25	35	45	55
BLOCK	1	2	3	4	5	6
TREATMENT	3	2	9	10	5	9
PLOT	6	16	26	36	46	56
BLOCK	1	2	3	4	5	6
TREATMENT	10	1	8	5	4	10
PLOT	7	17	27	37	47	57
BLOCK	1	2	3	4	5	6
TREATMENT	2	3	10	4	2	1
PLOT	8	18	28	38	48	58
BLOCK	1	2	3	4	5	6
TREATMENT	7	9	7	2	6	8
PLOT	9	19	29	39	49	59
BLOCK	1	2	3	4	5	6
TREATMENT	1	8	5	8	7	3
PLOT	10	20	30	40	50	60
BLOCK	1	2	3	4	5	6
TREATMENT	4	6	3	7	9	5

Appendix E – Copy of the Certificate of Official Recognition of Efficacy Testing Facility or Organisation



Certificate of

Official Recognition of Efficacy Testing Facilities or Organisations in the United Kingdom

This certifies that

ADAS UK Limited

complies with the minimum standards laid down in Regulation (EC) 1107/2009 for efficacy testing.

The above Facility/Organisation has been officially recognised as being competent to carry out efficacy trials/tests in the United Kingdom in the following categories:

Agriculture/Horticulture
Stored Crops
Biologicals and Semiochemicals

Date of issue: 10 May 2013
Effective date: 18 March 2013
Expiry date: 17 March 2018

Signature *Authorised signatory*

Certification Number
ORETO 339

HSE Chemicals Regulation Directorate

Department of Agriculture and Rural Development

Appendix F – Photographs

Figure 1. Vine weevil trial in polytunnel

Figure 2. Mixer used for mixing propagation mix for plugs and herbaceous mix for potting up

Figure 3. *Fuchsia erecta* cuttings

Figure 4. Trial assessment

