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[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.]

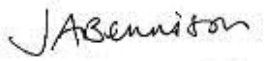
AUTHENTICATION

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.

Jude Bennison

Senior Research Entomologist

ADAS



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SCIENCE SECTION

Introduction

Vine weevil is currently the most serious pest of UK hardy nursery stock. Adult damage to leaves and presence of larvae around roots can make ornamental plants unmarketable. Root damage caused by larvae in ornamental leads to reduced plant vigour and if damage is severe, to plant death. Chemical control of larvae is now difficult due to the withdrawal of the most persistent products for use in growing media, and due to current EC restrictions on using one of the available neonicotinoid insecticides (imidacloprid e.g. Imidasect 5GR) on flowering plants and on label restrictions that do not permit using thiacloprid (Exemptor) in peat-free growing media. Vine weevil populations have been increasing recently on some HNS nurseries due to these restrictions. There is now more grower interest in using methods for control of weevil adults as well as larvae, and growers need more information on the efficacy and timinutesg of insecticide sprays that are compatible with Integrated Pest Management (IPM) programmes, linked with further knowledge on weevil activity and egg laying behaviour. Growers are under increasing pressures to reduce the use of pesticides, not only to meet retail demands but also to meet the requirements of the EC Sustainable Use Directive (SUD) which states that all growers must use IPM where practical and effective. Many growers of HNS are now adopting biological pest control methods within IPM programmes. Available biological methods for vine weevil control include the entomopathogenic fungus (Met52 Granular) for incorporation in growing media and entomopathogenic nematodes which are applied as drenches. However, growers lack confidence in the efficacy of Met52 and view current nematode application methods using drenches in HNS as labour-intensive and thus expensive. Growers now feel under-confident in the efficacy of currently available control measures for vine weevil. This project hopes to address grower needs by filling knowledge gaps in how to optimise best-practice use of available vine weevil control methods within IPM and to develop novel approaches to both monitoring and control.

Objective 1. Improve understanding of the impact of environmental conditions on vine weevil biology and behaviour in order to optimise application of plant protection products

Task 1.1. Egg laying activity of overwintered vine weevil adults under constant and fluctuating temperatures (Harper Adams, ADAS and Warwick, (Years 1 and 2)

Egg laying activity of vine weevil adults appears to cycle between periods of peak egg laying and periods where few or no eggs are laid. The causes of these cycles are not fully understood but are thought to relate to the nitrogen content in the host plant and to temperature (Moorhouse et al., 1992). There is conflicting information on the minimum temperature required for egg laying. Stenseth (1979) suggests that egg laying only occurs at temperatures above 12°C while Blackshaw (1992) reports egg laying at lower temperatures.

The importance of temperature and vine weevil egg laying is primarily a limiting factor in the spring and autumn. Overwintering adults start egg laying in May and June while newly emerging adults, emerging in June and July may not start to lay eggs until August (Blackshaw, 1996). As such spring temperatures are likely to determine the start of egg laying by overwintered adults, and autumn temperatures will determine the end of egg laying by adults completing their development during the growing season. Although the severity of the preceding winter will determine the numbers of weevils successfully overwintering, these individuals may contribute more than half of all the eggs laid in a season (Blackshaw, 1996).

The aim of this task is to determine the minimum temperature required for vine weevil adult feeding activity and egg laying. In addition, this work will investigate the impact of fluctuating temperatures above and below these temperature thresholds. There is currently some debate as to the minimum temperature for egg laying. A minimum temperature of 12°C has been suggested while other researchers report egg laying at lower temperatures. It has also been suggested that overwintered adults do not require a period of feeding before egg laying recommences, but this has not been confirmed. An added complication, not previously considered, is that for much of the year air temperatures fluctuate between warmer days and cooler nights (night-time temperatures below 12°C). Determining the minimum temperature at which vine weevil adults feed and lay eggs, the time between the start of feeding and egg laying and what, if any, impact temperature fluctuations has on feeding and egg laying is important if growers are to optimise timinutesg of applications of controls in outdoor, polytunnel and glasshouse grown crops.

Task 1.1.1. Determining the minimum temperature for vine weevil feeding and egg laying

Materials and methods

Site: This work was done in controlled environment cabinets in the Princess Margaret Laboratories at Harper Adams.

Insects: Vine weevil adults were collected during the summer of 2016 from commercial strawberry crops grown in Shropshire and Staffordshire. A population of vine weevil adults was maintained in temperature controlled rooms (Weiss Technik) in the Jean Jackson Entomology Laboratory at Harper Adams. Weevils were kept at 20°C, 60% RH with a 16:8 (L:D) photoperiod. Weevils were kept in BugDorms (MegaView Science, Taiwan) (47.5x47.5x47.5 cm), fed yew (*Taxus baccata*) leaves and provided with damp tissue paper as a source of moisture. Vine weevil adults were maintained in these conditions for a minimum of two months before being used in the experiment.

Insect conditioning: Prior to the start of the experiment, approximately 40 weevils were moved to a controlled temperature cabinet (Sanyo/Panasonic) set to a constant 5°C. Weevils were kept in a ventilated plastic box containing yew and damp tissue paper for a minimum of 7 days before the start of the experiment. At the start of the experiment 10 healthy weevils were selected at random and assigned to a controlled temperature cabinet (Sanyo/Panasonic) set to a constant 6, 9 or 12°C. Each weevil was placed in a ventilated Petri dish (90mm diameter) lined with damp Whatman No. 1 filter paper (Whatman Plc, UK).

Feeding assessments: Each weevil was fed a single leaf disc (19 mm diameter), cut from a fully unfurled strawberry leaf (cv. Elsanta) using a cork borer. Each week, for five consecutive weeks, each weevil was allowed to consume a leaf disc for a 48 hour period as their only food source. The leaf disc was then removed from the Petri dish and photographed against a white background and the area of the leaf consumed was calculated using ImageJ software (https://imagej.nih.gov/ij/). The leaf discs were replaced with an excess of strawberry leaf material to feed the weevils until the following week's assessment.

Egg laying assessments: Egg laying was recorded at the same time as the feeding assessments. Once a week, after the completion of the leaf disc 48 hour feeding period, eggs were collected. This was done by moving the individual weevils to a new Petri dish prepared as previously described and assessing the eggs laid in the 'old' dish over a 40 day period. The total number of eggs laid by each weevil was recorded as well as the number of eggs that turned brown (indicating viability) over time. Eggs were stored in the same controlled

temperature cabinet, at the same conditions as the parent weevil. The egg colour was assessed once a week. The filter papers were kept damp throughout.

In addition to the described feeding and egg laying assessment, egg laying and feeding was briefly assessed at 5°C. Vine weevil feeding was assessed using 10 weevils, conditioned as previously described, over a single 2 day period and egg laying was assessed over a two week period.

Statistical analysis: Numbers of vine weevil eggs laid and leaf area consumed was analysed using generalised linear modelling (GLM) with quasipoisson errors or linear modelling using square root transformed data or untransformed data, as appropriate. Post-hoc analysis using Tukey's tests were used to test for differences between individual treatment means. All analyses were done in R.3.2.2 (R Core Team, 2015).

Results

Temperature and humidity conditions in each of the controlled temperature cabinets was verified using iButton (HomeChip, UK) data loggers. The temperature and humidity data are summarised in Table 1.

Table 1. Temperature and humidity conditions within controlled temperature cabinets

Cabinet temperature set point (˚C)	Actual mean temperature (˚C)	Humidity (%RH)
5	5.8	n.d.
6	6.4	85
9	9.2	82
12	12.6	54

Egg laying and leaf area consumption data are summarised in Figure s 1 and 2. Data for weevils held at 5°C is presented alongside the rest of the data for illustrative purposes only. Analysis of the egg laying data indicates that temperature significantly affects the total number of eggs laid at each temperature (x^2 = 122.99, *P* <0.001), see Figure 1. Post-hoc analysis, however, does not show significant differences between individual treatment means. Similarly, analysis of the egg laying data indicates that temperature significantly affects the number of viable (brown) eggs recorded at each temperature (x^2 = 115.97, *P* <0.001). Post-hoc analysis, indicates that significantly more viable eggs were recorded at 9°C than at 6°C or 12°C. There was no difference in numbers of viable eggs recorded 6°C and 12°C. Despite the higher number of viable eggs recorded at 9°C, there was no difference between the

proportions of viable eggs recorded at the different temperatures tested (F = 1.75, P = n.s.). Of the eggs that were laid, 0%, 20%, 11%, and 31% hatched at 5, 6, 9 and 12°C respectively. Data on time to eggs turning brown and/or hatching is presented in Figure 3.

Analysis of leaf area consumption data indicates that temperature did not affect (F = 2.69, P = n.s.) leaf area consumed.

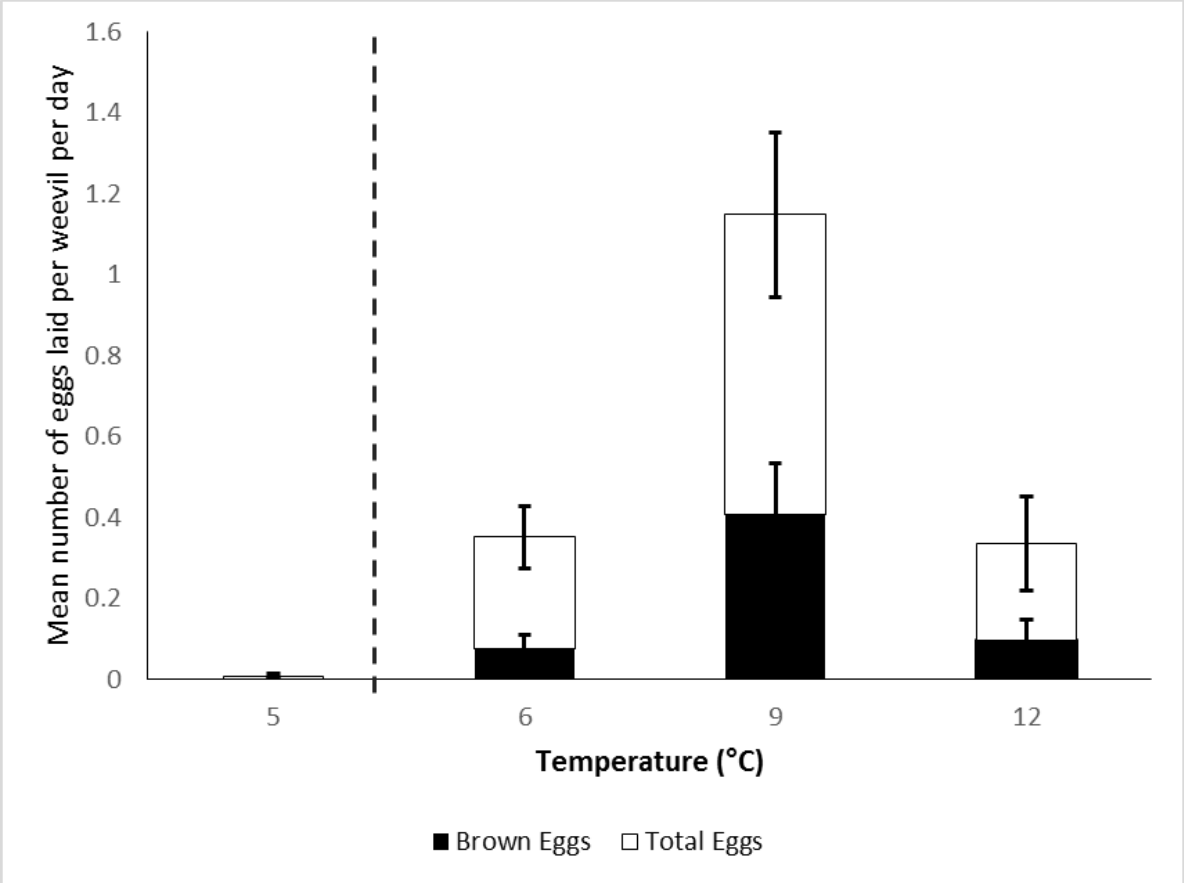

Figure 1. Numbers of vine weevil eggs laid per weevil per day at four constant temperatures over a 5 week period* (*data for weevils at 5°C collected over a 2 week period). Means +/- SE, n = 10.

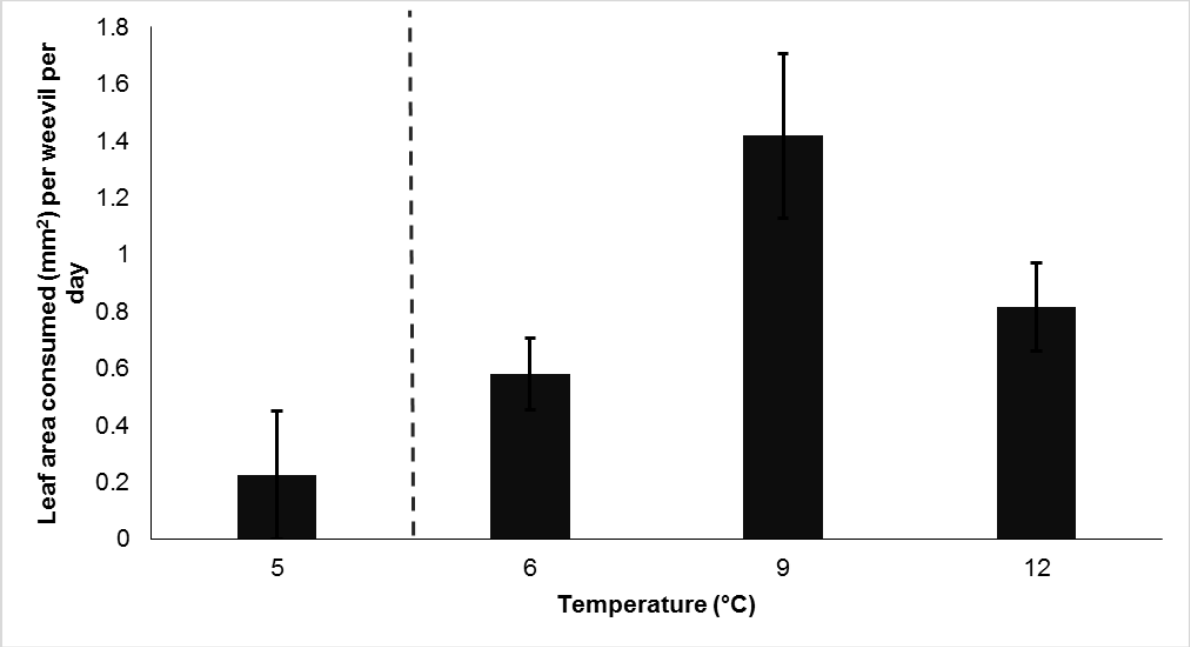

Figure 2. Area of leaf consumed per weevil per day at various temperatures over 5* weeks (*except 5°C). Means +/- SE, *n* = 10.

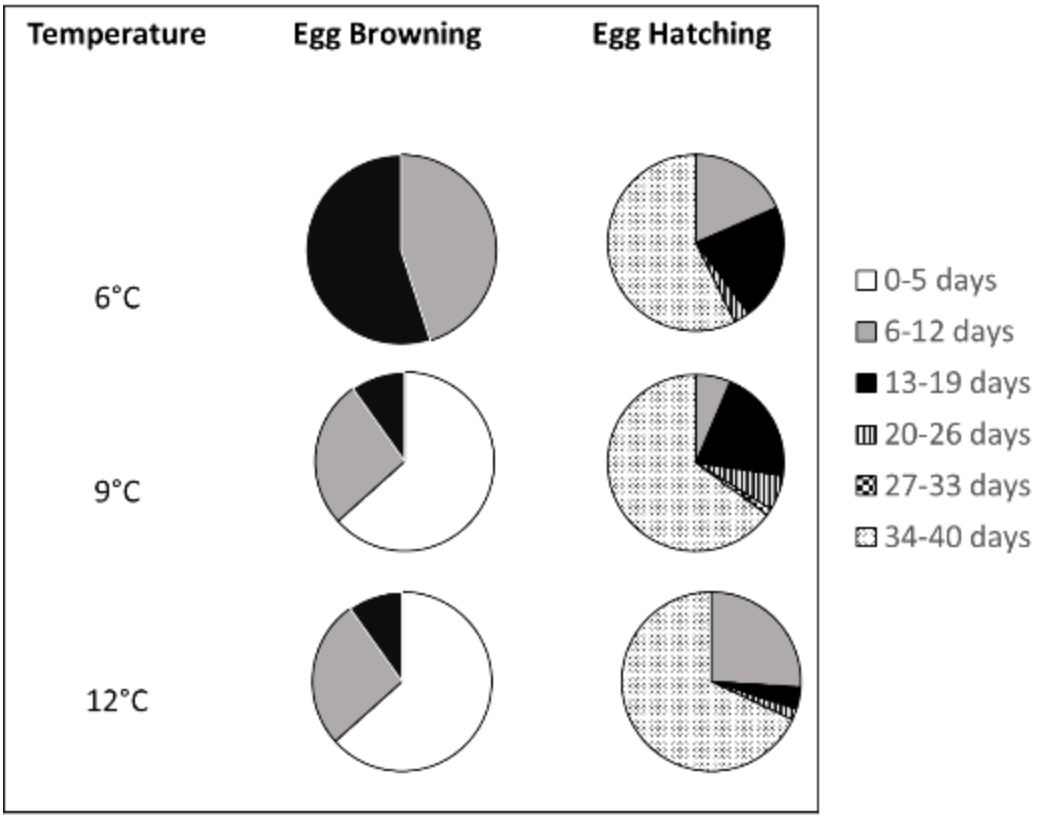

Figure 3. The time to eggs browning and hatching.

Discussion

The literature on vine weevil biology provides conflicting information on the minimum temperature required for egg laying. Stenseth (1979) suggests that egg laying only occurs at temperatures above 12°C while Blackshaw (1992) reports egg laying at lower temperatures. Results presented here support the work of Blackshaw (1992) by indicating that egg laying may continue at temperatures below 12°C. Egg laying and feeding by vine weevil adults continues at temperatures as low as 6°C (6 out of 10 vine weevil adults laid viable eggs at this temperature) but, egg laying at least, appears to stop at a temperature of 5°C (no viable eggs laid at this temperature). In addition, egg hatch was recorded at all temperatures tested, with the exception of 5°C, which appears to be in line with previous estimates of the lower temperature threshold for egg development 6.3°C (Masaki & Ohto, 1995). As the lower temperature threshold for development of larvae is thought to be just 2.4°C, it can be expected that hatched larvae would develop at the low temperatures tested. It is, however, important to remember that at low temperatures weevil development is slow; larval development is thought to take 231 days at a constant 12°C (Son & Lewis, 2005).

It is notable from the results presented that more eggs were laid and a greater area of leaf consumed at 9°C than at 12°C. Although the controlled temperature cabinets maintained constant temperatures effectively the humidity at 12°C was lower than at 9°C. Although less important, humidity is known to still be an important factor in determining rates of vine weevil development (Son & Lewis, 2005; La Lone & Clarke, 1981). It is, however, also true that vine weevil adults cycle both in terms of feeding and egg laying activity. This was reflected in the results with weevils at 9°C laying 0-63 eggs in the five week study period while weevils at 12°C laid 0-31 eggs in the same period. Similar variability was also apparent for leaf area consumption. These cycles may then explain differences recorded between temperatures but not the main finding that egg laying and feeding continue at temperatures at or above 6°C. The repeat of the presented work using vine weevil adults collected at the end of the winter will be useful in confirming the trends observed in this experiment.

Conclusions

- Vine weevil adults are capable of laying eggs and feeding at temperature below 12°C.
- Vine weevil adults appear to cease egg laying and feeding at a temperature of approx. 5°C.

- Egg laying and feeding by vine weevil adults varies greatly between individuals collected from the same population that had been maintained under similarly conditions before the start of the experiment.

Future work

- *Task 1.1.1. Determining the minimum temperature for vine weevil feeding and egg laying* - A repeat of the presented work will be completed using overwintered vine weevil adults. Vine weevil adults were placed in a semi-field environment within a ventilated polytunnel at Harper Adams in November 2016 and will be collected for use in the experiment in February/March 2017. Overwintered vine weevil adults will be placed in controlled temperature cabinets set to constant temperatures, similar to those described above.
- *Task 1.1.2. Determining the impact of fluctuating temperatures on vine weevil egg laying* - In addition to the further work described above, additional overwintered vine weevil adults will be placed in controlled temperature cabinets set to fluctuating temperatures. Cabinets will be set to temperatures above and below those required for egg laying and feeding activity.
- *Task 1.2. Estimating the period during which vine weevil egg laying may occur in outdoor, polytunnel and glasshouse grown crops* – Based on results from Tasks 1.1.1. and 1.1.2. the period during the year when vine weevil adults may be laying eggs and feeding will be estimated. This will be determined using air temperature and substrate temperature data from commercial nurseries (temperature data kindly supplied by Fargro Ltd).

Objective 2. Develop practical methods for monitoring adults in order to detect early infestations and inform control methods

Task 2.1 Relative effectiveness of monitoring tools with which to detect activity of vine weevil adults within a crop. (Harper Adams, Year 1)

Presence of vine weevil adults may be determined either directly or indirectly. Directly monitoring vine weevil adults can be done by simply searching through leaf litter, under pots or other suitable refuges during the day (Buxton, 2003; Raffle, 2003). After dusk the crop may be searched for the presence of adults. A torch may be used to search the crop, although

care needs to be taken so as not to disturb the adults as they have a tendency to drop from the plant and feign death. Unfortunately these direct monitoring approaches are time consuminutesg and must be repeated at regular intervals throughout the season.

Trapping of vine weevil adults reduces amount of time required to monitor crops at a more convenient time. Several techniques have been developed, including grooved boards placed on the ground within crops (Li et al., 1995; Gordon et al., 2003) and corrugated cardboard wrapped around the stems of larger bushes or trees (Phillips, 1989) which exploit the fact that vine weevil seek out refuges during the day. Similarly, simple plastic crawling insect traps are readily used as refuges by vine weevil (Pope et al., 2013). Pitfall traps consisting of a plastic cup sunk into the ground so that the lip of the cup is at soil level may also be used to effectively monitor for the presence of vine weevil adults (e.g. Casteels et al., 1995; Solomon, 2000; Buxton, 2003).

Instead of directly counting numbers of vine weevil adults, the characteristic leaf notching damage caused by adults feeding on leaf margins can be used to monitor this pest (Buxton, 2003; Raffle, 2003). Often this leaf notching is the only sign of an infestation but even when checks are made, early stages of an infestation can go unnoticed. In order to focus monitoring efforts indicator plants that are particularly attractive to vine weevil (such as *Primula*) can be placed within the crop. These plants can be checked regularly and any damage removed so that fresh damage is immediately obvious. Presence of the larvae feeding on the roots of plants only becomes apparent when plants wilt as the main roots are severed and plants are easily lifted. Unfortunately, detecting the presence of vine weevil larvae through evidence of plant stress means that there has already been significant damage to the crop before control measures can be applied.

The aim of this task is to assess the relative effectiveness of a range of traps and indicator plants that may already be used or which could be easily adopted by growers to detect activity of adult vine weevil within crops. This comparison will be completed under standardised conditions using simulated crop environments and known numbers of adult weevils. Information from this work will provide growers with information on the selection of suitable monitoring tools on which to base crop protection decisions.

Materials and methods

Site: This work was done in a ventilated research polytunnel at Harper Adams (Crop and Environment Research Centre). The sides of the polytunnel were raised or lowered in order to maintain ‘warm’ conditions within the tunnel, suitable for insect movement.

Insects: Vine weevil adults were collected during the summer from commercial strawberry crops grown in Shropshire and Staffordshire as previously described.

Experimental Designs: Four separate experiments were completed, each simulating an ornamental crop environment. This was achieved by using large tent cages (1.45 m x 1.45 m x 1.52 m) (Insectopia, UK) placed within a research polytunnel at Harper Adam. Potted (12 cm diameter pots) strawberry plants (cv. Elstanta) were used to simulate a susceptible host plant species. Adult vine weevils will then be released into each cage and their positions recorded on subsequent days. A single monitoring tool/trap was then randomly allocated to each tent cage. The tent cage to which each monitoring tool/trap was allocated was re-randomised each day in order to exclude the effect of tent cage position, weevil population and/or simulated crop.

Experiment 1: This experiment tested the efficacy of six different monitoring tools/trap types (Table 2). Each monitoring tool was used as supplied by the manufacturer with the exception of the pitfall trap, modified red palm weevil trap and the vine weevil trap where a small piece of corrugated cardboard (approx. 10cm x 10cm) was placed inside the trap. The card was used to provide a suitable refuge within the plastic traps. This piece of card helped to reduce weevil mortality in this experiment as on hot days the card allowed the weevils to avoid direct exposure to the sun or to come into direct contact with hot plastic surfaces. The pitfall trap was further modified by painting the top of the catching box with PFTE paint (Fluon^{TM}) to prevent weevil escape.

Table 2. Adult vine weevil monitoring tools/traps types tested.

Monitoring tool/trap type	**Supplier/manufacturer**
Corrugated cardboard roll (approx. 5 cm diameter x 30 cm long)	n/a
Grooved board	e-nema, Schwentinental, Germany
Pitfall trap	CSalomon, Budapest, Hungary
Roguard trap	BASF, Cheadle Hulme, UK
Modified red palm weevil trap	Sentomol, Monmouth, UK
Commercial vine weevil trap	ChemTica, Heredia, Costa Rica

Each monitoring tool was placed in a tent cage with a known population of 40 weevils (approx. 19 weevils/m^2) and five potted strawberry plants, providing both a food source and a range of alternative refuges e.g. under pots, around rims, within compost. The efficacy of each monitoring tool was assessed on 12 occasions (between 9th and 14th August), recording numbers of weevils within or underneath the monitoring tool/trap type in the morning (9am to 12 noon) (see Figure 4).

Figure 4. Arrangement of large tent cages used for testing efficacy of monitoring tools/traps types. Tents are within a ventilated polytunnel at Harper Adams.

Experiment 2: This experiment tested the best performing monitoring tools identified in Experiment 1. These were: Pitfall traps, the base of the modified red palm weevil trap (30 cm diameter Stewart Plastics plant tray) and the commercial vine weevil trap. Tent cages were prepared as before but vine weevil numbers were varied between 9 (approx. 4 weevils/m^2), 22 (approx. 10 weevils/m^2) and 34 (approx. 16 weevils/m^2). The efficacy of each monitoring tool at each weevil density was assessed on 9 occasions (between 19th and 28th August), recording numbers of weevils within or underneath the monitoring tool in the morning (9am to 12 noon).

Experiment 3: This experiment tested the best performing monitoring tools identified in Experiments 1 and 2. These were: Pitfall traps and the commercial vine weevil trap. Tent cages were prepared as before but plant numbers were varied between 5 (approx. 2/m^2), 10 (approx. 5/m^2) and 15 (approx. 7/m^2). Vine weevil numbers were standardised at 40 (approx.

19 weevils/m^2) in each tent cage. The efficacy of each monitoring tool at plant density was assessed on 12 occasions (between 28th October and 8th November), recording numbers of weevils within or underneath the monitoring tool in the morning (9am to 12 noon).

Experiment 4: This experiment tested the best performing monitoring tool identified in Experiments 1, 2 and 3. This was: the commercial vine weevil trap. Tent cages were prepared as described for Experiment 1 with five strawberry plants (approx. 2/m^2) and 40 vine weevil adults (approx. 19 weevils/m^2) in each tent cage. The efficacy of the commercial vine weevil trap was compared with each of two indicator plants: *Euonymus fortunei* and *Primula* (polyanthus). For both indicator plants, a single potted plant was placed in the centre of a tent cage in place of a trap. Each commercial vine weevil trap and indicator plant was assessed on 10 occasions (between 9th November and 15th November), recording numbers of weevils within or under the commercial vine weevil trap or plant. Additionally, indicator plants were assessed for signs on notching on leaves (damaged leaves were removed after assessment). As for the other experiments, assessments were completed in the morning (9am to 12 noon).

Statistical analysis: Numbers of vine weevil adults caught within or found underneath each monitoring tool/trap type on each assessment day were analysed using generalised linear modelling (GLM) with quasipoisson errors. Post-hoc analysis using Tukey's tests were used to test for differences between individual treatment means. All analyses were done in R.3.2.2 (R Core Team, 2015). Results in Experiment 4 were not analysed statistically due to the limited data collected for the indicator plants.

Results

Experiment 1: Results from this experiment indicated that monitoring tool significantly affected the numbers of weevils recorded on each assessment day (x^2 = 249.71, *P* <0.001) (Figure 5). Post-hoc analysis confirmed that significantly more weevils were recorded in the commercial vine weevil trap (approx. 10 weevils per trap per day) than in any other monitoring tool/trap type. There were no significant differences between other monitoring tools/trap types tested. Pitfall, Roguard and modified red palm weevil traps caught 2-3 weevils per trap per day, while fewer than 1 weevil per trap per day were recorded in the cardboard roll or grooved boards. The mean daytime (0530 to 2030 BST) temperature between 9th August and 14th August was 23.7°C (max = 32.2°C, minutes = 15.6°C) and the mean night-time temperature was 14.5°C (max = 19.1°C, minutes = 8.6°C).

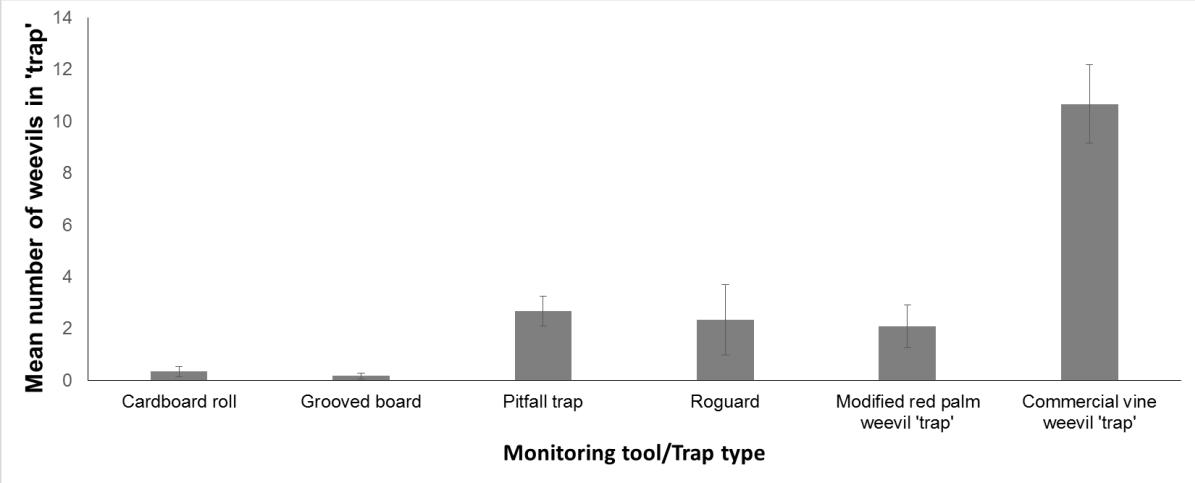

Figure 5. Numbers of vine weevil adults recorded within each monitoring tool/trap type on each assessment day. Means +/- SE, *n* = 12.

In completing each assessment it was noticed that approximately twice as many vine weevil adults were recorded underneath the modified red palm weevil trap than within the trap, while small numbers of weevils were found underneath other monitoring tools. The data were, therefore, reanalysed to combine numbers of weevils within and underneath each monitoring tool on each assessment day. This analysis again showed that monitoring tool significantly affected the numbers of weevils recorded on each assessment day (x^2 = 268.53, *P* <0.001) (Figure 6). Post-hoc analysis confirmed that significantly more weevils were recorded within or underneath the commercial vine weevil trap (approx. 10 weevils per trap per day) than in any other monitoring tool/trap type, except the modified red palm weevil trap (approx. 6 weevils per trap per day). There were significantly more weevils within or underneath the modified red palm weevil trap than the cardboard roll or grooved board but not the pitfall or Roguard traps. There were again no significant differences between other monitoring tools/trap types tested. There 2-3 weevils per trap per day within or underneath the pitfall and Roguard traps, while fewer than 1 weevil per trap per day was found within or underneath the cardboard roll or grooved boards.

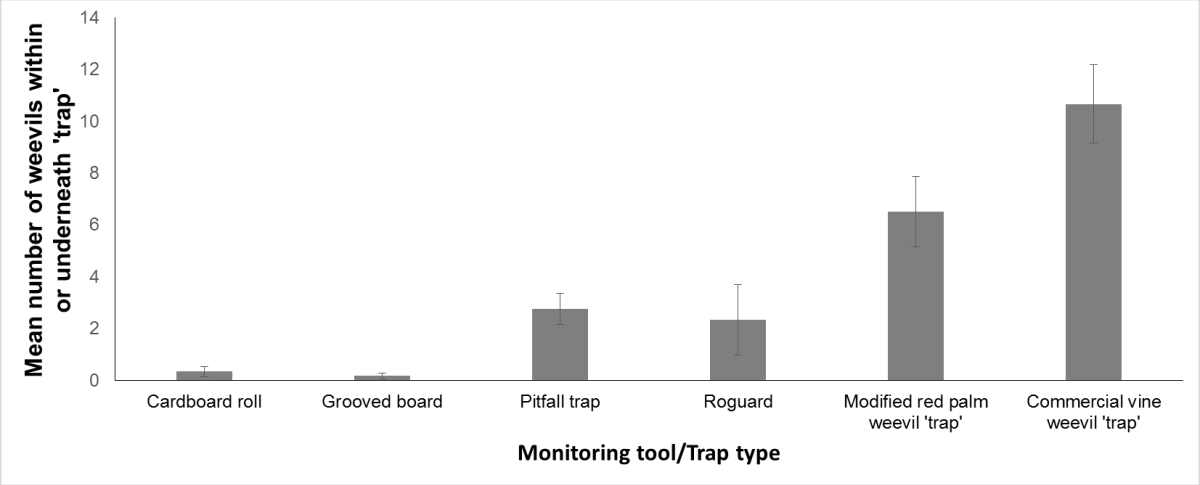

Figure 6. Numbers of vine weevil adults recorded within or underneath each monitoring tool/trap type on each assessment day. Means +/- SE, *n* = 12.

Weevil numbers within or underneath each monitoring tool were assessed on 12 occasions. The number of times that weevils were recorded within or underneath a monitoring tool (expressed as percentage i.e. reliability) are illustrated in Figure 7. The modified red palm weevil and commercial vine weevil trap were both 100% reliable in that weevils were recorded within or underneath these traps each time they were assessed. Pitfall traps were 91% reliable, Roguard traps were 42% reliable, corrugated cardboard rolls were 25% reliable and the grooved boards 17% reliable.

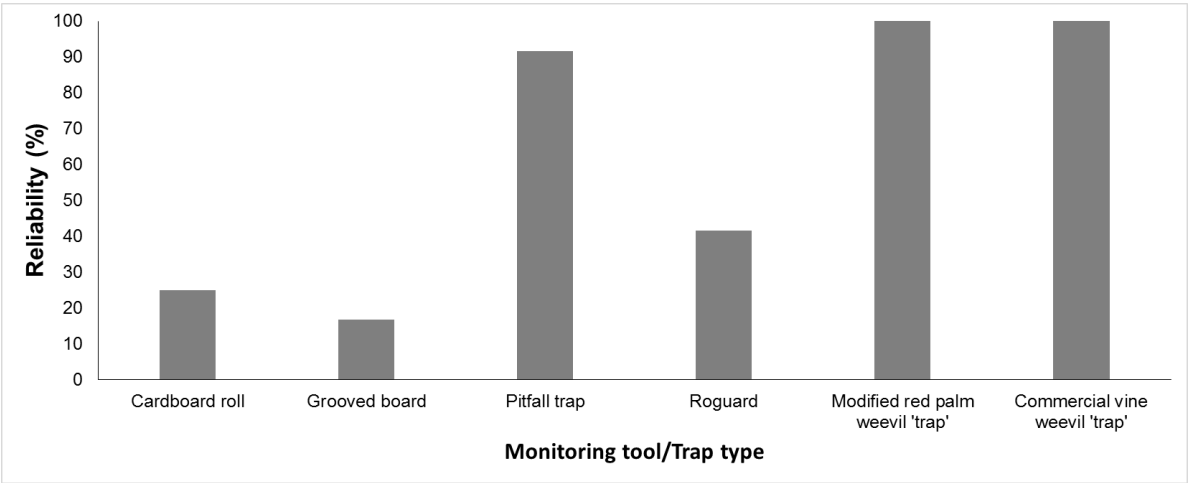

Figure 7. Reliability expressed as a percentage for the number of times out 12 assessments completed in which vine weevil adults were recorded in the monitoring tool/trap type (*n* = 12).

Experiment 2: Results from this experiment indicated that both monitoring tool (x^2 = 39.881, *P* <0.001) and weevil density (x^2 = 60.040, *P* <0.001) significantly affected the numbers of weevils recorded on each assessment day, see Figure 8. There was, however, no interaction between monitoring tool and weevil density. Post-hoc analysis confirmed that significantly more weevils were recorded in the commercial vine weevil trap (1.2, 3.7 and 6.9 weevils per trap per day at weevil densities of 4, 10 and 16/m^2, respectively, than either the pitfall or base of the red palm weevil trap. There was no significant difference in numbers of weevils recorded in/beneath the pitfall or base of the red palm weevil trap. The mean daytime (0550 to 2010 BST) temperature between 19th August and 28th August was 22.9°C (max = 36.6°C, minutes = 15.6°C) and the mean night-time temperature was 17.3°C (max = 24.1°C, minutes = 13.6°C).

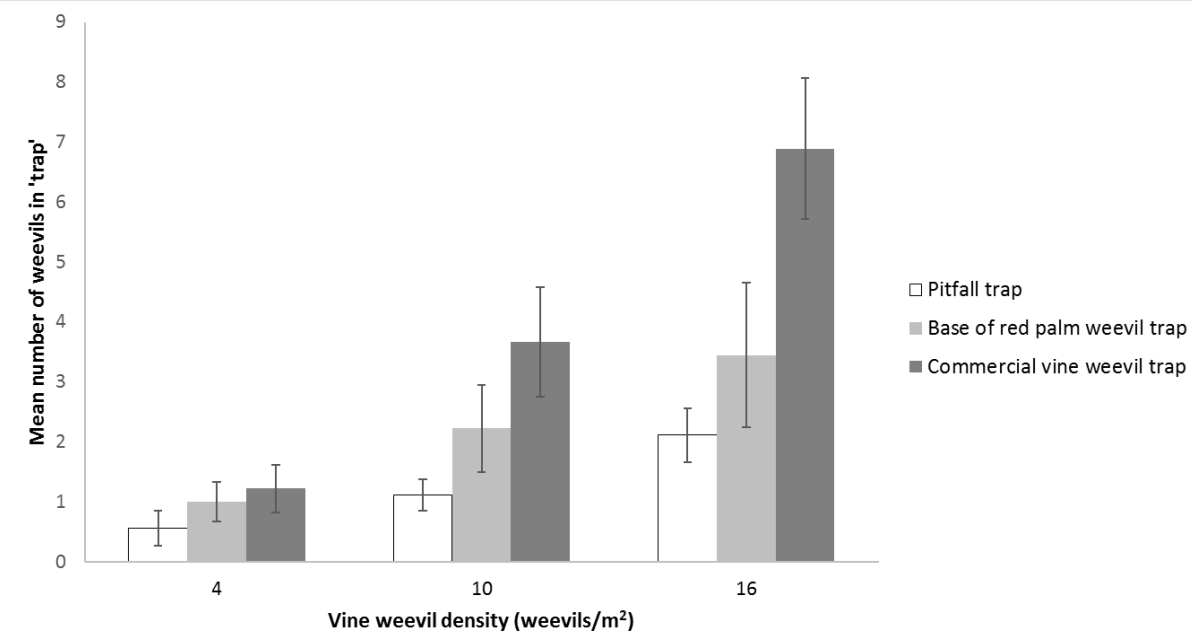

Figure 8. Numbers of vine weevil adults recorded within or underneath each monitoring tool/trap type at each weevil density on each assessment day. Means +/- SE, *n* = 9.

Experiment 3: Results from this experiment indicated that monitoring tool (x^2 = 78.403, *P* <0.001) but not plant density (x^2 = 0.438, *P* = n.s.) significantly affected the numbers of weevils recorded on each assessment day (Figure 9). This result confirmed that significantly more weevils were recorded in the commercial vine weevil trap (5.5, 6.6 and 5.3 weevils per trap per day at plant densities of 2, 4 and 7/m^2, respectively) than the pitfall traps (2.3, 1.3

and 1.6 weevils per trap per day at the same plant densities). The mean daytime (0650 to 1640 GMT) temperature between 28th October and 8th November was 12.3°C (max = 29.5°C, minutes = -1.0°C) and the mean night-time temperature was 7.3°C (max = 14.0°C, minutes = -1.0°C).

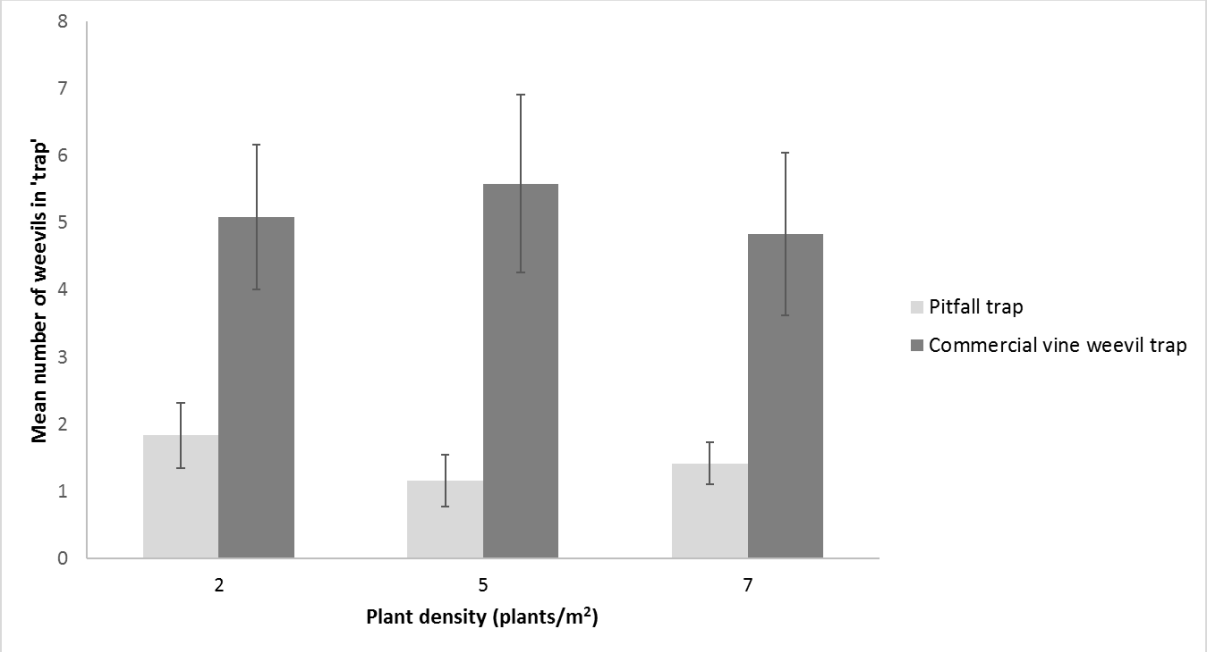

Figure 9. Numbers of vine weevil adults recorded within or underneath each monitoring tool/trap type at each plant density on each assessment day. Means +/- SE, *n* = 12.

Experiment 4: Weevil numbers and weevil feeding damage was assessed on 10 occasions. The number of times that weevils or weevil feeding damage was recorded (expressed as percentage i.e. reliability) is illustrated in Figure 10. The commercial vine weevil trap was again 100% reliable in that weevils were recorded in these traps each time they were assessed. *Euonymus* plants indicated the presence of vine weevil adults 40% while the *Primula* plants gave no evidence that vine weevil adults were present in the semi-field environments. The mean daytime (0700 to 1600 GMT) temperature between 9th November and 15th November was 9.7°C (max = 15.0°C, minutes = 5.1°C) and the mean night-time temperature was 6.7°C (max = 13.2°C, minutes = -1.1).

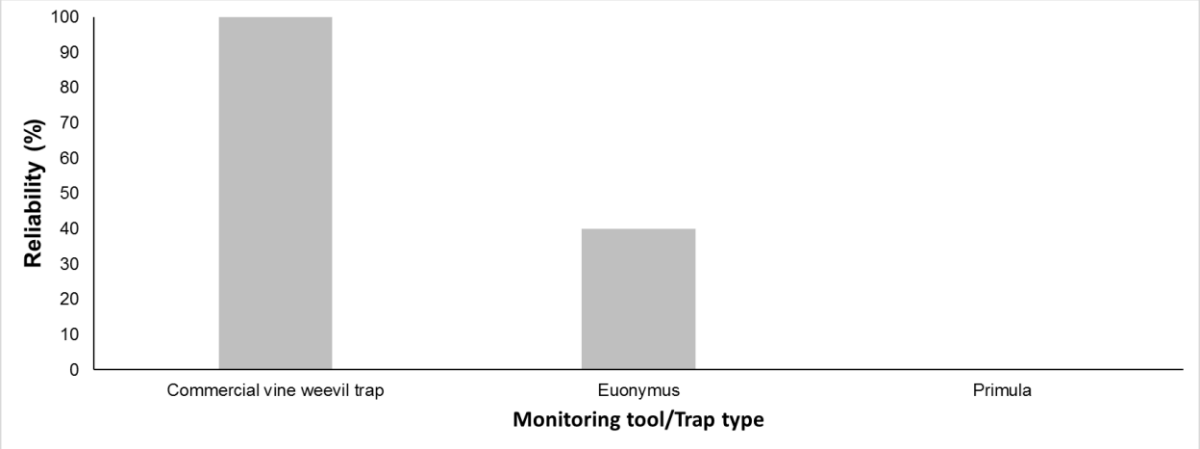

Figure 10. Reliability expressed as a percentage for the number of times out 10 assessments completed in which vine weevil adults or feeding damage were recorded (*n* = 10).

Discussion

A wide range of techniques have been developed in order to monitor for the presence of vine weevil adults within crops. Until now, however, a direct comparison between these techniques had not been completed and nor had traps been tested when vine weevil or plant densities had been varied. This is because most previous work had involved field testing, where weevil densities were unknown. Results from this work confirm that each of the established methods for determining the presence of vine weevil adults within crops work to a greater or lesser extent. There were, however, large differences in terms of numbers of weevil found and the reliability of each technique.

Grooved boards placed on the ground within crops (Li et al., 1995; Gordon et al., 2003) and corrugated cardboard (Phillips, 1989) are simple approaches for growers to use but were the least effective of the refuges or traps tested. Both techniques caught few weevils and often caught no weevils at all, despite weevils being present within crops. More effective than either the corrugated cardboard or grooved boards was an upturned plastic plant tray (e.g. http://stewart-garden.co.uk/products/terracotta/30_cm/multi-purpose-saucer/). Weevils were found to use these refuges where an uneven surface created gaps for weevils to crawl beneath the tray (on flat surfaces a small stone may be used to lift one side of the tray). Simple plastic crawling insect traps (Roguard traps) and pitfall traps were found to be more effective than either corrugated cardboard or grooved boards. Pitfall traps typically consist of a plastic cup sunk into the ground so that the lip of the cup is at soil level (e.g. Casteels et al., 1995; Solomon, 2000; Buxton, 2003). This often makes pitfall traps impractical for growers to use. In the present study, however, a modified pitfall trap that can be placed on the ground and does not require any digging was used. These traps, available from CSalomon

(http://www.csalomontraps.com/), were found to reliably indicate the presence of vine weevil adults. The commercial trap, supplied specifically for this research by ChemTica, Costa Rica, and otherwise not currently available in the UK, were the most effective of the traps studied. It should be noted, however, that the efficacy of these traps is likely to be impart due to the fact that weevils are unable to leave these traps once they have entered.

The characteristic leaf notching damage caused by adults feeding on leaf margins can be used to monitor this pest and the use of indicator plants can be used to help focus monitoring efforts (Buxton, 2003; Raffle, 2003). Although *Primula* has previously been suggested as an indicator plant, no damage was recorded on these plants in this study. In contrast *Euonymus* plants were found to be more effective indicator plants both because leaf notching was seen but also because adult weevils were often found associated with these plants. In previous work vine weevil adults have been shown to respond positively to the odour of *Euonymus* but not strawberry (the simulated crop plant used in this study) (van Tol et al., 2002). The relative attraction between the crop and indicator plant is likely to be key in determining the efficacy of this type of monitoring. Although, *Euonymus* indicated the presence of vine weevil adults 40% of the time, the commercial vine weevil trap indicated presence of vine weevil adults with 100% reliability in this experiment.

Conclusions

- The commercial vine weevil trap supplied by ChemTica was more effective in detecting the presence of vine weevil adults than all other monitoring tools or trap types tested.
- Modified pitfall traps and crawling insect traps may be usefully used to detect the presence of vine weevil adults within a crop.
- An upturned plastic plant tray more effectively detects the presence of vine weevil adults than grooved boards or rolls of corrugated card.
- *Euonymus* indicator plants are more effective than *Primula* indicator plants at detecting the presence of vine weevil adults. The efficacy of indicator plants will, however, be determined at least in part by the relative attractiveness to adult weevils of the crop being grown.

Task 2.2 Potential of lures to improve monitoring of vine weevil adults (Harper Adams and NRI, Years 1 and 2)

Research on attractants for vine weevil adults has focussed on potential aggregation pheromones produced by the live weevils, volatiles produced by host plants and weevil frass. van Tol et al. (2004), using a still-air olfactometer, reported that, rather surprisingly, vine weevil adults were attracted to odours from the clay coloured weevil (*Otiorhynchus salicicola*) but not to odours from conspecifics. Vine weevil adults showed some attraction to the frass of both species. These authors had previously reported that vine weevil adults were attracted to odours from yew, *Taxus baccata*, and *Euonymus fortunei*, but not to odours from *Rhododendron* or strawberry (van Tol et al., 2002). van Tol and Visser (2002) screened a wide range of compounds by electroantennogram (EAG) recording from vine weevil adults and found 14 compounds, which elicited a significant EAG response. Combining the results of these reports, van Tol et al. (2012) collected volatiles and extracts from a preferred host-plant, *Euonymus fortunei*, and analysed these by gas chromatography (GC) coupled to EAG recording from the weevil antenna. Eight compounds which elicited EAG responses were identified. The three most active compounds were tested in field trapping tests with boll weevil traps. No weevils were caught in the traps, but more weevils were found within a radius of 60 cm of traps containing (*Z*)-2-pentenol with or without methyl eugenol than round traps containing (*E*)-2-hexenol alone or mixed with the other two compounds. These results formed the basis of a patent application (Bruck et al., 2011) in spite of the fact that no weevils were caught in the traps and no data was provided on release rates of the compounds used. Interestingly, Borden et al. (2006) and Elmhirst et al. (2007) evaluated some of these compounds in trapping experiments in Canada, but few weevils were caught, probably because of very low populations.

In our own work, initial studies in laboratory arenas indicated that vine weevil adults spent more time in the part of the arena adjacent to conspecific weevils than in the part with no weevils. This was followed up in HDC project SF/HNS 127 (2011-2012). Tests in a moving-air olfactometer failed to confirm the attraction of vine weevil adults to conspecific weevils or frass, but they were attracted to damaged strawberry leaves. Volatiles collected from strawberry leaves and weevil frass were collected by solid-phase microextraction (SPME) and analysed by GC coupled to mass spectrometry (MS). Volatiles from leaves were dominutesated by “green-leaf” volatiles and those from frass by sesquiterpenes, particularly *β*-caryophyllene, germacrene-D and *α*-farnesene, most likely derived from plant food. A range of synthetic volatiles was tested by GC-EAG and reproducible EAG responses were recorded to (*E*)-2-pentenol and (*Z*)-2-pentenol, (*E*)-2-hexenol, (*Z*)-3-hexenol, 1-octen-3-ol, linalool and methyl salicylate. An occasional response to *α*-farnesene and to methyleugenol was

observed. No responses were observed to germacrene-D or *β*-caryophyllene. Volatiles were also collected from *Euonymus fortunei* on Porapak and analysed by GC-MS and GC-EAG. EAG responses were observed to 1-hexanol, (*Z*)-3-hexenol, (*E*)-2-hexenol and methyl salicylate. When candidate attractants were tested in the moving air olfactometer, (*E*)-2-hexenol was attractive but 1-hexanol and (*Z*)-3-hexenol were repellent at the concentrations tested.

In addition to potential role of volatile compounds, the lipid layer on the cuticle of the weevils may play a role in intraspecific communication. The lipid layer of the insect cuticle is essential for their survival, preserving the insect from desiccation, cuticle abrasion and infection (Lockey 1988). However, across many insect taxa, cuticular lipids, in particular the hydrocarbons, have evolved to become part of their communication system acting as short-range/contact pheromones involved in species and sex recognition (Blomquist et al., 1998; Howard and Blomquist, 2005; Blomquist and Bagnères 2010; Prestwich and Blomquist 2014).

Given the aggregation behaviour of vine weevil, it is perhaps surprising that their cuticular lipids have apparently not been studied previously with only a study of the cuticular hydrocarbons of the related weevil, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) being apparent (Lapointe et al., 2004). A study was, therefore, undertaken to investigate the cuticular hydrocarbons of vine weevil adults and to determine if their composition is species-specific and hence may play a role in their behaviour, particularly in aggregation. If this is the case then it may be possible to use them to increase aggregation of the weevils in traps/refuges.

The aim of this task is to assess the potential of lures based on plant volatiles to improve the efficacy of the best performing vine weevil traps tested in Task 1. The purpose of this work is to improve the reliability and sensitivity of traps so that vine weevil infestations can be detected earlier and when populations are lower. This information would allow growers to implement control measures before adults have had time to cause significant leaf notching damage and would minutesimise the period in which eggs could be laid. Demonstrating that an attractant can lure adult weevils to a monitoring trap would also provide a vital step in developing a novel lure and kill approach to controlling this pest (see Objective 4, novel approaches to control).

Materials and methods

Insects: Vine weevil adults were maintained as previously described at Harper Adams with samples sent to NRI as required. In addition, a sample of an unknown species of weevil collected by Jude Bennison, ADAS, from a commercial soft fruit crop was also sent to NRI.

Solid phase microextraction (SPME)-GC-MS analysis of volatile compounds produced by vine weevil adults: Fifty vine weevil adults were placed into a 2 litre glass sample container sealed with a PTFE-lined screw cap lid retrospectively fitted with a PTFE septa (Figure 11). Vine weevil adults were starved for at least 24 hours prior to sampling to reduce the quantity of frass and associated plants volatiles produced. At the bottom of the sample container, two filter papers moistened with distilled water and a Roguard trap (see Objective 1) were placed to provide hydration and a refuge site. Gauze was inserted at the top of the container to prevent physical contact between the wine weevil adults and the SPME fibre. After an equilibration period of 4 hours at 20°C and 60% RH, a conditioned SPME fibre (75 µm film thickness, CAR/PDMS, stableflex, 24Ga) (Supelco, UK) was exposed to the headspace of the container for 16 hours (overnight approx. 5pm to 9am in order to cover the period during which aggregation behaviour typically occurs) to entrain VOCs. Collected VOCs were released from the SPME fibre by thermal desorption over 5 minutes directly within the inlet port of the GC-MS used for VOC analysis [GC (Agilent 7890B fitted with a HP-5MS column: 30 m x 0.32 mm x 0.25 µm, injection temperature: 285°C, splitless injection); MS (Agilent 5977A mass selective detector, 70 EV, scan range: 50 - 500 M/Z, source temperature: 230°C, quadrople temperature: 150°C, solvent delay: 0 minutes)]. The temperature program of the GC-MS was from 40°C (5 minute hold) to 240°C (20 minute hold) at 10°C/minutes (total run time: 42 minutes). Tentative identification of VOCs was undertaken by comparing the spectra and retention indices of the VOCs with a mass spectra database (NIST MS search 2.0; National Institute of Standards and Technology, USA). Chemical compound carry over between samples, as well as peaks originating from the SPME fibre were regularly assessed by running blank samples. After each desorption the SPME fibre was immediately reconditioned in a GC injector port for 10 minutes at 300^0C to prevent contaminutesation. Periodic blank headspace vials were used as control to ascertain system impurities. Each sample collection of VOCs and GC-MS analysis was repeated four times with new samples. The sample container, lid, and cockroach trap were cleaned between each replicate by rinsing with 100 ml of deionised water and 100 ml of HPLC-grade acetone (Sigma-Aldrich, UK) before heating in an oven set to 100°C for 15 minutes.

Figure 11. A two litre glass sample container sealed with a PTFE-lined screw cap lid retrospectively fitted with a PTFE septa with SPME fibre inserted into container.

Thermal desorption (TD)-GC-MS Analysis of vine weevil volatiles: One hundred adult vine weevil adults were placed into a clean BugDorm cage (47.5x47.5x47.5 cm) containing a Roguard trap and lined with tissue paper moistened with distilled water. Vine weevil were starved for at least 24 hours prior to sampling to reduce the quantity of frass and associated plant volatiles produced (see Figure 12). A stainless steel sorbent tube containing 200 mg of Tenax-TA sorbent (Markes International, Llantrisant, United Kingdom) was placed at the entrance of the cockroach trap. Air was drawn through the sorbent tube at 50 ml/minutes for 16 hours using a membrane pump (Rothamsted Research, Harpenden, United Kingdom). The membrane pump and sorbent tube were connected using PTFE tubing with an outside diameter of 3 mm (Airline Fittings, Matlock, United Kingdom) and 6 mm to 3 mm brass tube reducing unions (Swagelok, Manchester, United Kingdom). Prior to undertaking entrainments, the BugDorm cage was cleaned with 100 ml of deionised water and 100 ml of HPLC-grade acetone (Sigma-Aldrich, UK). Entrainments were completed overnight approx. 5pm to 9am in order cover the period during which aggregation behaviour typically occurs.

Entrained volatiles were desorbed from the Tenax-TA via a UNITY series 2 thermal desorption unit (Markes International, Llantrisant, United Kingdom) by heating the sorbent tube to 250°C for 10 minutes under a He flow at a rate of 20 ml/minutes. The desorbed volatile compounds were collected in a general-purpose C4 – C32 carbon cold trap (Markes International, Llantrisant, United Kingdom) at − 10°C before ballistic heating to 300°C to ensure that a sharp injection of the volatiles into the capillary column of the gas chromatograph-mass spectrometer (GC-MS) was achieved. The temperature program of the GC-MS was from 40°C (5 minute hold) to 240°C (20 minute hold) at 10 °C/minutes (total run time: 42 minutes) [GC (Agilent 7890B with an HP-5MS column: 30 m x 0.32 mm x 0.25 µm, injection temperature: 250 °C, splitless injection); MS (Agilent 5977A mass selective detector, 70 EV, scan range: 50 - 500 M/Z, source temperature: 230 °C, quadropole temperature: 150°C, solvent delay: 0 minutes)]. Identification of VOCs was undertaken by comparing the spectra with a mass spectra database (NIST MS search 2.2; National Institute of Standards and Technology, USA). After each desorption the sorbent tubes were immediately reconditioned in the UNITY series 2 thermal desorption unit for 15 minutes at 300°C to prevent chemical compound carry over. Periodic entrainments of empty BugDorms and Tenax-TA tubes were used as controls to ascertain system impurities and to assess chemical compound carry over between samples.

Figure 12. Vine weevil adults aggregating in Roguard trap placed inside a BugDorm cage.

Isolation and analysis of cuticular hydrocarbons: This work was completed at the NRI. Most of the work was done on solvent extracts of the cuticular hydrocarbons. Vine weevil adults and adults of the unknown species of weevil were extracted individually in hexane (Pesticide Residue Grade, Fisher; 1 ml) for 1 h with occasional shaking. The solvent was removed and the carcass washed with a further aliquot (0.5 ml) of hexane. The combined extract was concentrated to approximately 0.2 ml under a gentle stream of purified nitrogen.

Solid-phase microextraction (SPME) was also examined. A SPME needle (100 µ polydimethylsiloxane; Supelco) was stroked across the cuticle of a live weevil for 1 minute, and then inserted directly into the injector of the gas chromatograph used for analysis.

Components were quantified by gas chromatography (GC) with flame ionisation detection (FID) and identified by GC coupled to mass spectrometry (GC-MS)

Analyses by GC-FID were carried out on a HP6850 instrument (Agilent Technologies) fitted with a fused silica capillary column (30 m x 0.32 mm i.d. x 0.125 µm film thickness) coated with HP5 (Agilent). Carrier gas was helium (2.4 ml/minute), injector 280°C, detector 320°C and oven temperature programmed from 60°C for 2 minutes then at 10°C/minutes to 300° and held for 5 minutes. Injection was splitless.

Analyses by GC-MS were carried out on a HP6890N GC coupled to a HP5973 quadrupole mass selective detector (Agilent), fitted with a fused silica capillary column (30 m x 0.25 mm i.d. x 0.125 µm film thickness) coated with DB5 (Agilent). Carrier gas was helium (1 ml/minutes), injector 280°C, and oven temperature programmed from 60°C for 2 minutes then at 10°C/minutes to 300° and held for 5 minutes. Injection was splitless.

GC retention times were converted to Retention Indices (RI) relative to the retention times of *n*-alkanes.

Results

Solid phase microextraction (SPME)-GC-MS and Thermal desorption (TD)-GC-MS analysis of volatile compounds produced by vine weevil adults: Volatile compounds produced by vine weevil adults were effectively sampled using SPME fibres. A large number of volatile compounds have been tentatively identified through mass spectrometry from samples collected using SPME fibres. The large number of volatile compounds identified reflects the large number of weevils sampled. The following GC trace (Figure 13) is typical of each of the four replicates completed.

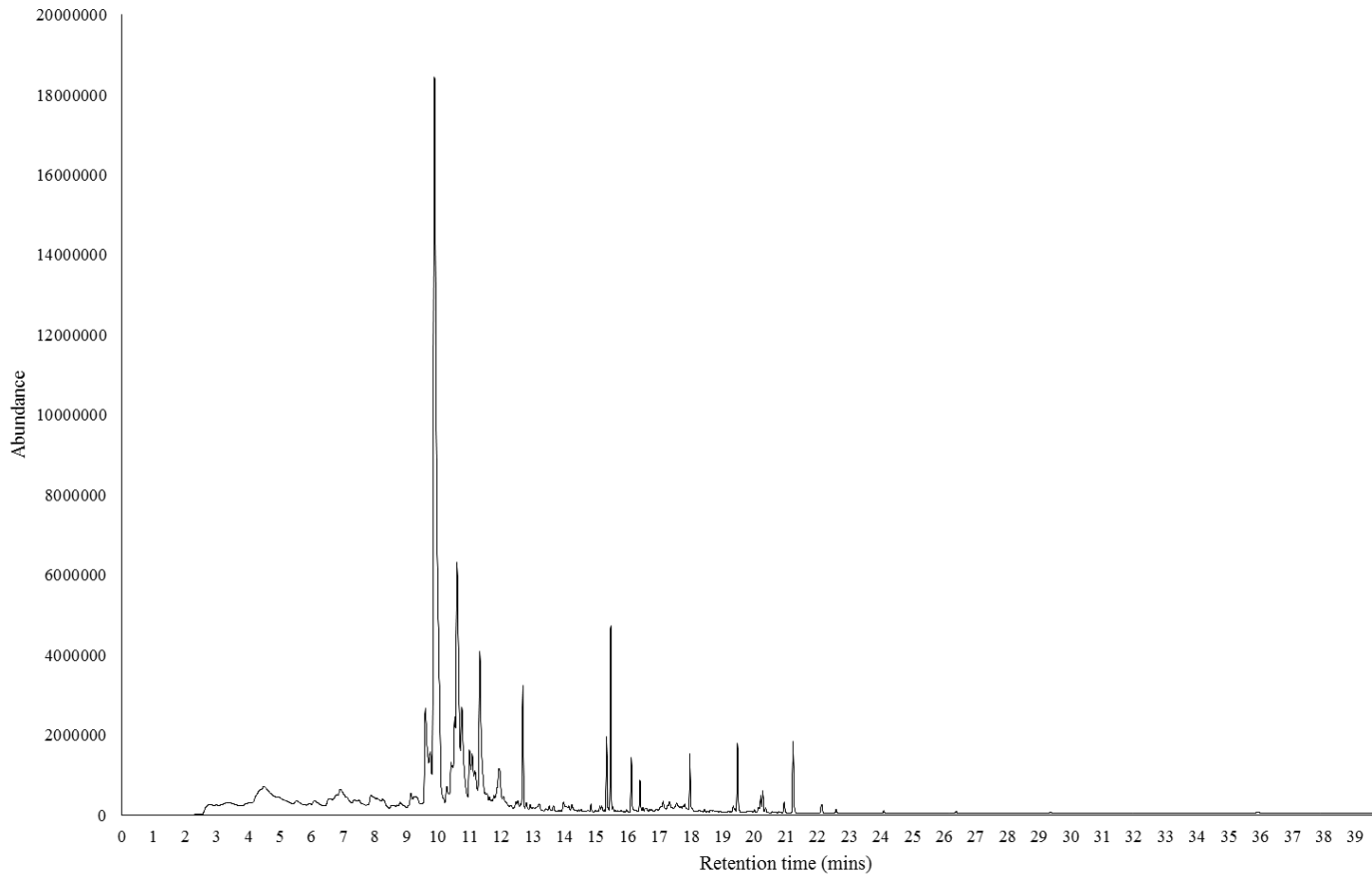

Figure 13. Illustrative GC-MS analyses of volatile compounds produced by vine weevil adults desorbed from a SPME fibre.

Many of the same volatile compounds have been tentatively identified through mass spectrometry from samples collected using sorbent tubes containing Tenax-TA as were identified from samples collected using SPME fibres. The relative abundance of the different volatile compounds, however, differed between the two techniques. The following GC trace (Figure 14) is illustrative.

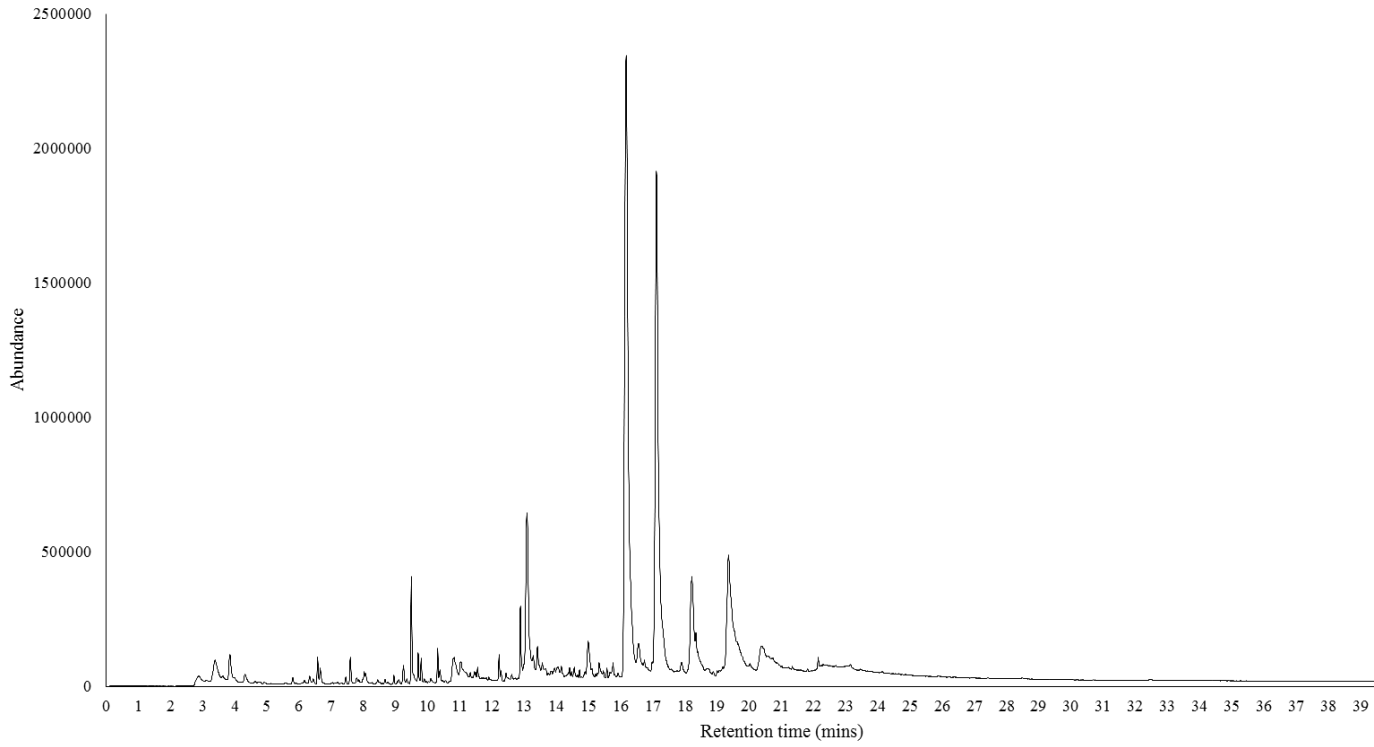

Figure 14. Illustrative GC-MS analyses of volatile compounds produced by vine weevil adults desorbed from a Tenax filters.

Isolation and analysis of cuticular hydrocarbons: Isolation of cuticular hydrocarbons of vine weevil by SPME gave very low amounts of material and this approach was not pursued although it has worked well for cerambycid beetles in our laboratory.

Extraction of the cuticular hydrocarbons of vine weevil adults and the unknown species of weevil for 1 h in hexane was adequate, and longer extraction and/or brief sonification did not improve extraction as indicated by GC-FID analyses. Amounts of material obtained were low relative to previous experience with cerambycid beetles and quantification against pentacosane as internal standard of the three extracts with most material indicated total hydrocarbons was 6.5 µg/weevil ± 1.0 µg (SE).

However, the relative amounts of the hydrocarbons in the extract were quite consistent (Figure 15), and some of these were identified in GC-MS analyses by means of their RI and mass spectra (Blomquist et al., 1987) (Table 3).

Analyses of extracts of cuticular hydrocarbons from adults of the unknown species showed many similar compounds to those from vine weevil adults, but their distribution was very different.

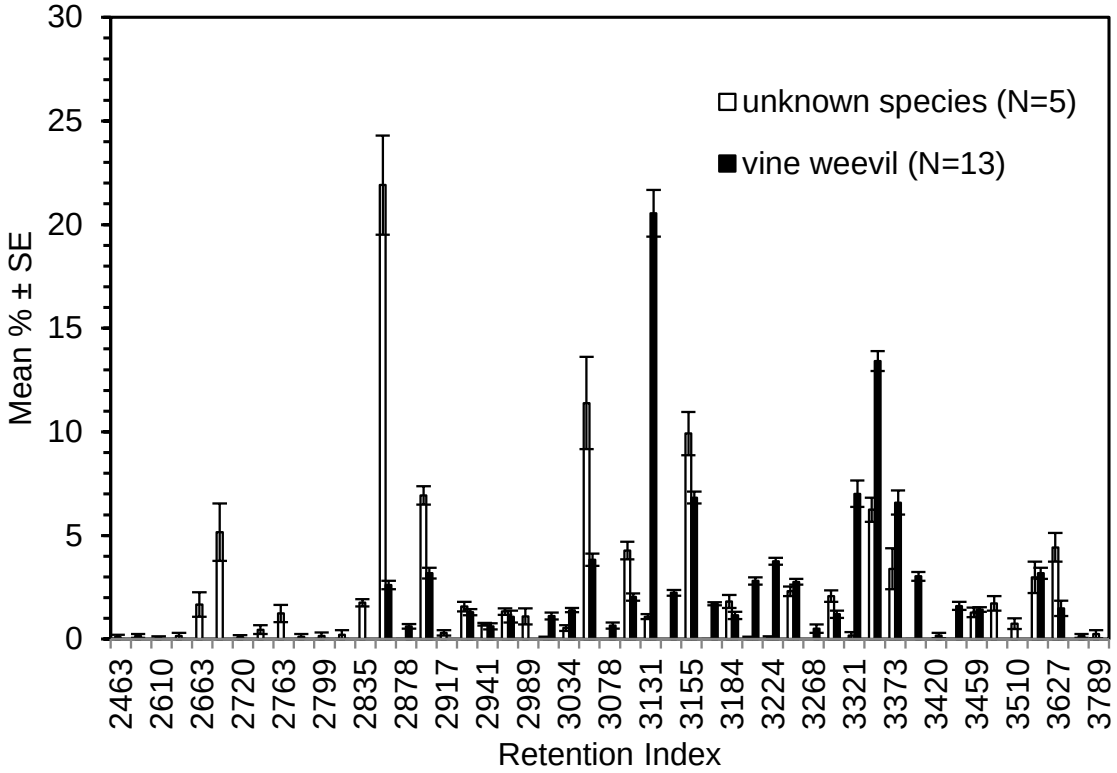

Figure 15. GC Analyses of cuticular hydrocarbons from vine weevil adults and adults of the unknown species of weevil.

Table 3. Compounds present in vine weevil but only at low levels in the unknown species were 13- and 15-methylhentriacontane (13/15Me-31H; RI 3131), 13-, 15- and 17-tritriacontane (13/15/17-33H; RI 3321), and 13,19-dimethyltritriacontane (13,19diMe-33H; RI 3346). Compounds present in the unknown species but only at low levels in vine weevil were 7,20-dimethylhexacosane (7,20diMe-26H; RI 2703), 4-methyloctacosane (4Me-28H; 2863) and 4-methyltriacontane (4Me-30H; RI 3064).

			Relative amount	
RI	Compound	Ions (m/z)	VW	UK
2703	7,20diMe-26H	113, 309, 379		++
2835	8,21diMe-28H?	127, 323, 407		+
2863	4Me-28H	365, 393, 408	+	+++++
2902	7,22diMe-28H	113, 337, 407	+	++
3064	4Me-30H	393, 421, 436	+	+++
3131	13/15Me-31H	435, 196/280, 224/252	+++++	+
3142	7Me-31H	435, 112/365	+	
3155	13,17diMe-31H	464, 449, 196/295, 224/267	++	++
3323	13,15,17Me-33H	477, 463, 196/309, 224/280, 224/267	++	

3346	15,19diMe-33H ?	224, 267, 295, 478	+++	++
3349	13,19diMe-33H	477, 196/323, 224/295		
3380	11,15,21-triMe-33H	491, 168/365, 239/295, 337/196	++	+

Discussion

Solid phase microextraction (SPME)-GC-MS and Thermal desorption (TD)-GC-MS analysis of volatile compounds produced by vine weevil adults: Previous work to identify volatile compounds associated with vine weevil adults has focused on frass and host plant volatiles (e.g. Karley et al., 2012; Pickett et al., 1996; van Tol et al., 2002, 2004, 2012; van Tol and Visser, 2002). This previous work has for example identified compounds such as (Z)-2-pentenol. This compound, which when released with or without methyl eugenol increases the number of weevils found within a radius of 60 cm of the lure but crucially did not increase trap catches (van Tol et al., 2012). As the weevils were starved before completing headspace sampling, few plant volatiles were recorded, regardless of the approach taken. A large number of volatile compounds (>100) have, however, been tentatively identified from a range of chemical groups. As these volatile compounds were sampled when weevils were actively aggregating (see Figure 12) at least some may be important in this process. Further work is required to confirm the identification of each of these volatile compounds but a number of volatiles compounds previously shown to be behaviourally active in other species of insect have been identified.

Identified volatile compounds associated with vine weevil adults will be tested for their physiological and behavioural activity. This will be done through use of electroantennograms (EAGs) and olfactometry using approaches developed in HDC project SF/HNS 127.

Isolation and analysis of cuticular hydrocarbons: Analyses of cuticular hydrocarbons from vine weevil adults and adults of the unknown species of weevil showed that their compositions were quite consistent and different from each other, providing evidence that they are species-specific and could be used for species recognition. The main components in the vine weevil cuticular hydrocarbons were monomethyl substituted hydrocarbons with an odd number of hydrocarbons and the substituents at odd-numbered carbons in the chain. The main components of the unknown species of weevil were methyl substituted hydrocarbons with even numbers of hydrocarbons and the substituent particularly in the 4-position.

Cuticular extracts will be tested for their ability to increase colonisation of refuges. The component hydrocarbons are not commercially available, but if the extracts show activity, the major components can be synthesised. They will be safe, stable and very persistent in use.

Conclusions

- A large number of volatile compounds have been identified from starved vine weevil adults, where there was no host plant leaf material and little frass.
- Analyses of cuticular hydrocarbons from vine weevil and an unknown species showed that their compositions are quite consistent and different from each other, providing evidence that they are species-specific and could be used for species recognition. These have potential to increase colonisation of refuges.

Future work

- *Task 2.2 Potential of lures to improve monitoring of vine weevil adults (Harper Adams and NRI, Years 1 and 2)*
    - Behavioural and electrophysiological assays will be completed to determine which of the identified volatile compounds are the most promising vine weevil attractants. This work will include use both of olfactometery and GC-EAG.
    - Behavioural assays will be completed using the identified cuticular hydrocarbons. This work will include use of choice tests in which vine weevil adults will be presented with refuges treated with cuticular hydrocarbons or left untreated refuges.
    - The most promising volatile compounds and/or cuticular hydrocarbons will be tested together with the best performing of the vine weevil traps identified in the work presented above. This work will use the semi-field conditions described in this report and test the efficacy of the traps with or without the addition of an attractant/arrestant at a range of weevil densities.

Objective 3. Improve best-practice IPM approaches including the use of entomopathogenic nematodes, fungi and IPM-compatible insecticides

Task 3.1. Alternative application method for entomopathogenic nematodes (ADAS, years 1 and 2)

Objective

The aim of this task is to provide growers with an alternative, less time-consuminutesg and more cost-effective application method than using drenches for reliable control of vine weevil larvae with entomopathogenic nematodes. The method will be based on a 'little and often' approach for maintaining control of vine weevil larvae through the season, applying reduced rates of entomopathogenic nematodes through overhead irrigation. The work will be done over two years, in the first year the method will be tested and nematode dose rates compared in a research poly tunnel at ADAS Boxworth and in the second year a selected reduced dose rate will be tested on a commercial nursery.

Task 3.1.1 Pilot experiment testing little and often nematodes at ADAS Boxworth (year 1).

Materials and methods

Site

Two adjacent poly tunnels, ADAS Boxworth, Cambridge.

Experimental plants

Fuchsia plugs were potted into 9 cm pots using untreated growing media at Darby Nursery Stock on 6 May 2016. The pots were surrounded by rows of blue sticky traps on the concrete floor of the glasshouse to prevent any resident vine weevil adults infesting the experimental plants. The pots were transported to ADAS Boxworth and potted on into 2 litre pots on 2nd June.

Treatments

The treatments are shown in Table 4. *Steinernema kraussei* (Nemasys L) was selected as the nematode species for the experiment as it would be effective if low temperatures occurred early and/or late in the season (recommended temperature range 5-30°C).

Table 4. Treatments

Treatment number	Treatment	Application method and water volume	Timinutesg	Total number nematodes applied through the season
1	Nemasys L full rate (500,000 per m^2)	Drench in 200 ml per 2L pot	2 applications (22 Sep & 21 October)	1,000,000 per m^2
2	Nemasys L full rate (500,000 per m^2)	Overhead irrigation in 4L water per m^2	2 applications (22 Sep & & 21 October)	1,000,000 per m^2
3	Nemasys L 40% rate (200,000 per m^2)	Overhead irrigation in 4L water per m^2	5 applications (1 July, 28 July, 31 Aug, 22 Sep, 21 Oct)	1,000,000 per m^2
4	Nemasys L 20% rate (100,000 per m^2)	Overhead irrigation in 4L water per m^2	5 applications (1 July, 28 July, 31 Aug, 22 Sep, 21 Oct)	500,000 per m^2
5	Water control	Drench 200 ml per pot	2 applications (22 Sep & & 21 October)	0
6	Water control	Overhead irrigation in 4L water per m^2	5 applications (1 July, 28 July, 31 Aug, 22 Sep, 21 Oct)	0

Experimental design

After potting into 2L pots, the Fuchsia plants were laid out in a randomised block design using six plants per plot and six replicate plots per treatment (Figure 16). In addition to the 216 experimental plants, an additional 84 ‘spare’ plants were used to monitor vine weevil larval development during the experiment period in order to help decide on numbers of eggs to infest the plants with and when to carry out the final destructive assessment. A border of duct

tape coated with Eco Tack® glue was used around the plants on the woven ground-cover matting on the floor of the tunnel in order to prevent any resident naturally-occurring vine weevil adults on site from infesting the experimental plants.

Figure 16. Experiment layout in poly tunnel

Plant husbandry

The pots were watered twice per day for five minutes on each occasion through the overhead irrigation. Pests other than vine weevil were controlled using biological control agents which were used preventively, including the parasitoid *Encarsia formosa* against whitefly, a mix of aphid parasitoids against aphids and the predatory mite *Phytoseiulus persimilis* against two-spotted spider mite.

Vine weevil egg infestation

Vine weevil adults were collected from commercial crops of HNS and strawberry between May and July and maintained in plastic boxes on damp tissue in a controlled environment room kept at 21°C. The weevils were fed with strawberry or yew leaves. The experimental plants and extra plants (300 in total) were infested with brown (embryonated) eggs on the following four dates, to mimic the natural vine weevil egg laying period:

- Five eggs per plant on 16 June and 15 July
- Fifteen eggs per plant on 10 August and 9 September

Therefore each plant was infested with a total of 40 eggs during the experimental period. On each infestation date, the required number of brown eggs was collected from the vine weevil

culture using a fine paintbrush and transferred to 300 pieces of damp filter paper, using either five or fifteen eggs per filter paper according to the infestation rate used. A small area of the topmost layer of growing media next to each plant was removed and the eggs were washed onto the growing media. The eggs were then covered lightly with damp growing media. Eggs were applied to all the control treatments first in order to avoid transferring any nematodes to untreated pots.

Vine weevil egg viability

Percentage egg viability was determined by collecting 100 additional brown eggs from the culture on each of the four infestation dates and assessing how many hatched in the laboratory.

Calibration of water application through sprinkler

Prior to nematode application, the volume of water applied through an overhead sprinkler was calculated in a 4.2 m x 4.2 m area around the sprinkler in an adjacent poly tunnel to the one used for the experimental plants. The test area was laid out in a grid with 49 plastic plant pot saucers (27.5 cm diameter) spaced 50 cm from centre to centre in seven rows of seven with the sprinkler in the centre of the grid (Figure 17). The 49 locations in the grid were labelled 1-7 from left to right and A-G from top to bottom. The sprinkler was run for ten minutes in order to apply 4L per m^2 water. The water in each saucer was then collected and the volume measured. This was replicated three times. The results were used to select five locations in the grid around the sprinkler where the volume of water applied was the most accurate and consistent. The experimental plants for each treatment application were placed in these five locations.

Figure 17. Plant pot saucers used to calibrate water application through sprinkler.

Calibration of nematode application through sprinkler

A pack of 50 million Nemasys L was used to assess how many nematodes were applied to the five selected locations around the sprinkler used for the calibration of water volume application. A 'stock' suspension of nematodes was mixed using one pack mixed with 4L water to give the full rate to use in the feeder bucket for the Dosatron®. This stock suspension was then diluted with water to give the 40% and 20% rates for use in the feeder bucket for the application of the reduced rates of Nemasys L (Table 5).

Table 5. Numbers of nematodes and water volumes added to feeder bucket for Dosatron® to make up the stock suspensions of Nemasys L at the three dose rates applied.

Nemasys L rate	Numbers of nematodes added	Volume of water added	Total nematode concentration in feeder bucket
100%	1 pack of Nemasys L	4L water	50,000,000 (12,500 nematodes/ml)
40%	1600ml of the 100% rate	2400ml water	20,000,000 (5,000 nematodes/ml)
20%	800ml of the 100% rate	3200ml water	10,000,000 (2,500 nematodes/ml)

Each rate of Nemasys L was delivered through the sprinkler at 4L/ha using a Dosatron® set at a 1% feed intake rate. The filters were removed from both the sprinkler nozzle (Rainbird 1800) and the Dosatron® to avoid nematode blockages and to allow the nematodes to move freely through the system. Before assessing the numbers of nematodes applied to the five selected locations in the grid, each of the three rates were applied through the Dosatron® and sprinkler for one minute to allow the nematode suspension to travel through the irrigation line and to start being applied through the sprinkler. The nematode suspension was kept agitated during application to prevent settling out. After the system had been primed for one minute, the sprinkler was turned off and a plastic pot (85mm diameter) was placed onto the five saucers in the five selected locations where the required volume of water was applied most accurately and consistently (Figure 18). The sprinkler system was then run for ten minutes for each of the three nematode rates, using fresh collection pots for each rate. The application of the lowest rate (20%) was replicated twice but as the numbers of nematodes delivered were found to be very accurate, the 40% and 100% rates were only applied once.

The collection pots were taken to the laboratory for assessment of nematode numbers per millilitre applied through the system. One ml of the nematode suspension was taken from

each pot using an Eppendorf pipette and transferred to a 'Doncaster' counting dish and examined under a low power binocular microscope. Numbers of active nematodes per millilitre were recorded. This procedure was replicated three times for each collection pot.

Figure 18. Plastic pots used to assess numbers of nematodes applied through sprinkler in the five selected locations where water volume application was most accurate and consistent.

Treatment application to experimental plants

Treatment application dates are shown in Table 4. The plants to be treated with each of the treatments applied through the overhead irrigation (Treatments 2-4 and 6) were taken in plastic crates into the adjacent poly tunnel. One treatment was applied at a time after placing the appropriate crates of plants onto the five selected locations around the sprinkler. Treatments were applied between 9 am and 1 pm on each treatment date.

A fresh 50 million pack of Nemasys L was used to apply all nematode rates (Treatments 1-4) on each application date. The nematodes were made up according to the manufacturer's recommendations. For the nematode treatments applied through the sprinkler (Treatments 2-4), the same procedure was used as that used to calibrate the nematode application through the sprinkler. The pack contents were first added to a small amount of water and mixed to a paste, then the required amount of water was added to make the nematode suspension in the feeder bucket for the Dosatron®. To confirm the numbers and viability of the nematodes, three replicate 1 ml samples of the nematode suspension for each of Treatments 1-4 were taken and transferred to a Hauxley haemocytometer slide in the laboratory. The samples were examined under a binocular microscope and numbers of active nematodes per millilitre were recorded. All other manufacturer's recommendations were followed during application.

All nematode applications made through the overhead irrigation (Treatments 2-4) and the water control (Treatment 6) were applied to the plants at 4L/m^2 in two 10 minute sessions. Following application water was applied for a further 30 seconds through the sprinkler to wash any nematodes remaining on the leaves into the growing media. During application of each

treatment on every treatment date a 20 ml specimen tube (9.1 cm long and 2.2 cm diameter) was inserted into the growing media at the edge of the pot in each of the five selected locations to collect nematodes, in order to check how many reached the growing media (Figure 19). The tubes were then taken to the laboratory and the volume of nematode suspension and numbers of nematodes per millilitre assessed, compared with the numbers expected in the area of the growing media occupied by the tube for each of the three application rates.

Figure 19. Specimen tube inserted into growing media (bottom right) to check nematode numbers reaching the growing media when applied through overhead irrigation.

The nematode treatment and water control applied as a drench (Treatments 1 and 5) were applied in 200 ml water per 2L pot as this was consistent with the volume most growers apply drenches at (10% of pot volume). The drench was applied to each pot using a syringe, after checking that the growing media was moist to enable the drench to be absorbed.

Destructive plant assessments

The experimental plants were destructively assessed between 28 November and 5 December. The growing media in each pot was searched for live vine weevil larvae and numbers per pot were recorded. The roots of each plant were then washed over a sieve to collect and remaining vine weevil larvae and the roots were assessed for vine weevil feeding damage, using a score between zero (no damage) to five (plant dead with no root system remaining), Figure 20.

Figure 20. Fuchsia plants with vine weevil damage to roots scored as 0 (no damage) to 5 (plant dead with no root system remaining).

Growing media temperatures

Growing media temperatures were monitored during the experiment period using two data loggers buried in the growing media in two of the spare Fuchsia pots.

Statistical analysis

The data on vine weevil larvae numbers were subjected to analysis of variance (ANOVA) using Genstat 14th Edition. The data on root damage scores was subjected to Regression Analysis using Genstat.

Results

Vine weevil egg viability

Vine weevil egg viability tests showed that 70%, 87.5%, 100% and 71% of the eggs successfully hatched on the four infestation dates respectively.

Calibration of water application through sprinkler

Following measurement of the water volumes collected in the three replicate saucers per grid location, locations A1, A2, A4, A6 and B1 were selected as the five locations where the required water volume needed to apply the nematodes at 4 litres/m^2 (238ml +/- 20 ml) was applied by the sprinkler most accurately and consistently (Table 6). The grids highlighted in yellow show the results given in the five selected locations which were then used for nematode application to the experimental plants. It was calculated that for accurate delivery of 4 litres/m^2, 238 ml should be collected in each saucer during the ten minute period the sprinkler was used.

Table 6. Mean volumes of water (ml) applied to three replicates of the 77 grids around the sprinkler (**X**) over a 10-minute period. Grids highlighted in yellow are the five selected locations for treatment application to experimental plants. Grids coloured green delivered the most accurate water volume, those coloured orange delivered less accurate volumes and those coloured red delivered the least accurate volumes.

Grid location	1	2	3	4	5	6	7
A	**223.3** (+/- 18)	**238** (+/- 14)	**194** (+/- 8)	**226** (+/-14)	**224.33** (+/- 39)	**228** (+/- 10)	**199** (+/- 46)
B	**229** (+/- 17)	**271** (+/- 103)	**169.7** (+/- 15)	**165** (+/- 19)	**171.67** (+/- 25)	**226** (+/- 52)	**208** (+/- 129)
C	**198** (+/- 19)	**167.67** (+/- 27)	**151.7** (+/- 22)	**114.67** (+/- 10)	**135.67** (+/- 42)	**146.67** (+/- 110)	**162.67** (+/- 106)
D	**170.67** (+/- 15)	**134.33** (+/- 13)	**88.33** (+/- 19)	**X**	**82.33** (+/- 72)	**129.67** (+/- 101)	**161** (+/- 92)
E	**124** (+/- 10)	**80.67** (+/- 12)	**60** (+/- 2)	**49.33** (+/- 5)	**103.67** (+/- 86)	**114.67** (+/- 103)	**135.33** (+/- 105)
F	**69.33** (+/- 6)	**99** (+/- 12)	**51.67** (+/- 10)	**150.67** (+/- 25)	**216.33** (+/- 49)	**161** (+/- 145)	**146.33** (+/- 98)
G	**118** (+/- 4)	**47.33** (+/- 5)	**99.67** (+/- 16)	**230.33** (+/- 25)	**211** (+/- 50)	**267.67** (+/- 29)	**189** (+/- 109)

Calibration of nematode application through sprinkler

Mean numbers of nematodes per millilitre collected in the pots placed on saucers in the five selected grid locations around the sprinkler for each Nemasys L dose rate and mean numbers

expected if nematodes were delivered accurately in 4L/m^2 water are shown in Table 7. Nematode delivery rate was very accurate at all rates and in all locations.

Table 7. Mean numbers of nematodes per millilitre collected in the pots in the five selected grid locations around the sprinkler when the three rates of Nemasys L were applied and mean numbers per millilitre expected if nematodes were delivered accurately in 4L/m^2 water

Grid	**20% rate**		**40% rate**	**100% rate**
	Rep 1 mean of 3 counts per millilitre	Rep 2 mean of 3 counts per millilitre	Mean of 3 counts per millilitre	Mean of 3 counts per millilitre
A 1	22.6	25.6	49	126.3
A 2	27.3	25.3	52	124.3
A 4	22.6	23	45.3	119.6
A 6	23	22.6	52.6	125.6
B 1	22.6	24	49.3	138
Mean	**23.6**	**24.1**	**49.6**	**126.8**
Expected	**25**	**25**	**50**	**125**

Nemasys L application to growing media

Mean numbers of nematodes per millilitre reaching the growing media when applied through the overhead irrigation (collected in plastic tubes pushed into the growing media during application) at each of the three rates are shown in Figure 6. The expected mean numbers of nematodes per millilitre (based on the area of the tube opening and the area of the top of the 2L pot) if all the nematodes applied reached the growing media are also shown in Figure 21.

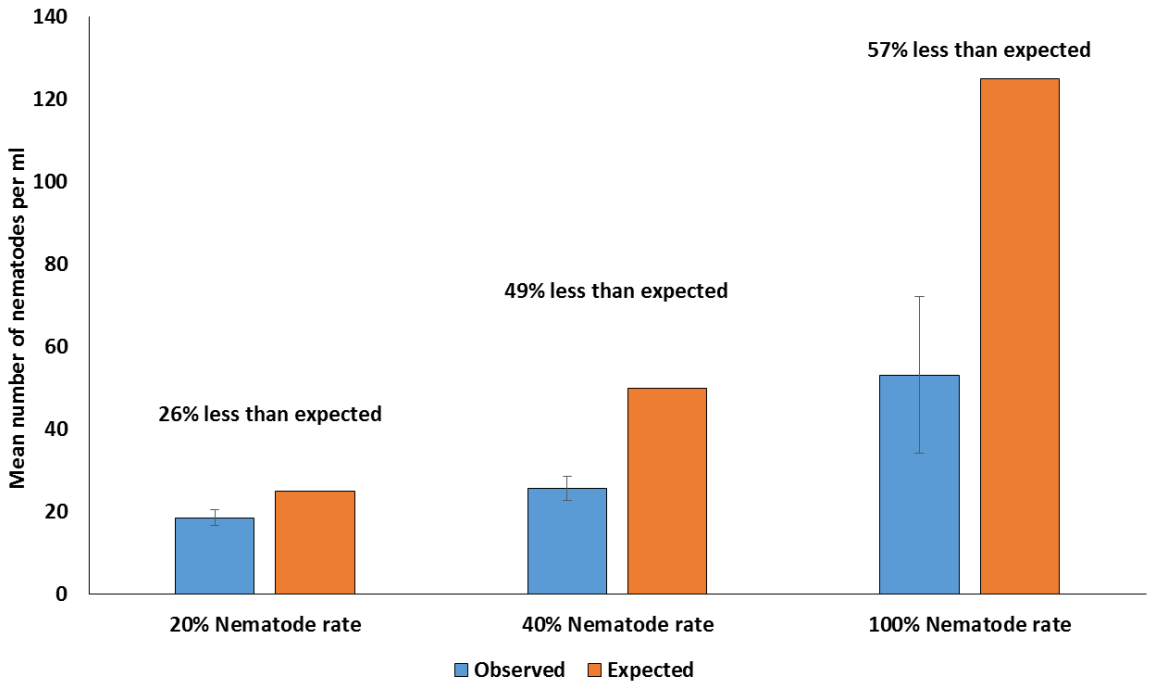

Figure 21. Mean observed and expected numbers of nematodes per millilitre collected in the tubes pushed into the growing media during application of the 20%, 40% and 100% nematode rates through the overhead irrigation. Means +/- SE.

The observed and expected mean numbers of nematodes per millilitre were very variable throughout the experiment. The greatest variability was given by the 100% Nemasys L application (Figure 21) with a maximum of 124 and a minimum of one nematode observed per millilitre. The greatest difference between observed and expected was also recorded with the 100% rate, with a mean of only 57% of the expected numbers observed. Observed numbers of nematodes were also lower than expected in both the 20% and 40% Nemasys L application rates, with mean numbers of observed nematodes being 25% and 49% less than those expected respectively. In the 20% rate a mean of 18.6 nematodes per millilitre were observed rather than the 25 nematodes per millilitre expected. In the 40% rate a mean of 25.7 nematodes per millilitre was observed whereas 50 nematodes per millilitre were expected. This indicates that a high percentage of nematodes (over half in some cases) did not reach the growing media.

The observed volumes of the nematode suspension collected in the tubes during Nemasys L application were much closer to the expected volumes (Figure 22). The mean observed and expected volumes in the five selected locations during application of all the treatments applied through the overhead irrigation were 1.43 and 1.64 ml, indicating that only 13% of the

nematode suspension did not reach the growing media. However, volumes recorded were very variable with a maximum of 7 ml and a minimum of 0 ml being recorded.

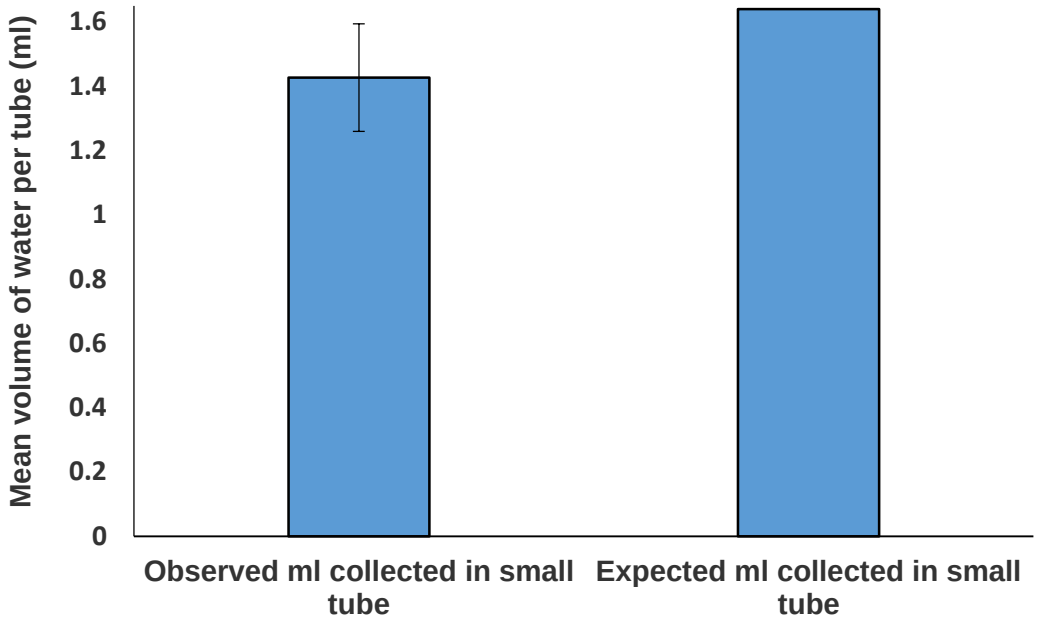

Figure 22. Mean observed and expected water volume (ml) collected in the tubes pushed into the growing media during application of all treatments through the overhead irrigation

Numbers of vine weevil larvae

Despite estimated mean numbers of nematodes reaching the growing media being lower than expected when applied through the overhead irrigation, all the Nemasys L treatments were equally effective in giving significant reductions in mean numbers of vine weevil larvae per pot compared with both water controls ($P<0.01$). Mean numbers of larvae per pot in the water control applied as a drench and through the overhead irrigation were seven and 8.4 respectively and were not significantly different from each other (Figure 23). Mean numbers of larvae per pot in the full rate Nemasys L applied as a drench in September and through the overhead irrigation five times between July and October were 1.8 and 2.8 respectively and mean numbers per pot in the 20% and 40% Nemasys L rates applied through the overhead irrigation five times were 2.2 and 1.6 respectively.

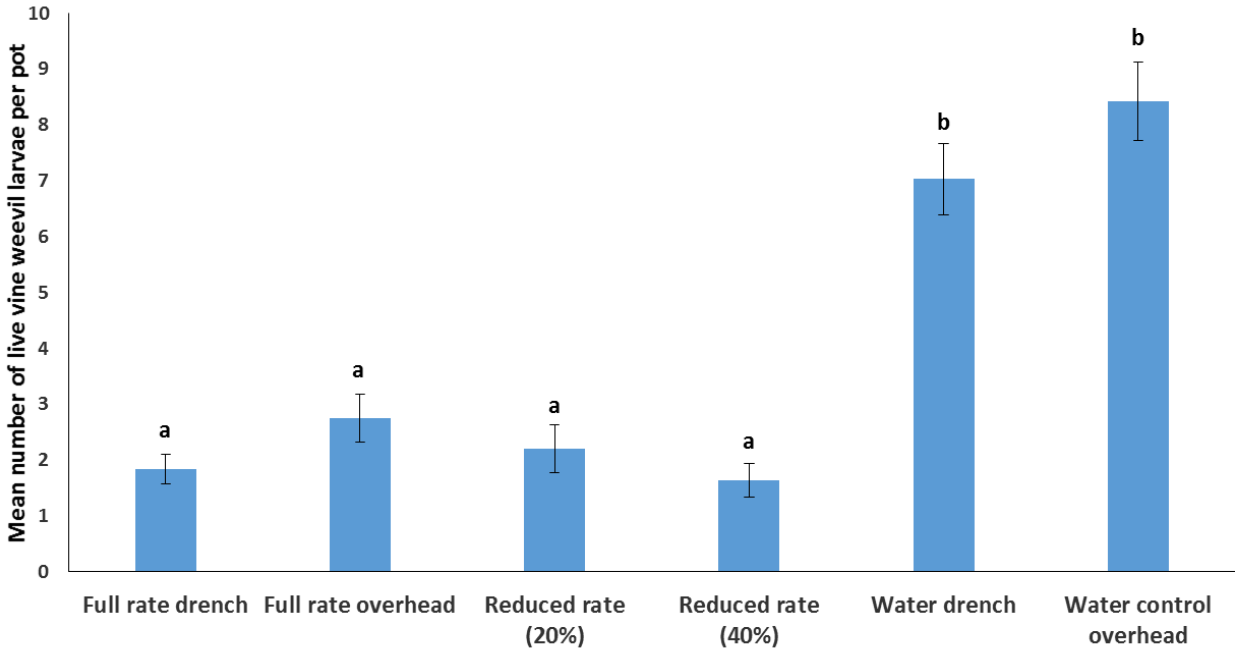

Figure 23 Mean numbers of vine weevil larvae per pot. Means +/- SE. Bars with different letters significantly different (P<0.01).

Percentage control of vine weevil larvae

Using the mean numbers of vine weevil larvae per pot in each treatment, the calculated percentage control of larvae by each Nemasys L treatment compared with its respective water control showed that the four treatments gave 67-81% control (Figure 24). The full rate drench and overhead treatments gave 73.9% and 67.3% control respectively and the 20% and 40% rate overhead treatments gave 73.9% and 80.5% control respectively.

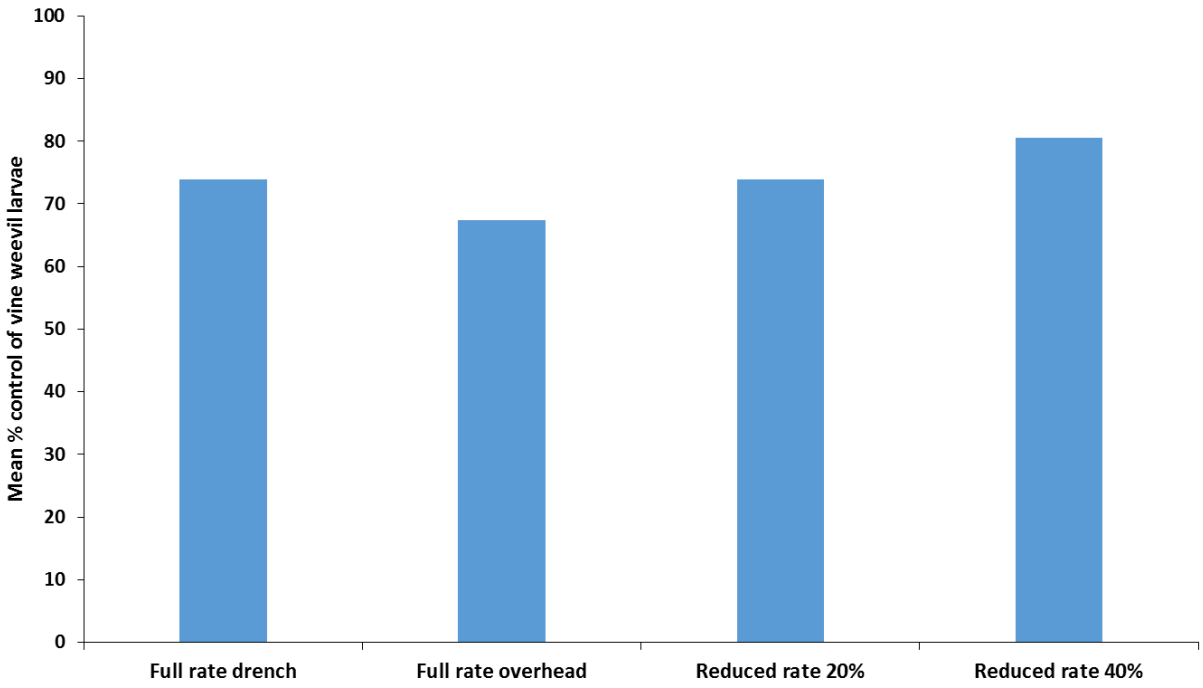

Figure 24 Mean % control of numbers of vine weevil larvae compared with the respective water control.

Root damage

The root damage scores allocated to each of the 36 plants per treatment (six plants in each of six replicate plots) were grouped, with plants scoring 0-2 being considered as still relatively healthy with only slight root damage and plants scoring 3-5 considered as having unsustainable, severely damaged root systems (Figure 20).

The mean percentage plants in each treatment with severe root damage (scores of 3-5) are shown in Figure 25. The water control applied as a drench and through the overhead irrigation led to 78% and 94% of plants with severe root damage. All Nemasys L treatments except for the full rate applied through the overhead irrigation five times between July and October significantly reduced the percentage plants with severe root damage compared with both water control treatments. The full rate overhead Nemasys L treatment was equally effective to the water control applied as a drench but significantly reduced the mean % plants with severely damaged root systems (53%) compared with the water control overhead treatment (94%). The Nemasys L applied at 40% rate five times through the overhead irrigation between July and October significantly reduced the mean percentage plants with severe root damage (11%) compared with the full rate Nemasys L applied using the same method and timinutesgs (53%) but was equally effective as the full rate drench applied twice in September and October (31%) and the 20% rate applied five times through the overhead irrigation (31%).

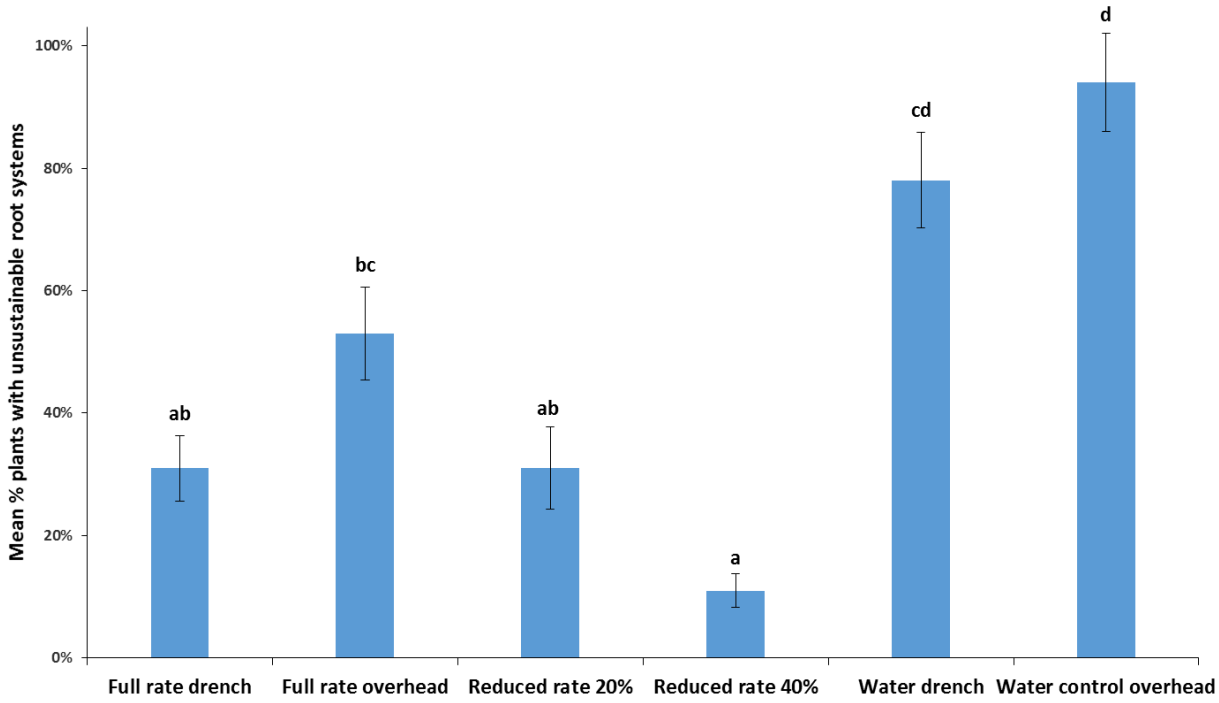

Fig 25. Mean % plants in each treatment with severe root damage (scores of 3-5, indicating unhealthy plants with unsustainable root systems). Means =/- SE. Bars sharing the same letters are not significantly different (P<0.01).

Growing media temperatures

Mean, maximum and minimum growing media temperatures throughout the experiment are shown in Figure 26, together with the recommended temperature range for Nemasys L (5-30°C) and the Nemasys L application dates. During the experimental period, maximum growing media temperatures rose above the maximum recommended temperature for Nemasys L on five dates; 24 June (for 0.5 hrs), 17 July (for 2.5 hrs), 18 July (5.5. hrs), 19 July (6.5 hrs) and 24 August (for 0.5 hrs). Minimum growing media temperatures fell to below the minimum recommended temperature for Nemasys L on several dates in November from 3 November.

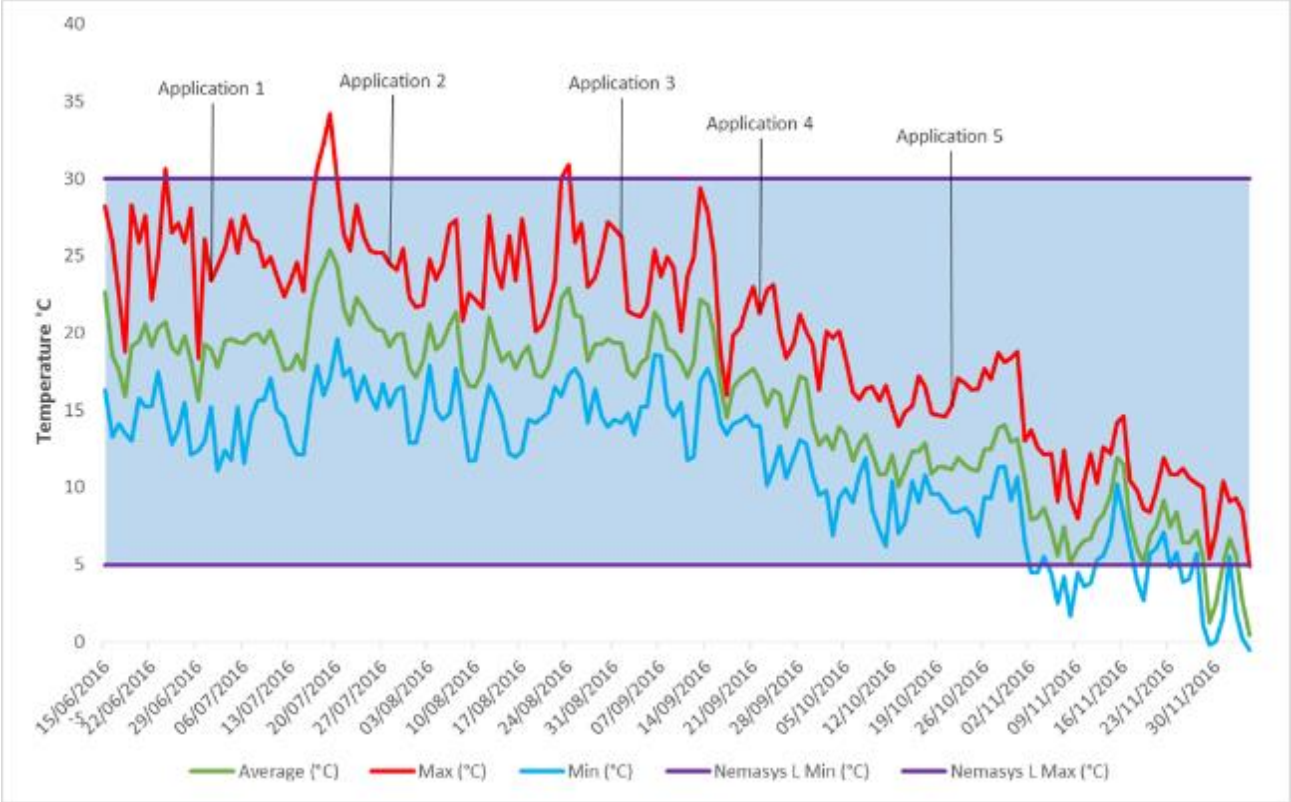

Figure 26. Mean, maximum and minimum growing media temperatures during the experiment. The horizontal lines indicate the recommended temperature range for Nemasys L (5-30°C).

Discussion

Calibration of water and nematode application through sprinkler

The results of the calibration of water volumes applied in different areas around the sprinkler demonstrated how variable the volumes were. However, in this pilot experiment only one sprinkler was used and in commercial practice the delivery of water through adjacent sprinklers in an overhead irrigation system will overlap, giving a different pattern of water volumes applied. In year 2 of the project it is planned to repeat the experiment on a commercial nursery, where the water volumes applied to the area used for the experiment will be calibrated. In this pilot experiment, the five locations selected to place the experimental plants during treatment application received very similar amounts of water and numbers of nematodes when applied at each rate, which allowed the efficacy of treatments to be compared.

Nemasys L application to growing media

The results of collecting the Nemasys L suspension in tubes inserted into the growing media during application through the overhead irrigation indicated that although the full rate of Nemasys L was being applied, estimated means of 26%, 49% and 57% of the nematodes in the 20%, 40% and 100% rates respectively did not reach the growing media. However, each tube represented only 1.94% of the overall surface area of the pot being treated and therefore may have given an unreliable estimate of nematode numbers. The results of measuring the volumes of nematode suspension collected in all the tubes inserted into the growing media demonstrated that 13% of the water did not reach the growing media which could account for some of the 'shortfall' in nematodes reaching the growing media. The initial calibration of nematodes delivered to collection pots via the overhead irrigation in the five selected areas around the sprinkler showed that numbers of nematodes delivered were very close to the expected numbers at all application rates. Therefore it is possible that some of the nematodes, particularly those used for the 40% and 100% rates may have remained on the foliage despite being rinsed off with water using the overhead irrigation following application. However, leaves sampled from spare plants after an application were examined under a microscope and no nematodes were observed on the upper or lower surface of the leaves. Another possibility is that correct nematode numbers reached the surface but due to the coverage of the plant canopy they were not evenly distributed over the growing media but only reached areas under gaps in the canopy or where the nematode suspension dripped from leaves. This would explain the variability in numbers of nematodes collected in the tubes.

Despite this result, all nematode treatments applied through the overhead irrigation were as effective in reducing numbers of vine weevil larvae per plant compared with the water controls and with the full rate of Nemasys L applied as drenches. Future work should consider more accurate recording of nematode numbers reaching the growing media when applied through overhead irrigation and should also consider plant species of different architectures which will affect nematode delivery through the crop canopy.

Effect of growing media temperature on nematodes and vine weevil

During the experimental period, maximum growing media temperatures rose above the maximum recommended temperature for Nemasys L on five dates between late June and late August. These high temperatures could have affected Nemasys L efficacy on these dates. Minimum growing media temperatures fell to below the minimum recommended temperature for Nemasys L between 3 November and the end of the experiment. The final

Nemasys L application date was on 21 October, two weeks before these low temperatures were recorded. The Nemasys L recommendations are that the growing media temperatures should be between 5°C and 30°C for at least two weeks after application, therefore these low temperatures should not have affected efficacy.

The survival of vine weevil larvae is also affected by high temperatures. Son & Lewis (2005) reported that temperatures above 27°C are lethal to larvae if exposed for over eight hours. During this experiment, temperatures were above 27°C for nine hours on 18 July and this could have led to some vine weevil mortality in all treatments but on other dates where this temperature was exceeded the time period was less than eight hours.

Control of vine weevil numbers and root damage

The results demonstrated that all the Nemasys L treatments were equally effective in reducing numbers of vine weevil larvae per pot compared with both the water controls. The full rate drench and overhead treatments gave means of 74% and 67% control respectively and the 20% and 40% overhead treatments gave means of 74% and 81% control respectively. In an experiment carried out in AHDB Horticulture project CP 124 'Managing ornamental plants sustainably' (MOPS), a single drench of full rate Nemasys L applied in mid-September to Fuchsia plants following a single infestation of 15 vine weevil eggs per pot on 1 August, gave 87% control of vine weevil larvae compared with a water control drench (Hough, 2015). Most nematode suppliers and consultants expect 80-90% control from a single, well-timed drench of any nematode species at the recommended rate (Bennison *et al*, 2014).

In the experiment reported here, all Nemasys L treatments except for the full rate applied through the overhead irrigation were also equally effective in reducing the number of severely damaged root systems compared with both the water controls. The full rate of Nemasys L applied through the overhead irrigation reduced the numbers of severely damaged root systems compared with the water control applied through the overhead irrigation but not when compared with the water control applied as a drench.

The results of this experiment highlight the potential of using a 'little and often' system of applying entomopathogenic nematodes through overhead irrigation. Various entomopathogenic nematode species and products are available for vine weevil control (see AHDB Horticulture Factsheet 24/16). Many growers choose to use *Heterorhabditis bacteriophora* when growing media temperatures are suitable (minimum 12-14°C depending on product) and *Steinernema kraussei* at lower temperatures (minimum 5°C). It is estimated that it takes five hours labour to apply a high volume drench of nematodes to an area of 1000m^2 with 3L pots but only one hour to apply them through the overhead irrigation. Taking

into account the costs of two consecutive drenches of nematodes at recommended rates (one of *H. bacteriophora* and one of *S. kraussei),* it is estimated that applying 40% rates of the same products five times through the overhead irrigation (four applications of *H. bacteriophora* and one application of *S. kraussei*) would be equal to the cost of two full-rate drenches whereas applying 20% rates five times through the overhead irrigation would save 44% of the cost. Cost savings of applying reduced rates of nematodes five times through the overhead irrigation would be greater if growers currently apply three consecutive drenches of nematodes at recommended rates (two of *H. bacteriophora* and one of *S. kraussei*) i.e. a saving of 30% if using 40% rate and 60% if using 20% rate.

This research was inspired by recent experience on tunnel-grown substrate-grown strawberry in Scotland where growers have been experimenting with a 'little and often' approach for nematode application through dripline irrigation for vine weevil control, after unreliable control had been given using the label-recommended dose at the 'normal' timinutesgs in early autumn and late spring (Caroline Reid, Bioline AgroSciences, personal communication). This strategy has been to apply *Steinernema kraussei* at one fifth of the recommended rate (5,000 per plant) each month, through dripline irrigation to substrate-grown strawberry, although higher rates than this are sometimes used depending on the number of vine weevil larvae present and the time of year. Grower feedback has been that this strategy has given more reliable control of vine weevil, whilst maintaining the cost of nematodes at an acceptable level. The reason for the improved control could be that nematodes are maintained in the substrate throughout the year and thus give improved control of vine weevil damage.

The 'little and often' system for nematode application through the overhead irrigation could offer growers of protected HNS a more cost-effective strategy for effective vine weevil control than using high volume drenches and the system will be validated on a commercial nursery in year 2 of the project.

Conclusions

- In an experiment in a research polytunnel at ADAS Boxworth, *Steinernema kraussei* (Nemasys L) applied through the overhead irrigation at reduced rates (20% and 40% of the label rate) five times between 1 July and 21 October was as effective in reducing numbers of vine weevil larvae per Fuchsia plant compared with water controls as the full label rate applied twice (in September and October) either as a drench or through the overhead irrigation.
- All Nemasys L treatments (except for the full rate applied through the overhead irrigation) were also equally effective in reducing numbers of severely damaged root

systems compared with both the water controls. The full rate of Nemasys L applied through the overhead irrigation reduced the numbers of severely damaged root systems compared with the water control applied through the overhead irrigation, but not when compared with the water control applied as a drench.

- A proportion of the nematodes applied at reduced rates through the overhead irrigation may not have reached the the growing media despite washing them off the foliage with water after application. However, vine weevil control was still as effective as the label rate drench. More accurate measurement of nematode numbers reaching the growing media will be done in the trial on the commercial nursery in year 2.
- A 'little and often' system for applying reduced rates of entomopathogenic nematodes through the overhead irrigation could offer growers of protected HNS a more cost-effective strategy for vine weevil control than using high volume drenches without compromising on efficacy.
- Nematode application through the overhead irrigation five times at 40% of the label rate (four applications of *Heterorhabditis bacteriophora* and one of *Steinernema feltiae*) would cost the same as two full rate high volume drenches (including labour) but would be 30% less than three full rate drenches. If using 20% rates, the same 'little and often' strategy using the overhead irrigation would save 44% of the cost of two full rate drenches and 60% of three full rate drenches.
- The water volumes applied through the overhead irrigation were very variable in different areas around the single sprinkler used for the experiment. Therefore the experimental plants were placed in locations around the sprinkler where water delivery during a set time period accurately and consistently applied water and nematodes at the required rates. Commercial sprinkler systems would need calibrating to ensure the required nematode rates are applied through the overhead irrigation.
- Nematode delivery to the growing media when applied through the overhead irrigation needs testing on HNS species with different plant architectures than Fuchsia.
- The system needs validating on a commercial nursery and this will be done in year 2.

Future work

- Task 3.1.2. Experiment testing little and often nematodes on commercial nursery

- An experiment will be done on a commercial HNS nursery in 2017 to validate the results of the pilot experiment completed at ADAS Boxworth in 2016. Rather than using *Steinernema kraussei* for all the nematode applications as in the pilot experiment, only the final application will use this species and the earlier applications will use *Heterorhabditis bacteriophora* to be consistent with standard commercial practice. The experimental plants will be infested with vine weevil eggs by ADAS as in the pilot experiment and ADAS will work with the host grower on each of the nematode application dates. The experimental plants will be taken to ADAS Boxworth for the final destructive assessment of vine weevil larvae therefore there will be no risk of larvae surviving on the nursery to present a risk to the host grower.

Knowledge and Technology Transfer

- Presentations were given at the following events:
- 12 July 2016 – Jude Bennison – HTA vine weevil summit, Banbury, Oxon
- 7 September 2016 – Tom Pope – Ento ’16 – Royal Entomological Society Annual Meeting, Harper Adams University, Shropshire
- 8 November 2016 – Jude Bennison and Sam Brown - AHDB Horticulture IPM on ornamentals day, Chichester
- 15 November 2016 – Jude Bennison and Sam Brown - British Herbs Trade Association technical meeting, St. Neots, Cambs
- 17 November 2016 – Jude Bennison, Sam Brown and Tom Pope - AAB conference: Advances in IPM, Grantham, Lincs
- 23 November 2016 – Jude Bennison, Sam Brown and Tom Pope – AHDB/EMRA soft fruit day at NIAB EMR, Kent

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Glossary

Entomopathogenic – capable of causing disease or death in insects

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