Project title:	Improving Vine weevil control in Hardy Nursery Stock
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Location of work:	ADAS Boxworth Harper Adams University University of Warwick Natural Resources Institute, University of Greenwich
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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.

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GROWER SUMMARY

Headline

- Overwintering vine weevil adults require a period of at least five weeks feeding before recommencing egg laying in the spring. Growers should monitor for adults and check for feeding damage from March onwards when temperatures rise above the activity threshold of 6°C.
- Vine weevil adults can be attracted by the odour of plant volatiles such as (*Z*)-2-pentenol and methyl eugenol.
- A 'little and often' system for applying reduced rates of entomopathogenic nematodes through the overhead irrigation was validated on a commercial nursery and offers up to 52% cost savings compared with using high volume drenches without compromising on efficacy against vine weevil larvae.
- A predictive day degree model estimates that Met52 will not kill vine weevil larvae at temperatures below 11.6°C. Investigations into the activity of cold tolerant fungal strains against vine weevils are underway.

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, Objective 2. Develop practical methods for monitoring adults in order to detect early infestations and inform control methods and Objective 4. Develop novel approaches to control.

Background

Minimum temperatures - Newly emerging adults are thought to require a pre-oviposition period during which weevils feed voraciously before starting to lay eggs. It is not known whether overwintered adults require a similar period in which they feed before recommencing egg laying in the spring. The aim of this objective is to determine whether overwintered adults recommence egg laying as soon as temperatures allow.

Period of vine weevil activity - Knowledge of the start and end of vine weevil feeding and egg laying activity allows for application of controls to be correctly timed and monitoring efforts to be focused on those times when weevils are likely to be active. The aim of this task is to take the information from work on minimum temperatures for egg laying and feeding and to use temperature data collected from commercial nurseries to estimate periods of vine weevil adult activity.

Potential of lures to improve monitoring - There is a need to improve our understanding of the chemical ecology of vine weevil adults in order to develop effective lures based on volatile compounds. A patent has recently been produced for a vine weevil attractant based on the plant volatiles (*Z*)-2-pentenol and methyl eugenol, but to date there is no evidence that weevils are attracted to these compounds. The aim of this task is to record responses of vine weevil adults to olfactory stimuli and to test these compounds for use as potential lures.

Develop novel approaches to control vine weevil – A gel formulation of the insect-pathogenic nematode species *Steinernema carpocapsae* has recently been developed by e-nema. The aim of this task is to assess the efficacy of a gel formulation of *Steinernema carpocapsae* produced by e-nema and applied to traps/or the crop for control of vine weevil adults under semi-field conditions.

Summary

Minimum temperatures – Overwintered vine weevil adults were placed individually in ventilated Petri dishes lined with damp filter paper and a strawberry leaf disc provided as a source of food. Petri dishes were placed into controlled temperature cabinets set to a constant 12, 15 or 18°C. Leaf area consumed and numbers of eggs laid were recorded. Intense vine weevil feeding was recorded at all temperatures tested, but feeding did not increase at higher temperatures. No eggs were recorded at any of the temperatures during the five weeks of the experiment. This result indicates that overwintering vine weevil adults require a period of at least five weeks before egg laying recommences in the spring.

Period of vine weevil activity – Air temperature data were recorded inside a polytunnel located in Pulbourough, West Sussex, in 2014 and an unheated glasshouse located in Walburton, West Sussex in 2015. Additional data of external air temperatures were obtained from a nearby meteorological stations in 2014 and 2015. These data indicate that a period of at least one hour would have been suitable for vine weevil activity during most nights of the year for the sites and years for which data were available.

Potential of lures to improve monitoring - Using a Y-tube olfactometer, the responses of vine weevil adults to the odour of plant volatiles were recorded as well as the odour produced by other vine weevil adults and their waste (frass). Results indicate that vine weevil adults are strongly attracted by the odour of the host plants yew and *Euonymus* as well as the odour of frass produced by other vine weevil adults that had fed on yew. The odour of only a few plant volatiles, most notably (*Z*)-2-pentenol and methyl eugenol, were attractive to vine weevil

adults. The addition of a lure releasing (*Z*)-2-pentenol and methyl eugenol, however, did not affect the numbers of weevils caught by traps. In these experiments, most weevils were found during the day to be aggregating underneath plant pots.

Develop novel approaches to control vine weevil – Results from an experiment in which 5 ml of a gel formulation of *Steinernema carpocapsae* applied to the base of plant pots and a second experiment where 5 ml of the gel was placed inside Roguard crawling insect bait stations, did not statistically significantly increase weevil mortality.

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

Background

Entomopathogenic nematodes – various species of entomopathogenic nematodes are used for vine weevil control in HNS, but as it is recommended to apply them using high volume drenches, growers view them as labour-intensive and thus expensive. In year 1 of the project, a pilot experiment was done on Fuchsia cv. Alice Hoffman in a research polytunnel at ADAS Boxworth to test a 'little and often' approach for maintaining control of vine weevil larvae through the season. The experiment compared applying reduced rates of nematodes through the overhead irrigation with the standard full rate drenches. Both 40% and 20% rates of *Steinernema kraussei* (Nemasys L) applied five times between 1 July and 21 October through the irrigation were as effective as two full rate drenches applied on 22 September and 21 October in reducing numbers of vine weevil larvae per plant and in reducing the percentage of plants with severe root damage.

Entomopathogenic fungi – The currently approved entomopathogenic fungus *Metarhizium brunneum* (*anisopliae*) (Met52 Granular Bioinsecticide) for control of vine weevil larvae needs warm temperatures (15-30°C) to be effective and laboratory experiments indicated that its optimum temperature is 27°C. It is recommended that the product be used as part of an IPM programme rather than as the only method for vine weevil control, and is best used in spring pottings than in the autumn, as temperatures will be too low for it to give good control in the autumn and the following early spring. A cold-tolerant entomopathogenic fungus would be useful for use in autumn pottings for vine weevil control at 5-15°C. The aim of the work in 2016 and 2017 was to provide growers with practical information on the effect of fluctuating temperatures on Met52 infection of vine weevil larvae in the laboratory and in pot experiments.

Summary

Entomopathogenic nematodes - The results of the pilot experiment testing the 'little and often' approach in year 1 were validated on a commercial nursery. The trial was done in a polytunnel on four Fuchsia varieties; Riccartonii, Mrs Popple, Hawkshead and Tom Thumb. The nematode species used were consistent with commercial practice i.e. using the cheaper species *Heterorhabditis bacteriophora* (Nemasys H was used in the trial but other products are available) when growing media temperatures were expected to be 12°C or above and the more expensive cold-tolerant species *Steinernema kraussei* (Nemasys L) when growing media temperatures were lower than 12°C. Nemasys H was applied at 100%, 40% or 20% rates through the overhead irrigation four times between 21 June and 14 September and the fifth application was Nemasys L on 11 October. Full rate drenches were done on both 14 September (Nemasys H) and on 11 October (Nemasys L). Water controls were applied in September and October either as a drench or through the overhead irrigation.

When the data from all four Fuchsia varieties were combined, all the nematode treatments gave significant reductions in mean numbers of vine weevil larvae per pot compared with the water controls (Figure 2). The full rate drench and the 40% rate overhead treatments were equally effective. The full rate drench treatment was more effective than the full rate and 20% rate overhead treatments. However the 40% rate overhead treatment was equally effective as the full rate and 20% rate overhead treatments.

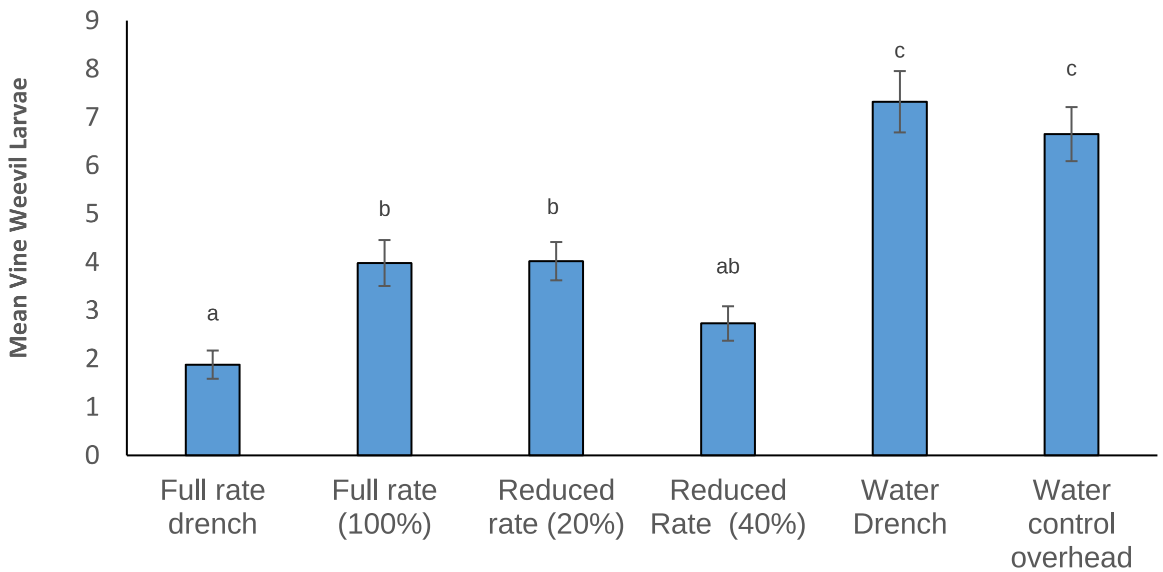

Figure 2: Mean numbers of vine weevil larvae per treatment when data from the four Fuchsia varieties were combined +/- SE. Bars sharing none of the same letters are significantly different (P<0.001).

When the data from all Fuchsia varieties was combined, all nematode treatments also significantly reduced percentage root area damaged compared with the water controls. The 40% rate overhead treatment was the most effective.

When the data from each Fuchsia variety were analysed separately, the full rate drench treatment and the 40% rate overhead treatment gave significant reductions in mean numbers of vine weevil larvae per pot compared with the water controls in all four varieties, but the 20% rate overhead treatment was equally as effective as the full rate drench and the 40% rate overhead treatments on only two varieties, Riccartonii and Tom Thumb. All the nematode treatments gave significantly lower percentage root damage on Mrs Popple and Hawkshead. None of the nematode treatments reduced root damage on Riccartonii and only the 40% overhead treatment reduced root damage on Tom Thumb despite all the other nematode treatments being as effective as the 40% overhead treatment in reducing numbes of larvae. A correlation between mean numbers of vine weevil larvae and percentage root damage indicated that root damage increased faster on Riccartonii than on the other three varieties as mean numbers of larvae increased, but root damage on Tom Thumb was the highest in the presence of the same mean numbers of larvae as the other three varieties.

Entomopathogenic fungi – Experiments in 2016 and 2017 evaluated the effect of temperature on the infectivity of Met52. Laboratory bioassays were done over 28 days at temperatures ranging from 12.5°C to 30°C. Second/ third instar larvae were kept in bioassay chambers in untreated or treated compost and assessed every other day for mortality. Mortality increased with temperature. A day degree model was fitted to the data to allow mortality predictions in the field. The model predicted that no control will occur at temperatures below 11.6°C. However, the Met52 spores will remain viable until temperatures are conducive for infection to re-occur. It also estimated that for 75% control 256 cumulative day degrees have to be achieved and analysis of historical temperature data recorded from UK nurseries suggests that this could be reached in the months of June, July and August in some years and locations.

Pot experiments were carried out in replicate plots of potted sedum plants infested with vine weevil eggs, in a research polytunnel and outside hardstanding at Warwick Crop Centre, Wellesbourne. Sedum plugs were potted up into untreated compost or compost containing Met52 at the recommended rate and weevil eggs added in early August, mid-August, mid-September and early October. Plants were sampled destructively after four weeks and plant health and number of weevils recovered were assessed. Low levels of control were observed during the experimental period due to the low mean temperatures in the compost but the *Metarhizium* conidia remained viable and were able to infect as temperatures increased.

Plants grown in Met52-treated compost weighed more and were generally of better quality than those grown in untreated compost.

Searching the scientific literature suggested that there are several entomopathogenic fungi that can germinate and grow at 5-15°C and hence could have potential for control of vine weevil at these temperatures, although infection of vine weevil larvae has not yet been studied. Eleven candidate cold-tolerant fungal strains are being tested at Warwick Crop Centre. These strains have been sourced from northern Europe and other 'cold' regions of the world and are being cultured in the laboratory. Current experiments are testing growth of the fungi on agar plates at a range of temperatures between 4°C and 40°C (Figure 3). Spore germination at different temperatures is also being assessed. Once these experiments are completed, selected fungal strains will be tested for infection and kill of vine weevil larvae at 5-20°C compared with Met52 at 20°C.

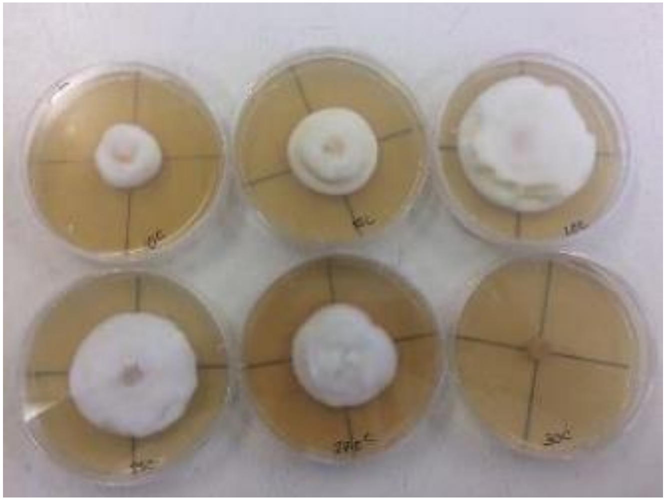

Figure 3. Growth of a *Beauveria bassiana* strain at six temperaures showing promising cold tolerance.

Financial Benefits

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 save 31% of the cost and using three applications of *H. bacteriophora* and two applications of *S. kraussei* (in a cold autumn) would save 26% of the cost. Cost savings of applying reduced rates of nematodes five times through the overhead irrigation would be even 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 52% if using four applications of *H. bacteriophora* and one of *S. kraussei* and a saving of 49% if using three applications of *H. bacteriophora* and two of *S. kraussei*. Cost savings would be even greater if using 20% rates of nematodes but using 40% rates is considered a safer option.

Action Points

- Monitoring for vine weevil adults should begin in spring when temperatures rise above the threshold of 6°C and continue until the autumn/winter when temperatures decline below this threshold once more.
- Overwintered adult vine weevils need a 5-week period of intense feeding before they recommence laying eggs. Growers should monitor for adults and check for feeding damage from March onwards and consider applying a plant protection product for adult control before egg laying starts (see AHDB Horticulture factsheet 24/16 ‘Vine weevil control in hardy nursery stock’ for more details).
- Consider using the ‘little and often’ system of applying entomopathogenic nematodes through the overhead irrigation between June and October, which is as effective as using two high volume drenches in September and October and is more cost-effective. If using this system it is very important to remove any internal or external filters from the dosing unit to avoid nematode blockages.