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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

- Catches of vine weevil adults in Chemtica traps can be increased by placing yew or *Euonymus fortunei* foliage inside traps.
- Spraying adult vine weevils with *Steinernema carpocapsae* (Nemasys C) and spraying both weevils and Euonymus leaves with AHDB 9933 led to temporary weevil abnormal behaviour but the weevils recovered and neither treatment led to effective kill or to reduced feeding or egg laying.
- Isolates of entomopathogenic fungi were identified with lower optimal temperatures for growth and germination than Met52. However, a predictive day degree model estimated that the cumulative day degrees needed for 50% mortality was similar or higher to that of Met52, suggesting that these fungi are less virulent to vine weevil larvae than Met52 which may offset the benefits of lower temperature development.

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

Background

The development of an effective vine weevil lure would improve the reliability and sensitivity of monitoring strategies and contribute to improved integrated pest management (IPM) of this pest. Several studies have shown that vine weevil adults detect plant-derived odours and these are used by weevils to locate suitable host plants for feeding and oviposition and may, therefore, also play a role in aggregation. Odours from yew (*Taxus baccata*) and *Euonymus fortunei*, for example, are known to be attractive to adult vine weevils. It is, however, not yet fully understood how vine weevil discriminate between the odours of potential host plants, although it is likely that the ratios of blends as well as concentrations of plant volatiles is important (see Year 2 annual report). Vine weevil adults also appear to be attracted by the odour of other vine weevils and specifically to the frass (droppings) produced by these weevils. Positive behavioural responses were also recorded in this study (see Year 2 annual report) to both other weevils and weevil frass. There is, however, conflicting evidence as to whether weevils use these cues to aggregate, and to date, weevil or plant odours have not been used to successfully increase catches of vine weevil adults in monitoring traps. Here

we: (1) seek to understand whether host plant material can be used to increase catches of vine weevil adults and (2) to further investigate the presence of a volatile aggregation pheromone.

Summary

Baiting Chemtica traps with plant material – using large tent cages set up within an unheated glasshouse at Harper Adams University, responses of vine weevil adults to traps containing a fine 'weevil proof' nylon mesh bag, which was either left empty or filled with 15 g of freshly cut *Euonymus fortunei* or yew foliage. Potted strawberry plants were placed into each of the cages to simulate a crop and to provide alternate refuges to the Chemtica traps. In this way vine weevil adults were presented with a choice between two Chemtica traps in each cage that differed only by the plant foliage inside each trap. Weevils entering a trap were not able to feed on the foliage inside the mesh bags. The cages were prepared as described and 15 weevils were released into each cage between 6pm and 8pm in the evening and traps assessed the following morning between 8am and 9am. Before being released into the tent cages, vine weevil adults were conditioned by providing either yew or *Euonymus fortunei* plant material as a food source for 10 days before the start of the experiment.

Results from this series of experiments indicated that the number of weevils found in Chemtica traps could be significantly increased by the addition of either type of plant material. This is the first time that plant volatiles have been used to increase catches of vine weevil adults in traps. When given a choice between traps containing different host-plant foliage, significantly more adult weevils were found in traps containing the host-plant foliage on which they had previously fed. These results indicate that previous feeding experience by vine weevil adults may have implications for designing effective monitoring strategies.

Aggregation pheromone – the volatile chemicals produced by 30 adult vine weevils were sampled (entrained) over a period of 30 minutes (before weevils had chance to start to produce frass) and then any volatile chemicals collected were washed off the filters using diethyl ether. This process was repeated both for weevils that had been fed on yew foliage or had first been starved for 72 hours before the sampling was started. Completed samples for fed and starved weevils were each analysed by gas chromatography-electroantennography (GC-EAG). For the EAG preparations, individual vine weevil adults were anaesthetised before excising their head and one antenna. The reference electrode was inserted into the back of the head and attached to a silver electrode held in micromanipulators on a portable EAG device. To complete the circuit, the tip of the one remaining antenna was excised and the recording glass electrode attached to the EAG device inserted. To confirm that the EAG

preparation method was suitable for recording electrophysiological responses from excised vine weevil heads, three individual preparations were tested against ylang ylang (*Cananga odorata*) essential oil. This essential oil is known to elicit electrophysiological responses in vine weevil adults. Samples containing chromatographic peaks evoking electrophysiological responses were further analysed using gas chromatograph-mass spectrometry (GC-MS). The diethyl ether extract from the samples was analysed and peak identification completed using a mass spectra database.

No consistent EAG responses were recorded for samples from fed or starved vine weevil adults. These results are consistent with most previously published research but do not explain the results presented in Year 2 where vine weevil adults respond positively to the odour produced by fed weevils. It remains possible that high molecular weight compounds, not detected using the set-up employed here, may be important as aggregation pheromones. Analysis of the ylang ylang essential oil by GC-EAG resulted in good electrophysiological responses to 16 chemical compounds, which were then identified by GC-MS. This result confirms previous work that identified responses to linalool present in this essential oil. Responses to other compounds also present in ylang ylang essential oil were also detected for the first time. These compounds include caryophyllene and germacrene D.

Previous work on analysis of volatiles from *Euonymouos fortunei* using GC-EAG was revisited. Additional compounds were identified, in particular *cis*-jasmone which caused a strong EAG response at low levels.

The electrophysiologically active compounds identified here may be important in the development of a vine weevil lure.

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

Background

Lethal and sub-lethal effects on adult weevils in response to direct application and to residues of IPM-compatible insecticides, entomopathogenic nematodes and a botanical pesticide

Many growers use entomopathogenic nematodes for the control of vine weevil larvae. However, control of adults is currently reliant on foliar sprays of insecticides. AHDB project SF HNS 112 showed that the IPM-compatible pesticides pymetrozine (Chess WG) and

indoxacarb (Steward) gave useful control of adults. However, pymetrozine approval will expire on 31 January 2020 and Steward currently only has an EAMU for use on outdoor ornamentals so cannot be used on protected HNS. Thus alternative IPM-compatible treatments are needed for adult control. Adult sprays are usually applied late in the day with the aim of direct contact with the nocturnal adults but it is not known whether spraying during the day could be as effective when adults pick up dried spray residues. As well as killing adults, some insecticides and biopesticides could have sub-lethal effects e.g. antifeedant activity, abnormal behaviour or egg laying or egg hatch inhibition. These sub-lethal effects could make an important contribution to vine weevil management.

Cold active entomopathogenic fungi

Laboratory experiments to quantify the effect of temperature on the virulence of Met52 to vine weevil larvae indicated strongly that this fungus has an optimum of about 27°C and has a low rate of infection at temperatures below 15°C. This is likely to be a problem when using the product on outdoor plants, as the soil temperatures in the autumn and spring when larvae are active and damaging plants are likely to be below 15°C. Fungal biopesticides can work very well against vine weevil larvae when the temperature is favourable, and they fit in well with IPM programmes. Hence there would be potential for a fungal strain that works well at the lower temperatures that typically occur in soil in the autumn and spring in the UK and other northern temperate countries where vine weevil is a problem. The aim of this new piece of work was to investigate the potential of 'cold active' EPF strains against vine weevil larvae, with the target temperatures for fungal activity being between 5 - 15°C.

Summary

Lethal and sub-lethal effects on adult weevils

Laboratory experiments were done to test the direct contact and leaf residue lethal and sub-lethal effects of candidate treatments against adult vine weevils. In the direct contact experiment, vine weevils were sprayed with each treatment then added to Euonymus leaves. In the leaf residue experiment, Euonymus plants were sprayed and vine weevils were added to detached sprayed leaves either when the spray deposit was still damp or when the leaves had dried one day later. Water was used as the control in both experiments. None of the treatments tested in the three experiments led to effective kill of vine weevil adults. One weevil died due to natural infection with the entomopathogenic fungus *Beauveria bassiana* and the other few deaths are likely to have been due to natural causes. A direct contact spray of pymetrozine (Tafari) significantly reduced egg hatch but only to 65% compared with 78% in the water control. A direct contact spray of *S. carpocapsae* (Nemasys C) applied in 0.1 L

water/ha as recommended for caterpillar control led to strong aversion behaviour by adult vine weevils, but only on the day of application. Treated weevils also laid significantly fewer eggs than those treated with *S. kraussei* (Nemasys L) or Nemasys C applied in 4 L water/ha as recommended as a drench for control of vine weevil larvae, but not fewer than in the water controls. Spraying leaves, or weevils and leaves with the coded insecticide AHDB 9933 led to abnormal behaviour of vine weevil adults (lying on their backs or hiding under leaves) for 2-3 days, after which the weevils recovered and behaved normally. The botanical biopesticide azadirachtin (Azatin) acts on ingestion and has antifeedant (reduced feeding) effects on some insects but neither damp nor dry residues of Azatin led to reduced feeding on treated Euonymus leaves.

Cold active entomopathogenic fungi

An analysis of the scientific literature on the thermal tolerance of entomopathogenic fungi was done. It identified a number of EPF strains which were able to germinate and grow adequately between 5 - 15°C and hence could have potential against vine weevil larvae at these temperatures. To date, 17 candidate isolates of fungi have been acquired for experiments from a variety of sources, and these have been catalogued and cryopreserved in the Warwick Crop Centre collection of entomopathogens. Laboratory experiments were done to measure the rate of spore germination and the rate of fungal colony extension on agar-based media at a range of temperatures between 4 - 30°C. Optimum temperatures for spore germination ranged from 20 - 28°C and for fungal colony extension ranged from 17.5 - 24.8°C. Only two isolates were able to germinate at temperatures below 10°C and only four isolates were able to grow at 4°C. The two most promising strains were screened in a laboratory bioassay (determines the concentration or potency of a substance) over 28 days, at temperatures ranging from 12.5 - 25°C and mortality fitted to a predictive model. The cumulative day degrees needed for 50% mortality was similar or higher to that of Met52, suggesting that they had a lower inherent virulence to vine weevil larvae than Met52 which offset the benefits obtained from these fungi being able to develop at lower temperatures than Met52.

Financial Benefits

- The value of the UK HNS industry is estimated at £933 million per year (Defra Horticultural Statistics 2017). Crop damage and crop rejections due to the presence of vine weevil larvae can cause up to 100% losses if control measures give inadequate control. Most of the growers of HNS interviewed in project CP 111 (Review of vine

weevil knowledge in order to design best-practice IPM protocols suitable for implementation in UK horticulture) reported that vine weevil caused significant crop losses, although only one grower estimated the losses, at 3-5%. Even at a conservative estimate of 3% losses due to vine weevil leading to crop damage or crop rejections, if improved control of vine weevil were achieved, this could be worth an extra £28 million per year to the industry.

- 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. Keep up to date with results of this project as further work on monitoring will be done during 2019 using prototype new traps which may become available in the UK.
- 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. No new effective products were identified in this project. In project SF/HNS 112, indoxacarb gave promising control of adults when used at 250 g/ha and three products (Explicit, Rumo and Steward) currently have EAMUs for use on both outdoor and protected ornamentals. However, the 250 g/ha application rate may only be used on outdoor ornamentals. For protected ornamentals the EAMU specifies that spray concentrations should not exceed 12.5g/100L and the efficacy of this rate was not tested in SF/HNS 112.

- Use entomopathogenic nematodes for control of larvae (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. See https://horticulture.ahdb.org.uk/video/vine-weevil-control-%E2%80%93-overhead-nematode-application

SCIENCE SECTION

Introduction

Vine weevil is currently the most serious pest of UK containerised hardy nursery stock. Adult damage to leaves and presence of larvae around roots can make ornamental plants unmarketable. Root damage caused by larvae 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 to current EC restrictions on using one of the available neonicotinoid insecticides, imidacloprid (Imidasect 5GR) on flowering plants. 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 timing 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 Bioinsecticide) for incorporation in growing media and entomopathogenic nematodes which are applied as drenches. However, growers lack confidence in the efficacy of Met52 due to its temperature requirements and view current nematode application methods using drenches in HNS as labour-intensive and thus expensive. This project will 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 2. Develop practical methods for monitoring adults in order to detect early infestations and inform control methods

Introduction

The development of an effective semiochemical lure would improve the reliability and sensitivity of vine weevil monitoring strategies and potentially lead to the development of novel control methods for this economically important pest. Identification of semiochemicals suitable for use in vine weevil monitoring strategies has previously proven difficult as adults do not produce a sex pheromone (van Tol *et al.*, 2012), which are often used as lures for

insect pests (e.g. Rowley *et al*., 2017; Gregg *et al.*, 2018). Adult weevils, however, display a strong aggregation behaviour and show attraction to plant odours (Pickett *et al*., 1996; van Tol *et al*., 2002; van Tol *et al.,* 2004; Kakizaki 2001; Nakamuta *et al.,* 2005). Several aggregation pheromones have been identified for weevil species around the world (van Tol *et al.*, 2002), however no study has yet identified such a pheromone for vine weevil adults.

Vine weevil adults appear to be attracted by the odour of other weevils of the same species (Nakamuta *et al.*, 2005), and specifically to the frass (droppings) produced by these weevils (van Tol *et al.*, 2004). Positive behavioural response were also recorded in this study (see Year 2 annual report) to both other unstarved weevils and weevil frass. There is, however, conflicting evidence as to whether weevils use these cues to aggregate. Pickett *et al*. (1996) noted that weevils were more likely to use refuges previously used by other weevils and therefore contain weevil frass (Pickett *et al.*, 1996), however, Nakamuta *et al.* (2005) found no such response.

Several studies have shown that vine weevil adults detect plant-derived odours and this is used by weevils to locate suitable host plants for feeding and oviposition and may, therefore, also play a role in aggregation. For example, odours of yew (*Taxus baccata*) and *Euonymus fortunei* damaged by adult vine weevil are attractive to other adult vine weevils, but *Rhododendron* and strawberry (*Fragaria* x *ananassa*) are not (van Tol *et al.*, 2002). It is not yet fully understood how vine weevil discriminate between the odours of potential host plants, as weevils appear to detect and respond to plant volatiles that are common to many plant species (van Tol & Visser, 2002; van Tol *et al.*, 2012; Karley, 2012). It is, however, likely that the ratios of blends of these plant volatiles is important in host plant detection (Bruce & Pickett, 2011). This suggestion is supported by the behavioural responses recorded in this study and also that the concentration of each plant volatile may be important in determining the nature of the response recorded (see Year 2 annual report).

Karley (2012) found plant derived cues, such as the plant volatile (*E*)-2-hexenol, were much more attractive to vine weevil than insect-derived cues. Several volatiles derived from *Euonymus fortunei* are known to attract vine weevils (van Tol *et al.*, 2002, 2012). A combination of two volatiles, methyl eugenol and (*Z*)-2-pentenol (1:1 ratio), when used as an attractant in traps were responsible for increased numbers of weevils close to the traps but did not increase trap catches (van Tol *et al.,* 2012). A similar result was recorded in this study (see Year 2 annual report) where weevils were attracted to a blend of these two volatiles under laboratory conditions, but release of these same two chemicals did not increase trap catches in semi-field conditions.

These studies suggest that vine weevil adults use olfactory cues for host plant location and aggregation. Identification of an effective attractant for vine weevil could significantly enhance existing trapping methods and improve monitoring of this pest. An attractant could also be exploited in a 'lure and kill' strategy involving biopesticide formulations, which would enable IPM-compatible control options to be developed. The aims of objective 2 in year 3 were twofold: (1) to investigate whether host plant material can be used to increase catches of vine weevil adults and (2) to further investigate the presence of a volatile aggregation pheromone.

In view of the work at Harper Adams confirming the strong attractiveness of *E. fortunei* to vine weevils, previous GC-EAG studies by NRI on volatiles from *E. fortunei* were revisited (Karley et al. 2012). Unidentified compounds in the previous analyses were identified, and the EAG responses of vine weevils to the newly-identified compounds were investigated. Further collections of volatiles from *E. fortunei* were made and analysed by GC coupled to EAG recording.

Materials and methods

Task 2.2 Potential of lures to improve monitoring of vine weevil adults

Insects

Adult vine weevils were collected from soft fruit crops (mainly strawberry) in Shropshire and Staffordshire in 2018 and kept at 20 °C in a controlled environment room (Fitotron, Weiss Technik, Ebbw Vale, Wales) under long-day conditions (L:D 16:8 h). Weevils were maintained on yew, *Taxus baccata* (Linnaeus), plants inside insect cages (47.5 x 47.5 x 47.5 cm, BugDorm, MegaView, Taiwan).

Semi-field 'tent' cages

Four large tent cages (1.45 m x 1.45 m x 1.52 m) (Insectopia, UK) were set up within an unheated glasshouse at Harper Adams University, each containing 15 weevils to test the response to each treatment combination. Four potted (12cm diameter pots) strawberry plants (cv. Elstanta) were placed into each of the cages along with two conical (Chemtica) traps (see Figure 2.1). Each trap contained a fine 'weevil proof' nylon mesh bag (30 x 20 cm), which was either left empty or filled with 15 g of freshly cut *Euonymus fortunei* or yew foliage. In this way *Euonymus* or yew odour was emitted from each trap in which plant material was placed but no odour was emitted from traps containing an empty nylon bag. Weevils entering a trap were not able to feed on the foliage inside the mesh bags. The cages were prepared as described and weevils released into cages between 6pm and 8pm in the evening and traps assessed

the following morning between 8am and 9am. The position of the traps both within and between cages was re-randomised each day. Count data was analysed in R statistics V3.1.5 (R Core Team, 2018) using exact binomial tests against the null hypothesis that the distribution of vine weevils in each Chemtica trap was 50:50. Prior to undertaking statistical analysis, the results from each experiment replicate were pooled.

Figure 2.1 Arrangement of traps and traps within each tent cage.

Conditioning of weevils

Weevils were conditioned by taking 20-30 adult vine weevil from the culture and placing these in Mini BugDorms (12.5x11.4 cm). Mini BugDorms consist of a mesh lid on a round plastic container (see Figure 2.2). Before the start of each experiment, weevils were conditioned with either yew or *Euonymus fortunei* plant material for 10 days. The cut end of the yew or *Euonymus* foliage was wrapped in damp tissue paper in order to keep this material fresh for as long as possible and to provide a source of moisture for the weevils. The foliage was placed inside each BugDorm along with a dry ball of tissue paper. The foliage was replaced every two to three days.

Figure 2.2 Mini BugDorm containing yew foliage, ball of dry tissue paper and vine weevil adults.

Experiments

- Weevils were conditioned on yew and presented with the following treatment combinations:

    a. Empty trap (no odour) versus Empty trap (no odour)
    b. Yew odour versus Empty trap (no odour)
    c. *Euonymus* odour versus Empty trap (no odour)
    d. *Euonymus* odour versus Yew odour

Ten replicates of each treatment combination was recorded between 4th and 13th July 2018.

- Weevils were conditioned on *Euonymus* and presented with the following treatment combinations:

    a. Empty trap (no odour) versus Empty trap (no odour)
    b. Yew odour versus Empty trap (no odour)
    c. *Euonymus* odour versus Empty trap (no odour)
    d. *Euonymus* odour versus Yew odour

Ten replicates of each treatment combination was recorded between 14th and 29th July 2018.

- Weevils were presented with a treatment combination of *Euonymus* odour versus yew odour having been previously conditioned on:

    a. Yew
    b. *Euonymus*

Twelve replicates of each treatment combination was recorded between 30th July and 8th August 2018.

Entrainment of vine weevil volatiles for analysis

Thirty adult vine weevils were carefully transferred into a triple cone and socket glassware set (Barry Pye, Hertfordshire, UK) (Fig. 2.3). Once the vine weevils had been transferred into the glassware, air was then pumped through the glassware using a Volatile Collection Kit (Barry Pye, Hertfordshire, UK) for 30 minutes at 200 ml/min to purge potential environmental contaminants from the system. After purging, a 150 mm glass tube (6 mm O.D., 4 mm I.D.) (Sci-Glass Consultancy, Bere Alston, UK) containing 200 mg of Porapak Q (80 - 100 mesh) (Sigma Aldrich, Gillingham, UK) was attached to the outlet of the glassware and a Volatile Collection Kit. Air was then drawn over the weevils and Porapak at 200 ml/min for 24 hours. Entrained volatiles were eluted from the Porapak using 500 µl of diethyl ether (97 % purity) (Sigma Aldrich, Gillingham, UK), which was then stored at - 20 °C until analysed. All glassware was thoroughly cleaned before use by first rinsing with distilled water followed by HPLC-grade acetone (Sigma Aldrich, Gillingham, UK) and baking at 200 °C for 30 minutes. Volatiles were entrained from vine weevils under two treatments: (1) fed on yew (*Taxus baccata*) and (2) starved for 72 hours. Each treatment type was entrained three times using a different vine weevil cohort.

Figure 2.3 Triple cone and socket glassware set used to contain the vine weevils during volatile entrainment.

Analysis by gas chromatography-electroantennography (GC-EAG)

Entrainment groups (unstarved and starved) were each analysed by GC-EAG in triplicate. Gas chromatography-electroantennography analyses were carried out on an Agilent 7820GC fitted with a capillary column (30 m x 0.32 mm i.d. x 0.25 µm film thickness) coated with a non-polar (HP5-MS) phase. The analyses were carried with splitless injection (285 °C), hydrogen carrier gas (2.4 ml/min) and the oven temperature programmed from 40 °C for 2 minutes, then ramped to 285 °C at 15 °C/min where it was held for 1 minute. The GC column effluent was split (1:1) with low-volume connector between the FID (300 °C) and through a heated outlet (300 °C) into a flow of purified air (500 ml/min) through a tube (4 mm I.D.) to the EAG preparation.

For the EAG preparation, individual vine weevils were anaesthetised by chilling at - 20 °C for 2 minutes before excising their head and one antenna using microscissors. The reference electrode, containing electrolyte (0.1 M potassium chloride with 10% polyvinylpyrrolidone) was inserted into the back of the head and attached to silver electrode held in micromanipulators on a portable EAG device (INR-02; Syntech, Hilversum, The Netherlands). To complete the circuit, the tip of the one remaining antenna was excised and the recording glass electrode attached to the EAG device inserted. Both FID and EAG signals were collected and analysed with EAD software (GCEAD v1.25; Syntech, Hilversum, The Netherlands).

To confirm that the EAG preparation method was suitable for recording electrophysiological responses from excised vine weevil heads, three individual preparations were tested against ylang ylang (*Cananga odorata*) essential oil diluted to a concentration of 1 mg/ml in HPLC-grade dichloromethane.

Analysis by gas chromatography-mass spectrometry (GC-MS)

Entrainments containing chromatographic peaks evoking electrophysiological responses were further analysed using GC-MS. The diethyl ether extract was injected into the inlet port of the GC-MS [GC (Agilent 7820B 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: 33 - 500 M/Z, source temperature: 230 °C, quad temperature: 150 °C, solvent delay: 5 minutes)]. The temperature programme of the GC-MS was from 40 °C (2 minute hold) to 285 °C (8.5 minute hold) at 10 °C/min (total run time: 35 minutes). Peak identification was undertaken by comparing the obtained spectra against a mass spectra

database (NIST MS search 2.0; National Institute of Standards and Technology, USA) and through comparison of calculated linear retention indices (LRIs) to those published in the literature. Chemical compound carry-over between samples, as well as peaks originating from the solvent or sample vials was assessed by running blank samples.

Collection of volatiles from *Euonymous fortunei* at NRI

Whereas in previous studies, volatiles were collected from intact plants, in the latest studies volatiles were collected from cut branches of *E. fortunei* (approx. 60 g) contained in round-bottomed flasks (3 litre) maintained in a controlled environment room with temperature 25°C, 12:12 h L:D cycle and relative humidity 50%. Air was drawn into the flask through an activated charcoal filter (20 cm x 2 cm; 10-18 mesh) and out through a filter containing Porapak Q (200 mg; Supelco) held between silanised glass wool plugs in a disposable pipette (4 mm i.d.). Trapped volatiles were eluted with dichloromethane (2 x 0.5 ml; Pesticide Residue Grade). Volatiles were collected for 24 h periods.

Analysis by gas chromatography linked to electroantennography (GC-EAG) at NRI

GC-EAG analyses were carried out with an HP 6890 instrument (Agilent) fitted with capillary GC columns (30 m x 0.32 mm i.d. x 0.25 µm film thickness) coated with polar (Wax10; Supelco) and non-polar (SPB1, Supelco) phases. The analyses were carried out on the polar column with splitless injection (220ºC), helium carrier gas (2.4 ml min^{-1}) and the oven temperature programmed from 50°C for 2 min, then at 20°C min^{-1} to 250°C. The ends of the GC columns were connected to a glass Y-piece with equal short lengths of deactivated fused silica tubing and this was similarly connected to a second Y-piece. One outlet of this Y-piece was connected with deactivated fused silica tubing to the FID (250°C). The other was connected to an equal length of deactivated silica tubing passing through a heated transfer line (250°C) into a glass tube (4 mm i.d.) through which air passed (500 ml/min) over the EAG preparation.

Vine weevils were provided by Tom Pope of Harper Adams University and were fed on yew. For the EAG preparation, the vine weevil was anaesthetised using carbon dioxide before excising an antenna. The cut end was inserted into the reference glass electrode, containing electrolyte (0.1 M potassium chloride with 10% polyvinylpyrrolidone and attached to a silver wire electrode held in micromanipulators on a portable EAG device (INR-02; Syntech, Hilversum, The Netherlands). The recording glass electrode was brought into contact with the distal end of the antenna to complete the circuit. Both FID and EAG signals were collected and analyzed with EZChrom software (Elite v3.0; Agilent).

Analysis by gas chromatography linked to mass spectrometry (GC-MS) at NRI

GC-MS analyses were carried out on a CP3500 GC (Varian) coupled to a CP2200 Ion Trap Detector (Varian). The fused silica capillary column (30 mm x 0.25 mm i.d. x 0.25 µm film) was coated with DBWax (Supelco) with splitless injection (220ºC) and oven temperature programmed from 40ºC for 2 min then at 10ºC min^{-1} to 240ºC. Compounds were initially identified from their mass spectra and comparison of their retention indices relative to the retention times of *n*-alkanes with data in the Pherobase (El-Sayed, 2019). Identifications were confirmed by comparison with the mass spectra and retention indices of authentic compounds.

Results

The results from *Experiment 1* where weevils had been fed on yew foliage before being released into the tent cages are shown in Figure 2.4. Significantly more weevils were recorded in traps containing either yew ($p < 0.001$) or *Euonymus* ($p < 0.001$) foliage when the other trap was empty. Where weevils were given a choice between traps containing yew or *Euonymus* foliage, significantly more were found in the yew baited traps ($p < 0.001$).

Task 2.2 Potential of lures to improve monitoring of vine weevil adults

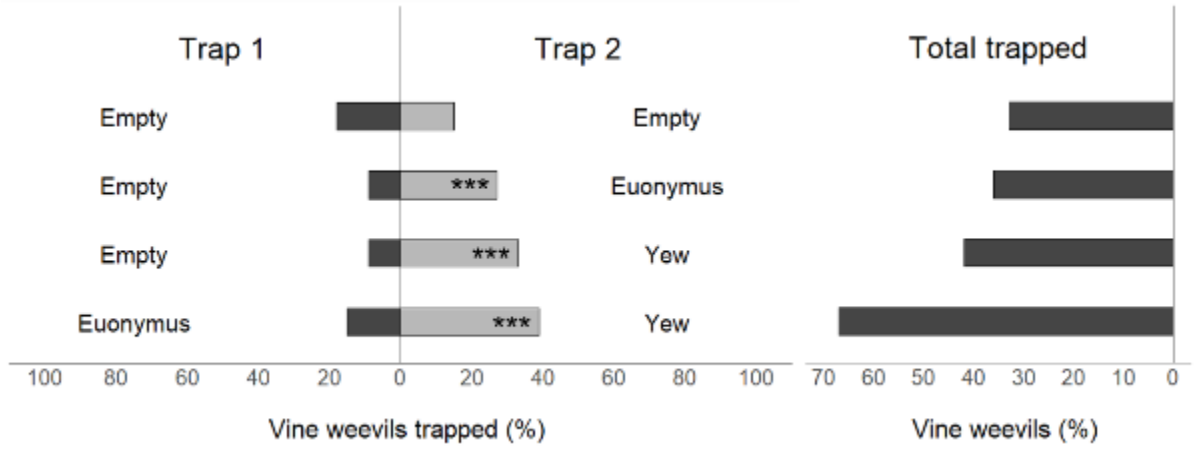


Figure 2.4 Percent of weevils caught in Chemtica traps either left empty or containing 15 g of yew or *Euonymus* foliage placed within a 'weevil proof' nylon mesh bag. The 15 weevils released into each tent cage had previous fed on yew foliage for at least 10 days prior to the start of the experiment ($n = 10$). Exact binomial test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The results from *Experiment 2* where weevils had been fed on *Euonymus* foliage before being released into the tent cages are shown in Figure 2.5. Significantly more weevils were recorded in traps containing either yew ($p < 0.001$) or *Euonymus* ($p < 0.001$) foliage when the other trap was empty. Where weevils were given a choice between traps containing yew or *Euonymus* foliage, significantly more were found in the *Euonymus* baited traps ($p < 0.001$).

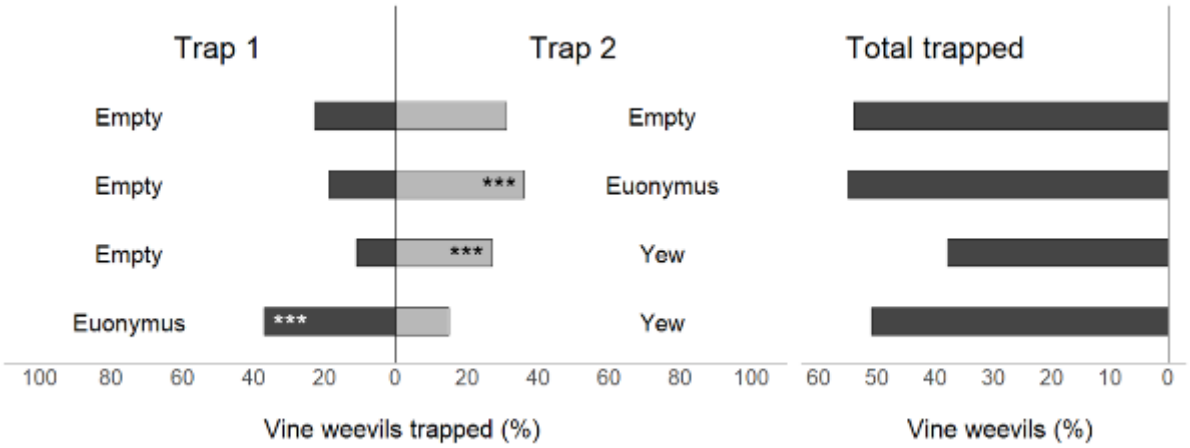


Figure 2.5 Percent of weevils caught in Chemtica traps either left empty or containing 15 g of yew or *Euonymus* foliage placed within a 'weevil proof' nylon mesh bag. The 15 weevils released into each tent cage had previous fed on *Euonymus* foliage for at least 10 days prior to the start of the experiment ($n = 10$). Exact binomial test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The results from *Experiment 1* where weevils had been fed either on yew or *Euonymus* foliage before being released into the tent cages are shown in Figure 2.6. Significantly more weevils were recorded in traps baited with yew foliage when previously fed on this host ($p < 0.001$) while significantly more weevils were recorded in traps baited with *Euonymus* foliage when previously fed on this host ($p < 0.01$).

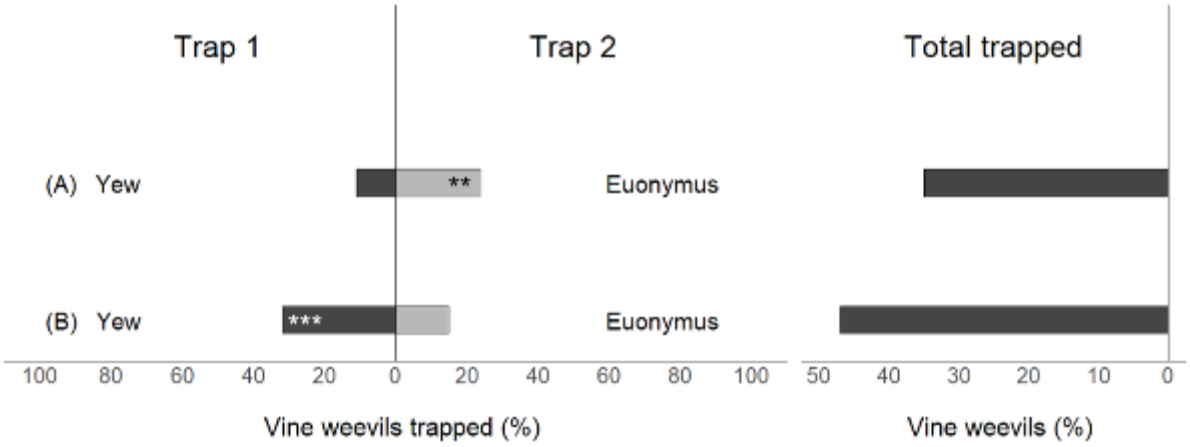


Figure 2.6 Percent of weevils caught in Chemtica traps either containing 15 g of yew or *Euonymus* foliage placed within a 'weevil proof' nylon mesh bag. The 15 weevils released into each tent cage had previous either fed on *Euonymus* (A) or yew (B) foliage for at least 10 days prior to the start of the experiment (n = 10). Exact binomial test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Analysis by GC-EAG and GC-MS

During GC-EAG analysis of the unstarved vine weevil entrainment the preparations provided a relatively stable baseline with little 'noise' but failed to show consistent EAG responses (Fig. 2.7).

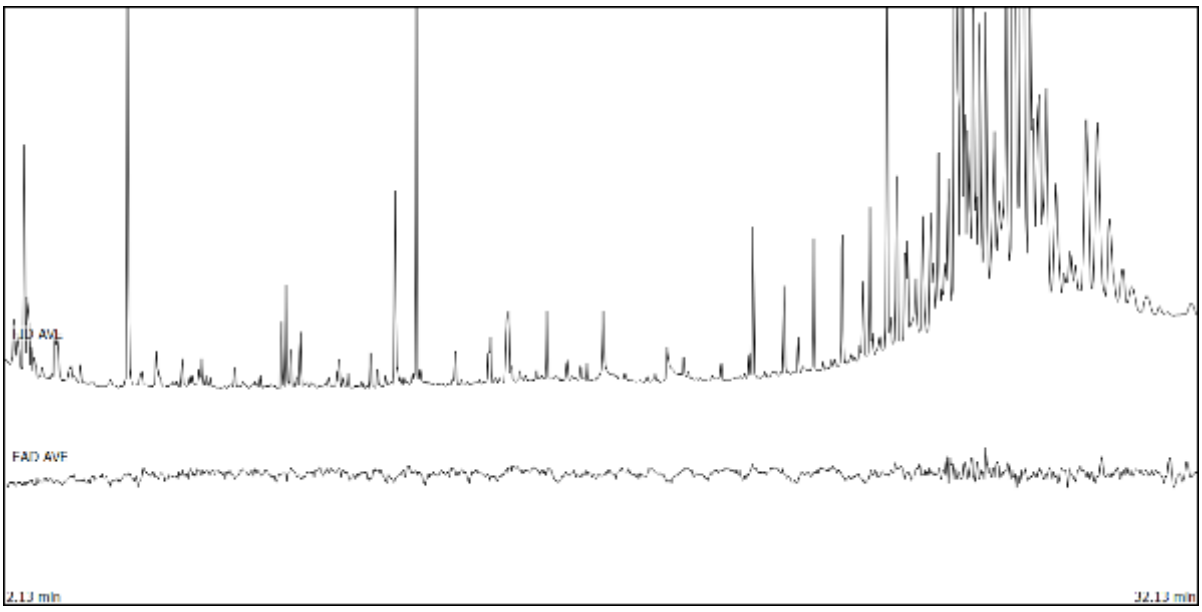


Figure 2.7 Mean GC-EAG traces for the unstarved vine weevil entrainments (n = *3*).

Analysis of the starved vine weevil entrainments by GC-EAG also provided a relatively stable baseline with little ‘noise’ but again failed to show consistent EAG responses (Fig. 2.8).

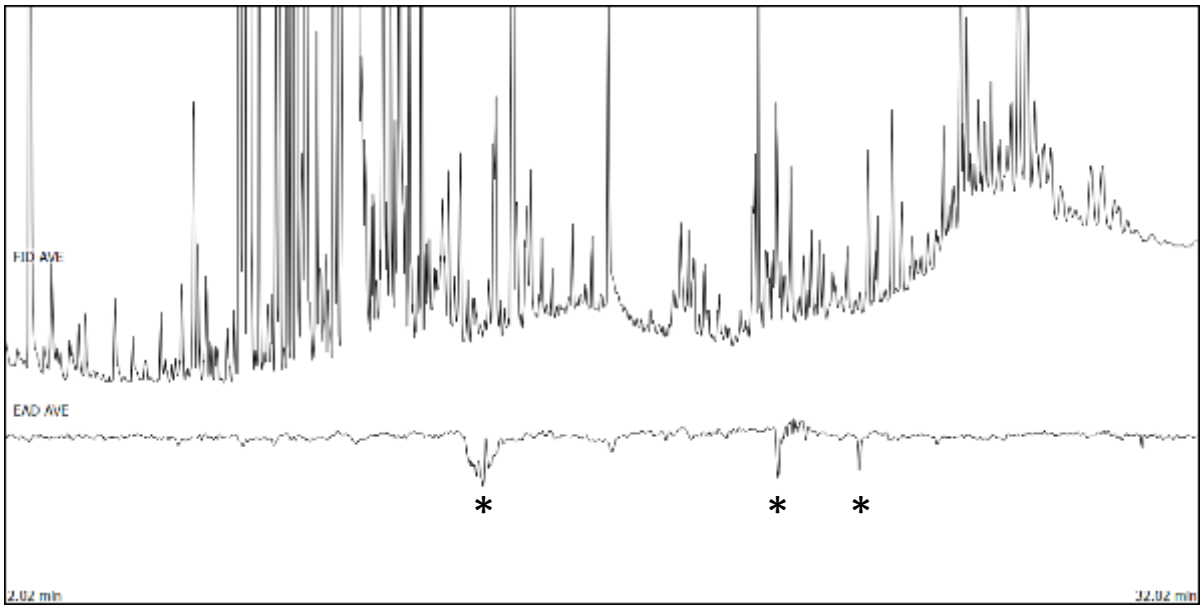


Figure 2.8. Mean GC-EAG traces for the starved vine weevil entrainments (*n* = *3*). Peaks denoted by an asterisks (*) in the EAG trace are artefacts resulting from vibrational disturbance during one of the analyses and not ‘true’ electrophysiological responses.

Analysis of the ylang ylang essential oil by GC-EAG resulted in good electrophysiological responses to 16 chemical compounds (Fig. 2.9), which were then identified by GC-MS (Table 2.1).

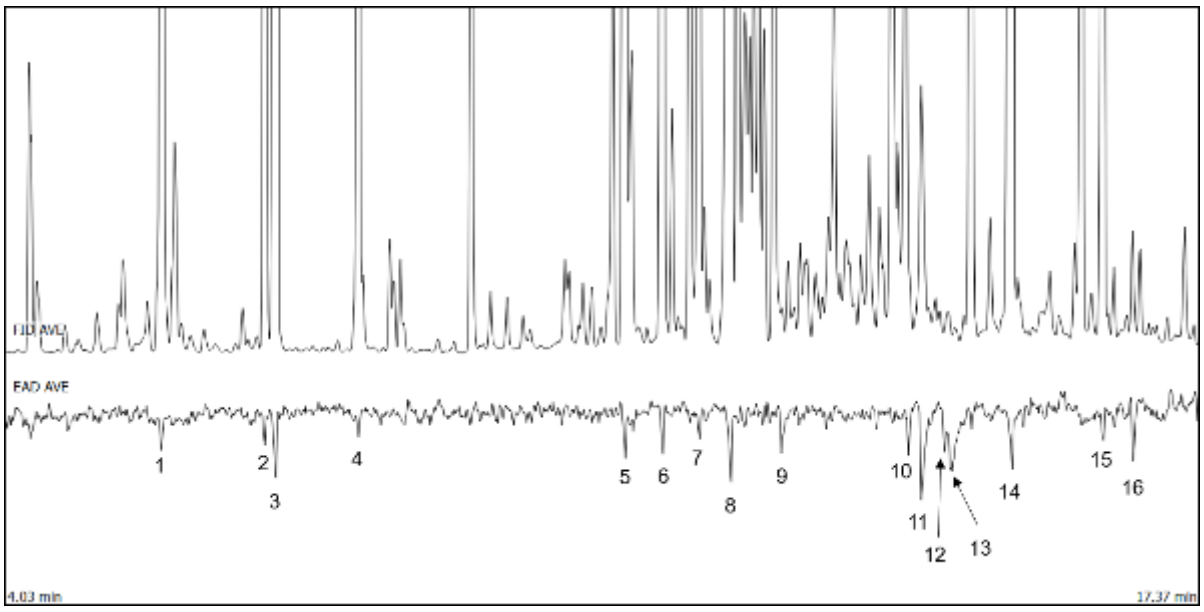


Figure 2.9 Mean GC-EAG traces for the ylang ylang essential oil at 1 mg/ml (*n = 3*). Peaks showing electrophysiological activity are: (1) p-cresol methyl ether, (2) methyl benzoate, (3) linalool, (4) acetic acid benzyl ester, (5) geranyl acetate, (6) caryophyllene, (7) ε-muurolene, (8) germacrene D, (9) γ-cadinene, (10) patchoulane, (11) unknown 1, (12) tau-cadinol, (13) (*E,E*)-3,7,11-Trimethyl-2,6,10-dodecatrienal, (14) benzyl benzoate, (15) (*E*)-cinnamyl benzoate, and (16) unknown 2.

Table 2.1. Electrophysiological responses of vine weevils to 1 mg/ml ylang ylang essential oil (*n = 3*). Compounds eliciting electrophysiological responses were identified by GC-MS.

Compound name	EAG response (±SEM) [-mV]
p-Cresol methyl ether	0.64 ± 0.36
Methyl benzoate	0.84 ± 0.29
Linalool	1.06 ± 0.09
Acetic acid benzyl ester	0.52 ± 0.35
Geranyl acetate	0.88 ± 0.02
Caryophyllene	0.99 ± 0.08
ε-Muurolene	0.28 ± 0.14
Germacrene D	1.17 ± 0.09
γ-Cadinene	0.29 ± 0.29
Patchoulane	0.29 ± 0.20
Unknown 1	0.61 ± 0.43
tau-Cadinol	0.76 ± 0.76
(*E,E*)-3,7,11-Trimethyl-2,6,10-dodecatrienal	0.88 ± 0.88
Benzyl benzoate	0.99 ± 0.04
(*E*)-Cinnamyl benzoate	0.47 ± 0.01
Unknown 2	0.37 ± 0.37

Re-examination of previous results from 2012

Results of previous GC-EAG and GC-MS analyses of volatiles from *E. fortunei* carried out in 2012 (Karley et al. 2012) were re-examined. A total of 23 reproducible EAG responses was observed in GC-EAG analyses on a polar GC column (Fig. 2.10), and the retention indices of the compounds responsible were correlated with those in GC-MS analyses on an essentially identical column.

Four of the previously unidentified compounds were identified (response 12 2-ethyl-1-hexanol, 15 muurolene, 18 (*E*)-geranyl acetone, 19 *cis*-jasmone) and two others were re-identified (4 (*E*)-2-hexenal, 16 ethylbenzaldehyde) (Table 2.2). The compound responsible for response 20 was also confirmed to be α-cadinol (Fig. 2.11) by comparison with the authentic compound isolated from hinoki essential oil.

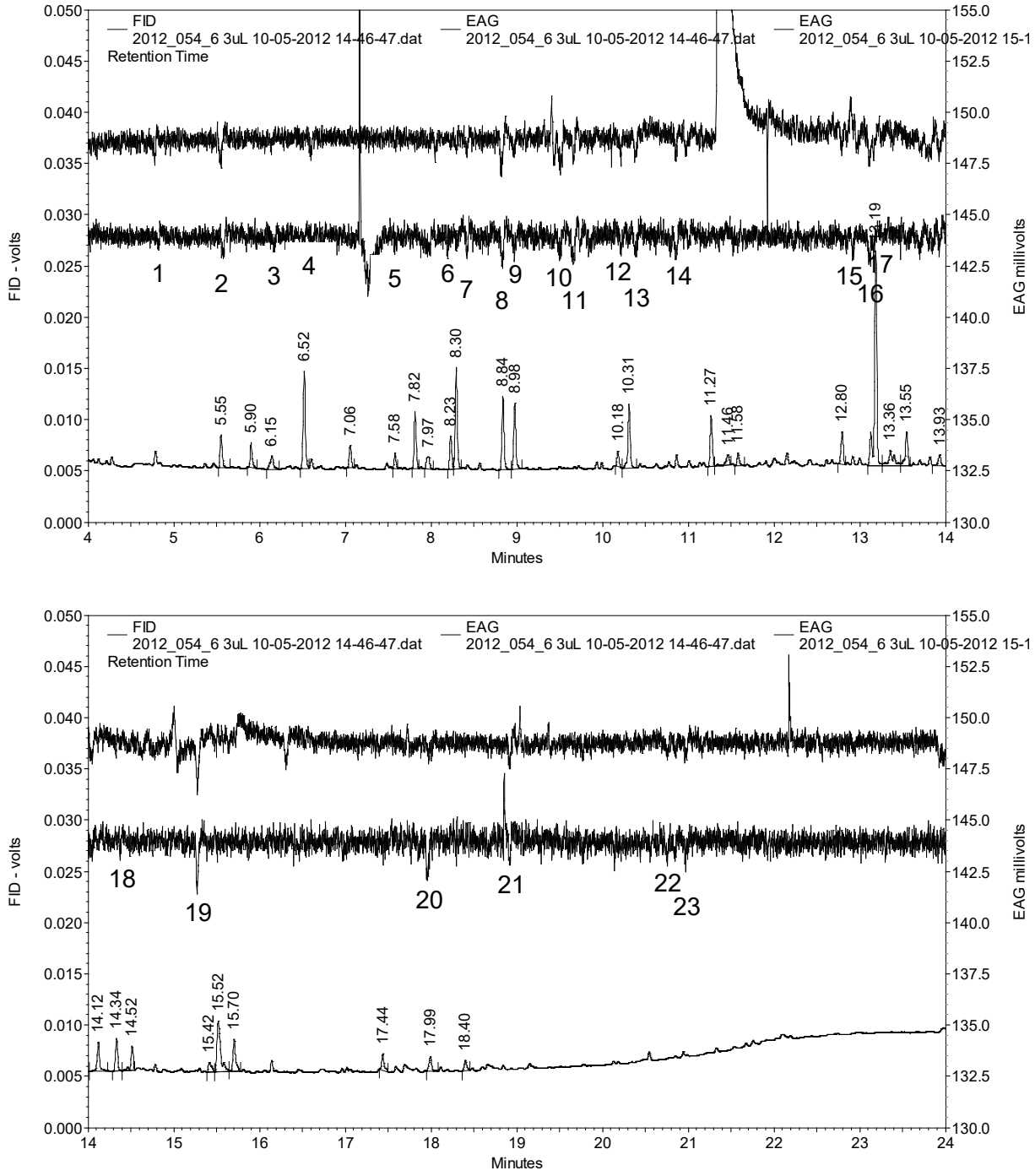


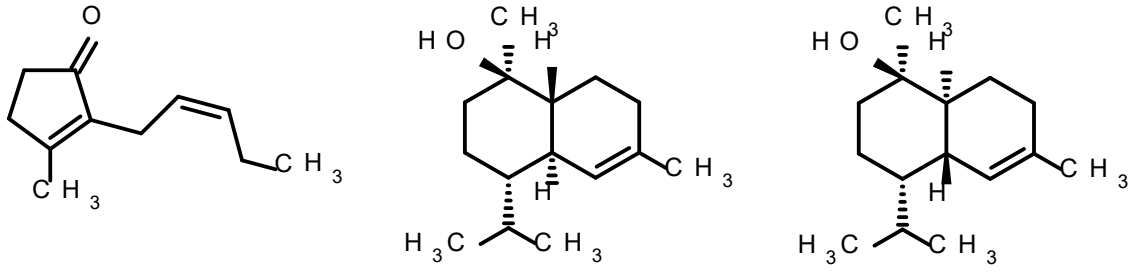
Fig. 2.10. GC-EAG analysis of volatiles from *Euonymus fortunei* (polar GC column) from 2012. (Upper traces are EAG responses in separate analyses, lower trace is FID; reproducible EAG responses are numbered as in Table 1).

Table 2.2. Compounds identified in GC-EAG analyses of volatiles from *Euonymous fortunei* during 2012 (EAG responses numbered as in Fig. 1; RI is retention index relative to retention times of *n*-alkanes on polar GC column; EAG is magnitude of EAG response; DMNT is 4,8-dimethyl-1,3,7-nonatriene; TMTT is 4,8,12-trimethyl-1,3,7,11-tridecatetraene)

EAG response	RT (min)	RI	Area (%)	Compound
1	4.79	1074	1.2	hexanal
2	5.55	1136	2.0	Silicon impurity
	5.90	1165	1.6	2-methyl-3-buten-2-ol
3	6.15	1185	1.4	heptanal
	6.52	1214	6.5	pinene oxide?
4	6.60	1220	0.7	(*E*)-2-hexenal
	7.06	1255	2.2	(*E*)-ocimene
5	7.57	1293	1.0	octanal
	7.82	1312	3.7	DMNT
	7.97	1323	1.3	(*Z*)-3-hexenyl acetate
6	8.23	1343	2.1	6-methyl-5-hepten-2-one
	8.30	1348	6.4	anisole
7	8.41	1356	0.0	hexanol
8	8.84	1389	4.7	(*Z*)-3-hexenol
9	8.98	1399	4.2	nonanal
10	9.50	1441	0.0	
11	9.66	1454	0.0	1-octen-3-ol
12	10.18	1496	1.6	2-ethyl-1-hexanol
13	10.31	1507	4.4	decanal
14	10.86	1551	0.8	linalool
	11.27	1585	3.4	DMSO?
	11.46	1600	1.7	hexadecane
	11.58	1610	1.3	β-caryophyllene
	12.80	1720	2.6	4-ethylbenzaldehyde
	12.92	1730	0.5	1,4-Dimethoxybenzene
15	13.00	1738	0.5	muurolene
16	13.14	1750	2.3	4-ethylbenzaldehyde
17	13.19	1754	15.6	α-farnesene
	13.36	1770	2.3	
	13.54	1786	2.7	methyl salicylate
	13.82	1811	0.6	TMTT
	13.93	1823	1.1	Silicon impurity

Table 2.2. (cont)

	14.12	1841	2.2	Porapak impurity
18	14.34	1863	2.1	(*E*)-geranyl acetone
	14.54	1883	1.7	Porapak impurity
19	15.28	1956	0.0	*cis*-jasmone
	15.42	1969	0.9	
	15.52	1979	5.6	hydroxyethylbenzaldehyde
	15.70	1997	2.5	hydroxyethylbenzaldehyde
	15.89	2017	0.0	methyl eugenol
	16.14	2044	0.8	(*E*)-nerolidol
	17.44	2183	1.8	tau-cadinol
20	17.99	2245	1.4	α-cadinol
	18.40	2293	0.8	squalene?
21	18.92	2353	0.0	
22/23	20.94	2588	0.0	



cis-jasmone α-cadinol tau-cadinol

Fig 2.11. Structures of new compounds identified

EAG Responses to synthetic compounds (2018)

To confirm the above findings, the EAG responses to the corresponding synthetic compounds were measured in coupled GC-EAG analyses. Strong EAG responses were recorded to authentic *cis*-jasmone and also to the small amount of *trans*-jasmone eluting just before the main peak (Fig. 2.12), similar in potency to hexanol which was previously considered to be the most potent stimulant.

No EAG response was recorded to α-cadinol, but a response was recorded to the tau-cadinol in the same sample isolated from hinoki essential oil (Fig. 2.13)

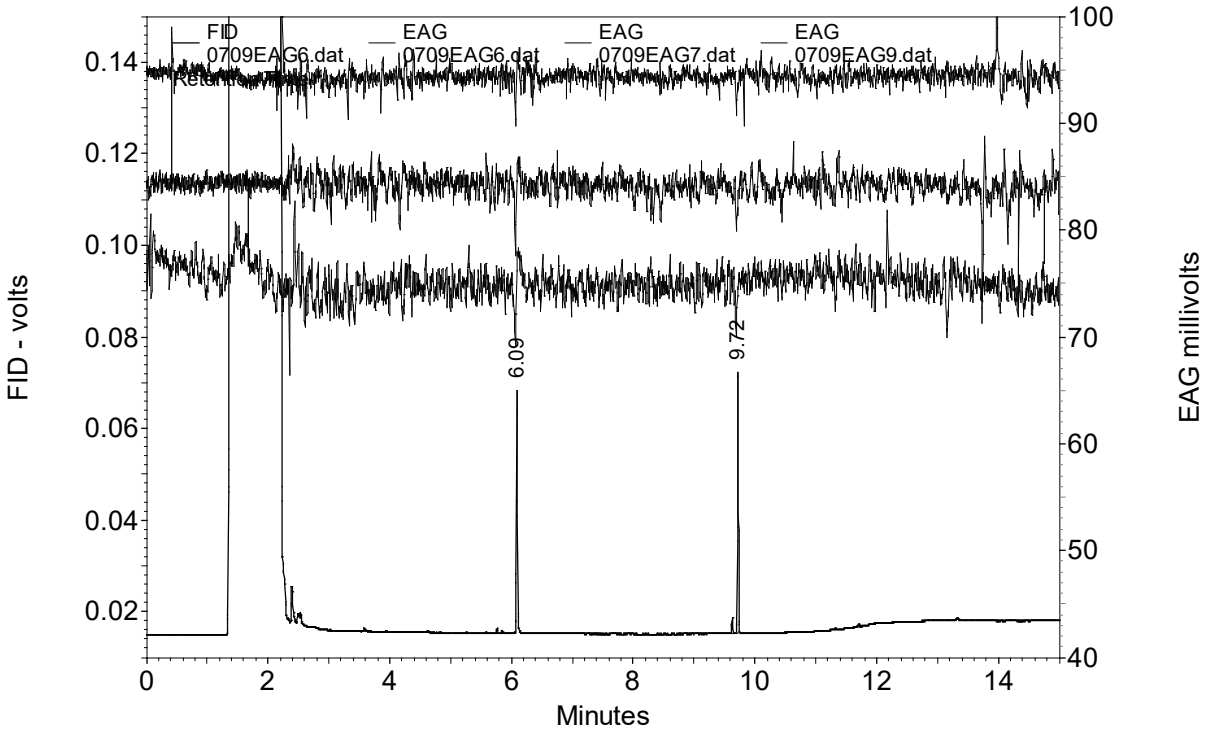


Fig. 2.12. EAG responses to 10 ng authentic hexanol (6.09 min) and *cis*-jasmone (9.72 min) (upper traces are EAG responses in three separate analyses, lower trace is FID)

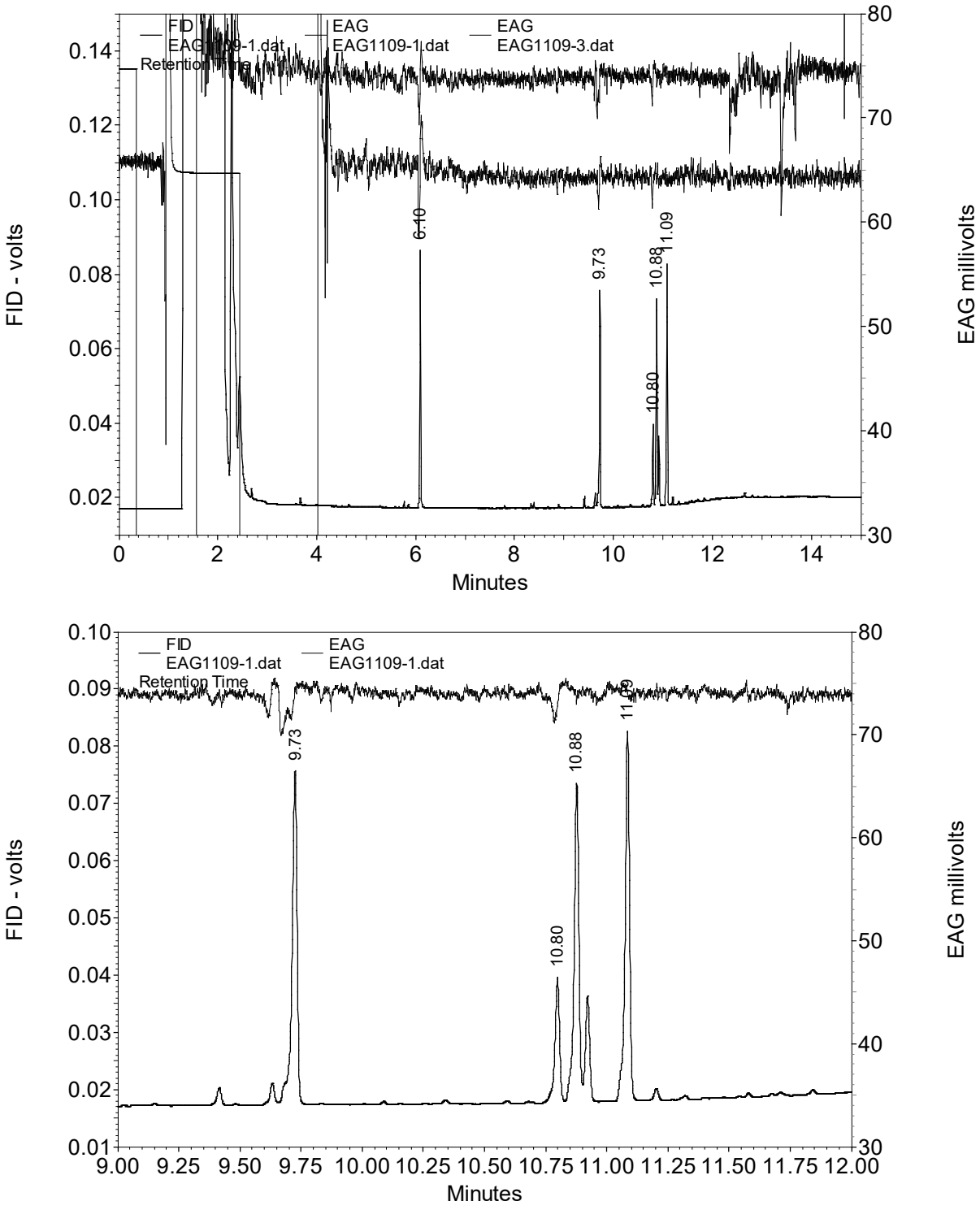


Fig. 2.14. EAG responses to 10 ng hexanol (6.10 min), cis-jasmone (9.73 min) and tau-cadinol (10.80 min) (upper traces are EAG responses in two separate analyses, lower is FID; α-cadinol 11.08 min)

GC-EAG Analyses of volatiles from *Euonymous fortunei* (2018)

As further confirmation of the new findings from the previous work, new collections of volatiles from *E. fortunei* were made using cut branches rather than the intact plants used in 2012. These collections were analysed by GC-EAG using an isolated antenna rather than insect head as this approach was found to give a more stable preparation. Results were similar, but not identical, to those obtained previously (Fig. 2.15 and Table 2.3).

In all, 20 EAG responses were observed. As previously, EAG responses were observed to 11 compounds: hexanal, heptanal, (*E*)-2-hexenal, octanal, hexanol, (*Z*)-3-hexenol, nonanal, decanal, linalool, (*E,E*)-α-farnesene and *cis*-jasmone. In addition, EAG responses to nine new compounds were observed: (*Z*)-3-hexenal, (*E*)-2-hexenol, germacrene D, methyl salicylate, benzyl alcohol, 2-phenylethanol, two unidentified acids, and indole. 1-Octen-3-ol and α-cadinol, compounds detected previously, were not observed.

As previously, (*Z*)-2-pentenol reported by van Tol et al. (2012) could not be detected in any of the samples.

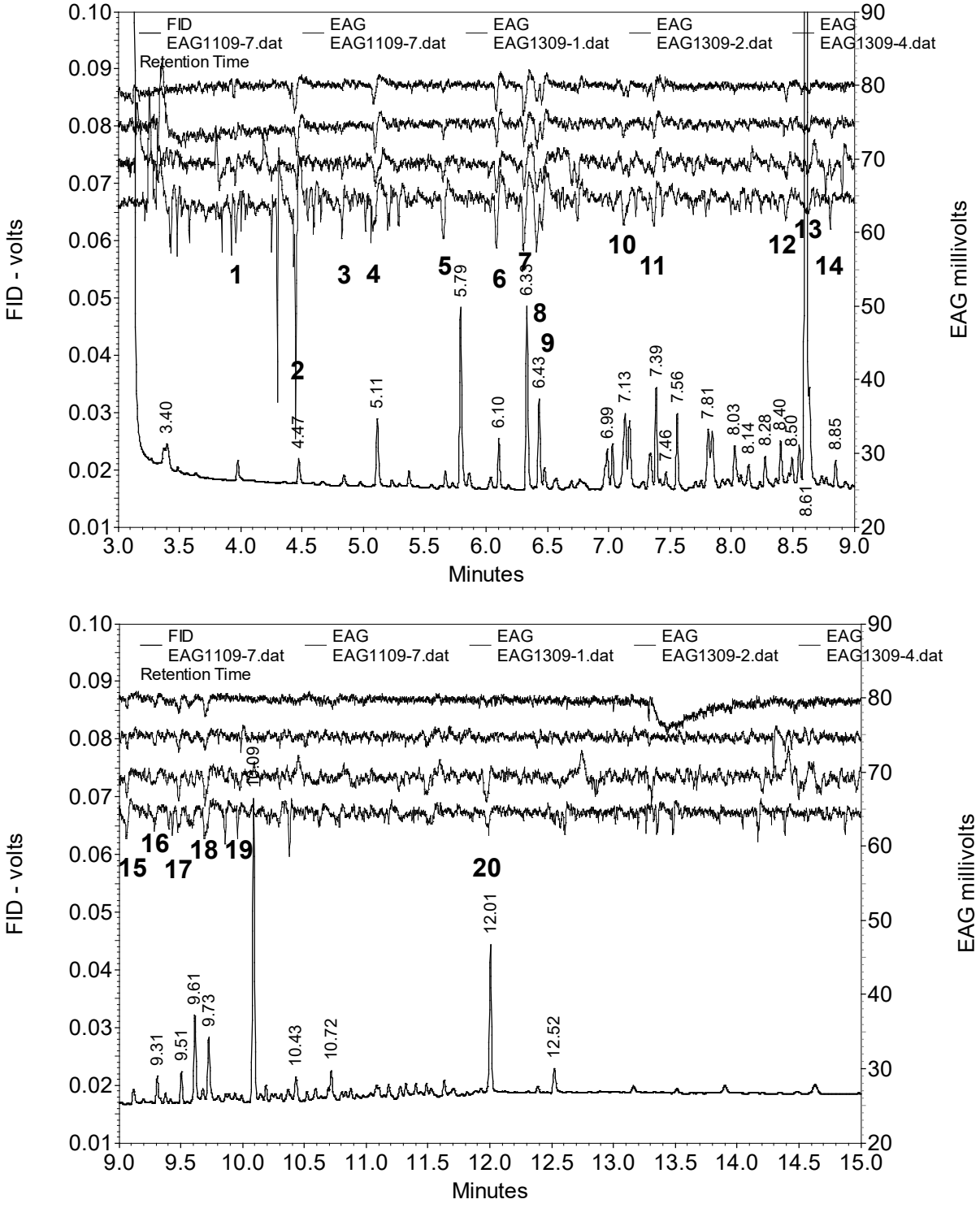


Fig. 2.15 GC-EAG analysis of volatiles from *Euonymus fortunei* (polar GC column) from 2018. (Upper traces are EAG responses in separate analyses, lower trace is FID; reproducible EAG responses are numbered as in Table 2).

Table 2.3. Compounds identified in GC-EAG analyses of volatiles from *Euonymous fortunei* during 2018 (EAG responses numbered as in Fig. 5; RI is retention index relative to retention times of *n*-alkanes on polar GC column; DMNT is 4,8-dimethyl-1,3,7-nonatriene)

EAG response	RT (min)	RI	Area (%)	Compound
1	3.97	1086	0.48	hexanal
2	4.47	1147	0.54	(*Z*)-3-hexenal
3	4.85	1193	0.31	heptanal
4	5.11	1226	1.58	(*E*)-2-hexenal
5	5.67	1301	0.32	octanal
	5.79	1317	3.98	DMNT
6	6.10	1358	1.07	hexanol
7	6.33	1388	3.63	(*Z*)-3-hexenol
8	6.43	1402	1.98	nonanal
9	6.48	1409	0.54	(*E*)-2-hexenol
	6.70	1443	0.35	
	6.99	1488	1.53	
	7.03	1495	1.07	2-ethyl-1-hexanol
10	7.13	1510	2.43	decanal
	7.17	1516	1.99	copaene
	7.35	1544	1.28	
11	7.39	1550	2.32	linalool
	7.46	1561	0.62	
	7.56	1577	1.64	
	7.81	1618	2.2	
	7.84	1623	1.79	β-caryophyllene
	8.03	1656	1.8	
	8.14	1675	0.85	
	8.28	1700	1.25	α-caryophyllene
	8.40	1721	1.56	
12	8.50	1739	1.28	Germacrene D
	8.55	1747	1.14	
13	8.61	1758	25.99	α-farnesene
14	8.85	1800	0.65	methyl salicylate
15	9.12	1852	0.45	acid?
16	9.31	1889	0.92	benzyl alcohol
17	9.51	1928	0.7	2-phenylethanol
	9.61	1948	1.94	benzyl cyanide

Table 2 (cont)

	9.70	1965	0.2	*trans*-jasmone
18	9.73	1971	1.99	*cis*-jasmone
19	9.89	2002	0.2	acid?
	10.09	2044	6.06	(*E*)-nerolidol
	10.19	2065	0.35	
	10.37	2103	0.5	
	10.43	2116	0.64	
	10.72	2177	1.13	
	11.08	2253	1.31	
20	12.01	2468	3.31	indole
	12.52	2584	0.63	

Discussion

Research on attractants for vine weevil adults has focused on potential aggregation pheromones produced by the live weevils, volatiles produced by host plants and weevil frass. Results presented here largely confirm previous research (see van Tol et al., 2002) by showing that vine weevil adults are strongly attracted to the odour of yew (*Taxus baccata*) and *Euonymus fortunei*. However, this is the first time that the odour of host-plant foliage has been used to increase catches of vine weevil adults in traps. In the first two experiments vine weevil adults always showed a preference towards the traps containing host-plant foliage compared to traps containing empty mesh bags. When given a choice between traps containing different host-plant foliage, significantly more adult weevils were found in traps containing the host-plant foliage which they were conditioned on. Experiment 3 confirmed results from the previously completed experiments. These results indicate experience-based behavioural plasticity among vine weevil adults. Such behavioural plasticity has been well studied and thoroughly reviewed by Papaj and Prokopy (1989). Previously reported examples of behavioural plasticity amongst herbivorous insects include the diamondback moth (*Plutella xylostella*) (Zhang *et al.*, 2007). In this earlier study, female diamondback moths were more likely to oviposit on non-host pea plants if they had been exposed to this plant before the start of the experiment. With respect to phytophagous Coleoptera, there are a number of examples in which previous feeding experience may influence feeding preference (Papaj and Prokopy, 1989). Perhaps most interestingly, innate host plant preferences can be modified in adult insects in a relatively short period of time (Takano *et al.,* 2010). Behavioural plasticity in vine

weevil may have implications for designing effective monitoring strategies used as part of future IPM programmes. It may be that any semiochemical lure based on plant volatiles would need to mimic the crop it is being used in to be effective, however, here the background crop used differed from either host plant used as a bait. Further work would be required to understand whether using the host plant on which the weevils are feeding as a bait remains effective when the crop in which the trap is based is the same species.

Although GC-EAG analysis of the volatiles entrained from unstarved and starved populations of vine weevils did not elicit any consistent electrophysiological responses, electrophysiological responses were identified to a number of volatiles present in the essential oil of ylang ylang (Figs. 7, 8, 9 and Table 2.1). The lack of electrophysiological responses to the unstarved vine weevil volatiles is in agreement with the previous GC-EAG results presented in the Year 2 annual report for this project. As there was no behavioural response to starved conspecifics in olfactometer bioassays in the Year 2 report for this project it is consistent that no electrophysiological response was recorded during GC-EAG analysis. The fact, however, that behavioural responses to unstarved conspecifics in olfactometer bioassays were recorded in the Year 2 report does suggest that electrophysiologically active compounds produced by these weevils are not currently being detected. It may be, for example, that high molecular weight compounds are not being detected using the approach described here. The good electrophysiological responses recorded to 16 chemicals present in the essential oil of ylang ylang (Table 2.1), with the largest responses being to linalool, caryophyllene, and germacrene D indicate that the approach used worked well for lower molecular weight compounds. Karley *et al.* (2012) previously tested the electrophysiological response of vine weevil to ylang ylang essential oil, with their results showing consistent responses to linalool only, with caryophyllene and germacrene D eliciting no response in 16 analyses.

The analyses of volatiles from *E. fortunei* by Karley et al. (2012) were revisited in order to identify more of the compounds eliciting EAG responses from vine weevils that were previously unidentified. Receptors on the vine weevil antenna responded to at least 20 compounds present. Most of these were “green leaf” volatiles common to many green plants. In addition responses were observed to the sesquiterpene hydrocarbons, germacrene D and α-farnesene. Of particular note was identification of cis-jasmone as a very minor component causing a strong EAG response. This was observed in collections made previously in 2012 and in new collections, and a strong EAG response to the synthetic compound was observed. cis-Jasmone is well-known as a signalling compound and has been shown to both attract and repel various insect species (e.g. Birkett et al. 2000; Matthes et al. 2011; Bayram and Tonga

2018). In the previous work a strong EAG response was observed to α-cadinol present in volatiles of E. fortunei. However, in subsequent results no EAG response was observed to an authentic standard, but an EAG response was observed to the stereoisomer, tau-cadinol.

Indole was detected in collections of volatiles from *E. fortunei* in 2018 and sometimes elicited an EAG response from vine weevil antennae.

In similar work on volatiles from *E. fortunei* by van Tol et al. (2012), EAG responses were recorded to (*E*)-2-hexenol, (*Z*)-3-hexenol, linalool, and (*E,E*)-α-farnesene, as above. van Tol et al. (2012) also recorded EAG responses to (*Z*)-2-pentenol, methyl benzoate, DMNT and methyl eugenol. In our work, DMNT was observed as a significant component but no EAG response was recorded. The other compounds were not detected.

The results presented here then highlight a wider range of physiological active plant volatiles that may be considered in the development of a vine weevil lure.

Conclusions

- Numbers of vine weevil adults caught in Chemtica traps can be increased by the simple addition of either yew (*Taxus baccata*) and *Euonymus fortunei* foliage placed in the traps.
- Responses of vine weevil adults to Chemtica traps containing yew or *Euonymus* foliage is influenced by previous feeding experience.
- No consistent EAG responses to entrainments from starved or unstarved vine weevil adults were recorded.
- Good electrophysiological responses were recorded to 16 chemicals present in the essential oil of ylang ylang.
- Receptors on the vine weevil antenna responded to at least 20 compounds present in volatiles collected from *Euonymous fortunei*. Most of these were “green leaf” volatiles common to many green plants, but a strong EAG response was also observed to small amounts of *cis*-jasmone present.

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

Task 3.2. Optimum time for spraying and the lethal and sub-lethal effects of IPM-compatible insecticides and a novel botanical biopesticide (ADAS)

Tasks 3.2.1 and 3.2.2. Lethal and sub-lethal effects on adult weevils in response to direct application and to residues of IPM compatible insecticides, a novel botanical pesticide and entoopathogenic nematodes (ADAS)

Objective

The aim of these tasks was to determine the lethal and sub-lethal effects of selected IPM-compatible chemical and biopesticide plant protection products and entomopathogenic nematodes on adult vine weevils as direct contact applications or as residues on leaves. Weevil survival and sub-lethal effects (behaviour, feeding activity, egg laying and egg hatch) were assessed.

Materials and methods

Site

ADAS Boxworth, Cambridge.

Experimental plants

Leaves were detached from *Euonymus fortunei*, cv Emerald Gaiety for the experiments. The plants were collected in plug trays from Darby Nursery Stock on 6 September 2018 and were potted into Levington M2 growing media.

Vine weevils

Vine weevil adults were collected from commercial crops of HNS and strawberry between May and July 2017, and maintained in plastic boxes on damp tissue in a controlled environment room kept at 21°C, and fed on yew leaves. Prior to direct contact Experiments 1 and 2, the weevil diet was changed from yew to Euonymus leaves for one week to allow them to adapt to a different food source. Weevils used in the residual effects Experiment 3 were fed on yew prior to the start of the experiment.

Treatments

The treatments for the direct application and the residual effects experiments used are shown in Table 3.1, Table 3.2 and 3.3.

Table 3.1. Treatments applied in direct contact experiment: Experiment 1.

Treatment	Treatment	Product rate	Water rate
1	Water- negative control)	-	4 L/m2
2	pymetrozine (Tafari) - positive control)	0.4 kg/ha in a max. conc. of 20 g/100 L	2,000 L/ha
3	*Steinernema kraussei* (Nemasys L)	500,000 per m^2	4 L/m2
4	*Heterorhabditis bacteriophora* (Nemasys L)	500,000 per m^2	4 L/m2
5	*Steinernema carpocapsae* (Nemasys C)	500,000 per m^2	0.1 L/m2
6	*Steinernema carpocapsae* (Nemasys C)	500,000 per m^2	4 L/m2

Table 3.2. Treatments applied in direct contact experiment: Experiment 2.

Treatment	Treatment	Product rate	Water rate
1	Water (negative control)	-	500 L/ha
2	AHDB 9933	10 g/100 L	500 L/ha

Table 3.3. Treatments applied in the residual effects experiment: Experiment 3. Vine weevil adults were added to treatments 1 - 4 while the leaves were still damp after application and were added to treatments 5 - 8 24 hours after application when the leaves had dried.

Treatment	Treatment	Product rate	Water rate
	"Damp" treatments – vine weevils added to freshly sprayed leaves		
1	Water (negative control)	-	500 L/ha
2	AHDB 9933	10 g/100 L	500 L/ha
3	Azatin	140 ml/100 L	500 L/ha
4	Dimilin Flo	13 ml/100 L	500 L/ha
	"Dry" treatments - vine weevils added to leaves 24 hours after spray application		
5	Water (negative control)	-	500 L/ha
6	AHDB 9933	10 g/100 L	500 L/ha
7	Azatin	140 ml/100 L	500 L/ha
8	Dimilin Flo	13 ml/100 L	500 L/ha

Experimental design

Direct contact Experiment 1

Sixty Petri dishes were set up (10 replicates for each of the six treatments) on 4 September 2019. After treatments had been applied (see below), one vine weevil adult was added to each Petri dish after adding a Euonymus leaf placed in a small block of damp Oasis®. This was placed onto a double layer of damp filter paper to keep the detached leaf alive during the experiment. All selected leaves were flat and of a similar-size. (Figure 3.1). Each vine weevil adult was moved to a new dish with fresh filter paper, a fresh leaf and Oasis® every three days.

Figure 3.1. Experiment 1 set up in Petri dish

Direct contact Experiment 2

Twenty Petri dishes were set up (10 for each of the two treatments on 20 November 2018. The set up was the same as for direct contact Experiment 1.

Residual effects Experiment 3

Eighty Petri dishes were set up (10 per treatments including both the damp and dry leaves) on 16 October 2018. The set up was the same as for direct contact Experiments 1 and 2 except that three Euonymus leaves were used per dish due to the smaller size of available leaves. If the leaves were too small to insert into the Oasis® block they were placed beneath it (Figure 3.2).

Figure 3.2. Example 3 set up in Petri dish.

Treatment application

Direct contact Experiments 1 and 2

Each of the treatments was applied to the ten replicate weevils for that treatment on a white plastic tray (Figure 3.3). After treatment each weevil was gently transferred onto its respective leaf in a Petri dish by hand, using a separate pair of disposable gloves for each treatment. The tray was cleaned using All Clear® Extra in between treatments.

Figure 3.3. Vine weevils on a white tray for direct contact treatment application.

Residual effects Experiment 3

"Damp" treatments (Treatments 1 – 4)

Selected leaves of similar size were detached from the Euonymus plants and scanned and labelled prior to treatment application so that accurate pre-treatment leaf area measurements of each leaf could be taken without getting spray residue on the scanner. Treatments 1 – 4 were applied to the detached leaves (Figure 3. **4**). The leaves were then returned to the correct petri dish and one vine weevil was added to each Petri dish with its damp leaf.

Every three days the leaf in each Petri dish was replaced with a fresh leaf removed from sprayed Euonymus plants (see "Dry" treatments below). Leaves were selected from the newest growth as these would have been more likely have taken up the systemic treatment.

"Dry" treatments (Treatments 5 – 8)

Treatments were applied to the Euonymus plants. The plants were not watered on the day of treatment or the day after to allow the spray residues to dry. Dry leaves were removed from

the plants 24 hours after treatment. The leaves were replaced every three days using additional leaves detached from the young growth on the sprayed plants.

Figure 3. 4. The application area for detached leaves for the ‘damp’ treatments in residual effects Experiment 3

Use of scanner and WinDIAS software to measure leaf area before and after treatment

A scanner and the WinDIAS v3.2 software were used to calculate leaf area (cm²) eaten by each vine weevil after each three day period. Firstly a scanned image of a ruler was used to calibrate the software by highlighting the length of 1 cm (Figure 3.5). After the software had been calibrated the leaf was scanned and the area was measured before treatment application (Figure 3.6).

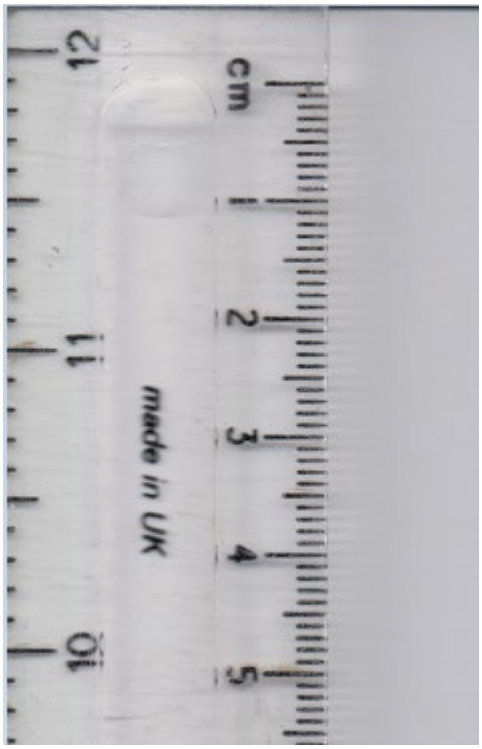


Figure 3.5. A scan of a ruler for calibrating the WinDIAS software.

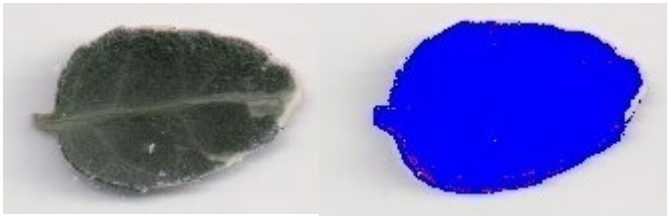


Figure 3.6. A scanned leaf prior to treatment application (left) and when viewed in the Win DIAS software (right).

The WinDIAS software measures leaf area using colour. All areas highlighted in either blue or red have been detected as leaf area. The pigments which the software highlights are manually selected and therefore all parts of the leaf, regardless of colour, can be highlighted. The software then calculates the area highlighted in cm^2.

After the leaves had been exposed to the vine weevil for three days it was scanned again and leaf area calculated (Figure 3.7). The area was then subtracted from the pre-treatment leaf area to give the area (cm^2) consumed by the vine weevil over the three day period.

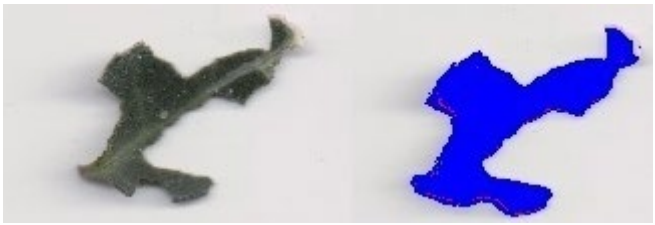


Figure 3.7. A scanned leaf three days after exposure to a vine weevil (left) and when viewed in the Win DIAS software (right).

Assessments

- Live and dead vine weevils were recorded 24 hours, 48 hours and 72 hours (3 days) after treatment application and again on day 6, 9, 12 and 15.
- If the vine weevil was still alive on the above assessment dates then any abnormal behaviour was recorded e.g. unco-ordinated movement, twitching, staggering, vomiting, 'frozen' (lying on its back with legs stuck out rather than retracted).
- Every three days each vine weevil was placed into a clean Petri dish with a fresh leaf, Oasis® block and damp filter paper. Leaf area was assessed before being placed into the Petri dishes and at the point of removal, using the scanner and the WinDIAS software. The leaf area eaten (cm^2) was calculated (or the amount of leaf shrinkage in dishes without weevils). This assessment was carried out every time a new leaf was added and whenever an old leaf was removed (five times).
- Any eggs laid in the dish (found during weevil transfer on every third day) were counted, kept on damp filter paper and assessed seven days later when numbers of brown and white eggs were recorded. After a further seven days (a total of 14 days from egg removal from the experimental dishes) the eggs were checked again and numbers of white, brown (embryonated) and hatched eggs/neonate larvae (Figure 3.9) recorded and percentage hatch calculated.

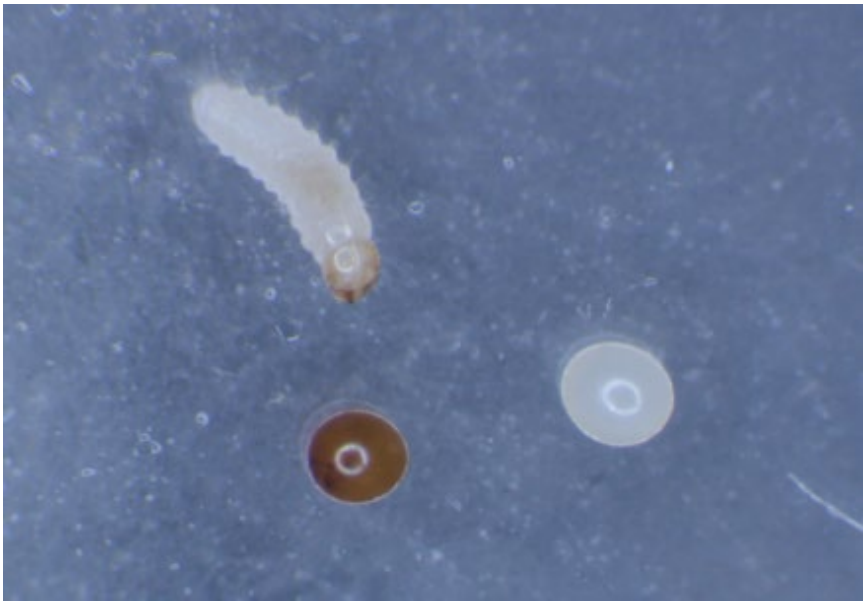


Figure 3.8. A neonate weevil larva, brown (embryonated) egg and a white egg.

Temperatures

Room temperatures were monitored during the experiment period using two data loggers.

Statistical analysis

The data on leaf area consumed were subjected to analysis of variance (ANOVA) using Genstat 18.2. The proportion of successfully hatched eggs was analysed using regression analysis.

Results

Direct contact Experiment 1

Weevil mortality

During the 15-day experiment, only six weevils died. One weevil (10%) treated with Tafari was dead on day 15, one (10%) treated with *S.kraussei* was dead on day 6 and two (20%) were dead by day 12 (Table 3.4). One weevil (10%) treated with *H. bacteriophora* was dead on day 15 and one (10%) treated with *S. carpocapsae* applied in 4 L water/m^2 was dead on day 12 with a further weevil recorded dead on day 15 giving a total of 20% dead. The dead weevil found on day 15 was infected with *Beauveria bassiana*. Dead weevils treated with nematode products were dissected but no nematodes were found inside the cadavers.

Table 3.4. Percentage dead weevils 3, 6, 9, 12 and 15 days after treatment (DAT).

Treatment	% dead 3 DAT	% dead 6 DAT	% dead 9 DAT	% dead !2 DAT	% dead 15 DAT
Water	0	0	0	0	0
Tafari	0	0	0	0	10%
Steinernema kraussei	0	10%	10%	20%	20%
Heterorhabditis bacteriophora	0	0	0	0	10%
Steinernema carpocapsae (0.1L/m^2)	0	0	0	0	0
Steinernema carpocapsae (4L/m^2)	0	0	0	10%	20%*

*One weevil (10%) infected with *Beauveria bassiana*

Abnormal behaviour

On the day of treatment application, weevils treated with the higher concentration of *Steinernema carpocapsae* (applied in 0.1 L water/m^2) immediately responded by defaecating and trying to brush the nematodes off their bodies with their legs (Figure 3.9). None of the other treatments had this effect, including the lower concentration of *S. carpocapsae* (applied in 4 L water/m^2). The immediate effect of the higher concentration of *S. carpocapsae* on weevil behaviour did not persist to the assessment 24 hours after treatment or to later assessments.

Figure 3.9. Vine weevil defaecating immediately after application of *Steinernema carpocapsae* applied at 0.1 L water/m^2.

Leaf area eaten

Significant differences in leaf area eaten over a 3-day period were only found on one assessment date, 12 days after treatment (Table 3.5 and Figure 3.10). While none of the treatments were significantly different from the water control, significantly less leaf area had been eaten by weevils treated with Tafari (0.11 cm^2) and *Steinernema carpocapsae* applied in 4 L water/m^2 (0.12 cm^2) compared to those treated with *S. kraussei* (0.24 cm^2) or *S. carpocapsae* applied in 0.1 L water/m^2 (0.24 cm^2), $P < 0.05$.

Table 3.5. Mean leaf area (cm²) eaten over a 3-day period 3, 6, 9, 12 and 15 days after treatment (DAT). Values not sharing the same letters are significantly different (P <0.05).

Treatment	Mean cm² eaten 3 DAT	Mean cm² eaten 6 DAT	Mean cm² eaten 9 DAT	Mean cm² eaten !2 DAT	Mean cm² eaten 15 DAT
Water	0.26	0.39	0.24	0.19 **ab**	0.40
Tafari	0.31	0.36	0.38	0.11 **a**	0.37
Steinernema kraussei	0.32	0.29	0.42	0.24 **b**	0.32
Heterorhabditis bacteriophora	0.34	0.46	0.56	0.20 **ab**	0.49
Steinernema carpocapsae (0.1L/m^2)	0.15	0.29	0.23	0.24 **b**	0.22
Steinernema carpocapsae (4L/m^2)	0.20	0.36	0.39	0.12 **a**	0.31

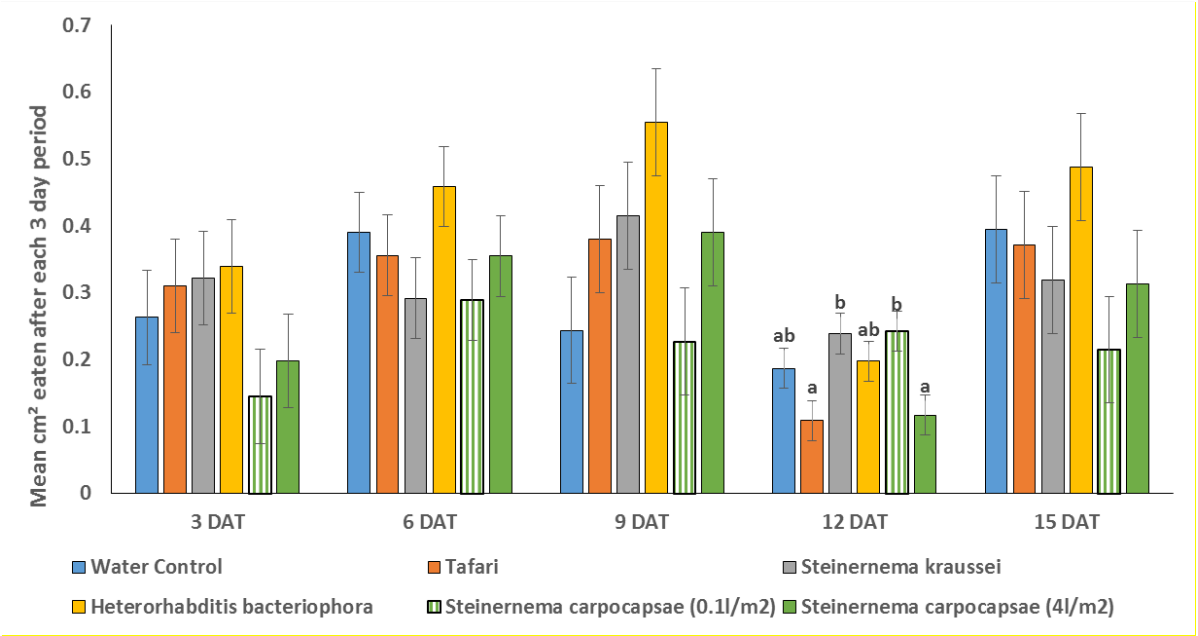


Figure 3.10. Mean leaf area (cm²) eaten over a 3-day period 3, 6, 9, 12 and 15 days after treatment (DAT). Values not sharing the same letters are significantly different (P <0.05)

When total leaf area eaten over the 15-day experimental period were compared, as for the individual assessment dates, none of the treatments were significantly different from the water control (Table 3.6 and Figure 3.11). However, significantly less leaf area was eaten by weevils treated with *S. carpocapsae* applied in 0.1 L water/m^2 (1.12 cm^2) compared to those treated with *S. kraussei* (1.72 cm^2) or *H. bacteriophora* (1.97 cm^2), P <0.05.

Table 3.6. Total mean leaf area (cm²) eaten* over the 15-day period. Values not sharing the same letters are significantly different (P <0.05).

Treatment	Mean cm² eaten throughout trial*
Water	1.41 **ab**
Tafari	1.53 **abc**
Steinernema kraussei	1.72 **bc**
Heterorhabditis bacteriophora	1.97 **c**
Steinernema carpocapsae (0.1 L/m^2)	1.12 **a**
Steinernema carpocapsae (4 L/m^2)	1.50 **abc**

*Due to six vine weevils dying in the trial Genstat calculated estimate values for any missing values in leaf area eaten, thus the sum of the means from the individual assessments (Table 3.) are not always equal to the total mean leaf area eaten over the 15-day period.

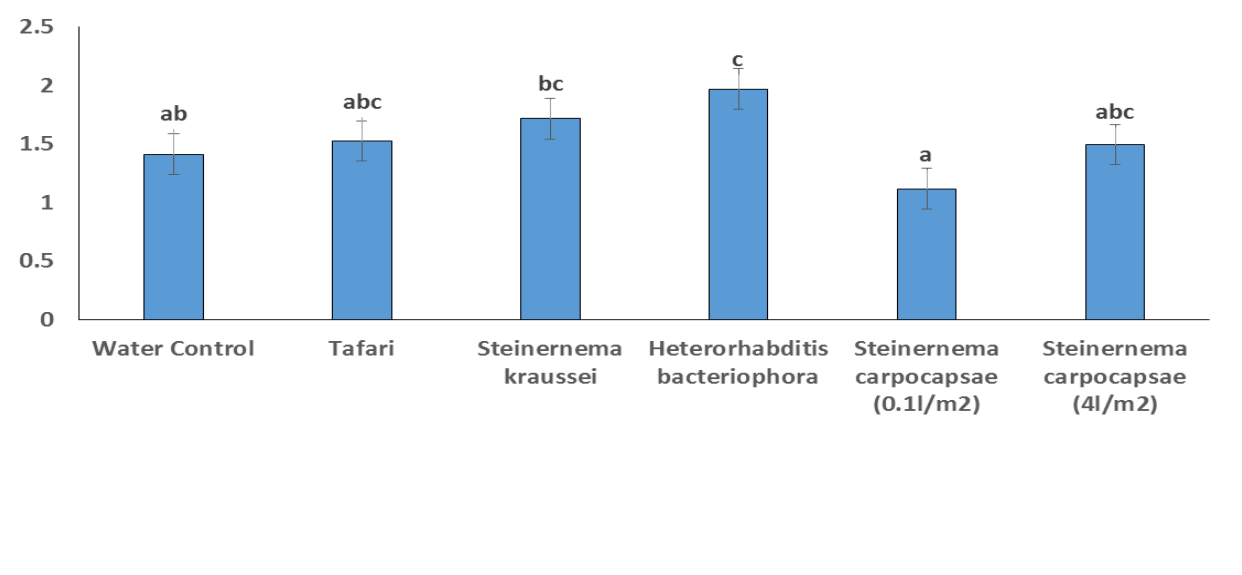


Figure 3.11. Total mean leaf area (cm²) eaten* over the 15-day period. Values not sharing the same letters are significantly different (P <0.05).

Number of eggs laid

There were no significant differences between any of the treatments in numbers of eggs laid over the 3-day period on any assessment date (Table 3.7 and Figure 3.12).

Table 3.7. Mean number of eggs laid on each 3-day period 3, 6, 9, 12 and 15 days after treatment (DAT).

Treatment	Mean no. eggs laid 3 DAT	Mean no. eggs laid 6 DAT	Mean no. eggs laid 9 DAT	Mean no. eggs laid 12 DAT	Mean no. eggs laid 15 DAT
Water	10.2	10.2	6.9	7.4	12.4
Tafari	7	11.2	7.9	8.5	9.4
Steinernema kraussei	9.2	13.6	10.9	9.61	14.9
Heterorhabditis bacteriophora	12.8	8.9	6.4	4.77	8.7
Steinernema carpocapsae (0.1L/m^2)	5.3	14.6	3.2	2.9	2.7

Steinernema carpocapsae (4L/m^2)	11.9	11.6	4.6	9.68	10

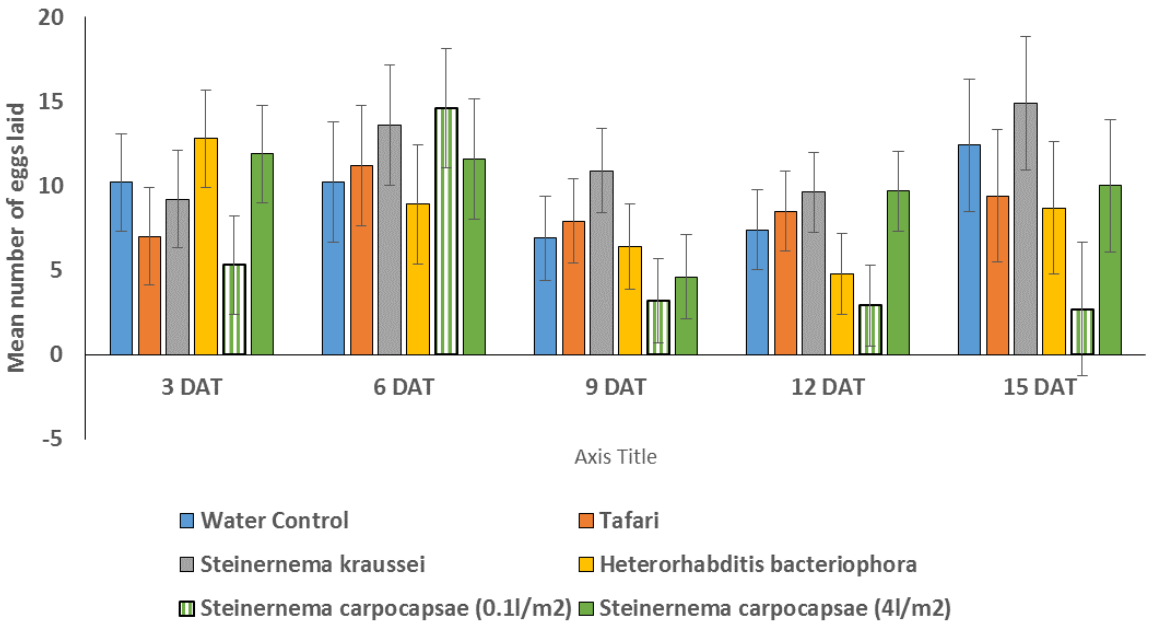


Figure 3.12. Mean number of eggs laid on each 3-day period 3, 6, 9, 12 and 15 days after treatment (DAT).

When the total mean number of eggs laid in each treatment over the 15-day period were compared, none of the treatments were significantly different from the water control (Table 3.8 and Figure 3.13). However, significantly fewer mean eggs were laid by weevils treated with *S. carpocapsae* applied in 0.1 L water/m^2 (28.7) than by those treated with *S. kraussei* (63.2) or *S. carpocapsae* applied in 4 L water/m^2 (52.6).

Table 3.8. Total mean number of eggs laid per treatment over the 15-day period*. Values not sharing the same letters are significantly different (P <0.05).

Treatment	Total mean eggs laid*
Water	47.1 **abc**
Tafari	44 **abc**
Steinernema kraussei	63.2 **c**
Heterorhabditis bacteriophora	39.1 **ab**
Steinernema carpocapsae (0.1 L/m^2)	28.7 **a**
Steinernema carpocapsae (4 L/m^2)	52.6 **bc**

*Due to six weevils dying in the experiment Genstat calculated estimate values for any missing values in leaf area eaten, thus the sum of the means from the individual assessments (Table 3) are not always equal to the total mean leaf area eaten over the 15-day period.

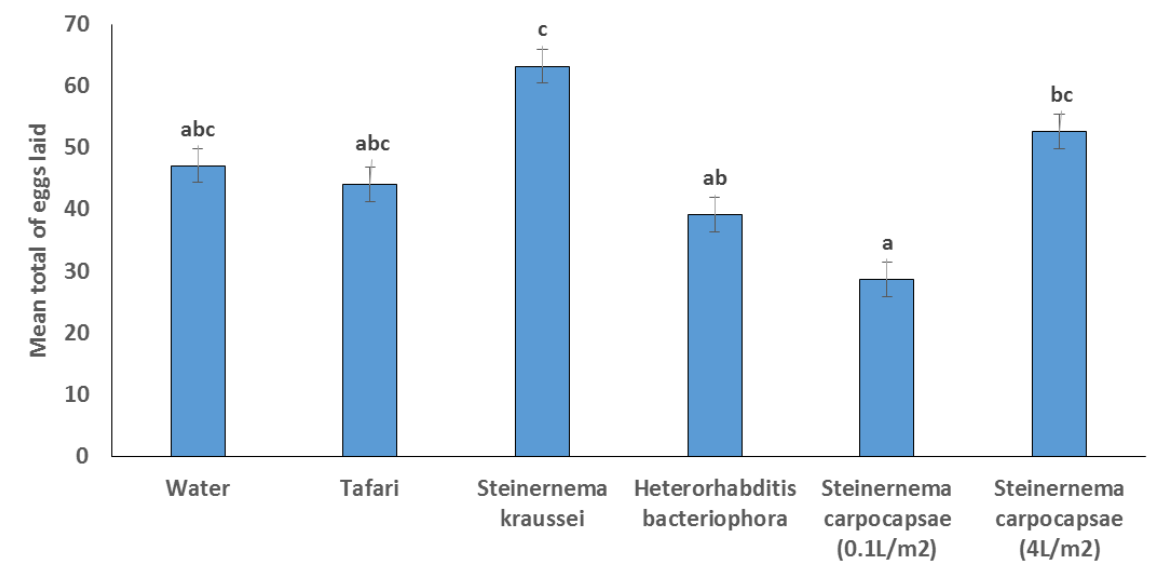


Figure 3.13. Total mean number of eggs laid per treatment over the 15-day period*. Values not sharing the same letters are significantly different (P <0.05).

Proportion of eggs hatching

Only one treatment, Tafari, significantly reduced the proportion of eggs hatching (65%) over the 15-day period when compared with the water control (78%), P <0.05 (Table 3.8) and Figure 3.14).

Table 3.8. Mean % hatch rate of eggs over the 15-day period. Values not sharing the same letters are significantly different (P <0.05).

Treatment	% eggs hatched
Water	78% **b**
Tafari	65% **a**
Steinernema kraussei	77% **b**
Heterorhabditis bacteriophora	75% **b**
Steinernema carpocapsae (0.1 L/m^2)	81% **b**
Steinernema carpocapsae (4 L/m^2)	87% **c**

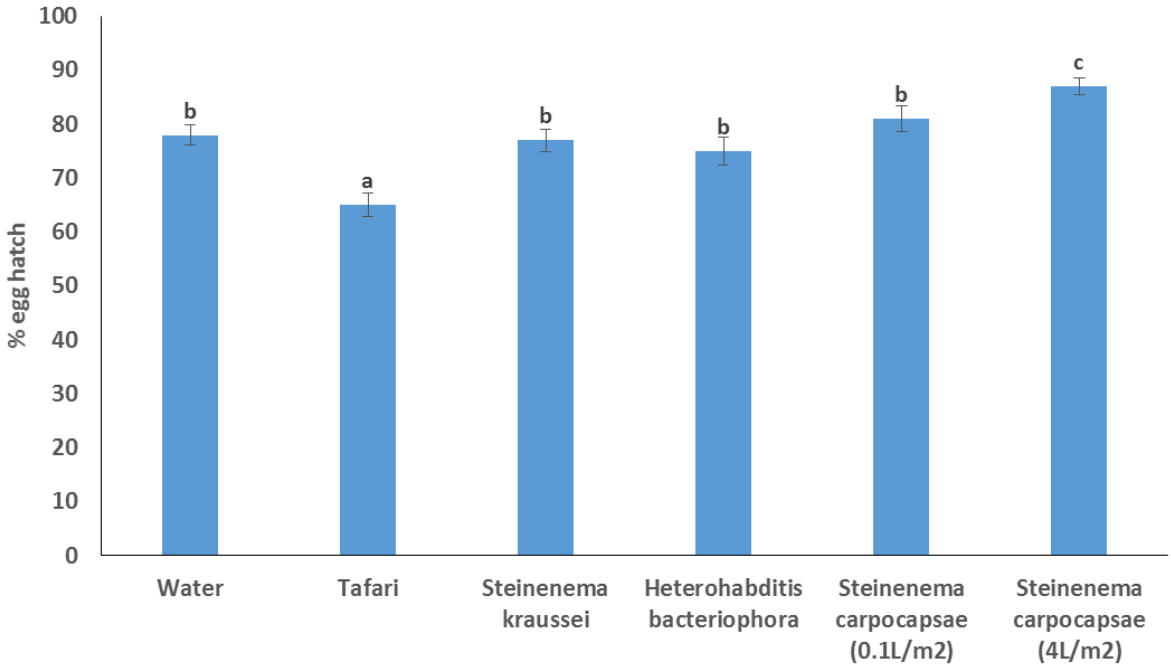


Figure 3.14. Mean % hatch rate of eggs over the 15-day period. Values not sharing the same letters are significantly different (P <0.05).

Direct contact Experiment 2

Weevil mortality

No dead weevils were recorded on any assessment date.

Abnormal behaviour

No weevils sprayed directly with the coded insecticide showed any behavioural abnormalities on any assessment date. However, following the abnormal behaviour of a few weevils in response to the 'damp' residue of the coded insecticide in the residual effects Experiment 3 (see below), an additional five weevils were sprayed directly and added to Euonymus leaves sprayed with the coded insecticide. These weevils vomited and defaecated immediately after the direct spray of the coded insecticide (Figure 3.15). The weevils then spent 2-3 days lying on their backs (Figure 3.16) and responded to being prodded by twitching their legs. Thereafter the affected weevils returned to feeding normally on the Euonymus leaves.

Fig. 3.15. Weevil vomiting and defecating immediately after direct contact application of the coded insecticide.

Figure 3.16. Vine weevil on its back 2 days after direct contact application of the coded insecticide.

Leaf area eaten

No significant differences in mean leaf area eaten (cm^2) were found on any of the assessment dates (Table 3.9).

Table 3.9. Mean leaf area (cm²) eaten over a 3-day period 3, 6, 9, 12 and 15 days after treatment (DAT).

Treatment	Mean cm² eaten 3 DAT	Mean cm² eaten 6 DAT	Mean cm² eaten 9 DAT	Mean cm² eaten 12 DAT	Mean cm² eaten 15 DAT
Water	0.0037	0.057	0.064	0.271	0.057
AHDB 9933	0.0047	0.129	0.164	0.315	0.076

Equally there were no significant differences between any treatments in total mean cm² eaten over the 15-day period (Table 3.10).

Table 3.10. Total mean leaf area (cm²) eaten over the 15-day period.

Treatment	Total mean cm² eaten
Water	0.453
AHDB 9933	0.689

Number of eggs laid and proportion of eggs hatching

No eggs were laid by any of the weevils in this experiment.

Residual effects Experiment 3

Weevil mortality

Only one dead weevil was found during the experiment, nine days after being treated with the coded insecticide.

Abnormal behaviour

No abnormal behaviour was seen in weevils added to the 'dry' leaves. Some of the weevils added to the 'damp' residues on leaves treated with the coded insecticide were found lying on their backs in the Petri dish, moving their legs slowly (similar to that shown in Figure 3.16), or crouched under the treated leaf, whereas the weevils in all other treatments were actively walking around the Petri dish. The first abnormal behaviour was recorded one day after treatment when 20% of the weevils were affected and by day 2 40% of the weevils were showing abnormal behaviour (Table 3.11). Only one (10%) of the weevils was still affected on day 3 and all the weevils had recovered by day 6. On day 9, two more weevils (20%) were showing abnormal behaviour but these weevils had recovered by day 12. Recovered weevils started behaving and feeding normally.

Table 3.11. Mean % weevils showing abnormal behaviour

Treatment	% showing abnormal behaviour 1 DAT	% showing abnormal behaviour 2 DAT	% showing abnormal behaviour 3 DAT	% showing abnormal behaviour 6 DAT	% showing abnormal behaviour 9 DAT	% showing abnormal behaviour 12 DAT	% showing abnormal behaviour 15 DAT
Water "Damp"	0	0	0	0	0	0	0
AHDB 9933 "Damp"	20%	40%	10%	0	20%	0	0
Azatin "Damp"	0	0	0	0	0	0	0

Dimilin Flo "Damp"	0	0	0	0	0	0	0

Leaf area eaten

Significant differences in leaf area eaten over a 3-day period were only given on the first assessment date, three days after treatment (Table 3.12 and Figure 3.17). On this date, weevils added to 'damp' residues of Azatin ate significantly more mean leaf area (0.3 cm^2) than those added to the water control leaves (0.02 cm^2).

Table 3.12. Mean leaf area (cm^2) eaten over a 3-day period 3, 6, 9, 12 and 15 days after treatment (DAT). Values not sharing the same letters are significantly different (P <0.05).

Treatment	Mean cm^2 eaten 3 DAT	Mean cm^2 eaten 6 DAT	Mean cm^2 eaten 9 DAT	Mean cm^2 eaten 12 DAT	Mean cm^2 eaten 15 DAT
Water "Damp"	0.02 **a**	0.05	0.08	0.11	0.01
AHDB 9933 "Damp"	0.01 **a**	0.01	0.02	0.01	0.01
Azatin "Damp"	0.30 **b**	0.01	0.01	0.03	0.004
Dimilin Flo "Damp"	0.06 **a**	0.1	0.08	0.13	0.01
Water "Dry"	0.03 **a**	0.01	0.10	0.31	0.15
AHDBB 9933 "Dry"	0.02 **a**	0.003	0.02	0.10	0.14
Azatin "Dry"	0.13 **ab**	0.05	0.13	0.11	0.04
Dimilin Flo "Dry"	0.03 **a**	0.01	0.02	0.19	0.10

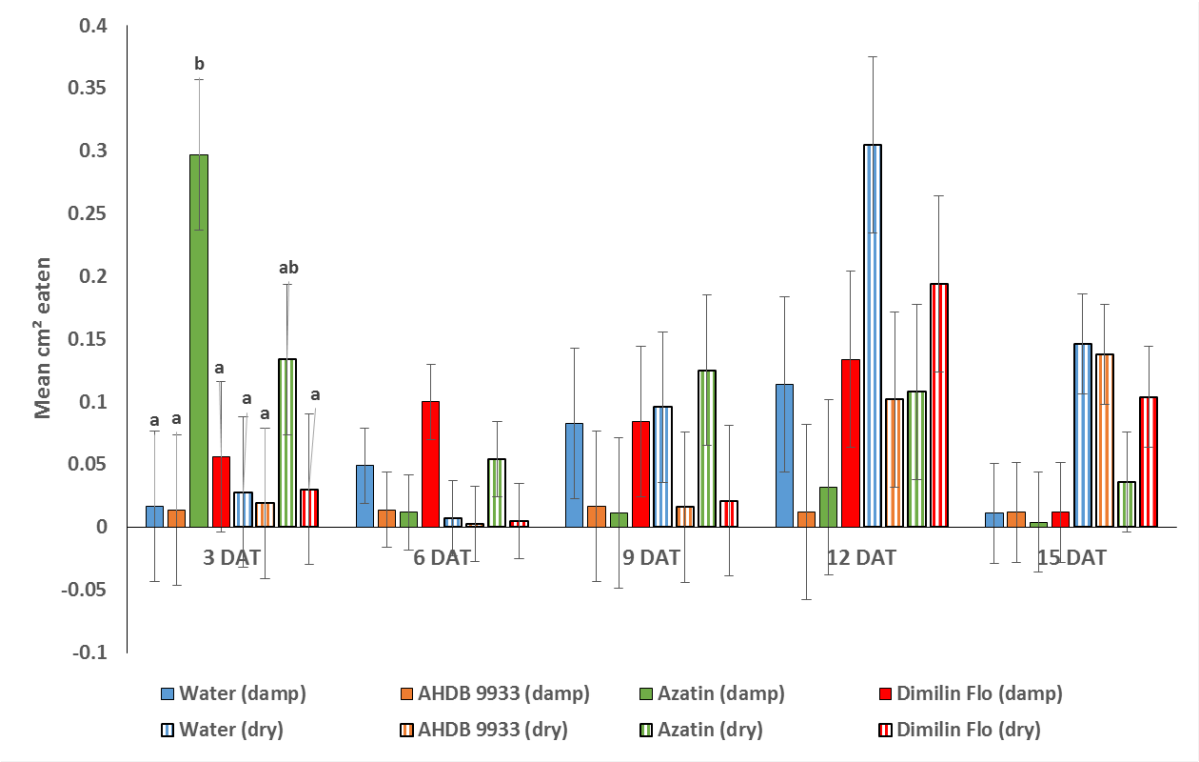


Figure 3.17. Mean cm² eaten for each treatment on each assessment date. Values marked with a letter are significantly different from other values on the same assessment date.

When total leaf area eaten over the 15-day experimental period was compared, there were no significant differences between any of the treatments (Table 3.13 and Figure 3.18).

Table 3.13. Total mean leaf area (cm²) eaten over the 15-day period.

Treatment	Total Mean cm² eaten throughout trial
Water “Damp”	0.274
AHDB 9933 “Damp”	0.064
Azatin “Damp”	0.356
Dimilin Flo “Damp”	0.387
Water “Dry”	0.581
AHDB 9933 “Dry”	0.278
Azatin “Dry”	0.458
Dimilin Flo “Dry”	0.355

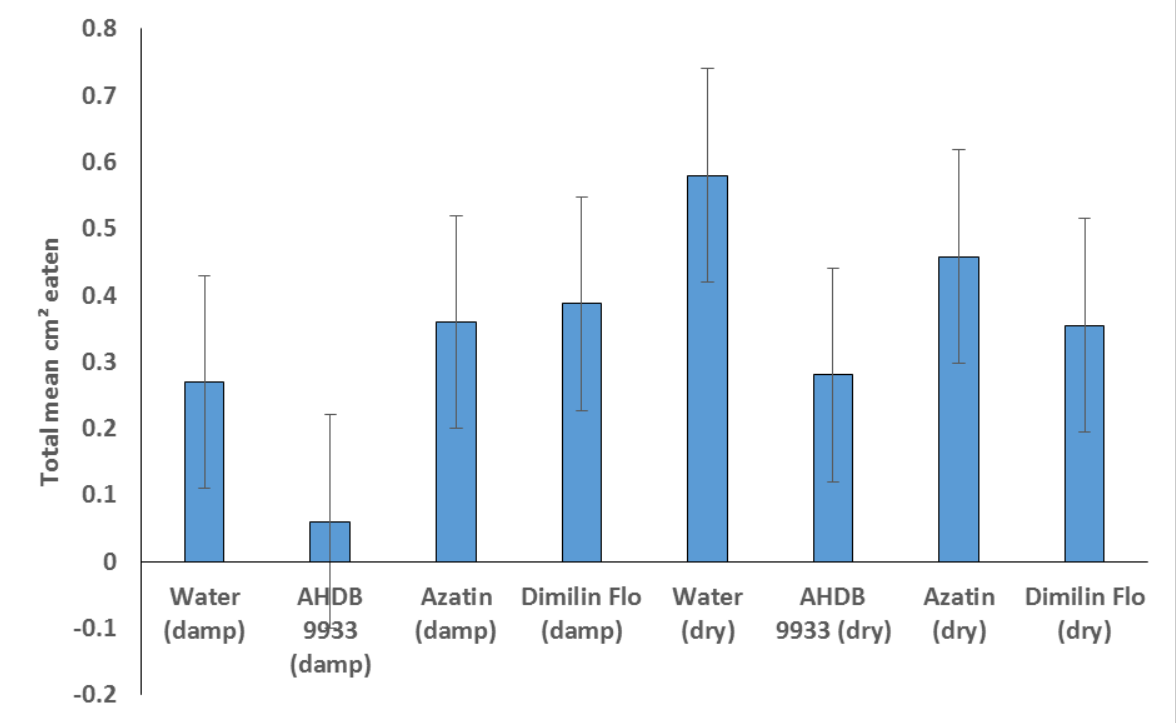


Figure 3.18. Total mean leaf area (cm²) eaten over the 15-day period.

Laboratory temperatures in the three experiments

Mean, maximum and minimum temperatures in the laboratory for the three experiments are shown in Figure 3.19.

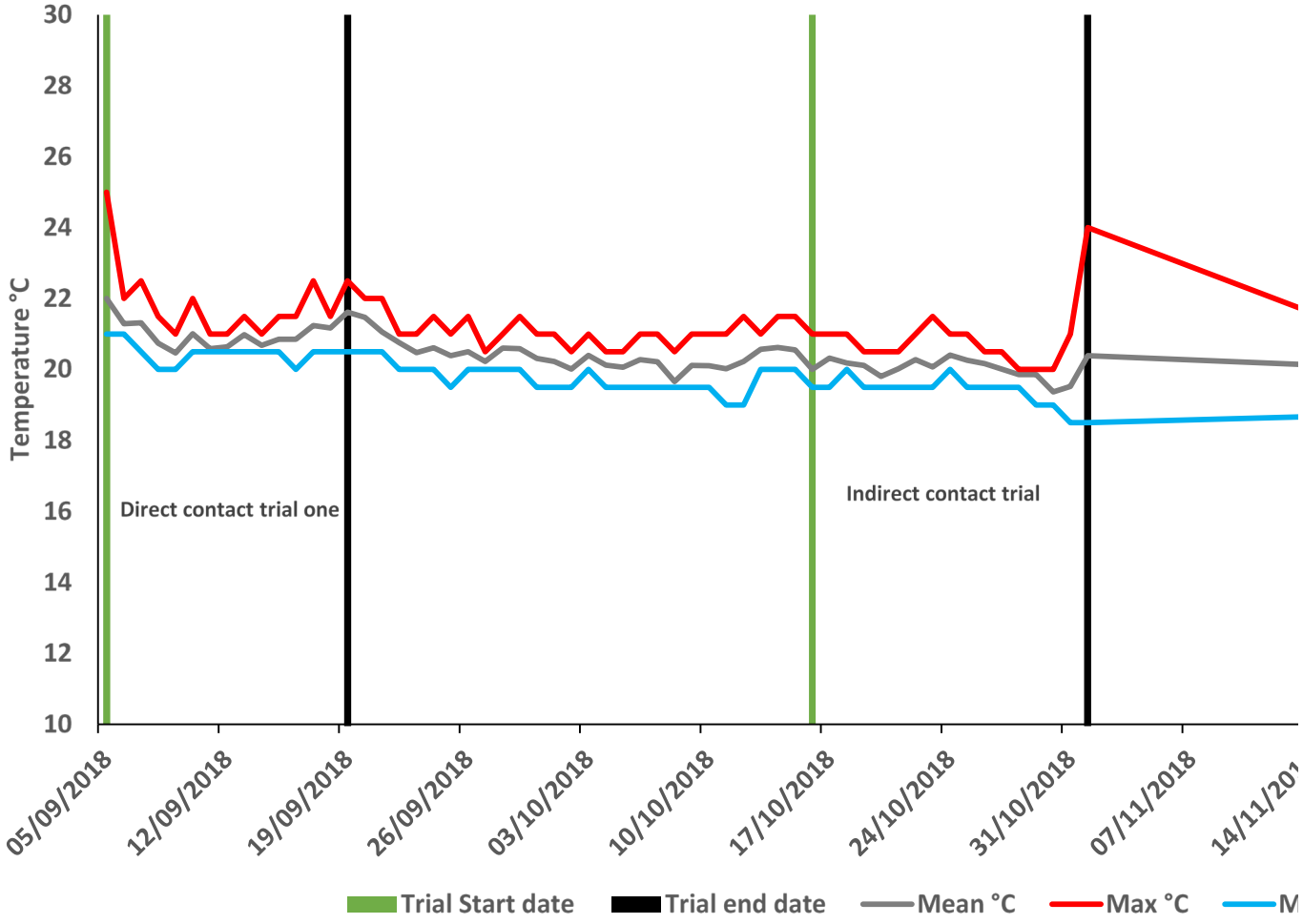


Figure 3.19. Mean, maximum and minimum laboratory temperatures in each of the experiments.

Discussion

Pymetrozine (Tafari) was used as the positive control in the direct contact Experiment 1 as although approval for its use expires on 31 January 2020, it was used in AHDB project SF HNS 112 (as Chess WG at the label rate of 20 g/100 L water) and gave promising control of adult vine weevils. In SF HNS 112, Chess WG was used at the recommended rate of 20 g/100 L and sprayed to both weevils and Euonymus plants in a semi-field experiment when it led to 60% mortality after 15 days (Buxton, 2011) although sub-lethal effects were not assessed. In the current project, Tafari was also used at the recommended maximum concentration of 20 g/100 L but was only applied in Experiment 1 to the weevils themselves before adding them to untreated Euonymus leaves, and this treatment only led to 10% weevil mortality after 15 days. Pymetrozine inhibits aphid feeding but in Experiment 1 Tafari did not reduce leaf area eaten compared with the water control, nor did it significantly reduce the mean number of eggs laid. This result was consistent with research in Germany when vine weevil mortality and egg laying rate were similar to untreated control weevils after being dipped in pymetrozine (Plenum 50 WG), Zieke & Losing, 2015). However, in Experiment 1 reported here, Tafari did significantly reduce the proportion of eggs hatching (65%) compared with the water control (78%).

The entomopathogenic nematode *Steinernema carpocapsae* is known to kill adult vine weevils when used in the e-nema 'weevil stop' traps, available on the home garden market (Bennison & Hough, 2013). In addition, dead adult weevils were found in the growing media of fuschia plants left over from the 'little and often' nematodes experiment in this project in spring 2018. The dead adults were confirmed to contain entomopathogenic nematodes on dissection. These plants had been treated with *S. kraussei* through the overhead irrigation during the previous season, indicating that this species might also kill adult vine weevils developing from any surviving pupae following a little and often programme of application through overhead irrigation. In the direct contact Experiment 1, *S. kraussei*, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* were used at the recommended rate for drenching ornamental plants for control of vine weevil larvae (first two species) or shore fly larvae (*S. carpocapsae*). *Steinernema carpocapsae* was also tested at a higher concentration in 0.1 L/m^2 as recommended for caterpillar control. Low proportions of dead weevils were found in the direct contact Experiment 1 after treatment with *S. kraussei* (20%), *H. bacteriophora* (10%) and *S. carpocapsae* applied in 4 L water/ha (20%) but no nematodes were found inside the cadavers on dissection and one of the weevils treated with *S. carpocapsae* was infected with *Beauveria bassiana* which is likely to have been the cause of death. The other dead weevils may have died of natural causes. Weevils treated with the more concentrated rate of *S. carpocapsae* (applied in 0.1 L water/ha) showed a strong

aversion to the treatment, immediately defaecating and attempting to brush the nematodes off their bodies. However, these responses only occurred on the day of treatment and none of the nematode treatments led to lower leaf area eaten compared with the water control. However, weevils treated with the more concentrated rate of *S. carpocapsae* ate significantly less leaf area compared with those treated with *S. kraussei* or *H. bacteriophora,* which is consistent with e-nema results indicating that a high rate of *S. carpocapsae* is needed (in the weevil-stop traps) to kill adult vine weevils, and that this species is the most effective against adult vine weevil (Arne Peters, personal communication). In direct contact Experiment 1 none of the nematode treatments significantly reduced egg laying compared with the water treated control. However, weevils treated with *S. carpocapsae* at the higher concentration (applied in 0.1 L water/ha) laid significantly fewer eggs (29) over the 15-day period than those treated with *S. kraussei* (63) or *S. carpocapsae* applied in 4 L water/ha (53), indicating that the higher concentration of this species might suppress egg laying, particularly if used at peak egg laying periods. Temperatures in the laboratory during Experiment 1 (maximum 22.5°C, minimum 20°C were within the recommended temperature range of all the nematode species tested (*S. kraussei* (Nemasys L) 5-30°C, *H. bacteriophora* (Nemasys H) 12-30°C, *S. carpocapsae* 14-30°C).

The coded conventional insecticide AHDB 9933 was used as a foliar spray in both the direct contact Experiment 2 and in the residual effects Experiment 3 as it has both contact and systemic activity. Although weevils added to 'damp' treated Euonymus leaves in the residual effects Experiment 3 had an adverse effect on some weevils for up to three days, where they either lay on their backs or hid beneath leaves moving their legs very slowly, the weevils recovered and behaved normally after a 2-3 day period and only one of the weevils (10%) died nine days after foliar treatment. In direct contact Experiment 1 none of the weevils died and abnormal behaviour (vomiting, defaecating and lying on their backs) only occurred when some additional weevils were sprayed directly and added to treated leaves. The period of abnormal behaviour did not lead to reduced leaf area eaten in either experiment. The same insecticide was included in the German research on controlling adult vine weevils where it did not increase mortality or decrease egg laying compared with untreated controls (Zielke & Losing, 2015). In the German research, the only insecticide tested that caused irreversible abnormal behaviour similar to the temporary behaviour shown by weevils treated with the coded insecticide in our research was Steward. This insecticide has already been shown in AHDB project SF HNS 112 to lead to abnormal behaviour and to kill 71% weevils 15 days after treatment (Buxton, 2011). Steward currently only has an EAMU for use on outdoor ornamentals so cannot be used on protected HNS. The same insecticide as AHDB 9933 was also used in research in the US where it was used as a drench on containerised plants of

various species to test its systemic activity, but it did not kill vine weevil adults. However, it suppressed feeding on yew but not on Euonymus, Heuchera, Rhododendron or Sedum (Reding & Ranger, 2011). This work reported variable systemic activity of the insecticides tested among different plant species. Future work could include testing candidate treatments on a range of plant species.

Azadirachtin (as the product NeemAzal) is reported to give a significant reduction in vine weevil egg laying when used as a foliar spray (Reineke & Hauck, 2012). Although NeemAzal is not approved in the UK, Azatin is now approved for use as a foliar spray on protected ornamentals for thrips control. Azatin was used only in the residual activity Experiment 3 as it acts on ingestion rather than on direct contact. No eggs were laid by weevils in Experiment 3, probably because this experiment started later (16 October) than direct Experiment 1 (4 September) in which eggs were laid and the weevils had stopped laying eggs by mid-October. Therefore the effect of Azatin on weevil egg laying and egg hatch could not be tested. Azadirachtin is also reported to have antifeedant activity, particularly on Lepidoptera and acts as an insect growth regulator (e.g. Mordue & Blackwell, 1993). In Experiment 3, weevils fed on 'damp' Azatin-treated leaves ate significantly more leaf area than those in the water controls but this only occurred on one assessment date, three days after treatment. These results indicated that Azatin did not have an antifeedant effect on vine weevil adults nor did it kill any weevils.

The insect growth regulator diflubenzuron (Dimilin Flo) is reported to slow vine weevil egg hatching rate when sprayed onto yew leaves and fed to adult weevils (Sol, 1985). Dimilin Flo was used only in the residual activity Experiment 3 as it acts on ingestion rather than on direct contact. No eggs were laid in this experiment therefore the effect on egg hatching rate could not be tested. Dimilin Flo did not kill any weevils in this experiment, nor did it cause any abnormal behaviour or reduce leaf area eaten.

Conclusions

- None of the treatments tested in the three experiments led to effective kill of vine weevil adults. One weevil died due to natural infection with the entomopathogenic fungus *Beauveria bassiana* and the other few deaths are likely to have been due to natural causes.
- A direct contact spray of Tafari significantly reduced egg hatch but only to 65% compared with 78% in the water control.
- A direct contact spray of *S. carpocapsae* (Nemasys C) applied in 0.1 L water/ha as recommended for caterpillar control led to strong aversion behaviour by adult vine

weevils but only on the day of application. Treated weevils also laid significantly fewer eggs than those treated with *S. kraussei* (Nemasys L) or *S. carpocapsae* applied in 4 L water/ha as recommended as a drench for control of vine weevil larvae, but not fewer than in the water controls.

- Spraying leaves, or weevils and leaves with AHDB 9933 led to abnormal behaviour of vine weevil adults for 2-3 days, after which the weevils recovered and behaved normally.
- The effect of Azatin on egg laying could not be tested as no eggs were laid in the residual effects Experiment 3 which started on 16 October after egg laying had stopped. Azatin did not act as a weevil antifeedant.
- The effect of Dimilin Flo on egg laying or egg hatch could not be tested as no eggs were laid in Experiment 3.

Objective 3. Improve best-practice IPM approaches including the use of entomopathogenic nematodes, fungi and IPM-compatible insecticides: Revision Task 3.3.2a: cold active entomopathogenic fungi

Revision: task 3.3.2a: cold active entomopathogenic fungi

Laboratory experiments to quantify the effect of temperature on the virulence of Met52 to vine weevil larvae indicated strongly that this fungus has an optimum of about 27°C and has low infectivity at temperatures below 15°C. This is likely to be a problem when using the product on outdoor plants, as the soil temperatures in the autumn and spring when larvae are active and damaging plants are likely to be below 15°C. Fungal biopesticides can work very well against vine weevil larvae when the temperature is favourable, and they fit in well with IPM programmes. Hence there would be potential for a fungal strain that works well at the lower temperatures that typically occur in soil in the autumn and spring in the UK and other northern temperate countries where vine weevil is a problem.

Objective

The aim of this new piece of work was to investigate the potential of ‘cold active’ EPF strains against vine weevil larvae, with the target temperatures for fungal activity being between 5 - 15°C. This was done by reviewing the scientific literature to identify and obtain EPF strains which are able to germinate and grow adequately at between 5 - 15°C and hence could have potential against vine weevil at these temperatures. The effect of temperature on the radial

extension and germination of the strains and the infectivity of the fungus was measured in a laboratory bioassay.

Materials & methods

Fungal culture

Stock cultures of the isolates were stored in liquid nitrogen (Chandler, 1994). Subcultures for laboratory experiments were grown on SDA from slant cultures and incubated in darkness at 23°C for 14 days prior to assay. Conidia were harvested in sterile 0.01% Triton X-100 and suspensions were filtered through milk filters (Lantor (UK), Bolton, UK) to remove hyphal fragments. Conidia were counted in an improved Neubauer hemacytometer and aliquots were prepared at concentrations of 10^7 in sterile 0.01% Triton X-100.

Fungal radial extension

A conidial suspension was prepared (10^7 spores per ml) and 100 µl was spread evenly over SDA in Petri dishes and incubated in the dark at 23°C for 48 h. Plugs (6 mm) cut from these plates with a flame-sterilised cork borer were then placed upside down in the centre of fresh SDA in Petri dishes, one plug per plate. The plates were incubated for 28 days in darkness at 4°C, 10°C, 15°C, 20°C, 25°C, 27.5°C, 30°C and 35°C with two plates for each isolate / temperature combination. Colony diameters were measured with a ruler using two cardinal diameters every 7 days for the duration of the experiment.

Fungal germination

A conidial suspension was prepared (10^7 conidia per ml) and 20 µl was pipetted onto three previously-marked circles (approx. 2 cm diameter) on plates of SDA. The plates were incubated at either 4°C, 10°C, 15°C, 20°C, 25°C, 27.5, 30°C and 35°C in darkness for 24 h. The germination assessment was carried out destructively by pipetting a drop of lactophenol methylene blue inside each circle. Treated plates were stored at 4°C before examination under a compound light microscope (Olympus BH-2, Tokyo, Japan) magnification x200. The numbers of germinated and ungerminated spores (conidia) were counted from a total sample of approximately 100 conidia per circle. Germination was defined as the point when an emerging germ tube was equal to, or longer than the width of the conidium.

Adult vine weevil culture

Field-collected adult vine weevils were collected from ornamental crops. Adult vine weevils were kept in 1.5 l plastic pots. The lids of these pots were perforated in order to provide ventilation. The base of each pot was lined with tissue paper, an additional ball of damp tissue

paper provided a source of moisture, a piece of corrugated cardboard provided a refuge and fresh yew leaves (*Taxus baccata*) provided a food source. Twenty-five to 30 weevils were placed into each pot, and maintained within a controlled environment room at 20°C; 16:8 L: D. Pots were cleaned once a week taking care to remove any dead or dying weevils.

Laboratory bioassay

Eggs were collected from the adult culture and larvae reared up to second / third instar (larval stage) on strawberry plants within a controlled environment room at 20°C; 16:8 L:D. Groups of ten weevil larvae were immersed for 10 seconds in 5 ml conidial suspension (UP1, UP4 02 275.82) or 0.01% Triton X-100 as a control. The larvae were added to plastic food boxes (11 x 8 x 7cm) containing 150 g of Levington M2 compost, along with carrot slices (Figure 1). The boxes were maintained at a range of constant temperatures, from 12.5°C to 25°C. The survival of the larvae was evaluated over time and the numbers of living and dead weevils counted. Dead weevils were removed and placed on damp filter paper in Petri dishes and incubated at 20°C and observed for the appearance of sporulating mycelium. All temperatures were assessed simultaneously on each occasion and the experiment was repeated on three occasions

Figure 1: Laboratory bioassay set up

Results

Fungal isolates

The review of the literature identified a number of EPF strains which were able to germinate and grow adequately between 5 - 15°C and hence could have potential against vine weevil at these temperatures. Seventeen candidate isolates of fungi, from three genera (*Beauveria*, *Isaria* and *Metarhizium*) have been acquired for experimentation and were catalogued and

cryopreserved in the Warwick Crop Centre collection of entomopathogenic fungal cultures (Table 3.1).

Table 3.1: Fungal isolates in this study

Species	Isolate	Host	Geographic origin
Beauveria bassiana	1789.17 (ARSEF 252)	*Hypothenemus hampei* (Coleoptera : Hyponomeutidae)	Brazil
	1790.17 (ARSEF 7552)	*Galleria mellonella* (Lepidoptera: Pyralidae)	Norway
	1791.17 (ARSEF 7554)	*Galleria mellonella (* Lepidoptera: Pyralidae)	Norway
	UP1		Poland
	UP2		Poland
Isaria farinosa	UP3		Poland
	UP4		Poland
Isaria tenuipes	UP6		Poland
Metarhizium anisopliae	108.82	*Wisena* sp. (Lepidoptera: Hepialidae)	New Zealand
	135.82	Oryctes rhinoceros (Coleoptera: Scarabaeidae)	France
	159.83	(Coleoptera: Scarabaeidae)	New Zealand
	1791.17 (ARSEF 4343)	-	Australia
	1792.17 (ARSEF 11661	*Galleria mellonella* (Lepidoptera: Pyralidae)	Norway
Metarhizium brunneum	1793.17 (ARSEF 6477)	*Phyllopertha horticola* (Coleoptera: Scarabaeidae)	Norway
	1794.17 (ARSEF 5626)	*Tenebrio molitor* (Coleoptera: Tenebrionidae)	Finland
Metarhizium flavoride	UP5		Poland
Metarhizium frigidum	1795.17 (ARSEF 4561)	-	Australia

Fungal growth

The temperature response of the isolates were characteristically asymmetric, with rapid inactivation at temperatures above the optimum (Figure 3.1). The isolates all grew slowly at 4°C and 10°C, one isolate (UP6) did not grow at 27.5°C and three isolates (UP4, UP6 and 108.82) did not grow at 30°C and 35°C. The data was fitted to a non-linear model (Briere et al (1999) in RStudio (version 0.99.903 – © 2009-2016 RStudio, Inc) using the package Minpack.lm (version 1.2-0) and the optimum temperature for fungal growth estimated. The model fitted the data well, with r^2 values ranging from 0.807 to 0.945. Optimum temperatures for growth ranged from 17.5°C (UP1) to 24.8°C (135.82) (Table 2).

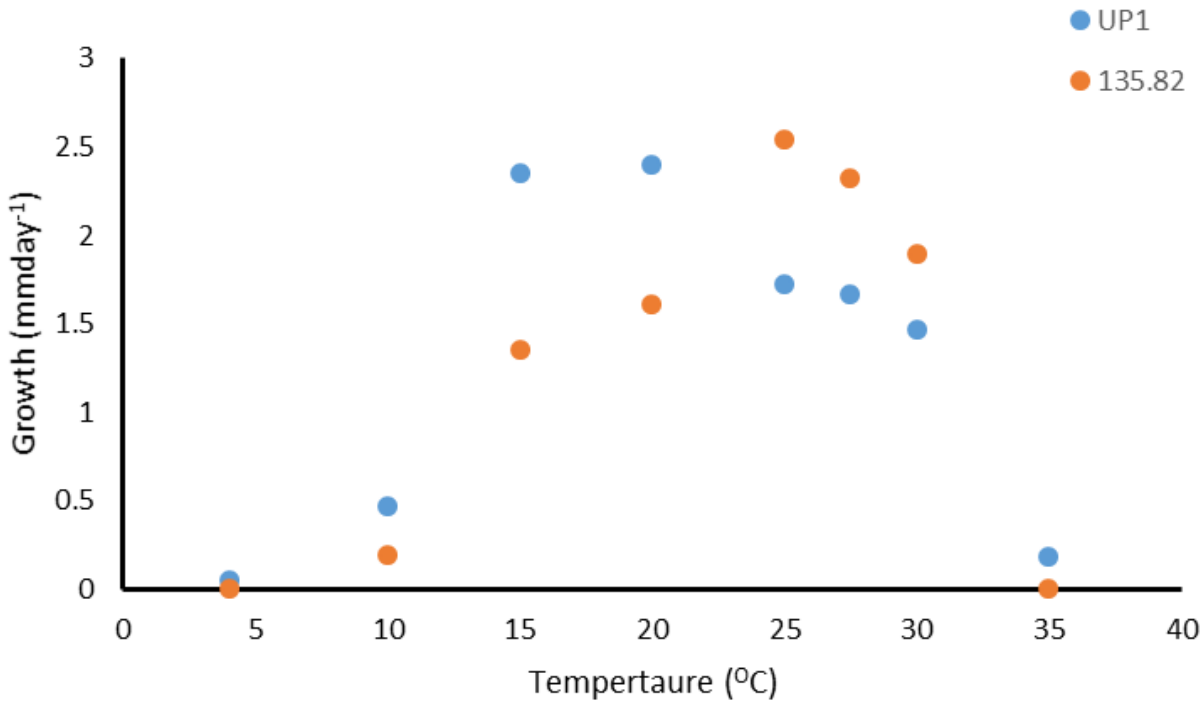


Figure 3.1: Growth response of *Beauveria bassiana* (UP1) and *Metarhizium anisopliae* (135.82) at eight temperatures ranging from 4-30°C.

Table 3.2: Predicted optimum temperatures (°C) for fungal growth.

Species	Isolate	Optimum Temperature (°C)
Beauveria bassiana	1789.17	21.9
	1790.17	23.2
	1791.17	23.6
	UP1	17.5
	UP2	21.7
Isaria farinosa	UP3	19.7
	UP4	18.3
Isaria tenuipes	UP6	18.2
Metarhizium anisopliae	108.82	19
	135.82	24.8
	159.83	22.4
	1796.17	23.5
	1792.17	22.1
Metarhizium brunneum	1793.17	23.3
	1794.17	23.7
	275.83	24.6

Metarhizium flavoride	UP5	21
Metarhizium frigidum	1795.17	20.7

Fungal germination

The temperature response of the isolates was characteristically asymmetric, with rapid inactivation at temperatures above the optimum (Figure 3.2). Only three isolates (UP1, UP2 and UP5) exhibited any germination at temperatures of 10°C or below. Three isolates (UP2, UP5 and 1795.17) exhibited more than 80% germination at 15°C. Two isolates (UP3 and UP6) and six isolates (UP3, UP4, UP6, 108.82, 159.83, and 1790.17) did not germinate at 30°C and 35°C respectively. Maximum temperatures at which germination still occurred were more than the maximum temperatures at which mycelial extension still occurred. The data was fitted to a non-linear model (Briere et al (1999) in RStudio (version 0.99.903 – © 2009-2016 RStudio, Inc) using the package Minpack.lm (version 1.2-0) and the optimum temperature for fungal germination estimated. The model fitted the data well, with r^2 values ranging from 0.645 to 0.846. Optimum temperatures for germination ranged from 20.2°C (UP3) to 28.1°C (1793.17) (Table 3).

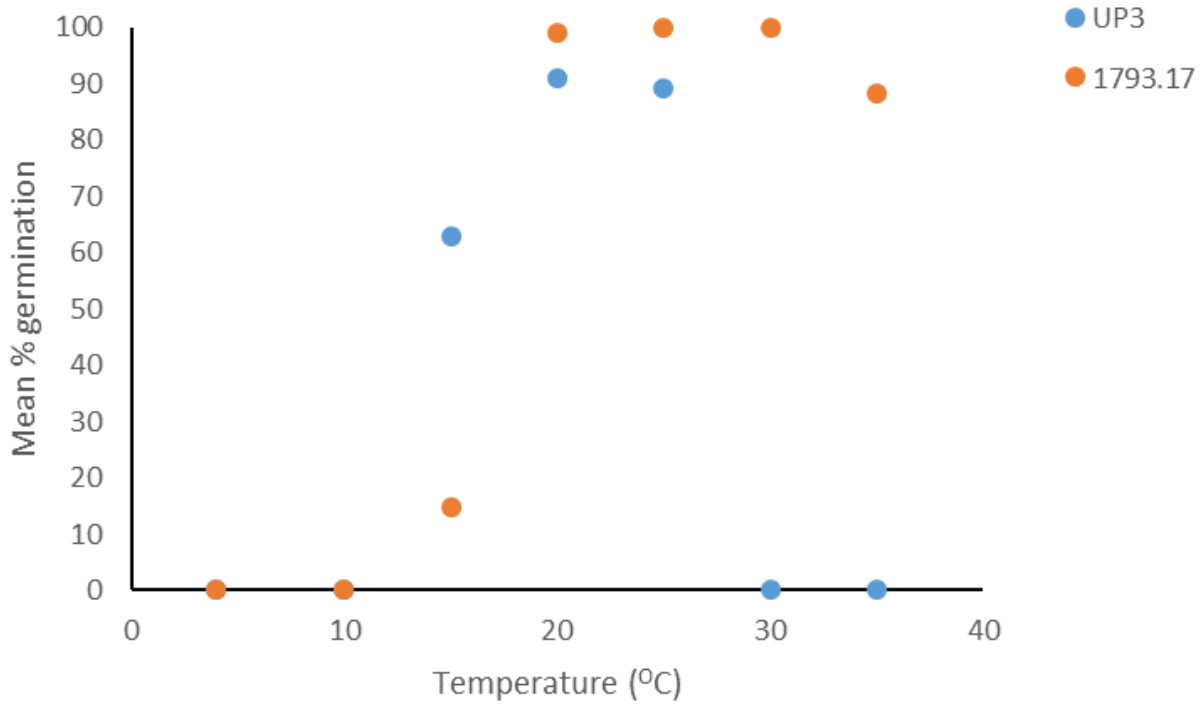


Figure 3.2: Germination response of *Isaria farinosa* (UP3) and *Metarhizium brunneum* (1793.17) at eight temperatures ranging from 4-35°C.

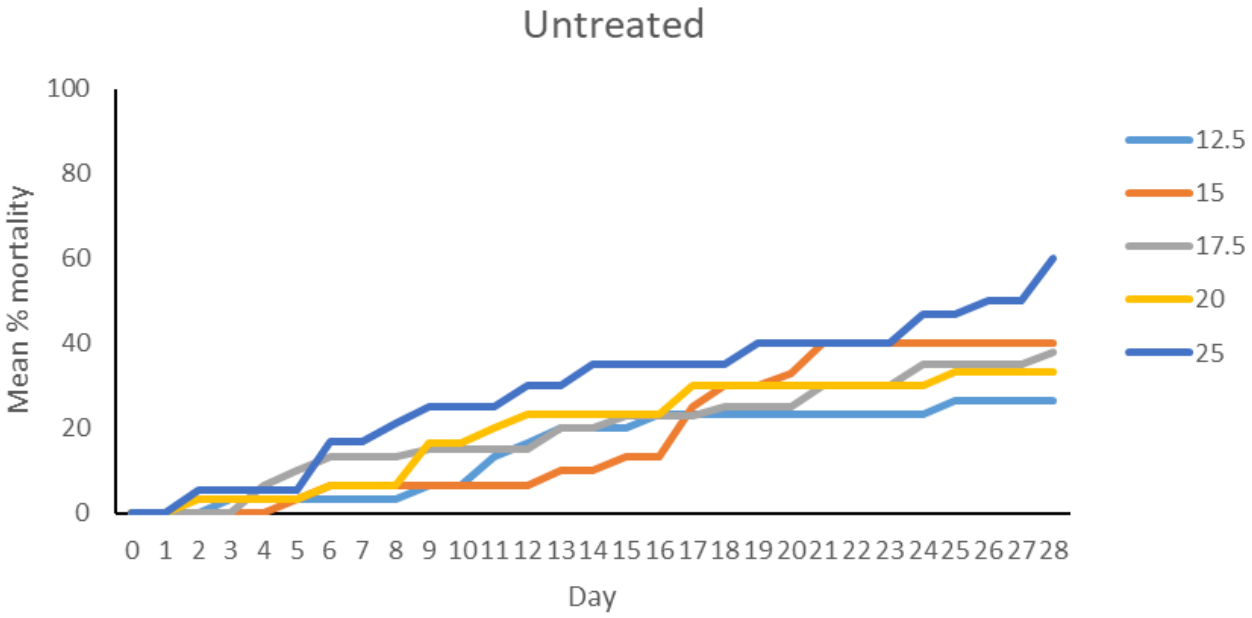
Table3. 3: Predicted optimum temperatures (^{O}C) for fungal germination.

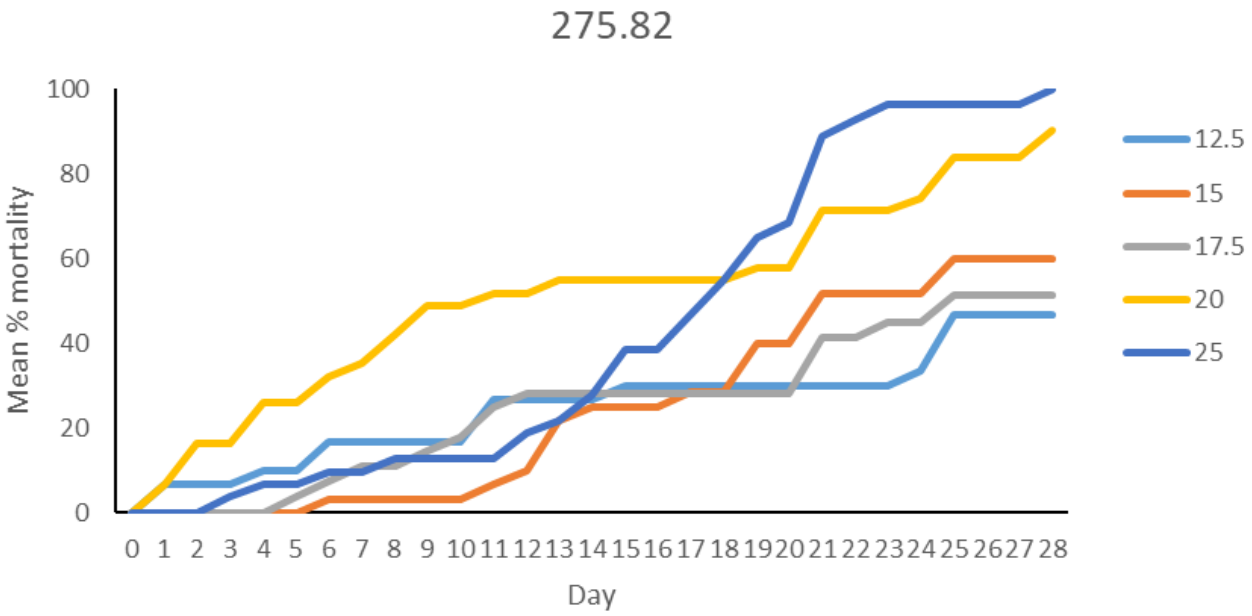
Species	Isolate	Optimum Temperature (^{O}C)
Beauveria bassiana	1789.17	25.5
	1790.17	24.7
	1791.17	24.9
	UP1	25.2
	UP2	24.5
Isaria farinosa	UP3	20.2
	UP4	20.5
Isaria tenuipes	UP6	20.7
Metarhizium anisopliae	108.82	24.5
	135.82	26.5
	159.83	23.3
	1796.17	27.0
	1792.17	25.8
Metarhizium brunneum	1793.17	28.1
	1794.17	23.6
	275.82	31.1
Metarhizium flavoride	UP5	23.0
Metarhizium frigidum	1795.17	26.7

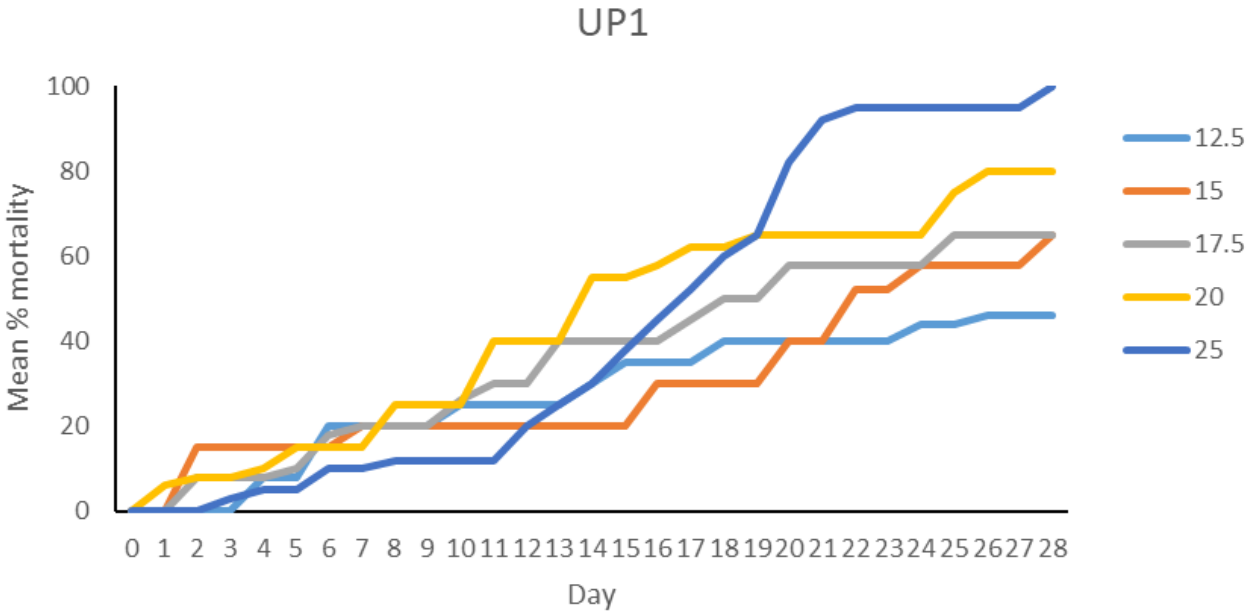
Laboratory bioassay

Two strains (UP1 and UP4) were selected for their lower optimum temperatures for germination and growth to be screened for virulence against second / third instar weevil larvae and compared with 275.82 (isolate used in the product Met52). Control (i.e. untreated) mortality varied with temperature. At day 28 the mortality in controls ranged from 27% (at 12.5°C) to 60% (at 25°C) (Figure 3.3a). The mean control mortality was 40%. The mortality of 275.83 treated vine weevils also varied with temperature, showing an overall trend for increased mortality with increasing temperature. At day 14, mean mortality in the 275.82 treated pots was 28% at 12.5°C, 26% at 15°C, 29% at 17.5°C, 55% at 20°C and 29% at 25°C (Figure 3.3b). By day 21, mean mortality in the 275.82 treated pots was 32% at 12.5°C, 54% at 15°C, 44% at 17.5, 71% at 20°C and 90% at 25°C. By day 28, 100% mean mortality was observed in the 275.82 treated pots held at 25°C. Similarly, the mortality of UP1 treated vine weevils also varied with temperature, showing an overall trend for increased mortality with increasing temperature. At day 14, mean mortality in the UP1 treated pots was 30% at 12.5°C, 20% at 15°C, 40% at 17.5°C, 55% at 20°C and 30% at 25°C (Figure 3.3c). By day 21, mean

mortality in the UP1 treated pots was 40% at 12.5°C, 40% at 15°C, 58% at 17.5, 65% at 20°C and 92% at 25°C. By day 28, 100% mean mortality was only observed in the UP1 treated pots held at 25°C. The mortality of UP4 treated vine weevils also varied with temperature, showing an overall trend for increased mortality with increasing temperature. At day 14, mean mortality in the UP4 treated pots was 30% at 12.5°C, 28% at 15°C, 36% at 17.5°C, 55% at 20°C and 25% at 25°C (Figure 3.3d). By day 21, mean mortality in the UP4 treated pots was 44% at 12.5°C, 50% at 15°C, 40% at 17.5, 70% at 20°C and 84% at 25°C. By day 28, 100% mean mortality was only observed in the UP4 treated pots held at 25°C.







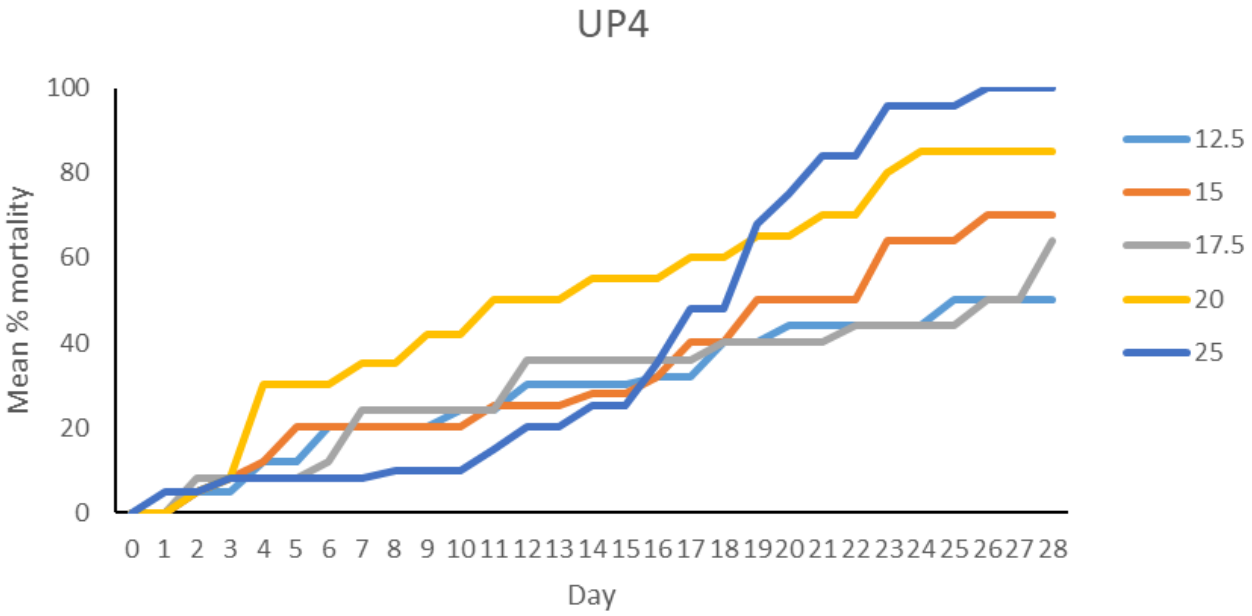


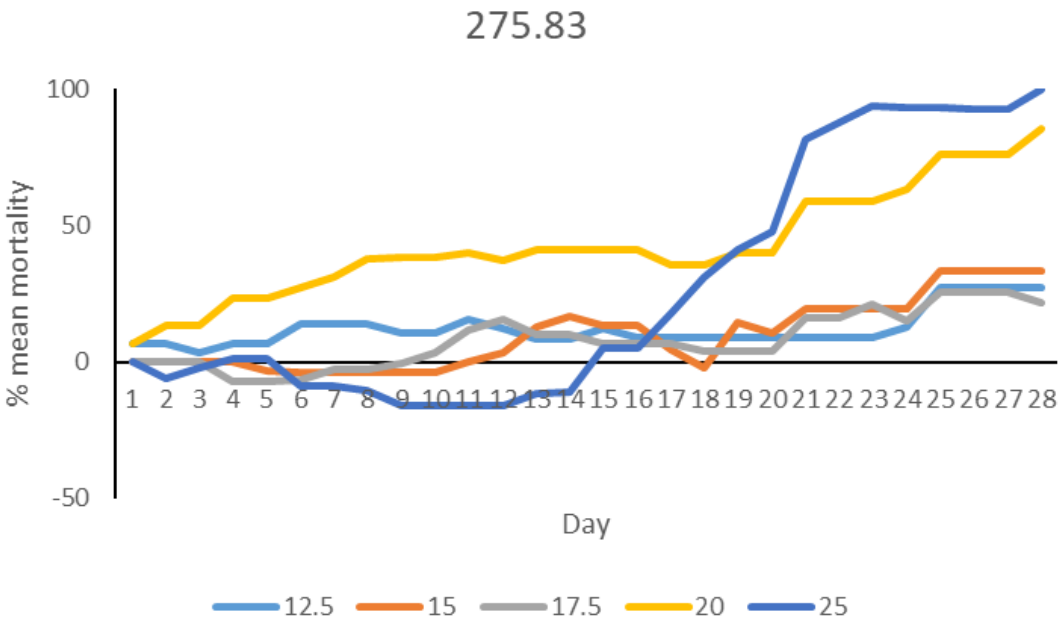
Figure 3: Mean % mortality of untreated weevils (a) and treated with (b) 275.82, (c) UP1 and (d) UP4 over 28 days at five temperatures.

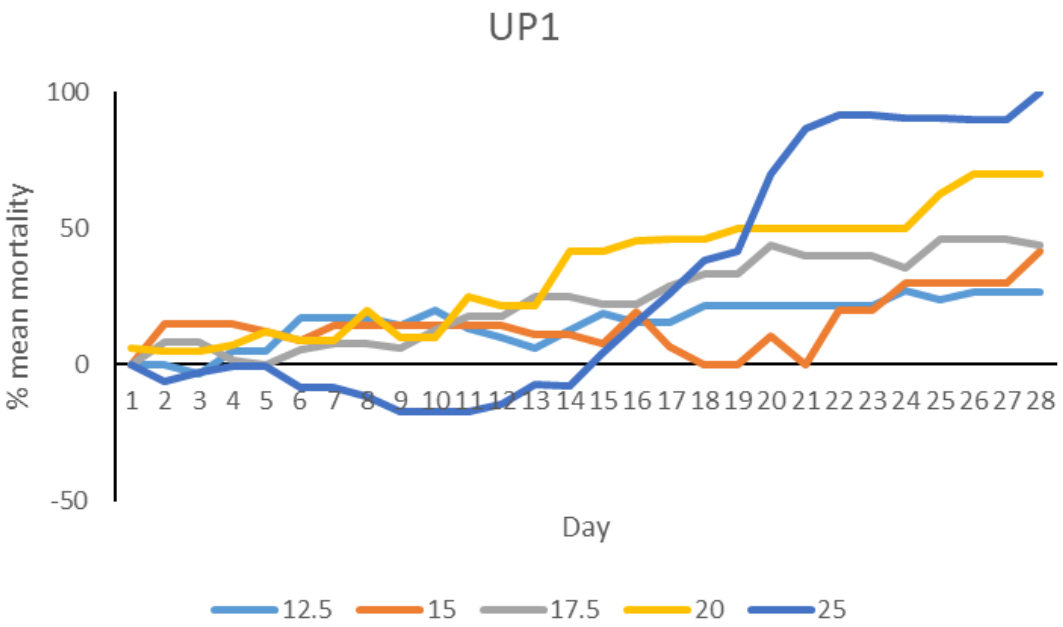
The data was adjusted to correct for the control mortality using Schneider-Orelli's formulae, as follows:

Corrected mortality (%) = $((a - b)/(100 - b)) * 100$

Where a is the percentage mortality data from the treated group and b is the percentage mortality from control group.

Corrected mortality increased with temperature (Figure 3.4). At temperatures below 15°C mortality remained low, reaching 27%, 26% and 32% at 12.5°C and 33%, 42% and 50% at 15°C at day 28 for 275.82, UP1 and UP4 treated weevils respectively. At day 21, mortality was 50% or greater for all treatments at 20°C and 25°C. At day 26, 100% mean mortality was observed at 25°C in weevils treated with UP4 and was observed in all treatments by day 28.





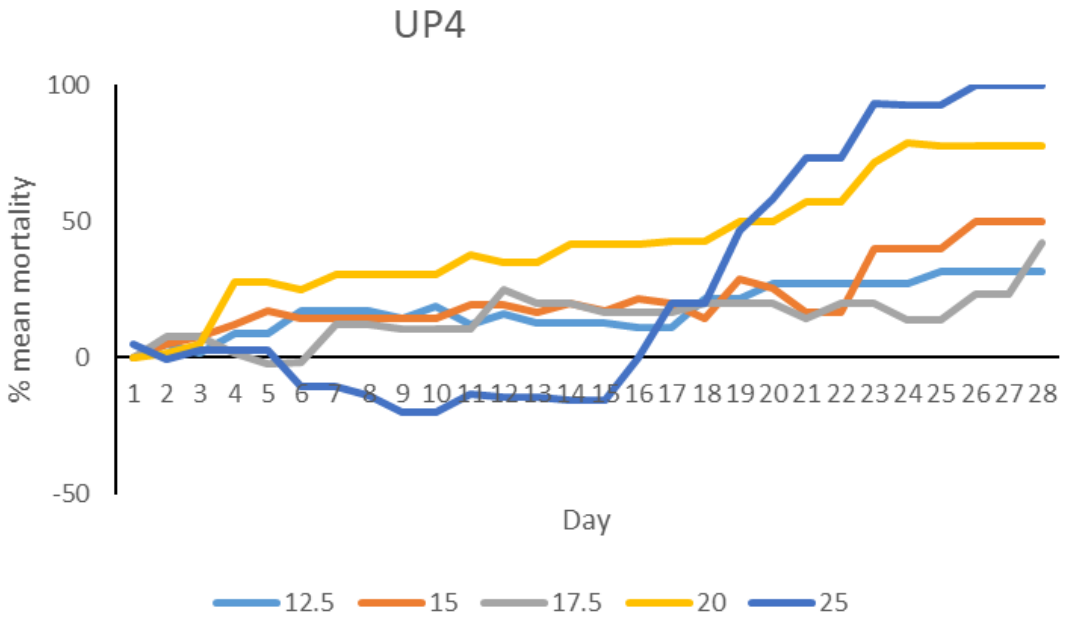
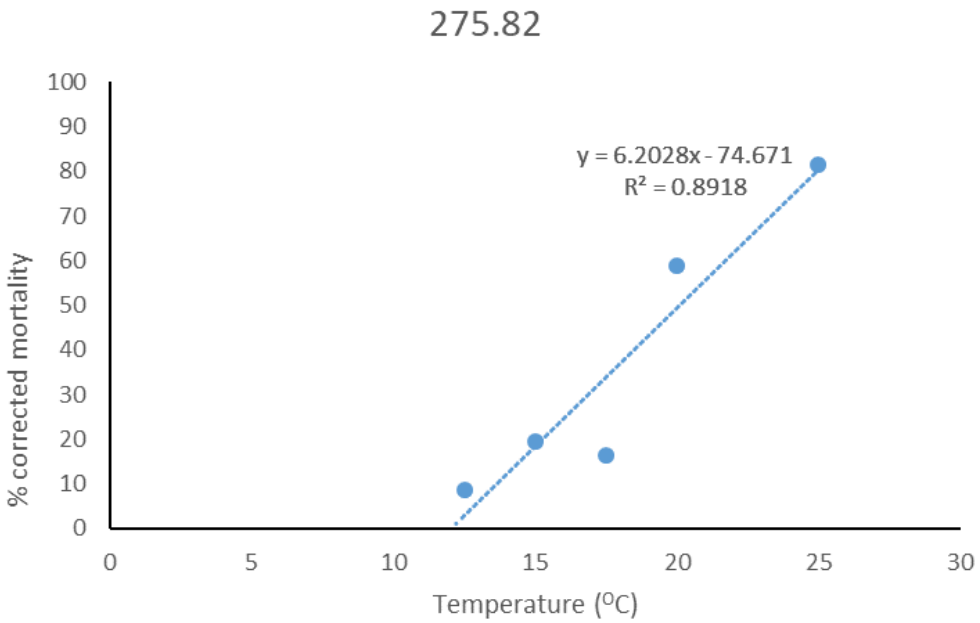
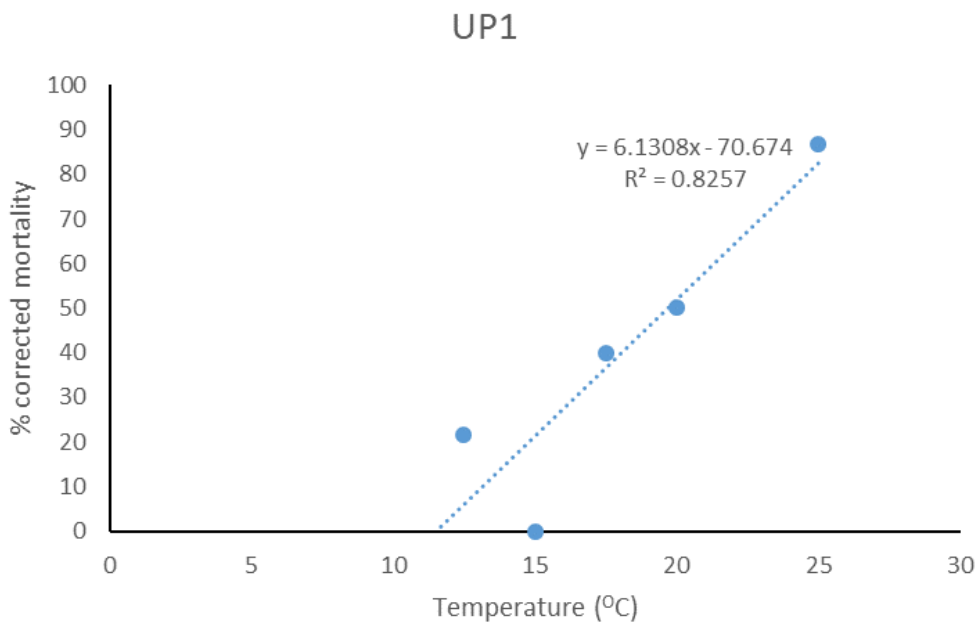


Figure 3.4: Mean % corrected mortality of weevils treated with 275.82, UP1 and UP4 over 28 days at five temperatures.

A day degree style of approach was used to develop a predictive model of the virulence of the treatments against third instar weevil larvae. A polynomial model was fitted to the corrected % mortality at 21 days and used to obtain the cardinal temperatures (*T0* and *Topt*) for use in the day degree model (Figure 5). The thermal minima (*T0*) was estimated at 12°C, 11.5°C and 9.75°C for 275.82, UP1 and UP4 respectively however the thermal optima could not be estimated, however it is unlikely that compost temperature would ever exceed the upper threshold. This value was used to calculate the thresholds for calculation of day degree. Day degrees would only be accumulated if temperatures were above the lower threshold. For each temperature assay, the number of day degrees accumulated, from days one to day 21 after treatment, was calculated using the Met. Office Formulae).





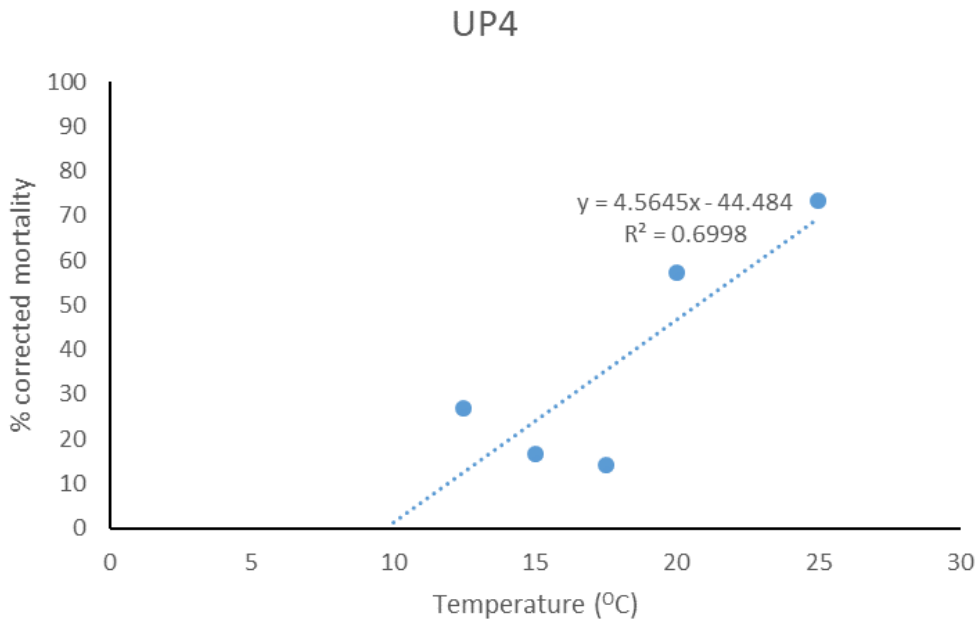
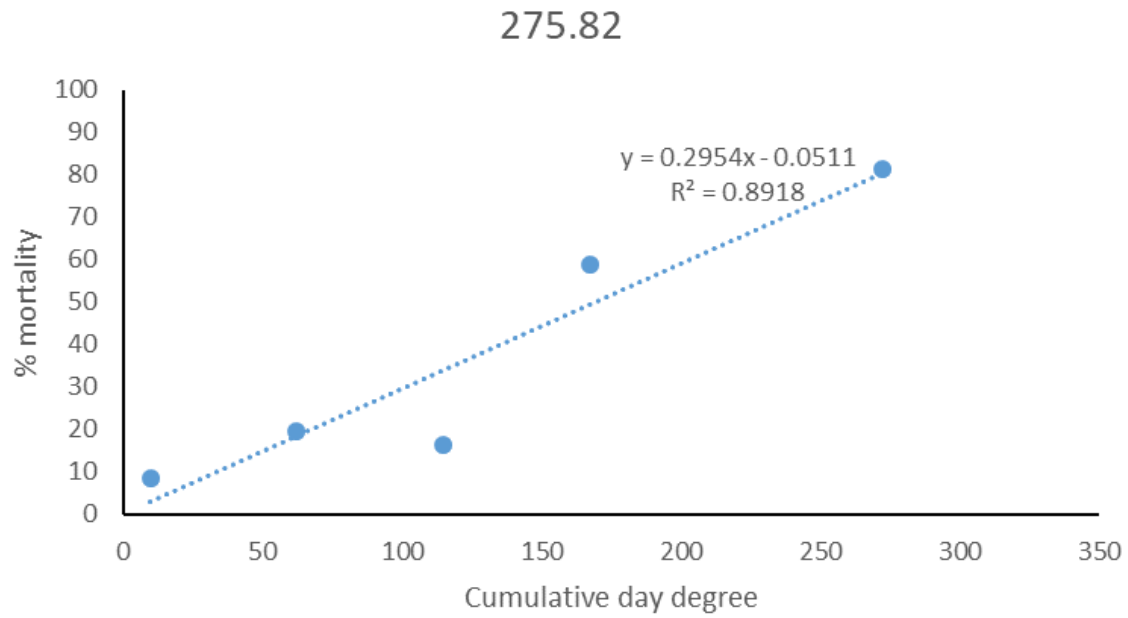
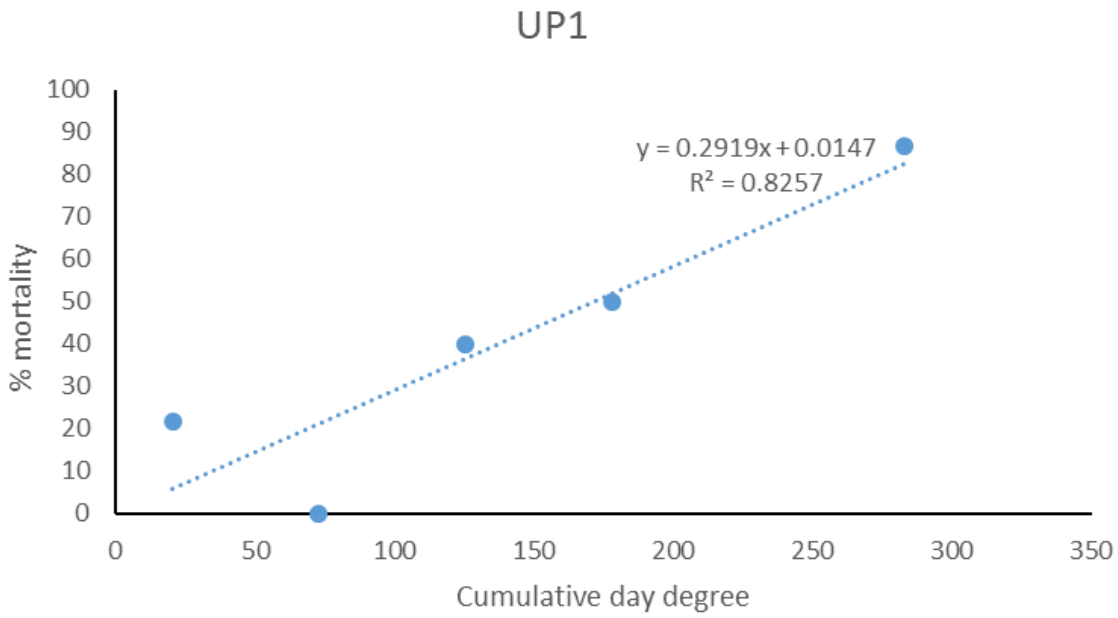


Figure 3.5: Regression model fitted to mean % corrected mortality of weevils treated with 275.82, UP1 and UP4 at 21 days at five temperatures.

Percentage mortality was then plotted against accumulated day degrees for each temperature and a regression model fitted to the relationship so that percentage mortality could be estimated on any given day (Figure 3.6; Table 3.4).





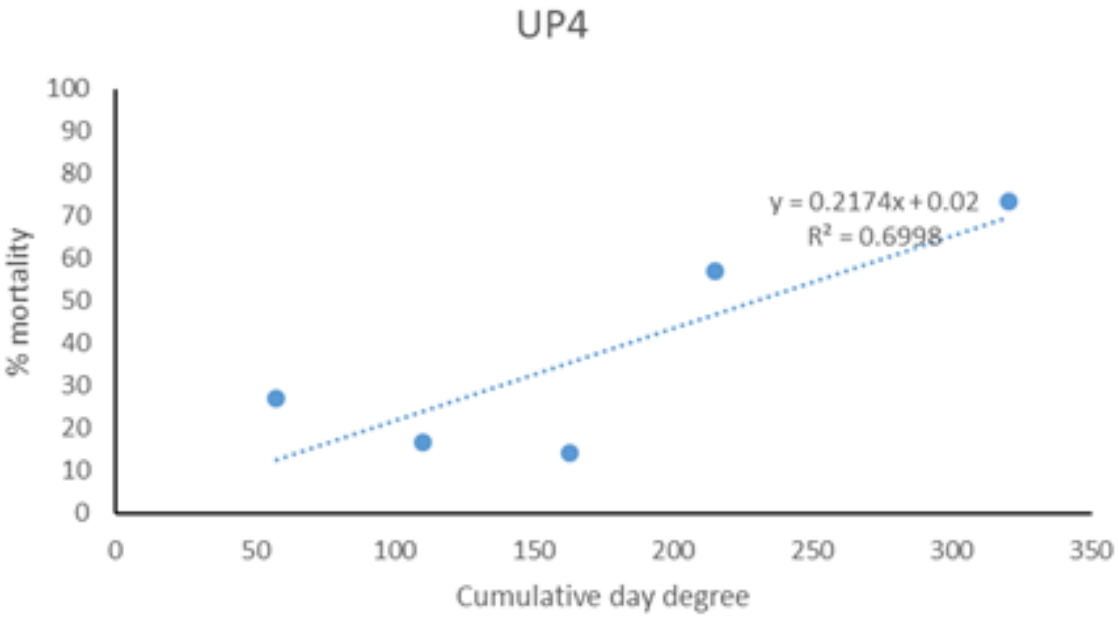


Figure 3.6: Polynomial model fitted to mean mean % corrected mortality of weevils treated with 275.82, UP1 and UP4 against cumulative day degrees.

Table 3.4: Predicted weevil mortality based on cumulative day degrees

	Estimated CDD		
% mortality	**275.82**	**UP1**	**UP4**
25	84.8	85.6	114.9
50	169.4	171.2	229.9

75	254.1	256.9	344.9
90	304.8	308.3	413.9
100	338.7	342.5	459.9

Discussion

Isolates of EPF have been identified which have a lower thermal profile than Met52 and can grow and germinate at 10°C. Analysis of the bioassay data suggests that no control will occur at temperatures below 10°C with any of the isolates tested in this study. However, the spores will remain viable until temperatures are conducive for infection to occur. Development of a day degree model estimates that for 75% control 255 CDD, 257 CDD and 344 CDD has to be achieved with 275.82, UP1 and UP4 respectively. Analysis of historical temperature data suggests that this could be reached in the months of June, July and August in some years and locations.

Conclusions

- The germination and mycelial (= colony) extension rates for 17 EPF isolates were quantified at a range of temperatures. This was done to provide an indication of the likely performance of the isolates under field conditions, and in particular to identify isolates able to develop well at 10 - 20°C, and which might therefore be suitable for use as a biopesticide against vine weevil under typical UK temperature conditions.

- Most of the 17 EPF isolates showed similar responses in terms of the effect of temperature on the germination of fungal conidia. Maximum germination occurred between 20 to 30°C. Most isolates showed only low levels of germination at 10°C. However, a number of fungal isolates were identified that had optimal temperatures that were significantly lower than that required for the growth and germination of Met52, which is an important proof of concept in terms of identifying low temperature EPF.

- An important caveat is that we measured spore germination at 24 h and used it as an indicator of spore viability. This is a standard system used by us and many other labs. However, it could be that the speed of germination is reduced at higher temperatures rather than an absolute reduction in viability. Thus, spores that have not germinated after 24 h at a particular temperature may still be viable and germinate after 48 h or longer. The downside for this is that a reduction in the time to germinate would be expected to reduce the virulence of the isolate to the target pest. However, this needs to be tested.

- Most isolates behaved similarly with respect to the effect of temperature on the rate of mycelial extension. Most EPF isolates showed fastest mycelial extension rates from 20 – 27.5°C. All isolates grew at 4°C and 10°C while one isolate (UP6) did not grow at 27.5°C and three isolates (UP4, UP6 and 108.82) did not grow at 30°C and 35°C.

- EPF isolates *B. bassiana* UP1 and *I. farinosa* UP4 showed 'good' patterns of growth and germination in the range 10 - 20°C. From this we would conclude that these EPF isolates would be able to operate under field temperature conditions when plants are most vulnerable to vine weevil.

- The effect of temperature on fungal virulence (= amount of weevil mortality) was measured for two selected EPF isolates (*B. bassiana* UP1 and *I. farinosa* UP4) compared to Met52. These EPF isolates exhibited a lower minimum temperature for weevil infection than Met52 but the CDD needed for 50% mortality was similar or higher. The data suggests that both UP1 and UP4 had a lower inherent virulence to vine weevil than Met52 which offset the benefits obtained from these fungi being able to develop at lower temperatures than Met52. We do not know what the natural insect host is for these two EPF; they were obtained from a source in Poland and it is possible that they were isolated either from soil or from a non-coleopteran host.

Conclusions from all the work in year 3

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

- Numbers of vine weevil adults caught in Chemtica traps can be increased by the simple addition of either yew (*Taxus baccata*) and *Euonymus fortunei* foliage placed in the traps.
- Responses of vine weevil adults to Chemtica traps containing yew or *Euonymus* foliage is influenced by previous feeding experience.
- No consistent EAG responses to entrainments from starved or unstarved vine weevil adults were recorded.
- Good electrophysiological responses were recorded to sixteen chemicals present in the essential oil of ylang ylang.
- Receptors on the vine weevil antenna responded to at least 20 compounds present in volatiles collected from *Euonymous fortunei*. Most of these were "green leaf" volatiles

common to many green plants, but a strong EAG response was also observed to small amounts of *cis*-jasmone present.

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

Lethal and sub-lethal effects on adult weevils in response to direct application and to residues of IPM compatible insecticides, entomopathogenic nematodes and a botanical pesticide

- None of the treatments tested in the three experiments led to effective kill of vine weevil adults. One weevil died due to natural infection with the entomopathogenic fungus *Beauveria bassiana* and the other few deaths are likely to have been due to natural causes.
- A direct contact spray of Tafari significantly reduced egg hatch but only to 65% compared with 78% in the water control.
- A direct contact spray of *S. carpocapsae* (Nemasys C) applied in 0.1L water/ha as recommended for caterpillar control led to strong aversion behaviour by adult vine weevils but only on the day of application. Treated weevils also laid significantly fewer eggs than those treated with *S. kraussei* (Nemasys L) or *S. carpocapsae* applied in 4L water/ha as recommended as a drench for control of vine weevil larvae, but not fewer than in the water controls.
- Spraying leaves, or weevils and leaves with AHDB 9933 led to abnormal behaviour of vine weevil adults for 2-3 days, after which the weevils recovered and behaved normally.
- The effect of Azatin on egg laying could not be tested as no eggs were laid in the residual effects Experiment 3 which started on 16 October after egg laying had stopped. Azatin did not act as a weevil antifeedant.
- The effect of Dimilin Flo on egg laying or egg hatch could not be tested as no eggs were laid in Experiment 3.

Cold active entomopathogenic fungi

- The germination and mycelial (= colony) extension rates for 17 EPF isolates were quantified at a range of temperatures. This was done to provide an indication of the likely performance of the isolates under field conditions, and in particular to identify isolates able to develop well at 10 - 20°C, and which might therefore be suitable for use as a biopesticide against vine weevil under typical UK temperature conditions.

- Most of the 17 EPF isolates showed similar responses in terms of the effect of temperature on the germination of fungal conidia. Maximum germination occurred between 20 to 30°C. Most isolates showed only low levels of germination at 10°C. However, a number of fungal isolates were identified that had optimal temperatures that were significantly lower than that required for the growth and germination of Met52, which is an important proof of concept in terms of identifying low temperature EPF.
- An important caveat is that we measured spore germination at 24 h and used it as an indicator of spore viability. This is a standard system used by us and many other labs. However, it could be that the speed of germination is reduced at higher temperatures rather than an absolute reduction in viability. Thus, spores that have not germinated after 24 h at a particular temperature may still be viable and germinate after 48 h or longer. The downside for this is that a reduction in the time to germinate would be expected to reduce the virulence of the isolate to the target pest. However, this needs to be tested.
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Knowledge and Technology Transfer

Presentations

- 20 February 2018 – AHDB Ornamentals Conference, Kenilworth.
- 7 June 2018 – Jude Bennison – HNS Panel Meeting, Chesford Grange.
- 6 December 2018 – Jude Bennison – AHDB hardy nursery stock event, Wyevale Nursery, Hereford.

Publications

Roberts, J. M., Kundun, J., Rowley, C., Hall, D. R., Shepherd, T., McLaren, R., Johnson, S. N., Karley, A. & Pope, T. W. (in prep) Behavioural and electrophysiological responses of vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae), adults to host plant odours. *Journal of Chemical Ecology*.

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