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Key staff:	Jude Bennison, ADAS Kerry Boardman, ADAS Diana Pooley, ADAS David Talbot, ADAS Chris Dyer (Statistical advice), ADAS Dr Tom Pope, Harper Adams University Dr Joe Roberts, Harper Adams University Prof David Hall, Natural Resources Institute, University of Greenwich Gill Prince, University of Warwick Dr Dave Chandler, University of Warwick
Location of project:	ADAS Boxworth Commercial nurseries Harper Adams University Natural Resources Institute, University of Greenwich
Industry Representative:	Alastair Hazell, Darby Nursery Stock Ian Nelson, Johnsons of Whixley
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[The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.]

AUTHENTICATION

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

Jude Bennison

Senior Research Entomologist

ADAS

JABennison

[Organisation]

Signature .... Date..........31.1.2020.................

[Name]

[Position]

[Organisation]

Signature .... Date ....

Report authorised by:

[Name]

[Position]

[Organisation]

Signature .... Date ....

[Name]

[Position]

[Organisation]

Signature .... Date ....

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GROWER SUMMARY

Headline

- Vine weevil activity can start in spring when temperatures rise above the threshold of 6°C and continue until temperatures drop below this in autumn/winter. Egg laying can start after weevils have fed for at least five weeks.
- Of the commercially available traps tested, the ChemTica vine weevil trap is the most effective and practical for use in vine weevil monitoring. Other vine weevil traps that are not yet commercially available are, however, at least as effective as the ChemTica vine weevil trap design.
- Catches of vine weevil adults in a range of traps can be increased by placing yew or *Euonymus fortunei* foliage inside but weevil responses to plant volatiles may be influenced by previous feeding experience. A commercial lure for use with traps developed in the Netherlands should be available in 2020 but this was not available for testing in this project.

Background

Vine weevil is currently the most serious pest of UK hardy nursery stock (HNS). With the imminent withdrawal of thiacloprid (Exemptor) growers will have no chemical plant protection products for use in growing media for control of vine weevil larvae. There is now more grower interest in controlling of weevil adults as well as larvae, and growers need more information on the efficacy and timing of treatments 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 chemical plant protection products, 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. However, Met52 needs warm temperatures to be effective and although in soft fruit production, nematodes for vine weevil control are applied quickly and easily through drip irrigation, most growers of HNS need to apply nematodes using high volume drenches which is labour-intensive and thus expensive. This project addresses 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.

Summary Years 1-4

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

A minimum temperature of 12°C has been suggested for vine weevil egg laying to occur but some other researchers report egg laying at lower temperatures. The aim of this objective was to investigate the minimum temperature for both egg laying and feeding to occur. Work in the first two years of the project showed that vine weevil adults are active and feed at 6°C and above. Overwintered vine weevil adults (rather than those that overwinter as larvae) are likely to become active and start feeding, even outside, as early as March, although egg laying may not start until they have fed intensively for at least five weeks. For further details see first and second annual reports, 2016 and 2017.

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

2.1 Relative effectiveness of monitoring tools with and without use of lures. (Harper Adams University and NRI)

Trapping of vine weevil adults reduces the amount of time required to monitor crops and can be done at a convenient time of day. Several techniques have been developed with which to monitor for the presence of vine weevil adults, such as use of grooved boards and corrugated cardboard. A commercial vine weevil trap produced by ChemTica has also been developed and this is available from ChemTica in Costa Rica or from Sentomol Ltd. in the UK.

In Year 1, a comparative study tested the efficacy of different vine weevil monitoring tools without the use of lures under semi-field conditions with known vine weevil populations and potted strawberry plants that simulated a crop. Significantly more vine weevil adults were recovered from the commercial vine weevil trap produced by ChemTica than any of the other monitoring tool designs tested, including corrugated cardboard roll, upturned plastic tray, grooved board, pitfall trap, Roguard cockroach trap and modified palm weevil trap. The ChemTica trap was also the most reliable in terms of indicating the presence of a vine weevil population when there were weevils present in the simulated crops. For other monitoring tool designs, such as the grooved boards or corrugated card, few weevils were recovered and the presence of a weevil population was only confirmed in between 20 and 30% of cases.

In semi-field conditions in Year 4, however, other insect traps supplied by the UK company Sentomol Ltd. such as a different cockroach trap and a banana weevil trap were found to be at least as effective as the ChemTica vine weevil trap design for use in monitoring for the presence of vine weevil adults. In addition, two novel vine weevil trap designs produced by Russell IPM Ltd in the UK were also found to be at least as effective as the ChemTica vine weevil trap design for use in monitoring for the presence of vine weevil adults. These results indicate that, while the ChemTica trap is an effective monitoring tool, alternative designs that are at least as effective as this may be developed and made available in the UK. By contrast, the WeevilGrip, a fabric vine weevil refuge, produced by Agri Gripping, The Netherlands, was found to be less effective than the ChemTica vine weevil trap design under the semi-field conditions used in this study. It is expected, however, that the WeevilGrip vine weevil refuge will be sold together with a 'Weevil Lure', which was not available to test in this project.

The potential to increase the effectiveness of vine weevil monitoring tools through the use of a plant lure was investigated in this project. *Euonymus fortunei* and yew (*Taxus baccata*) foliage are particularly attractive to vine weevils, and, in Year 3 it was shown in the tent cages that addition of *Euonymus* or yew foliage to the ChemTica traps significantly increased catches of vine weevils, regardless of their previous feeding experience. However, the relative attractiveness of the two baits depended upon the prior experience of the weevils with a preference shown for the plant species on which the weevil had previously been feeding. In Year 4 the addition of plant material similarly increased numbers of vine weevil adults found in both the cockroach trap supplied by Sentomol Ltd. and the novel Russell IPM Ltd. design tested.

Also in Year 4, the four most promising trap designs were tested in containerised HNS crops on two commercial nurseries by ADAS with assistance from the host growers. Designs tested were the ChemTica trap, cockroach trap, Russell IPM novel trap and the WeevilGrip trap. No weevils were caught, probably due to very low populations, but useful insights into the practical use of such traps were obtained.

For further details on work investigating monitoring tools used with or without use of lures to detect the presence of vine weevil adults see first, second and third annual reports, 2017, 2018 and 2019.

2.2. *Potential of lures to improve monitoring of vine weevil adults (Harper Adams and NRI)*

Research on attractants for vine weevil adults has to date focussed on the volatile compounds produced by live weevils, those in weevil frass and volatiles produced by host plants. Recently a vine weevil attractant, marketed as Weevil Lure and based on the plant volatile (Z)-2-pentenol, has been developed by Agri Gripping (see https://agri-gripping.com/).

In Year 2 of this project, a series of laboratory experiments using a glass Y-tube olfactometer confirmed positive behavioural responses to live weevils, weevil frass, host plant odours as well as individual plant volatiles. A key finding was that behavioural responses of adult weevils to physiologically active host plant volatiles are affected by the concentration of the volatile(s) and the combination of volatiles that the vine weevil detects. Although the individual components were variously attractive or repellent at different doses, blends of (Z)-2-pentenol + methyl eugenol or of (*Z*)-2-pentenol + methyl salicylate + 1-octenol + (*E*)-2-hexenol + (*Z*)-3-hexenol + 1-hexanol + (*E*)-2-pentenol were very attractive. However, in a preliminary test in Year 2 addition of the former blend to ChemTica traps gave no increase in numbers of vine weevils caught. Catches were very low, but development of an effective lure will thus probably require careful formulation to be effective.

In laboratory work, volatiles were collected from various sources and analysed by gas chromatography (GC) coupled to electroantennographic (EAG) recording from receptors on vine weevil antennae to determine and identify which compounds are detected by the weevils and are hence candidate attractants. In Year 2, no consistent EAG responses were shown to volatiles collected from unstarved weevils. In Year 3, at least 23 compounds produced by *Euonymus fortunei* were shown to elicit EAG responses from vine weevils. Similar work in Year 4 showed that 20 compounds in volatiles from yew elicited EAG responses, with 10 compounds in common. Identified plant volatiles were mostly fairly ubiquitous plant volatiles and are strong candidates for use as attractants for vine weevil adults. Both (*E*)- and (*Z*)-2-pentenol and methyl eugenol have been reported by other workers to be produced by *Euonymus fortunei* but could not be detected in this work. Other EAG-active plant volatiles, such as cis-jasmone, 1.2-dimethoxybenzene, eugenol and myrtenol were, however, detected and may also be useful as part of a vine weevil lure.

In Year 2, cuticular hydrocarbons from *Otiorhynchus sulcatus* and a new species, *O. lavandus,* were collected and analysed. Analyses showed that their compositions were quite consistent but different from each other, providing evidence that they are species-specific and could be used for species recognition. These have potential to increase colonisation of refuges, but experiments in Year 3 showed no evidence that they could elicit trail following in vine weevils.

Various controlled-release dispensing systems for candidate attractants were investigated under laboratory conditions, including pipette tips, polyethylene vials and sealed polyethylene disposable pipettes.

For further details see first, second and third annual reports, 2017, 2018 and 2019.

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

3.1. Little and often application of nematodes (ADAS)

A 'little and often' system for applying reduced rates of entomopathogenic nematodes through the overhead irrigation was tested in a research polytunnel in Year 1 and validated on a commercial nursery in Year 2. Application of nematodes at 40% rate five times between June and October was equally as effective in reducing mean numbers of vine weevil larvae per plant as two conventional full rate drench applications in September and October. Using 40% rates five times between June and October offers up to 52% cost savings compared with using standard high volume drenches due to reduced labour time without compromising on efficacy. For further details see first and second annual reports, 2016 and 2017.

3.2. Lethal and sub-lethal effects of IPM-compatible products against adult weevils (ADAS)

Control of adult vine weevil 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. Chess WG is no longer available and the EAMU for Steward and other indoxacarb products allows application at the rate shown to be effective against vine weevil on outdoor ornamentals but only at a lower rate for protected ornamentals. Laboratory experiments in Year 3 tested the lethal and sub-lethal effects of candidate IPM-compatible treatments against adult vine weevils. None of the treatments gave effective weevil kill. Pymetrozine (Tafari) significantly reduced egg hatch but only to 65% compared with 78% in the water control. A spray of *Steinernema carpocapsae* (Nemasys C) and the coded insecticide AHDB 9933 led to short-term abnormal behaviour after which the weevils recovered. The botanical biopesticide azadirachtin (Azatin) acts on ingestion and has antifeedant effects on some insects but neither damp nor dry residues led to reduced weevil feeding on treated Euonymus leaves. No new IPM-compatible controls for adult weevils were identified. For further details see third annual report, 2018.

3.3 Effects of temperature on entomopathogenic fungi (Warwick University)

Experiments in Years 1 and 2 tested the effect of temperature (12.5-30°C) on the infectivity of Met52 to vine weevil larvae. Mortality increased with temperature. A predictive day degree model was developed to predict Met52 infection and this estimated that no kill will occur below 11.6°C and that for 75% kill 256 cumulative day degrees are needed, which could be reached between June and August in some years and locations. A cold-tolerant fungal strain would be useful. Experiments were done on 17 cold-tolerant isolates of fungi. Only two isolates germinated below 10°C and only four isolates grew at 4°C. The two most promising strains were tested against vine weevil larvae. A predictive model indicated that although these fungi could develop at lower temperatures than Met52 they were less virulent to vine weevil larvae so did not offer opportunities for further development. For further details see second and third year annual reports, 2017 and 2018.

Objective 4. Develop novel approaches to control vine weevil (Harper Adams University)

A wooden trap with grooves filled with a gel containing *Steinernema carpocapsae* is available from e-nema for control of adult vine weevils that seek refuge under the traps during the day, become infected and subsequently die. The traps were tested in another AHDB project (see 2013 report of CP 89) and led to 92% weevil kill within four weeks. The traps are currently sold for home garden use but are too expensive for commercial use. An alternative cost-effective lure and kill approach could potentially be developed. In experiments where a gel formulation of *S. carpocapsae* was either applied to the base of plant pots or placed inside Roguard crawling insect traps, adult vine weevil mortality was not statistically significantly increased. For further details see second year annual report, 2017.

Objective 5. Disseminate new knowledge and updated best-practice control methods to growers and other industry members (all partners)

Throughout the project the results of the project have been disseminated extensively to growers, other industry members, the scientific community and the general public. Communication methods ranged from presentations at industry events and scientific conferences, a video on the AHDB website, radio and TV presentations and scientific papers. For further details see first, second and third year annual reports, 2016, 2017 and 2018.

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. 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. 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 6°C and continue until the autumn/winter when temperatures decline below this threshold once more. Traps are available which may help with monitoring. The Chemtica trap is commercially available from Costa Rica http://www.chemtica.com/ at an approximate price of $7 plus shipping from Sentomol Ltd. in the UK http://sentomol.com at an approximate price of £15. The WeevilGrip trap and Weevil Lure may be available during 2020 https://agri-gripping.com/

- Overwintered adult vine weevils need a 5-week period of intense feeding before they recommence laying eggs. 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 application 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

- Do not rely on Met52 as the sole method for controlling vine weevil larvae, use as part of an IPM programme for vine weevil management (see AHDB factsheet 24/16).
- Be aware that garlic (Pitcher GR) now has an EAMU for use on both protected and outdoor ornamentals for control of vine weevil and leaf and bud nematodes. This product was not tested in this project.

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. As the use of imidacloprid (Imidasect 5GR) is no longer approved and the approval for thiacloprid (Exemptor) is due to be withdrawn (sale up to October 2012 and use-up date October 2022), growers will soon have no chemical plant protection products for use in growing media for control of vine weevil larvae. 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 treatments 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 chemical plant protection products, 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, Met52 needs warm temperatures to be effective and although in soft fruit production, nematodes for vine weevil control are applied quickly and easily through drip irrigation, most growers of HNS need to apply nematodes using high volume drenches which is labour-intensive and thus expensive. This project addresses 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

Monitoring for the presence of vine weevil adults using traps and artificial refuges reduces the length of time required and increases the reliability of crop inspections to detect pest presence. Several techniques have previously been developed to monitor for the presence of adult vine weevils, including grooved boards placed on the ground within crops (Li et al., 1995; Gordon et al., 2003), corrugated cardboard (Phillips, 1989), simple plastic crawling insect traps (Pope et al., 2013) and pitfall traps (e.g. Casteels et al., 1995; Solomon, 2000; Buxton, 2003). In a direct comparison of these established techniques used by UK growers to monitor for the presence of vine weevil adults none were found to be reliable and each only 'caught' low numbers of adult weevils under semi-field conditions (see Year 1 report and Roberts *et al.,* 2019a). For comparison, in the same study the commercially produced ChemTica vine weevil trap was found to catch significantly higher numbers of weevils and to more reliably indicate the presence of weevils under semi-field conditions than the established monitoring techniques. This trap, however, is not widely available in the UK. More recently a vine weevil monitoring system has been developed in the Netherlands (see https://agri-gripping.com/). This system consists of the 'WeevilGrip', a fabric'ruffle' refuge that exploits the aggregating behaviour of adult weevils. The 'WeevilGrip' refuge may be used alone or together with a lure, which is based on the work of van Tol et al. (2012). The Agri-Gripping vine weevil monitoring system may be available to growers in 2020.

Development of an effective semiochemical lure would improve the reliability and sensitivity of vine weevil monitoring strategies and potentially lead to 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 reproduce parthenogenetically and therefore 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). 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; Roberts *et al.,* 2019: van Tol *et al.,* 2020. 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 responses have also been recorded to both satiated conspecifics and weevil frass (see Year 2 annual report). 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 use plant-derived odours to locate suitable host plants for feeding and oviposition and it is hypothesised that these may 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 adults 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 report and Roberts *et al.,* 2019b).

It has been reported that a combination of two volatiles, methyl eugenol and (*Z*)-2-pentenol (1:1 ratio), used as an attractant in traps increased vine weevil numbers close to the baited traps but did not increase the number of weevils entering traps (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. These behavioural responses were recorded despite the fact that in subsequent work (*Z*)-2-pentenol has not been detected in the headspace of *Euonymus fortune* (see Year 3 annual report and Roberts *et al.,* 2019b).

Recent work in this study (see Year 3 annual report and Robert *et al.,* 2019a) has shown that the numbers of weevils caught in a ChemTica vine weevil trap can be significantly increased through the addition of host plant foliage. These responses are, however, complicated by the fact that the behavioural responses of vine weevil adults appear to be flexible and determined, at least to some extent, by previous feeding experience.

The aim of objective 2 in year 4 was to investigate the potential of novel trap designs, that may be made readily available to UK growers, used with or without the addition of synthetic lures under semi-field conditions and to assess the most promising trap designs under commercial conditions. Additional work was carried out to identify host-plant volatiles that are candidate attractants for vine weevil and to develop lures containing blends of the synthetic chemicals.

Task 2.1. Potential of novel trap designs with or without the addition of lures for vine weevil monitoring (Harper and NRI)

Materials and Methods

Insects

Adult vine weevils were collected from strawberry crops in Shropshire and Staffordshire in 2019 and maintained at 20 °C and 60 % RH in a controlled environment room (Fitotron, Weiss Technik, Ebbw Vale, Wales) under long-day conditions (L:D 16:8 h). Weevils were cultured in groups of 20-30 adults in Mini BugDorms (12.5x11.4 cm) (Bugdorm, MegaView, Taiwan), which consist of a mesh lid on a round plastic container (see Figure 1). Before the start of each experiment, weevils were conditioned with yew plant material for a minimum of ten days. This was done by wrapping the cut end of the yew branches in damp tissue paper to ensure the foliage remained 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, with foliage being replaced every three to four days.

Fig. 1. Mini BugDorm containing yew foliage, ball of dry tissue paper and vine weevil adults.

Trap designs

1. Chemtica trap – commercially produced vine weevil trap but not widely available in the UK. Available from: http://www.chemtica.com/. Cone shaped black plastic trap (19 cm diameter base, 15 cm high)
2. Cockroach trap supplied by Sentomol Ltd – commercially produced cockroach trap not currently used as a vine weevil trap. Rectangular white plastic trap (21 x 39 x 7 cm).
3. Banana weevil trap - supplied by Sentomol Ltd – commercially produced banana weevil trap not currently used as a vine weevil trap. Square yellow plastic trap (30 x 30 x 17 cm).

4. Novel design 1 – supplied by Russell IPM Ltd - short – novel vine weevil trap design produced using 3D printing technology. Cone shaped black plastic trap (18 cm diameter base, 9 cm high).
5. Novel design 2 – tall supplied by Russell IPM – novel vine weevil trap design produced using 3D printing technology. Cone shaped black plastic trap (18 cm diameter base, 14 cm high).
6. WeevilGrip – 'ruffle' – commercially produced vine weevil monitoring tool likely to become widely available in the UK in 2020. Available from: https://agri-gripping.com/. Fabric ruffle (4 x 58 cm).

Trap design efficacy

Experiments one to four tested trap design efficacy in a no-choice scenario under semi-field conditions (Table 1). These semi-field conditions consisted of six large tent cages (1.45 m x 1.45 m x 1.52 m) (Insectopia, UK) set up in an unheated glasshouse at Harper Adams University, with each tent cage containing four potted (12cm diameter pots) strawberry plants (cv. Elstanta) along with one trap and 40 adult weevils (Fig. 2). The strawberry plants were watered as required, watering from above to avoid making the base of the tent cages wet. All traps in these experiments were un-baited (i.e. they contained no plant foliage or synthetic lure). Traps were placed into the cages between 5pm and 6pm in the evening and the number of weevils within each trap was assessed the following morning between 8am and 10am. The position of the traps both within and between cages was re-randomised each day. This series of experiments was completed between 6th June and 26th October 2019.

Fig. 2. Arrangement of traps within each tent cage (two trap arrangement).

Lure formulation efficacy

Experiments five to seven tested lure formulation efficacy in a dual-choice scenario under the same semi-field conditions described above. However, each cage contained two traps with one being baited and the other un-baited (Table 1).

Table 1. Summary of treatments tested in each experiment of this study

Exp.	Trial	Trap 1	Trap 2	Lure 1	Lure 2	Reps
1	1	Chemtica	N/A	N/A	N/A	20
	2	Banana weevil	N/A	N/A	N/A	20
	3	Cockroach	N/A	N/A	N/A	20
2	1	Chemtica	N/A	N/A	N/A	20
	2	Novel 1	N/A	N/A	N/A	20
	3	Novel 2	N/A	N/A	N/A	20
3	1	Chemtica	N/A	N/A	N/A	18
	2	Cockroach	N/A	N/A	N/A	18
	3	Novel 1	N/A	N/A	N/A	18
4	1	Chemtica	N/A	N/A	N/A	21
	2	Weevil Grip	N/A	N/A	N/A	21
5	1	Chemtica	Chemtica	Empty bag	Yew	22
	2	Cockroach	Cockroach	Empty bag	Yew	22
	3	Novel 1	Novel 1	Empty bag	Yew	22
6	1	Novel 1	Novel 1	Paraffin oil [a]	*cis*-jasmone [b]	10
	2	Novel 1	Novel 1	Paraffin oil [a]	1,2 dimethoxy benzene [b]	10
7	1	Novel 1	Novel 1	Paraffin oil [a]	*cis-jasmone* [b]	11
	2	Novel 1	Novel 1	Paraffin oil [a]	van Tol blend [c]	11

[a] 100 µl of pure paraffin oil

[b] 100 µl of synthetic chemical (100 mg mL^{-1})

[c] 100 µl of methyl eugenol and (*Z*)-2-pentenol (1:1 ratio; 100 mg mL^{-1})

Experiments using plant material baited traps contained 15 g of fresh yew foliage that was enclosed within a fine 'weevil proof' nylon mesh bag (30 x 20 cm) before being placed into the trap. An empty nylon mesh bag was used as a control in the un-baited trap in these experiments. In this way the 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 unable to feed on the foliage inside the mesh bags. For experiments where traps contained synthetic chemicals lures these were constructed from opaque, polypropylene pipette tips (1 ml; Fisher Scientific, UK) with a 0.2 mm aperture. To prepare the lures, synthetic chemicals were dissolved in paraffin oil (Fisher Scientific, UK) to make concentrations of 100 mg/ml. A total volume of 100 µl of each of these chemicals were then individually impregnated onto a separate cellulose acetate cigarette filter (14 × 6 mm; Swan, UK) and placed into pipette tips. These pipette tips were then sealed with a Teflon-lined crimp seal (11 mm; Chromatography Direct, UK) and stored at - 20°C until used.

Vine weevil numbers per cage varied with each experiment as follows: experiment five = 30, experiment six = 20 and experiment 7 = 12. Traps were placed into the cages between 5pm and 6pm in the evening and the number of weevils within the traps was assessed the following morning between 8am and 10am. The position of the traps both within and between cages was re-randomised each day. This series of experiments was completed between 6th June and 26th October 2019.

All statistical analyses were performed using R 3.6.1 (R Core Team, 2019). No choice experiments testing trap performance (i.e. experiments one to four) were evaluated using a with a general linear model (GLM) with a quasipoisson probability distribution and 'trap type' as a factor using the *glm* function from the *stats* R package (R Core Team, 2019). Multiple comparisons for the GLM were evaluated by Tukey's HSD tests implemented in the *HSD.test* function in the R package *agricolae* (de Mendiburu, 2019). Dual choice experiments testing lure performance (i.e. experiments five to seven) were analysed using exact binomial tests against the null hypothesis that the number of vine weevils in each trap had a 50:50 distribution using the *binom.test* function in the *stats* R package. The replicated results were pooled for each trial and un-trapped individuals were excluded from statistical analyses.

Results

Trap design efficacy

In Experiment 1, where two commercially available trap designs (banana weevil and cockroach) were tested against the Chemtica design, trap efficacy did not differ between the three designs as each trap captured ~ 9 % of the introduced weevils (generalised linear model: X_2^2 = 0.009, *df* = 57, *P* = 0.995) (Fig. 3). This experiment was completed between 5 and 15th June 2019. Mean daytime temperature during this period was 21°C and mean night-time temperature was 15°C.

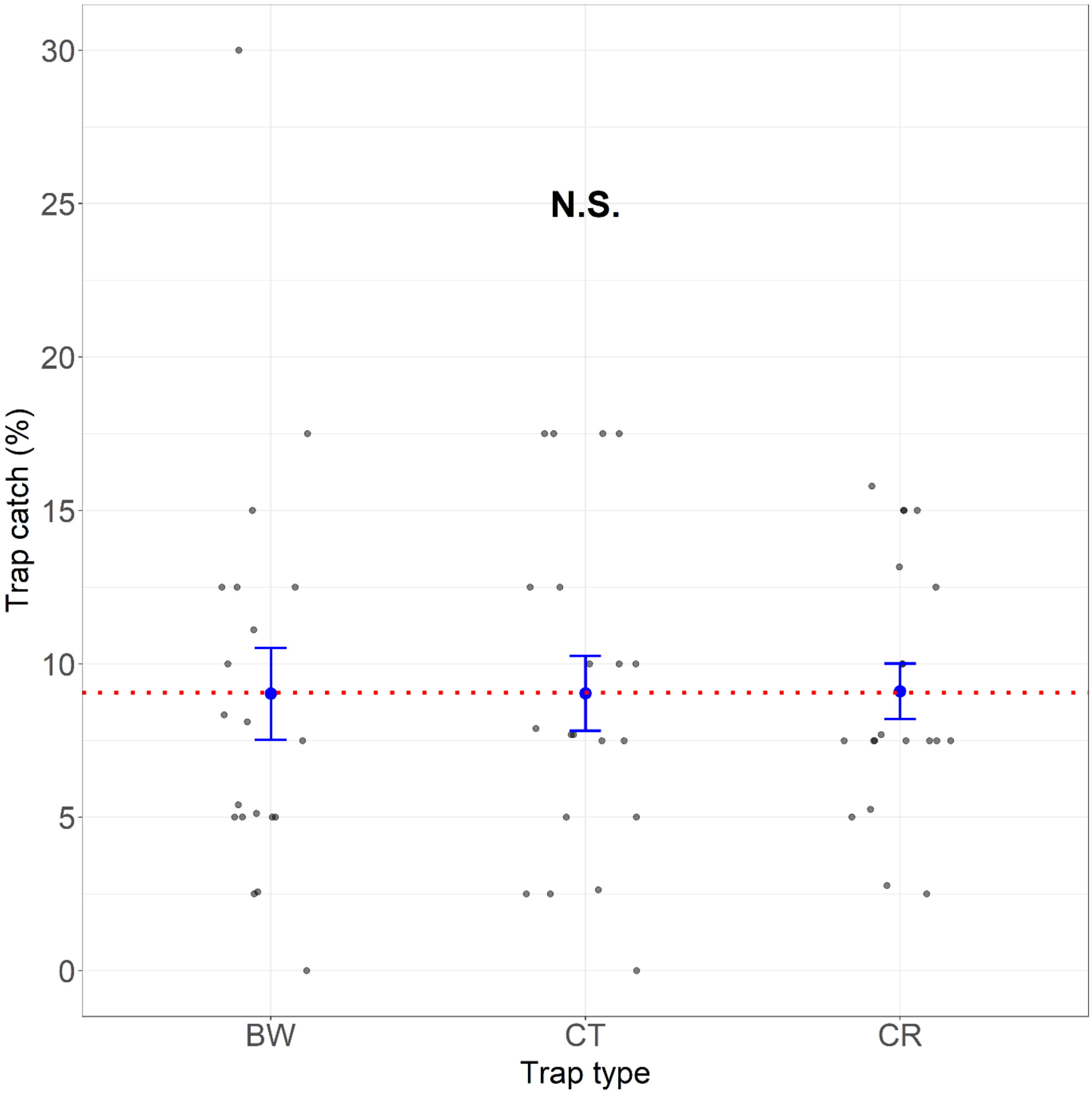

Fig. 3. Mean (± SE) trap catch of populations of 40 adult vine weevils. Trap types: BW = banana weevil, CT = Chemtica and CR = cockroach. Grey dots are individual data points, blue dots with error bars are treatments means and the dashed red line is the grand mean. N.S. indicates that there was no significant difference in trap catch between the three trap designs (generalised linear model: X_2^2 = 0.009, *df* = 57, *P* > 0.05).

In Experiment 2, where two novel trap designs were tested against the Chemtica design, overall trap efficacy differed between the three designs (generalised linear model: X_2^2 = 14.449, *df* = 57, *P* < 0.01). Both novel designs outperformed the Chemtica design, but performed similarly to one another and trapped ~ 8 % of the introduced vine weevil population (Fig. 4). This experiment was completed between 25th June and 6th July 2019. Mean daytime temperature during this period was 26°C and mean night-time temperature was 18°C.

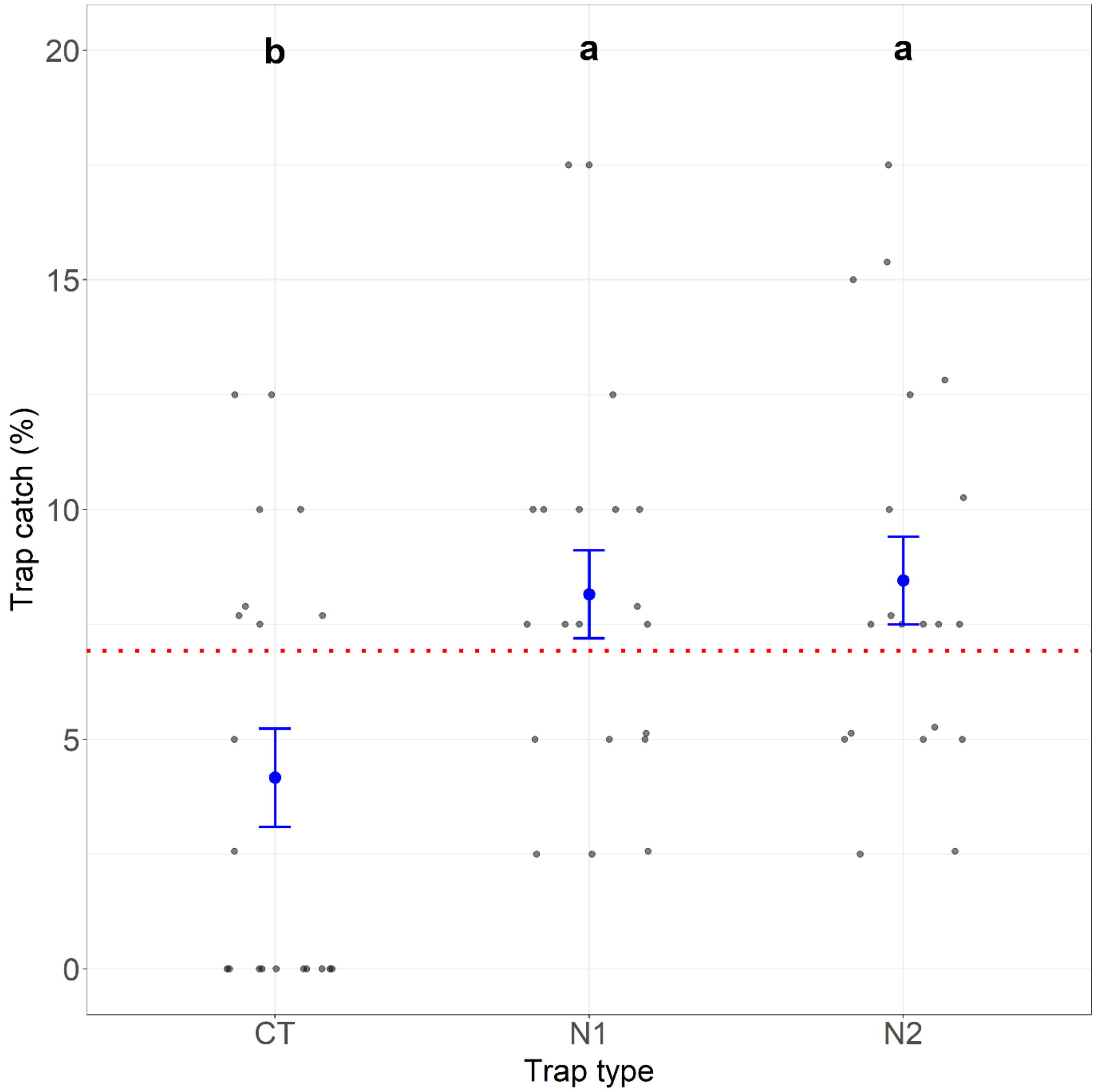

Fig. 4. Mean (± SE) trap catch of populations of 40 adult vine weevils. Trap types: CT = Chemtica, N1 = novel design 1 and N2 = novel design 2. Grey dots are individual data points, blue dots with error bars are treatments means and the dashed red line is the grand mean. Treatments capped with different letters are significantly different (generalised linear model:(generalised linear model: X_2^2 = 14.449, *df* = 57, *P* < 0.01; Tukey's HSD: *P* < 0.05).

In Experiment 3, where one novel trap design was tested against one commercially available design (cockroach) and the Chemtica design, overall trap efficacy differed between the three designs (generalised linear model: X_2^2 = 10.246, *df* = 51, *P* < 0.05). The commercially available cockroach design performed best as it trapped 5.8 % of the weevil population, compared to ~ 2.8 % for both the novel and Chemtica designs (Fig. 5). This experiment was completed between 8 and 17th July 2019. Mean daytime temperature during this period was 26°C and mean night-time temperature was 19°C.

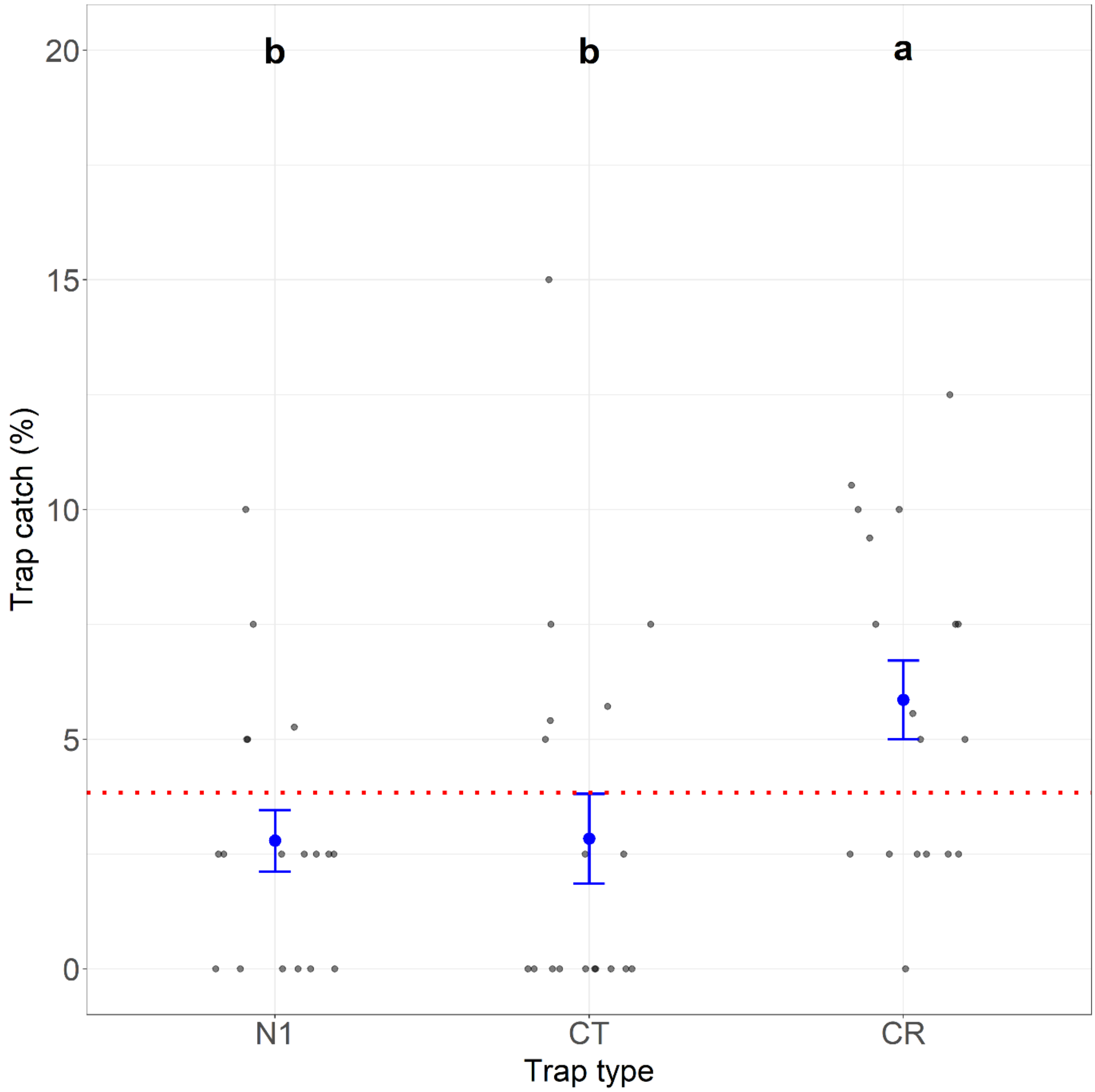

Fig. 5. Mean (± SE) trap catch of populations of 40 adult vine weevils. Trap types: N1 = novel design 1, CT = Chemtica and CR = cockroach. Grey dots are individual data points, blue dots with error bars are treatments means and the dashed red line is the grand mean. Treatments capped with different letters are significantly different (generalised linear model:(generalised linear model: X^2_2 = 10.246, *df* = 51, *P* < 0.05; Tukey's HSD: *P* < 0.05).

In Experiment 4, where two commercially available trap designs (Chemtica and WeevilGrip) were tested against one another, overall trap efficacy differed between the two designs (generalised linear model: X^2_1 = 23.169, *df* = 40, *P* < 0.001). The Chemtica design performed best, trapping 5.25 % of the weevil population compared to 1.14 % for the WeevilGrip design (Fig. 6). This experiment was completed between 18 and 29th July 2019. Mean daytime temperature during this period was 26°C and mean night-time temperature was 20°C.

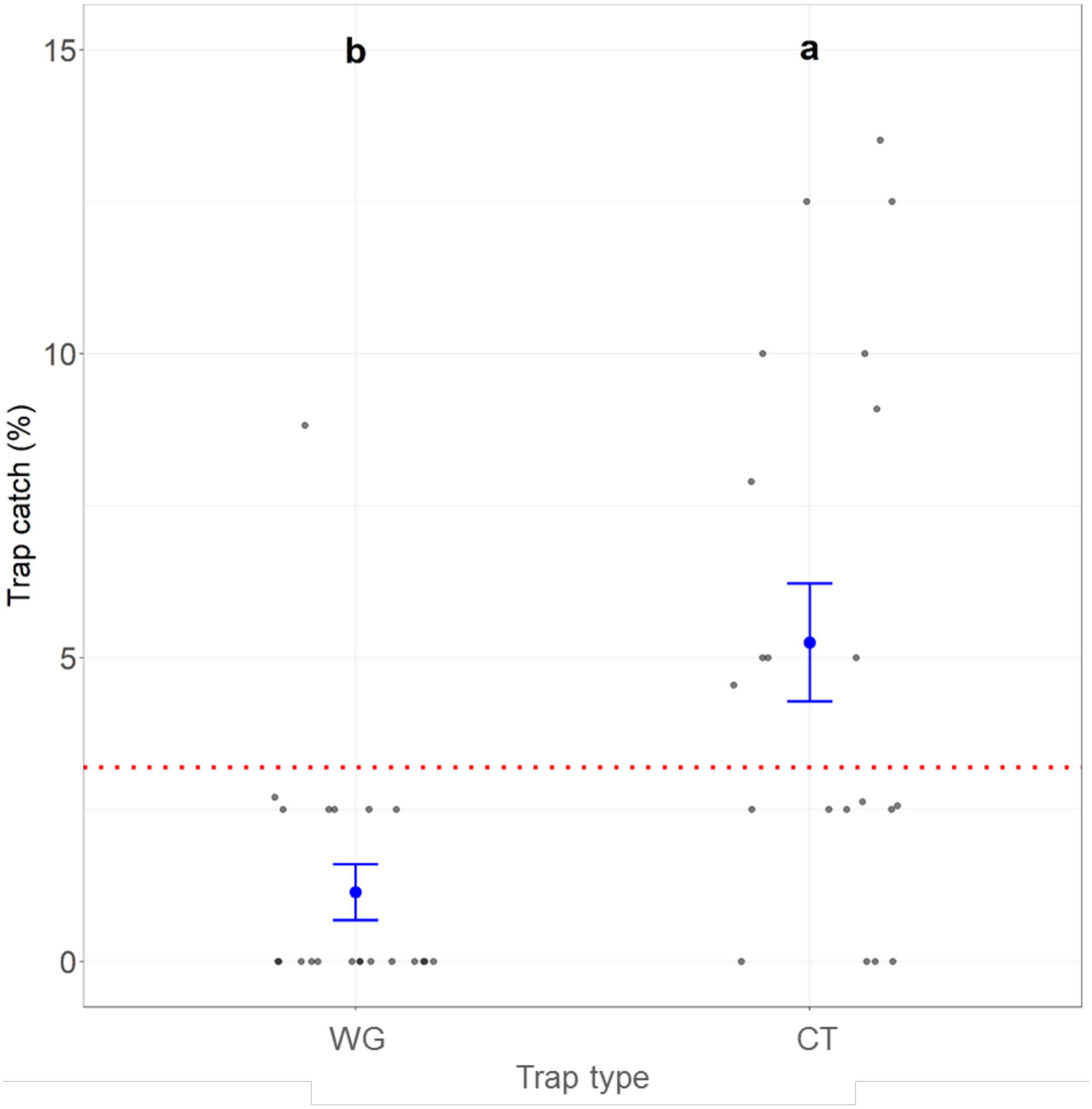

Fig. 6. Mean (± SE) trap catch of populations of 40 adult vine weevils. Trap types: WG = WeevilGrip and CT = Chemtica. Grey dots are individual data points, blue dots with error bars are treatments means and the dashed red line is the grand mean. Treatments capped with different letters are significantly different (generalised linear model:(generalised linear model: X_1^2 = 23.169, *df* = 40, *P* < 0.001; Tukey's HSD: *P* < 0.05).

Lure formulation efficacy

In Experiment 5, vine weevils showed varying levels of preference for three trap designs baited with yew foliage when offered a choice against an un-baited trap (Chemtica, exact binomial test: *P* > 0.05; cockroach, exact binomial test: *P* < 0.01; novel design 1, binomial exact test: *P* < 0.001) (Fig. 7). This experiment was completed between 4 and 20th September

2019. Mean daytime temperature during this period was 23°C and mean night-time temperature was 15°C.

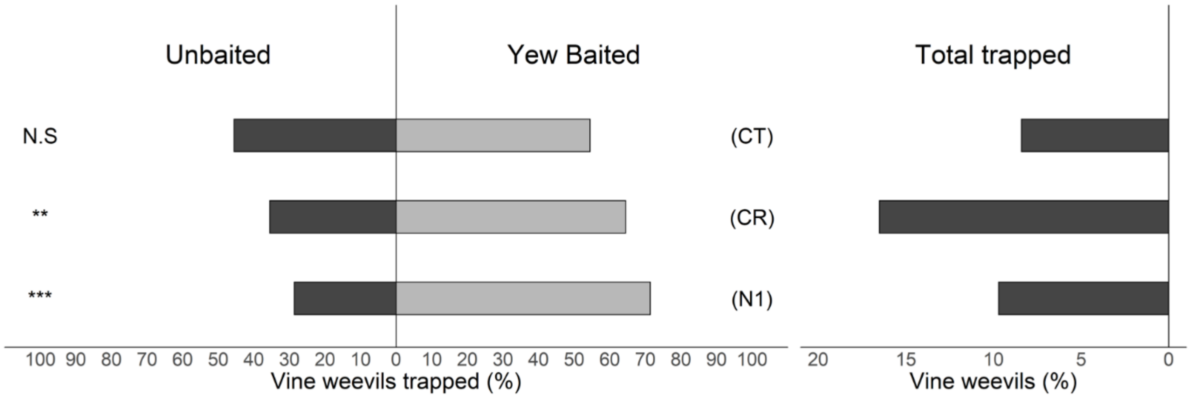

Fig. 7. Behavioural responses of adult vine weevils toward three trap designs baited with yew foliage. Letters in parentheses indicate trap type, with CT = ChemTica, CR = cockroach and N1 = novel. Asterisks indicate significance levels calculated using binomial exact tests: ** P < 0.01; *** P < 0.001; N.S. = no significance.

In Experiment 6, vine weevils showed no preference for novel 1 trap designs baited with *cis*-jasmone lures (exact binomial test: $P > 0.05$) and showed a negative behavioural response toward traps baited with 1,2 dimethoxybenzene (exact binomial test: $P < 0.05$, Fig. 8). This experiment was completed between 9 and 26th October 2019. Mean daytime temperature during this period was 19°C and mean night-time temperature was 14°C.

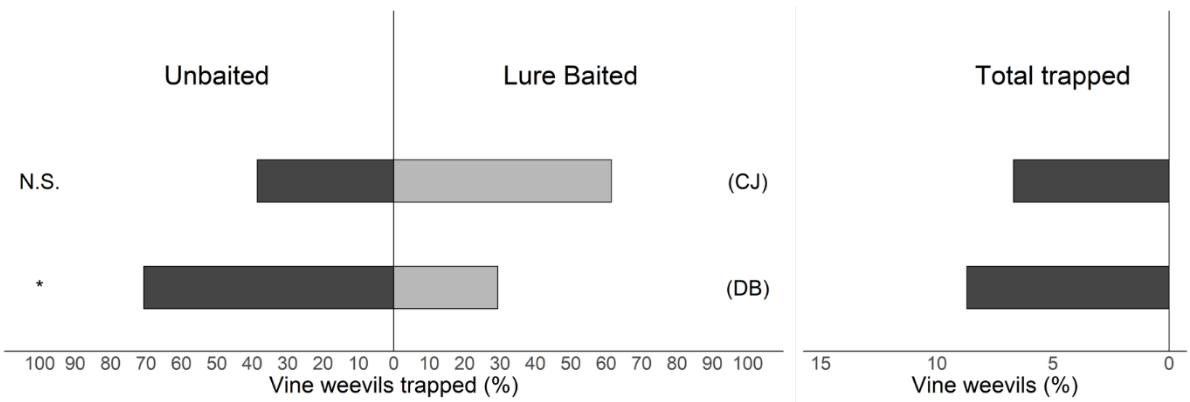

Fig. 8. Behavioural responses of adult vine weevils toward traps (novel design 1) baited with synthetic chemical lures. Letters in parentheses indicate lure, with CJ = *cis*-jasmone and DB = 1,2 dimethoxy benzene. Asterisks indicate significance levels calculated using binomial exact tests: * P < 0.05; N.S. = no significance.

In Experiment 7, vine weevils showed no preference for novel 1 trap designs baited with *cis*-jasmone lures (exact binomial test: $P > 0.05$) and showed a negative behavioural response toward traps baited with a 1:1 blend of methyl eugenol and (*Z*)-2-pentenol (exact binomial test: $P < 0.05$, Fig. 9). This experiment was completed between 26th September and 4th October 2019. Mean daytime temperature during this period was 21°C and mean night-time temperature was 15°C.

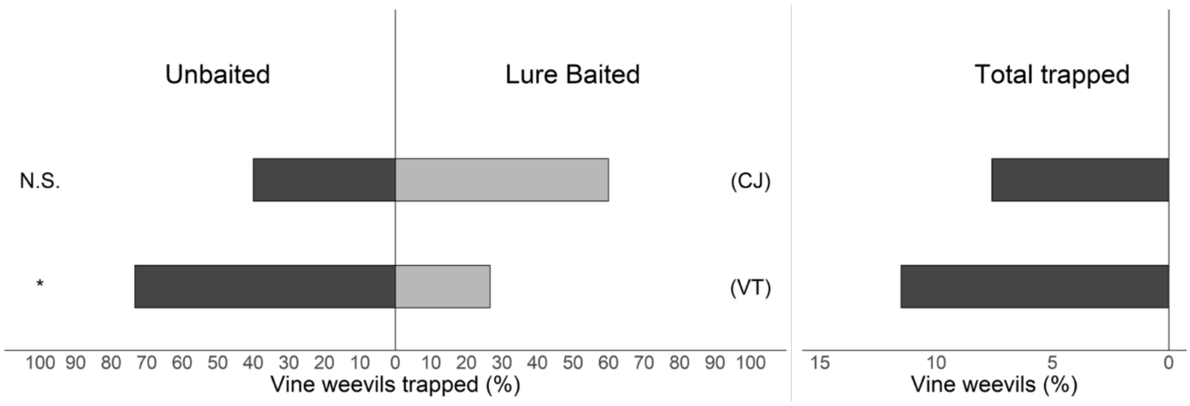

Fig. 9. Behavioural responses of adult vine weevils toward traps (novel trap design 1) baited with synthetic chemical lures. Letters in parentheses indicate lure, with CJ = *cis*-jasmone and VT = van Tol blend of a 1:1 ratio of methyl eugenol and (Z)-2-pentenol. Asterisks indicate significance levels calculated using binomial exact tests: * P < 0.05; N.S. = no significance.

Discussion

Work completed in Year 1 of this study (see Year 1 report and Roberts *et al.,* 2019a) indicated that the ChemTica vine weevil trap was a more effective and reliable means of monitoring for the presence of vine weevil adults than monitoring techniques currently more available to growers. The monitoring tools typically available to growers include use of grooved boards (Li et al., 1995; Gordon et al., 2003; van Tol et al., 2020), corrugated cardboard (Phillips, 1989), plastic crawling insect traps (Pope et al., 2013) and pitfall traps (e.g. Casteels et al., 1995; Solomon, 2000; Buxton, 2003). Despite this, in the work presented here, other trap designs were found to be as, or in some cases, more effective than the ChemTica trap design, based on numbers of vine weevil recorded in traps. These other trap designs included a commercially available cockroach trap and a banana weevil trap. In addition, two novel vine weevil traps produced by Russell IPM using 3D print technology were found to be at least as effective as the ChemTica trap design. A notable feature of the results presented here is that numbers of weevils recorded in each trap design were lower than those reported in Year 1 of this study. While weevil numbers recorded in the ChemTica trap in Year 1 of this study ranged between ~ 20 and 25% of those released, here the number of weevils recorded in ChemTica traps ranged between ~ 3 and 9%. In addition, while the ChemTica trap was 100% reliable in Year 1 of this study, here reliability varied between 44% and 95%. It is unclear why the efficacy of the ChemTica trap design was lower than had previously been reported in this study. It should also be noted that while reliability and numbers of weevils recorded in this trap were lower than had been reported in Year 1 of this study, the results presented here for the ChemTica trap are still better than those reported for the grooved boards (0.4%) or corrugated cardboard (0.8%) in Year 1 of this study (see Year 1 report).

It remains unclear from the work presented here as to why each of the designs tested as a vine weevil trap was effective. Colour, for example, may be an important factor in determining the effectiveness of a vine weevil trap. Dark traps may, for example, intuitively be thought to be more effective than lighter coloured traps. Despite this, here the cockroach trap design used was white while the banana weevil trap design was yellow. Both of the novel trap designs tested were, however, black. Each of the traps used in this study, including the ChemTica design shared the common feature of allowing entry into the trap from all sides. Aside from this, the traps varied greatly in overall size, with the banana weevil trap occupying approximately 900 cm^2 of floor space while the two novel trap design occupy just 254 cm^2. It is likely that growers would find the smaller trap advantageous, particularly where the traps have the same size footprint as the pots being used so that a trap could simply be used in place of a pot.

Results from this study demonstrate that, like in Year 3 of this study, the addition of yew foliage to the trap has the effect of significantly increasing the numbers of weevils recorded inside the trap (see Year 3 report and Roberts *et al.,* 2019a). This was demonstrated for both the cockroach trap design and Novel trap design 1. Interestingly, although the same trend was recorded for the ChemTica trap design the addition of yew foliage did not result in a statistically significant increase in the number of weevils recorded inside the trap.

Previous work has shown that vine weevil adults respond positively to blends of (*Z*)-2-pentenol and methyl eugenol as well as a blend consisting of (*Z*)-2-pentenol, methyl salicylate, 1-octenol, (*E*)-2-hexenol, (*Z*)-3-hexenol, 1-hexanol, (*E*)-2-pentenol) (see Year 2 report and Roberts *et al.,* 2019b). Despite this, there is a lack of evidence of a synthetic lure increasing catches of weevils within traps (see Year 2 report and van Tol *et al.,* 2012). Here we investigated whether release of 1,2-dimethoxybenzene and cis-jasmone, two volatiles produced by *Euonymus fortunei* that reliably elicit strong electrophysiological responses in vine weevil adults. *Cis*-jasmone was found to have no effect on numbers of adult weevils recorded inside Novel trap design 1, while 1,2-dimethoxybenzene was found to reduce numbers of weevils inside the trap. These results are likely to illustrate the importance of presenting a blend of plant volatiles. As reported for vine weevil in Year 2 of this project and other insects (see Bruce & Pickett, 2011) individual compounds may not elicit a positive behavioural response when presented on their own, but may still represent an important component of a blend of volatiles that does elicit a positive behavioural response. As such, neither *cis*-jasmone nor 1,2-dimethoxybenzene should be ruled out as being potentially important components of a vine weevil lure based on these results alone. The blend of (*Z*)-2-pentenol and methyl eugenol, as previously reported by van Tol et al. (2012), did not increase the numbers of vine weevil adults recorded inside a trap. Indeed here, the addition of this lure reduced numbers of weevils entering the trap. However, as relatively few weevils were trapped, this result should be interpreted with some caution.

Conclusions

- In previous work, more vine weevils were caught in the trap from ChemTica, Costa Rica, than in other designs. However, the ChemTica trap is not readily available in the UK.
- In this year's work in semi-field, tent cages, the banana weevil trap and cockroach trap performed similarly to the ChemTica trap. Furthermore, prototype designs from the UK company Russell IPM caught at least as many weevils as the ChemTica trap, and in one experiment significantly more.
- Addition of yew foliage to the cockroach trap and the Russell IPM trap significantly increased catches of vine weevils.
- 1,2-Dimethoxybenzene and cis-jasmone were previously in volatiles from Euonymous fortunei and shown to elicit strong electroantennogram (EAG) responses from vine weevil antennae. Addition of cis-jasmone to traps increased catches of vine weevils but not significantly. Addition of 1,2-dimethoxybenzene decreased catches. Addition of a blend of (Z)-2-pentenol and methyleugenol, reported to be attractive to vine weevil also reduced catches.

Task 2.2. Efficacy of novel trap designs for monitoring vine weevil adults within a commercial nursery (ADAS and Harper)

Materials and Methods

Four trap designs were tested in containerised HNS crops on two commercial nurseries by ADAS with assistance from the host growers and in consultation with Harper Adams. The trap designs were selected using the results of work in semi-field conditions at Harper Adams University.

Trap designs

1. Chemtica trap (Fig. 10) – commercially produced vine weevil trap but not widely available in the UK. Available from: http://www.chemtica.com/. Cone shaped black plastic trap (19 cm diameter base, 15 cm high)
2. Cockroach trap (Fig. 11) – commercially produced cockroach trap not currently used as a vine weevil trap. Rectangular white plastic trap (21 x 39 x 7 cm).
3. Novel design 1 – short – novel vine weevil trap design produced using 3D printing technology. Cone shaped black plastic trap (18 cm diameter base, 9 cm high).
4. WeevilGrip (Fig. 12) – 'ruffle' – commercially produced vine weevil monitoring tool likely to become widely available in the UK in 2020. Available from: https://agri-gripping.com/. Fabric ruffle (4 x XX cm).

Fig. 10. Chemtica trap

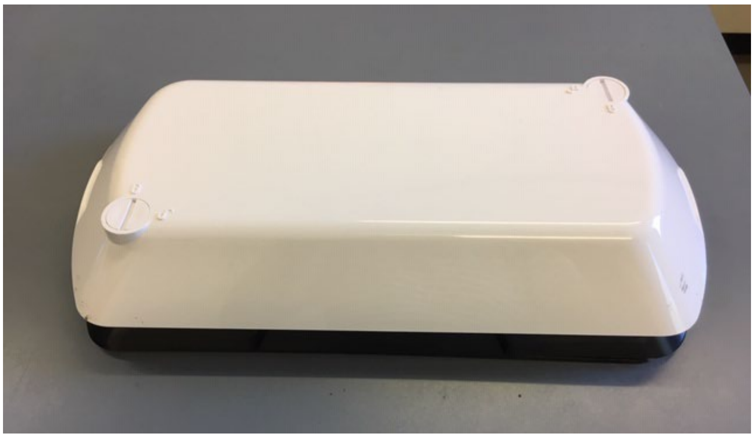

Fig. 11. Cockroach trap

Fig. 12. ‘WeevilGrip’ ruffle trap

Trap design efficacy

At Site 1, the four trap designs were tested in five replicate beds of containerised Photinia in 3L pots stood on sandbeds in a glasshouse, cv. Little Red Devil and cv. Scarlet Blaze. Photinia is one of the host plants found to be susceptible to vine weevil on the nursery. At Site 2, the four trap designs were tested in five replicate beds of containerised hardy nursery stock in 2L pots stood on outdoor gravel beds, two beds of *Skimmia japonica* (under shaded tunnels with open sides), two beds of *Euonymus fortunei* Emerald n Gold and one bed of *Bergenia* Silberlicht. *Bergenia, Euonymus* and *Skimmia* are host plants found to be susceptible to vine weevil on the nursery. At both sites, each replicate bed was 4x14m and one of each of the four traps was placed in each of the five beds at equal distances (2.8m) apart up the middle of the bed. The positions of the traps in each of the five beds were randomised. One pot was removed to make room for each of the Chemtica and novel design traps and two pots were removed for the larger cockroach trap. The WeevilGrip trap was wrapped around the base of the pots at Site 1 on the day the traps were set up and thereafter and at Site 2 the trap was placed around the base of the stem of the plant on the growing media to avoid getting too wet. The traps were set up on Monday morning 2 September 2019 at Site 1 and on 23 September at Site 2 and were checked for vine weevils each morning for four consecutive days until the Friday of each respective week. On each day at both sites, after checking the traps and removing any vine weevils, the positions of the four traps in each of the four beds were re-randomised in order to account for any patchy distribution of vine weevils.

Temperatures

Temperatures were recorded at crop canopy height using the grower's 30 MHz sensor at Site 1 and with a USB datalogger at Site 2.

Results

Trap design efficacy

No vine weevils were recorded in any of the traps on any date at either site.

Temperatures

At Site 1, mean 24-hour temperatures during the trapping period were 14-19°C and mean minimum temperatures were 9-14°C. At Site 2, mean 24-hour temperatures during the trapping period were 13-18°C and mean minimum temperatures were 12-15°C. Thus at both sites, mean temperatures remained above the 6°C 'threshold' for vine weevil activity.

Discussion

The four trap designs were shown to trap vine weevil adults when used in semi-field experiments where 40 weevils were released into tent cages with potted strawberry (see Task 2.1. above), with 1-9% of weevils being caught over a 24 hour period, depending on the trap type and the experiment. However, no weevils were caught in any of the traps during the 4-day period they were tested at the two commercial sites with a history of resident vine weevils on the nurseries. This could have been due to various potential reasons including the density of weevils being much higher in the semi-field tent cages than at the commercial sites and host plant density being lower in the semi-field experiments than at the commercial sites where any weevils would have more access to daytime refuges under pots and amongst host plants. Mean day and night temperatures were suitable for weevil activity in both the semi-field experiments and at the commercial sites.

Trap practicality was discussed with the two host growers at the commercial sites. The Chemtica trap was considered to be practical at both sites although the clips holding the upper section to the lower section were considered difficult to use. The cockroach trap was considered to be too large for use in containerised HNS. The Novel trap design 1 was considered easier to assemble than the Chemtica trap but impractical for use outdoors or where overhead irrigation is used due to the inside of the trap getting wet during rain or irrigation. In discussion with the supplier of the Novel trap 1 (Russell IPM), as this was an initial prototype design, an adapted trap without a hole could easily be made. The WeevilGrip trap also became wet and covered with sand and growing media when stood on sandbeds or during rain or irrigation when wrapped round the stem bases on the surface of the growing media and this may have made it less attractive as a weevil refuge. However, even when the WeevilGrip remained dry in the semi-field experiments, it trapped significantly fewer mean numbers of weevils over 24 hours (1%) than the Chemtica trap (5%). In addition, the design of the WeevilGrip is very similar to a hair 'scrunchie' used to secure a pony tail and nursery staff at Site 1 picked one of them up mistaking it for a lost item of clothing, although this could be avoided by appropriate staff training.

Conclusions

- In this experiment, four trap designs were tested under field conditions. Unfortunately no weevils were caught over four days, probably due to very low populations.
- Insights were obtained into the practicalities of using the traps. The ChemTica and new Russell IPM trap were considered easy to use. The cockroach trap was thought to be too large for use in containerised HNS. Apart from the fact that the WeevilGrip "trap" is really only a refuge, allowing the weevils to enter and exit, it became wet and covered with sand and growing media when stood on sandbeds or during rain or irrigation.

Task 2.3 Additional work on potential vine weevil lures (NRI)

Introduction

Several studies have shown that vine weevil adults use plant-derived odours to locate suitable host plants for feeding and oviposition and it is hypothesised that these may also play a role in aggregation. For example, odours of yew, *Taxus baccata,* and spindle, *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 adults 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).

In previous work in this project, volatiles were collected from both intact and cut plants of *Euonymous fortunei.* These were then analysed by gas chromatography (GC) coupled to electroantennographic (EAG) recording of responses from a vine weevil antenna to detect compounds that stimulated olfactory receptors on the antenna which were thus candidate attractants. Up to 22 EAG-active compounds were detected and identified by GC coupled to mass spectrometry and other methods as required (Year 3 Annual Report) and this work has now been published (Roberts *et al.,* 2019b).

Work in the current year aimed to carry out similar work to collect and analyse volatiles from yew which has also been reported to be attractive to vine weevil and has been used in bioassay work during this project. Detection and identification of components which elicited EAG responses from vine weevil antennae would provide candidate attractants which could be compared with those from *E. fortunei*. The two attractive plant sources belong to totally different families, and compounds in common from the two plant sources might be particularly strong candidates for those responsible for this attraction.

Controlled-release dispensers for use with some of the candidate attractants in laboratory and field bioassays were also investigated and release rates measured under laboratory conditions.

Materials and Methods

Plant Material and Collection of Volatiles

Yew branches were obtained from two gardens in Kent. Samples (approx. 70 g) were placed in silanised glass bolt-head flasks (5 l) maintained in a controlled environment room at 25°C, 60% RH and 12:12 L:D cycle. Volatiles were collected on Porapak resin in the same way as used for *E. fortunei* (Year 3 Annual Report; Roberts *et al.,* 2019b). For the two samples, volatiles were collected from 0-6 h and 6-24 h and analysed separately.

Analysis by Gas Chromatography Coupled to Electroantennographic Recording (GC-EAG)

Collections of volatiles were eluted from the Porapak with dichloromethane and analysed by GC-EAG using a polar DBWax GC column (Agilent; 30 m x 0.32 mm i.d. x 0.125 µ film thickness) and oven temperature programme of 50ºC for 2 min then at 20ºC/min to 250ºC for 3 min as previously (Year 3 Annual Report; Roberts *et al.,* 2019b).

For EAG recordings, the vine weevil was anaesthetised with carbon dioxide and an antenna excised with a surgical blade. The cut end of the antenna was inserted into a glass capillary base electrode containing electrolyte (0.1 N KCl with 1% polyvinylpyrrolidine to retard evaporation) and mounted on a silver wire electrode. The distal end was touched with a similar recording electrode to complete the circuit. EAG recordings were made and processed with EZChrom Elite software as described previously (Year 3 Annual Report; Roberts *et al.,* 2019b)

EAG responses to synthetic compounds were recorded similarly using 10 ng injected, i.e. 5 ng to the EAG preparation. Synthetic compounds tested here were 1-hexanol, 1,2-, 1,3- and 1,4-dimethoxybenzene, methyleugenol, eugenol, myrtenal and myrtenol (Figure 13).

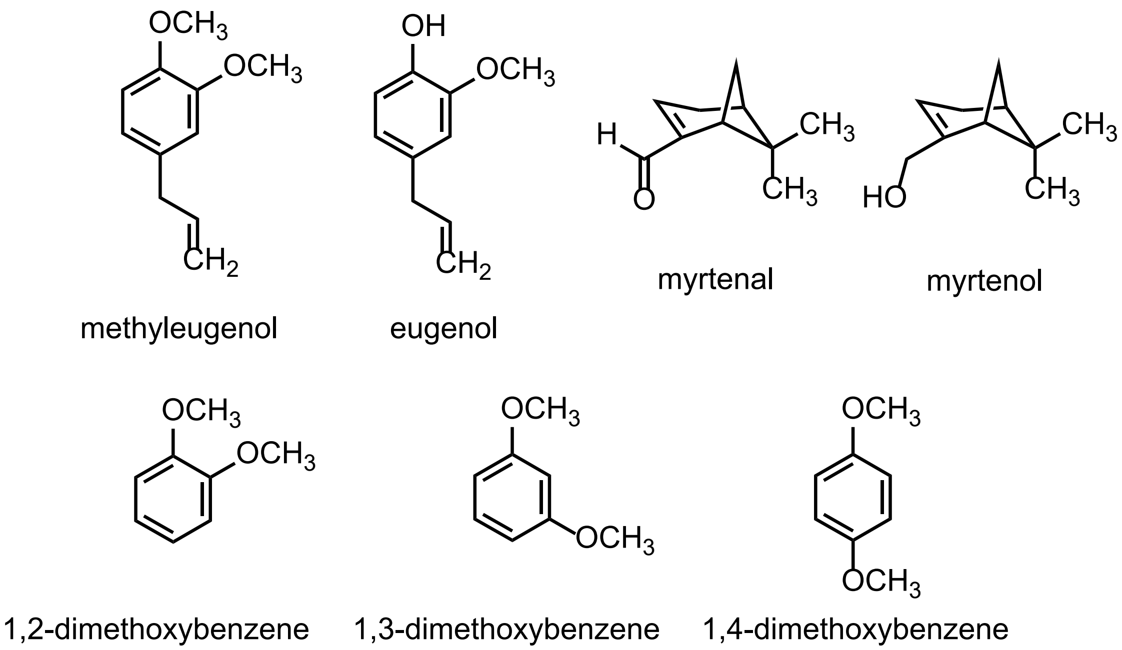

Fig. 13. Structures of synthetic compounds tested for EAG responses from vine weevil antennae

Analysis by Gas Chromatography Coupled to Mass Spectrometric Recording (GC-MS)

GC-MS analyses were carried out on a CP3500 GC (Varian) coupled to a CP2200 Ion Trap Detector (Varian) as previously. The fused silica capillary column (30 mm x 0.25 mm i.d. x 0.25 µm film) was coated with polar 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 (RI) with data in the Pherobase (El-Sayed, 2019). Identifications were confirmed by comparison with the mass spectra and retention indices of authentic compounds.

Controlled Release Dispensers

1,2-Dimethoxybenzene and *cis*-jasmone were formulated as the neat material (100 µl) on a cigarette filter in a sealed polyethylene vial (22 mm x 8 mm x 1.5 mm thick; Just Plastics, London, UK). Two vials for each compound were maintained in a windtunnel (27°C and 8 km/h windspeed) and release rates were measured by weight loss and occasional trapping of volatiles on Porapak followed by GC analysis as described for collection of plant volatiles.

A mixture of equal quantities of 1-hexanol, (*Z*)-3-hexenol, (*E*)-2-hexenol and 1-octanol (0.5 ml) was formulated in a sealed, 1 ml disposable pipette (400 µ thick; Figure 14) and maintained in a laboratory fume hood at 20-22°C. Release rates were measured by periodic weighing of duplicate samples and also by collection of volatiles and quantitative GC analysis against an internal standard (decyl acetate, 5 µg), as described for plant volatiles.

The release rate of (*Z*)-2-pentenol (200 µl) from similar sealed 1 ml disposable pipettes was measured under the same conditions by periodic weighing.

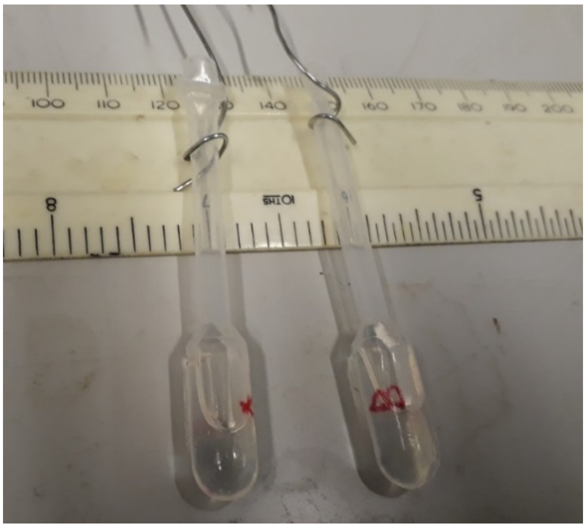

Fig. 14. Sealed 1 ml disposable pipette dispensers

Results

Collection and Analysis of Yew Volatiles

In GC-EAG analyses of volatiles collected from cut yew branches, consistent responses were observed to 20 components, as shown in Figures 15 and 16 and Table 2. Of these, 10 were also detected as EAG-active components of volatiles from *E. fortunei* (shaded in Table 2; Year 3 Annual Report; Roberts *et al.,* 2019b). These were the aldehydes hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, heptanal, octanal, nonanal and decanal, the alcohols 1-hexanol and (*Z*)-3-hexenol, and the sesquiterpene, (*E,E*-)-α-farnesene.

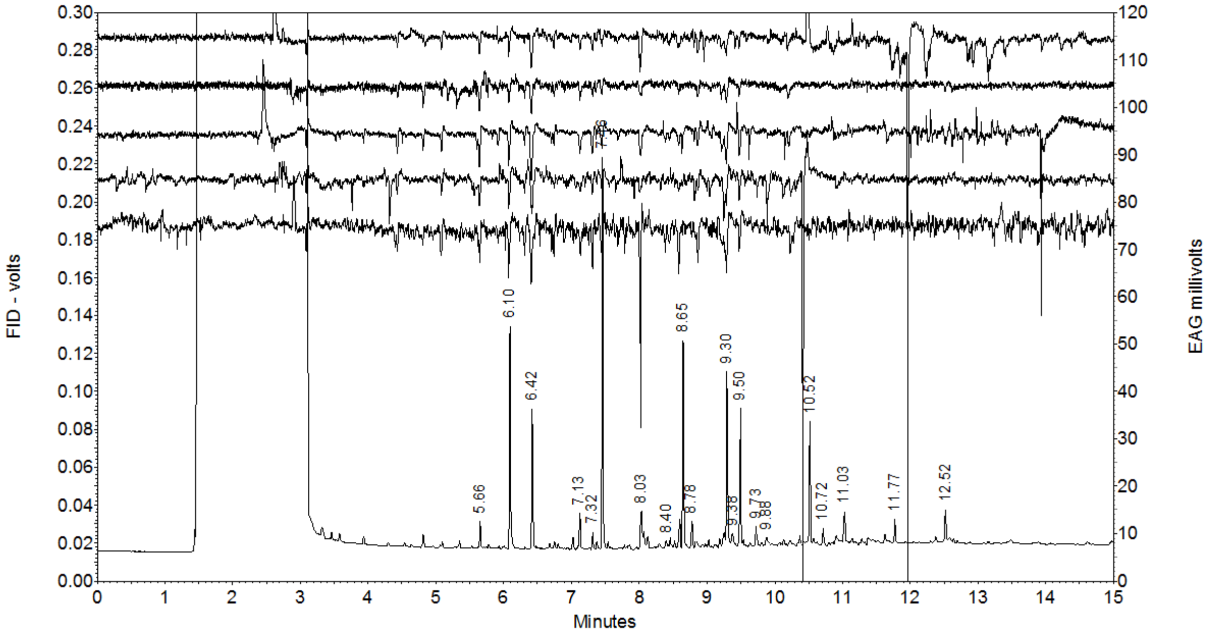

Fig. 15. GC-EAG Analyses of yew volatiles on a polar GC column with vine weevil antenna; upper traces show EAG responses from 5 different antennae; lower trace is FID trace

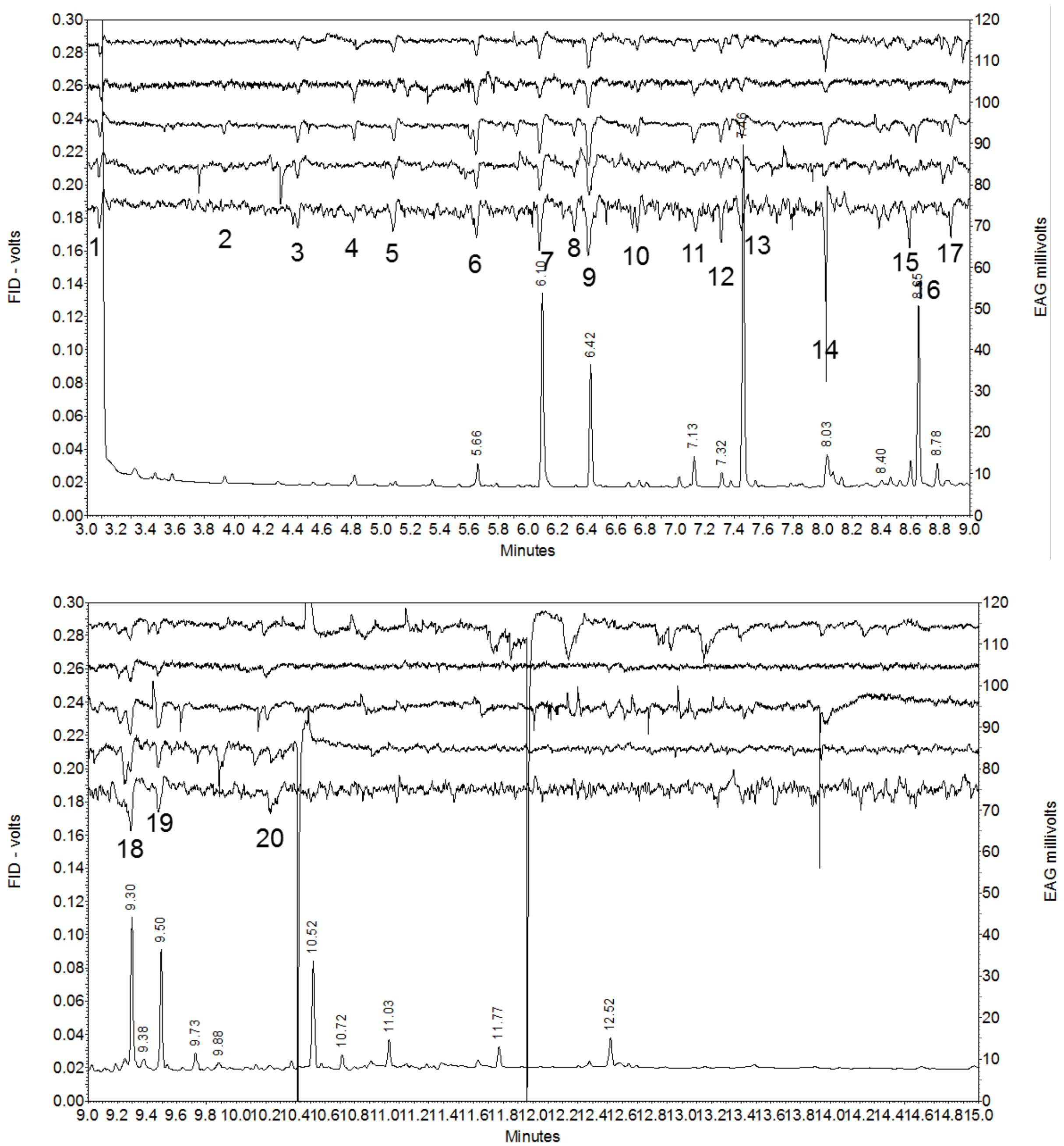

Fig. 16. GC-EAG Analyses of yew volatiles on polar GC column with vine weevil antenna expanded with EAG responses labelled as in Table 1

Table 2. Results of GC-EAG analyses of volatiles collected from yew showing those eliciting an EAG response from receptors on a vine weevil antenna numbered as in Fig. 4, retention times (RT) and retention indices (RI) relative to retention times for *n*-alkanes on a polar GC column, identification and mean relative amounts in four samples (*N* = 4); shaded compounds were observed as EAG-active compounds in volatiles from *Euonymous fortunei* (Year 3 Annual Report; Roberts *et al.,* 2019b).

EAG response	RT (min)	RI	Compound	Area (%; *N*=4) mean	SE
1	3.09	980	pentanal	0.0	0.0
	3.31	1007		0.6	0.4
2	3.93	1083	hexanal	2.1	0.9
3	4.43	1143	(*Z*)-3-hexenal	0.3	0.3
4	4.81	1188	heptanal	1.5	0.3
5	5.11	1227	(*E*)-2-hexenal	0.5	0.4
	5.34	1257		0.9	0.4
6	5.64	1297	octanal	2.5	0.7
7	6.08	1356	hexanol	15.0	3.9
8	6.31	1387	(*Z*)-3-hexenol	2.0	1.4
9	6.41	1400	nonanal	12.5	3.9
10	6.74	1444	1-octen-3-ol	0.4	0.3
	7.01	1480	2-ethylhexanol	0.8	0.1
11	7.12	1510	decanal	2.4	0.3
12	7.31	1540	benzaldehyde	1.0	0.1
13	7.45	1561	octanol	13.0	1.7
	7.70	1600		0.5	0.5
	7.83	1623	β-caryophyllene	1.8	0.9
	7.97	1647		0.2	0.2
14	8.03	1658	phenylacetaldehyde	1.5	0.7
	8.49	1739	germacrene D	0.7	0.4
	8.51	1742	bisabolene	0.1	0.1
	8.57	1753		0.3	0.3
15	8.60	1758	(*E,E*)-α-farnesene	0.0	0.0
16	8.64	1765	decanol	6.6	1.3
	8.77	1788	bisabolene	0.3	0.3
17	8.83	1798	methyl salicylate/myrtenol	1.3	0.3
	9.02	1835		0.7	0.4
	9.34	1897	hexanoic acid	0.1	0.1
18	9.29	1887	benzyl alcohol	6.0	0.9

	9.37	1903	nonadecane	2.5	0.8
19	9.49	1926	2-phenylethanol	4.7	0.7
	9.72	1971	dodecanol	1.8	0.2
	9.80	1986		0.2	0.2
	9.87	2000	eicosane	0.7	0.2
20	10.35	2101	octanoic acid	0.3	0.3
	10.52	2137	phytone	3.9	1.2
	10.63	2160	nonanoic acid	0.2	0.2
	10.71	2177	tetradecanol	1.5	0.3
	10.92	2223		0.4	0.3
	11.03	2248	isopropyl hexadecanoate	1.7	0.6
	11.14	2273	decanoic acid	0.2	0.2
	11.26	2300	tricosane	1.7	0.8
	11.52	2359	di-t-butyl-phenol	0.3	0.3
	11.63	2384	hexadecanol	0.2	0.2
	11.68	2395		0.1	0.1
	11.77	2416	hexadecanolide	0.4	0.4
	12.52	2586	octadecanol	2.7	0.8
	12.58	2600		0.5	0.5
	12.65	2616	phytol	0.2	0.2
	13.64	2732	eicosanol	0.2	0.2

The most abundant compounds were mostly primary alcohols. 1-Hexanol, (*Z*)-3-hexenol, octanol, decanol, benzyl alcohol and 2-phenylethanol all elicited EAG responses. Myrtenol was detected and may have been responsible for EAG response 17, although it chromatographed very close to methyl salicylate. 1-Dodecanol, tetradecanol, hexadecanol, octadecanol and eicosanol were also present. Other significant components were isopropyl hexadecanoate, hexadecanolide and 6,10,14-trimethylpentadecan-2-one (phytone), but these did not elicit EAG responses.

(*E*)- And (*Z*)-2-pentenol, compounds reported to be present in volatiles from *E. fortunei* and attractive to vine weevils by van Tol et al. (2012) could not be detected (RT 5.76 min and 5.82 min respectively; RI 1313 and 1321 respectively). Similarly, methyleugenol and eugenol (RT 9.97 min and 10.73 min; RI 2015 and 2175 respectively) also could not be detected.

EAG Responses to Synthetic Compounds

EAG responses to synthetic compounds were measured using the GC-EAG system to ensure complete volatilisation of the compound. Test compounds were co-injected with 1-hexanol to confirm that the preparation was responsive.

No consistent EAG response was observed to methyleugenol, although a small EAG response was elicited by eugenol (Figure 5).

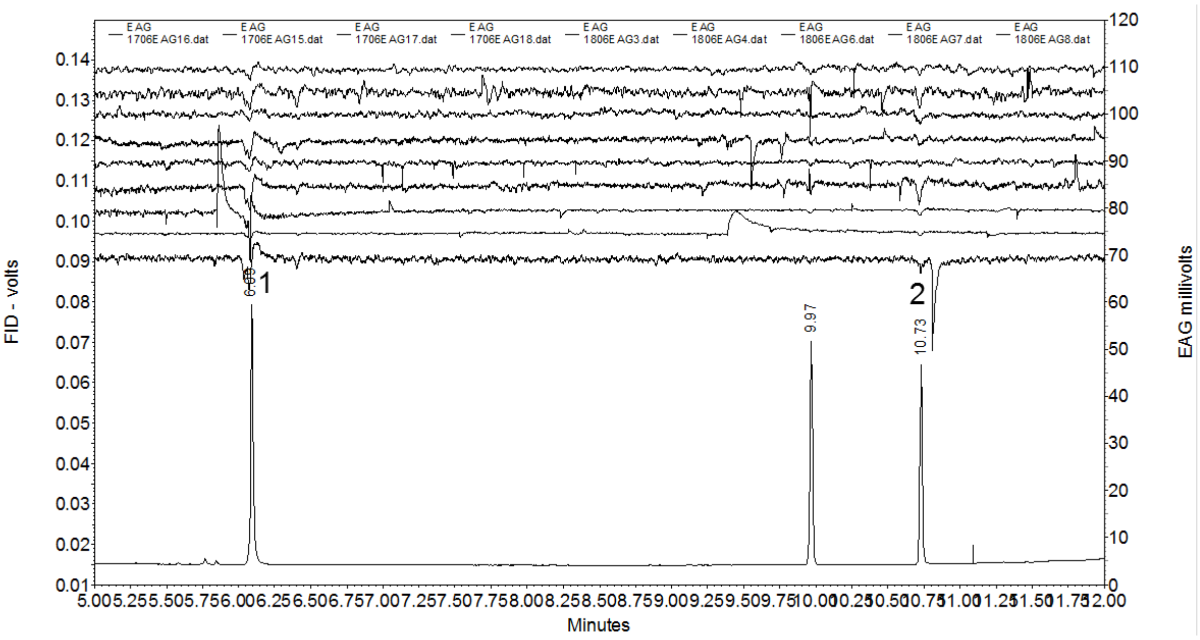

Fig. 17. GC-EAG Analyses of 1-hexanol (6.09 min), methyleugenol (9.97 min) and eugenol (10.73 min) showing EAG responses to 1-hexanol (1) and eugenol (2) but only occasional possible small response to methyleugenol.

1,2-Dimethoxybenzene (veratrole) was previously detected in volatiles from *E. fortunei* and elicited an EAG response from vine weevil antennae at very low levels (Year 3 Annual Report; Roberts *et al.,* 2019b). The synthetic compound also elicited a strong EAG response (Figure 18) and, remarkably, the 1,4- and 1,3-isomers did not. Initially all three isomers were analysed together (Figure 18). The 1,2-isomer eluted first and it was thought possible that the antenna had not recovered by the time the other two isomers eluted, but no response was observed to these when they were analysed separately (Figures 19 and 20).

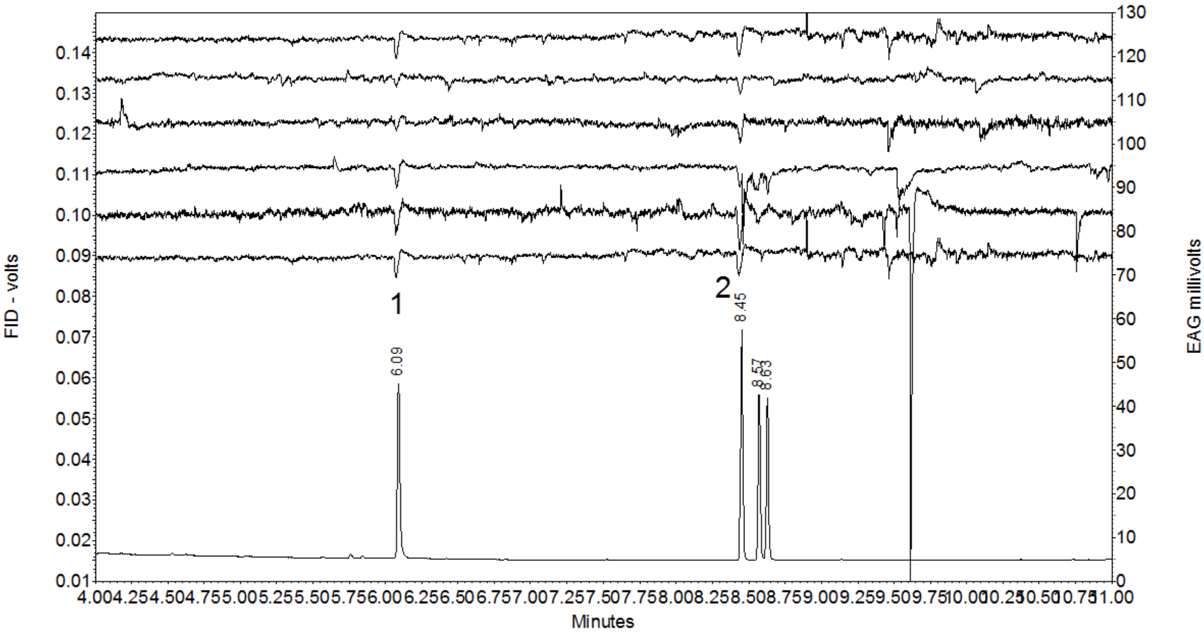

Fig. 18. GC-EAG Analyses of 1-hexanol (6.09 min), 1,2-dimethoxybenzene (8.45 min), 1,4-dimethoxybenzene (8.57 min) and 1,3-dimethoxybenzene (8.63 min) showing EAG responses to 1-hexanol (1) and 1,2-dimethoxybenzene (2).

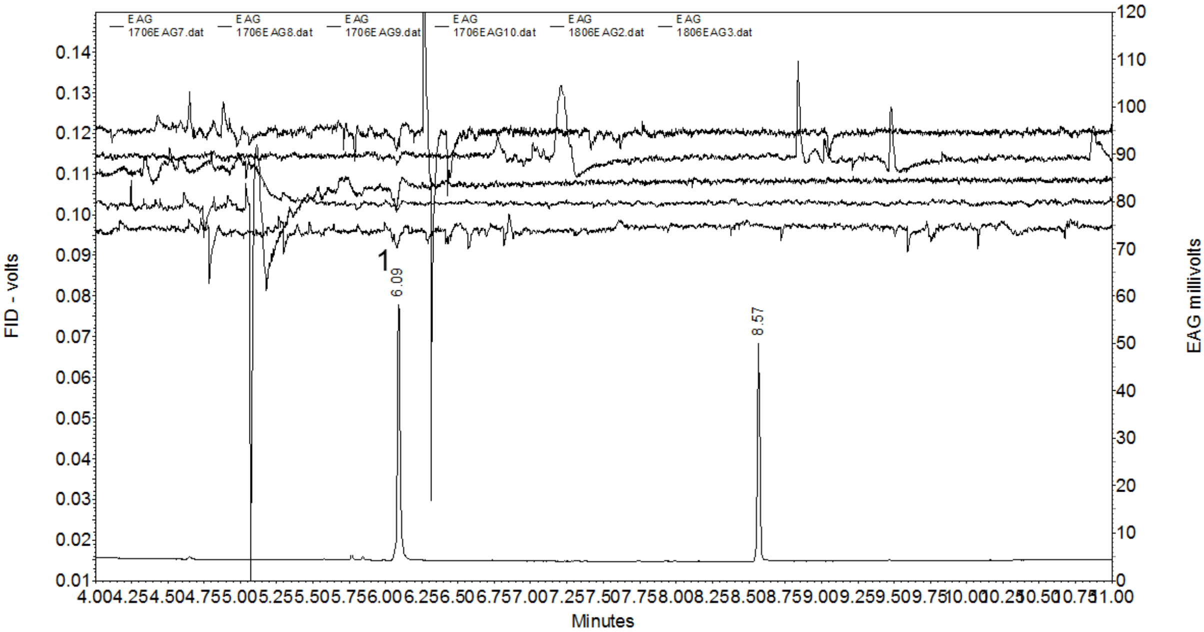

Fig. 19. GC-EAG Analyses of 1-hexanol (6.09 min) and 1,4-dimethoxybenzene (8.57 min) showing EAG response to 1-hexanol (1) but not to 1,4-dimethoxybenzene.

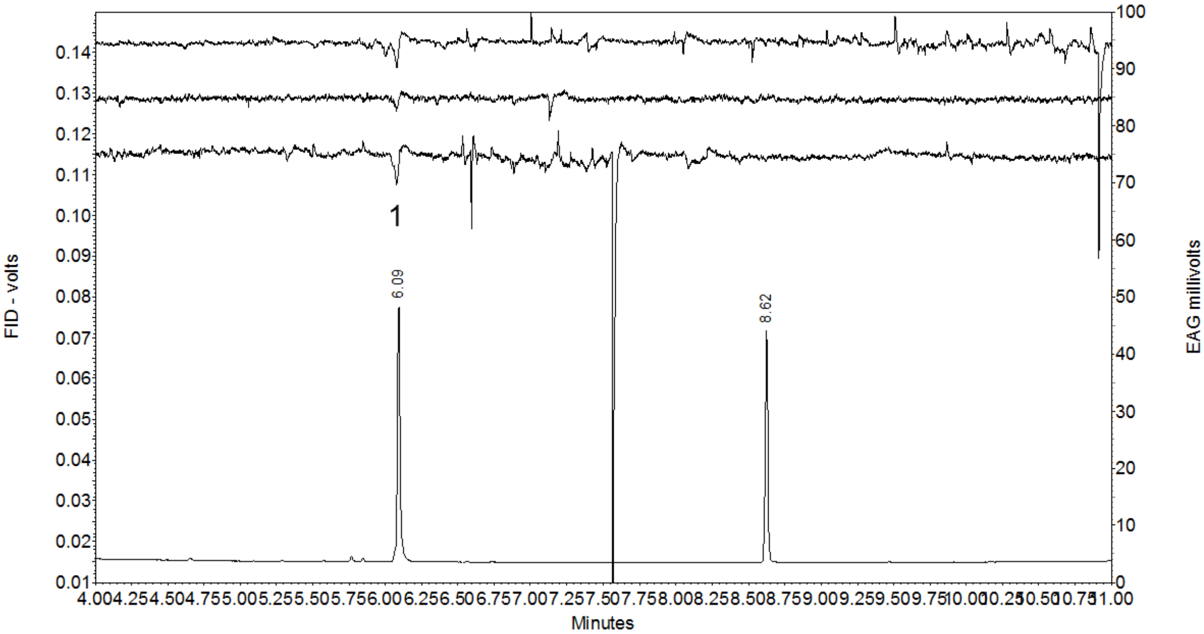

Fig. 20. GC-EAG Analyses of 1-hexanol (6.09 min) and 1,3-dimethoxybenzene (8.62 min) showing EAG response to 1-hexanol (1) but not to 1,3-dimethoxybenzene.

Myrtenol elicited an EAG response from vine weevil antennae (Figure 9), adding further evidence that this was present in the volatile collections from yew and may have been responsible for EAG response 17 (Table 1 and comment above). The corresponding aldehyde, myrtenal, elicited an EAG response in some runs (Figure 21).

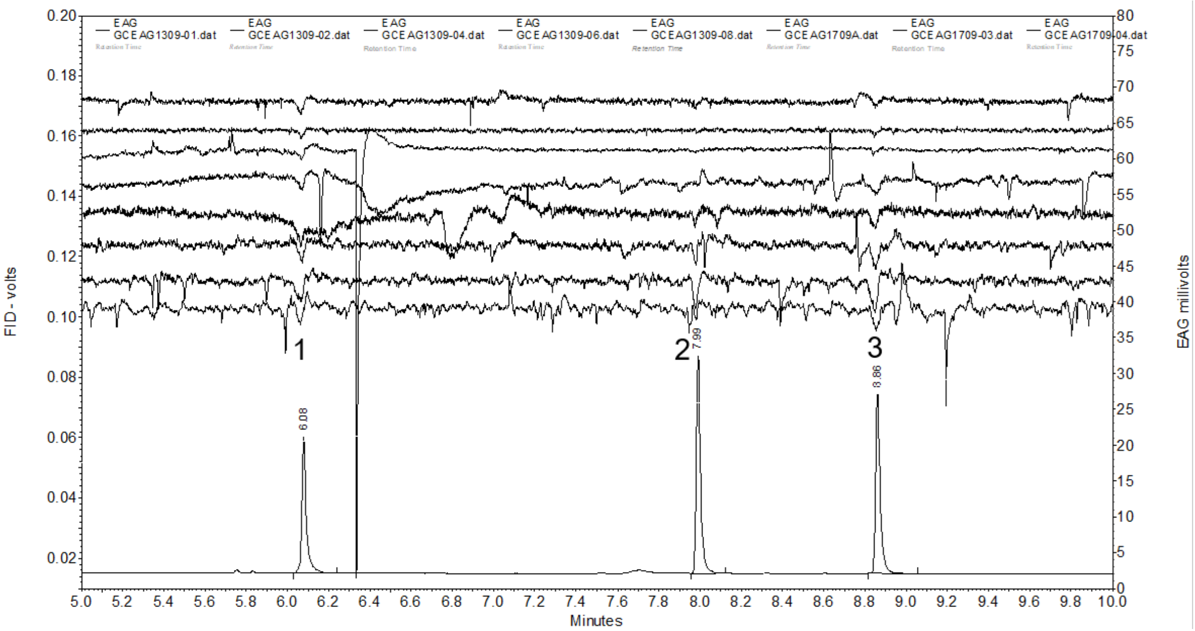

Fig. 21. GC-EAG Analyses of 1-hexanol (6.08 min), myrtenal (7.99 min) and myrtenol (8.86 min) showing consistent EAG responses to 1-hexanol (1) and myrtenol (3) and occasional responses to myrtenal (2).

Release Rate Studies

1,2-Dimethoxybenzene was released uniformly at 0.74 mg/d from polyethylene vials for over 100 d at 27°C and 8 km/h windspeed (Figure 10). *cis*-Jasmone was released uniformly at 0.52 mg/d for over 50 d but then seemed to flatten out (Figure 22).

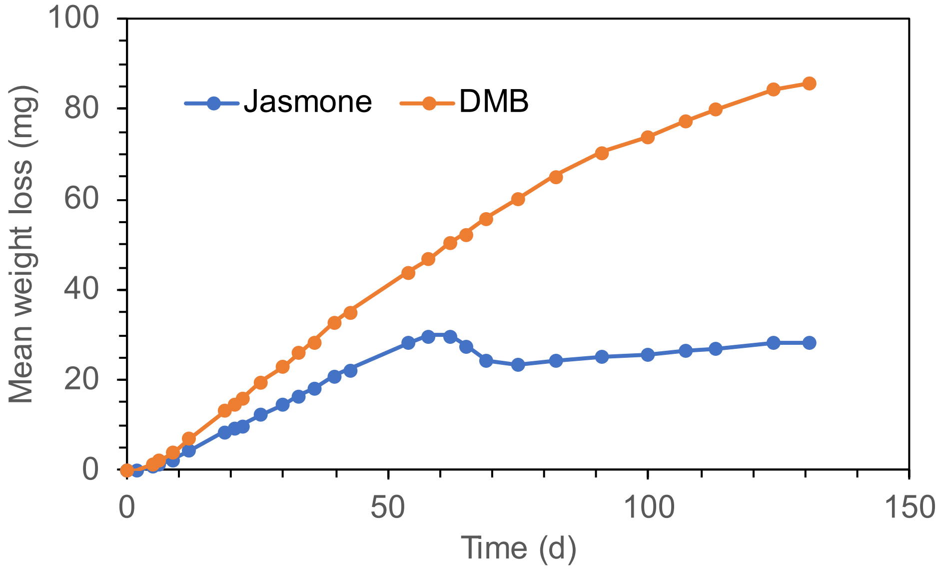

Fig. 22. Release of 1,2-dimethoxybenzene (DMB) and *cis*-jasmone (jasmone) from polyethylene vials (100 µl on cigarette filter) at 27ºC and 8 km/h windspeed as measured by weight loss

(*Z*)-2-Pentenol was released uniformly at 1.1 mg/d from 1 ml disposable pipettes at 20-22°C (Figure 23).

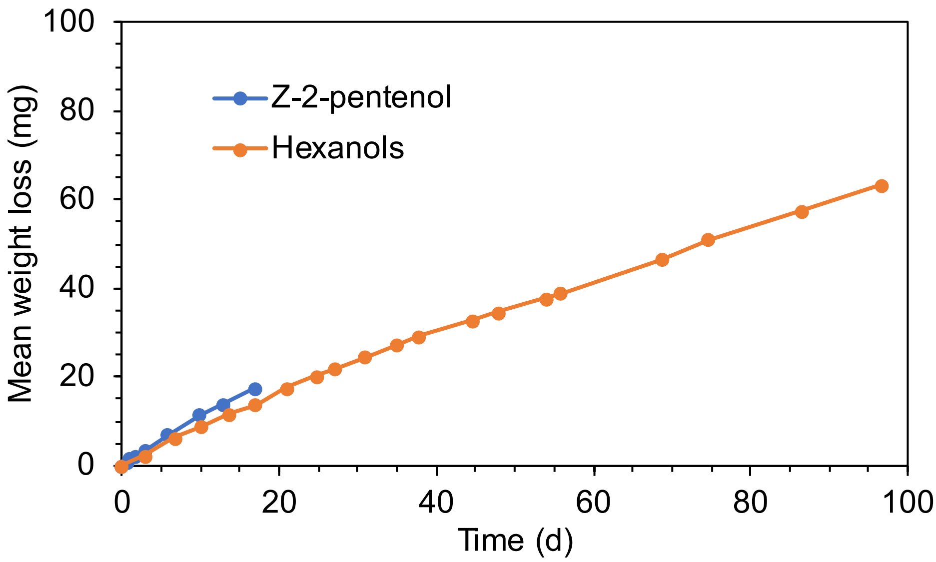

Fig. 23. Release of (*Z*)-2-pentenol (Z-2-pentenol) and a mixture of equal quantities of 1-hexanol, (Z)-3-hexenol, (E)-2-hexenol and 1-octanol (hexanols) from 1 ml disposable pipettes at 20-22°C as measured by weight loss

A mixture of equal quantities of 1-hexanol, (Z)-3-hexenol, (E)-2-hexenol and 1-octanol was released at 0.7 mg/d for over 100 d from the pipettes under the same conditions (Figure 23). Trapping and analysis of the volatiles released showed that the relative release rates of the compounds remained remarkably constant (Figure 24).

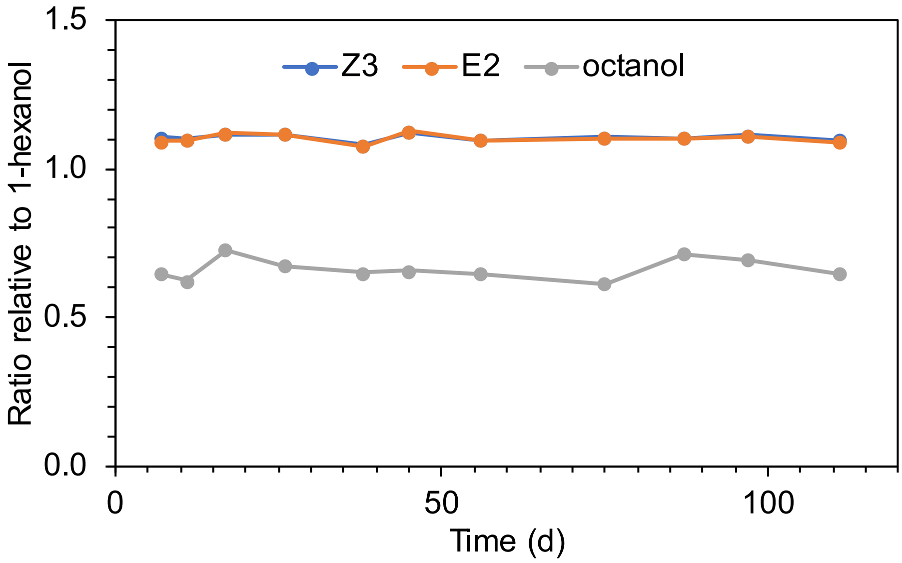

Fig. 24. Release rates of (*Z*)-3-hexenol (Z3), (*E*)-2-hexenol (E2) and 1-octanol (octanol) relative to the release rate of 1-hexanol from 1 ml disposable pipettes at 20-22°C

Discussion

Cut branches of yew, *Taxus baccata*, have been shown to be attractive to vine weevils in this work and by van Tol et al. (2002) and above in Task 2.1. In GC-EAG analyses of volatiles collected from yew, 20 components were observed to elicit EAG responses from vine weevil antennae. Of these, 10 were previously observed in volatiles from *E. fortunei*, another plant attractive to vine weevil (Year 3 Annual Report; Roberts *et al.,* 2019b). These were mainly simple aldehydes and primary alcohols which are fairly ubiquitous plant volatiles, consistent with the fact that vine weevils are attracted to a wide range of host plants and that they are attracted to blends of compounds rather than to single compounds (Roberts et al., 2019b).

Somewhat surprisingly, there is little literature on the composition of volatiles from yew, presumably due to the toxicity of the plant which is due to non-volatile alkaloids. Yasar (2013) analysed essential oils from yew leaves from Turkey and found the most abundant compounds were primary and secondary aliphatic alcohols, consistent with the findings here. The most abundant component was 1-octen-3-ol which was observed as a minor, EAG-active component in our work. Yasar (2013) also reported 1-hexanol, (*Z*)-3-hexenol and (*E*)-2-hexenol, observed in our work, but only small amounts of the aldehydes were observed. It is possible they were oxidised to the acids during distillation of the essential oil used by Yasar (2013). Radulović et al. (2010) and Stefanović et al. (2016) reported similar results from Serbian populations of yew.

More unusual compounds identified in volatiles from yew during our work were isopropyl hexadecanoate, hexadecanolide, 6,10,14-trimethyl-2-pentadecanone (hexahydofarnesyl acetone, phytone), and myrtenol, although only the latter elicited an EAG response from vine weevil antennae. Radulović et al. (2010), Yassar (2013) and Stefanović et al. (2016) also observed significant amounts of phytone and myrtenol in yew essential oils.

In all our work on volatiles from *E. fortunei* and yew, neither (*E*)- nor (Z)-2-pentenol could be detected, although these were well resolved from each other and the solvent front on the polar GC columns. These were reported to be present in volatiles from *E. fortunei* by van Tol et al. (2012). The synthetic compounds were previously shown to elicit EAG responses from vine weevil antennae (Year 3 Annual Report; Roberts *et al.,* 2019b), like other aliphatic, primary alcohols such as the six-carbon analogues. Similarly we could not detect methyleugenol, also reported by van Tol et al. (2012), and the synthetic compound did not elicit a significant EAG response, although eugenol did.

Among other synthetic compounds tested here, myrtenol elicited an EAG response from vine weevil antennae, consistent with that observed from the compound in yew volatiles.

Vine weevil antennae give EAG responses to a wide range of compounds, particularly primary alcohols and aldehydes. However, they do show a remarkable specificity to the isomeric dimethoxybenzenes. A strong response is elicited by the 1,2-isomer which was observed as a minor component in volatiles from *E. fortunei* (Year 3 Annual Report; Roberts *et al.,* 2019b), but the 1,3- and 1,4- isomers elicited no detectable response. In the bioassay work reported here, 1.2-dimethoxybenzene seemed to be repellent to vine weevils at the dose tested.

Sealed polyethylene vials could be useful controlled release dispensing devices for *cis*-jasmone and 1,2-dimethoxybenzene and the release rates were probably much higher than from the pipette tip dispensers used in the bioassay work reported here. Sealed disposable pipettes were also shown to be good dispensers for a blend of primary alcohols that could be tested in future bioassay work.

Conclusions

- In previous work, volatiles were collected from *Euonymous fortunei*, known to be attractive to vine weevils, and collections were analysed by GC coupled to EAG recording from vine weevil antennae to detect candidate attractants. This year, volatiles were similarly collected and analysed from yew, also demonstrated to be attractive to vine weevils. At least 20 compounds in volatiles from yew elicited EAG responses, with 10 compounds in common with those from *E. fortunei*.. These were mostly aliphatic aldehydes and alcohols which are fairly ubiquitous plant volatiles and are strong candidates for attractants for vine weevil.
- (*E*)- and (*Z*)-2-Pentenol and methyleugenol were reported by other workers to be present in volatiles from *E. fortunei* and responsible for its attractiveness to vine weevil, but these compounds could not be detected in this work, either from *E. fortunei* or yew.
- Apart from aliphatic alcohols and aldehydes, other compounds shown to elicit EAG responses from vine weevil were *cis*-jasmone, 1.2-dimethoxybenzene, eugenol and myrtenol.
- Various controlled-release dispensing systems for candidate attractants were investigated under laboratory conditions, including pipette tips, polyethylene vials and sealed polyethylene disposable pipettes

Knowledge and Technology Transfer

Industry Presentations

13 February 2019 – AHDB Hardy Nursery Stock Research and Development update, Stirling, Scotland (ADAS)

4 July 2019 – Herbaceous Perennial Technical Discussion Group, Syngenta, Jealotts Hill (ADAS)

16 January 2020 – HTA Contact Conference (Harper Adams)

Publications

Roberts, J. M., Jahir, A., Graham, J. & Pope, T. W. (2019) Catch me if you can: the influence of refuge / trap design, previous feeding experience, and semiochemical lures on vine weevil (Coleoptera: Curculionidae) monitoring success. Pest Management Science. https://doi.org/10.1002/ps.5545

Roberts, J. M., Kundun, J., Rowley, C., Hall, D. R., Douglas, P. & Pope, T. W. (2019) Electrophysiological and behavioural responses of adult vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae), to host plant odors. Journal of Chemical Ecology. 45: 858-868.

Media coverage

20th November 2019 – BBC Midlands Today (Harper Adams)

20 November 2019 – BBC Radio Shropshire (Harper Adams)

27 November 2019 – BBC Farming Today (Harper Adams)

27 November 2019 – BBC Radio Four – You and Yours (Harper Adams)

16 January 2020 – HTA Contact Conference (Harper Adams)

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