Project title: Characterising vine weevil aggregation pheromone for use in traps at soft fruit and nursery sites

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Project leader: Dr Alison Karley, The James Hutton Institute, Dundee

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Key staff: Dr Tom Shepherd, JHI
Professor David Hall, NRI
Robbie McLaren, JHI
Dr Scott Johnson (former JHI project leader)

Location of project: The James Hutton Institute and NRI

Industry Representative: Richard Stanley, Farmline, Rectory Farm, Stanton St John, Oxford, OX33 1HF

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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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[Organisation]

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[Name]
[Position]
[Organisation]

Signature ....   Date ....

Report authorised by:

[Name]
[Position]
[Organisation]

Signature ....   Date ....

[Name]
[Position]
[Organisation]

Signature ....   Date ....

CONTENTS

GROWER SUMMARY

Headline

- Candidate plant-derived compounds were identified that either attracted ((*E*)-2-hexenol) or repelled (1-hexanol and (*Z*)-3-hexenol) vine weevils.

Background and expected deliverables

Vine weevils (Figure 1a) remain damaging pests of soft fruit and hardy nursery stock. Heavily infested plantations can reduce fruit yield by 50–60% if untreated and can increase the incidence of problems such as crumbly fruit (Figure 1b). Similar reductions in woody plant growth are also apparent with ameliorative measures. Developing effective control strategies is therefore needed. One possibility is the inclusion of chemical attractants that can be incorporated into lure-and-kill traps, which circumvents increasingly stringent legislation on the use of insecticides.

Figure 1.

(a) Adult vine weevil *Otiorhynchus sulcatus*. (b) Damage by weevil feeding leads to 'crumbly fruit'.

This pilot project aimed to identify chemical compounds that could be used in lure-and-kill traps, with an initial hypothesis that an aggregation pheromone might be released and transmitted between adult vine weevils.

Summary of the project and main conclusions

It was found that odours from frass did not attract other weevils, as previously thought, but increased the activity of weevils. The frass emitted high levels of α-farnesene, β-caryophyllene and germacrene-D which may underpin this.

A typical problem found when attracting vine weevils into traps is that a high proportion of the insect population is unresponsive and do not readily move, .which emphasises the need to identify effective attractant compounds. It was found that plant-derived cues, which were dominated by green leaf volatile compounds, were far more attractive than insect-derived volatiles and showed greater potential for use in traps, which became the main focus of this research.

Detailed analysis of the volatile composition of leaf, insect and frass material identified groups of chemicals that might provide cues for insect behaviour, particularly green leaf volatiles (in leaves) and sesquiterpenes (in frass). The analysis was refined by examining antennal electrical responses to volatiles emitted in blends derived from plant material and from purified synthetic sources. Strong and reproducible antennal responses were detected to three green leaf volatile compounds, 1-hexanol, (*E*)-2-hexenol and (*Z*)-3-hexenol.

Synthetic sources of the green leaf volatiles 1-hexanol, (*E*)-2-hexenol and (*Z*)-3-hexenol were tested in a series of behavioural experiments. At intermediate concentrations, (*E*)-2-hexenol attracted 100% of responsive weevils (i.e. those moving) into the chamber containing this compound, and 55% of all weevils tested overall. The compounds 1-hexanol and (*Z*)-3-hexenol were generally repellent to insects. While repellent compounds are unsuitable for use in lure-and-kill traps, this initial work suggests that they might be of use in deterring colonization of weevils from outside the main crop, whilst attractants might be used in traps within the crop.

Financial benefits

As this was a pilot study, it is difficult to identify financial benefits. However, based on the estimates of damage within a four year large scale field trial using raspberry (Clark *et al.*, 2012), some figures can be calculated. If the successful attraction of 55% of all vine weevils by (*E*)-2-hexenol into chambers with this compound were to be directly extrapolated into field captures, this would reduce damage to plants and limit yield losses (Figure 2).

Figure 2. Projected protection to yield loss and reduced plant vigour if traps captured and removed weevils in a directly equivalent manner. Figures based on Clark *et al.* (2012) field trials.

Action points for growers

- The nature of this pilot study is inappropriate for making prescriptive management recommendations, although several aspects can be developed that may lead to effective control recommendations.

- The attractive compound might be included in traps for catching adult weevils already within the crop, potentially supplemented with the mobility stimulating compounds (identified from the excrement).

- Trap captures of vine weevils are frequently low and this needs to be addressed if this approach is to be successful. A recent trial (Van Tol *et al.*, 2012), for example, included weevils in the vicinity of traps in the trap count, but these insects could still contribute to crop damage.

- Prevention of invading weevils may benefit from inclusion of repellent compounds in dispensers at the perimeter of the crop.

SCIENCE SECTION

Introduction

The problem

Vine weevil (*Otiorhynchus sulcatus*) is a significant pest of soft fruit and ornamental horticultural production, and accounts for significant losses in both sectors. This situation is likely to worsen with the withdrawal of many pesticides, either because of new legislation or, in the case of edible crops, with the demand to produce residue-free fruit. For example, a four year study (Clark *et al.*, 2012) at the James Hutton Institute quantified the effects of vine weevils on raspberry yield in the absence of control measures and showed a massive decrease in yield (Figure 3) in Glen Ample (66%) and Glen Rosa (49%). While these losses represent an extreme scenario in which no preventative or ameliorative action is taken, it illustrates the potential damage that vine weevils can cause (e.g. £50 million per annum in raspberry alone) in the absence of control measures. Moreover, weevils reduced berry size by 45% in Glen Ample, which increases picking time and costs. Added to this, the widespread use of polytunnels for growing soft fruit significantly speeds up the development time and reproduction of vine weevils (Johnson *et al.*, 2010).

Low vine weevils ○   High vine weevils ●

Figure 3. Effects of vine weevil infestation on raspberry yield three years after being introduced to tunnels. Yield losses indicate those that would occur if no attempt was made to control populations.

In nursery production, estimates of damage are less precise because of the diversity of crop species grown in nursery environments. However, extrapolating the effects of vine weevils on growth of other woody plants (e.g. raspberry) indicates that the loss of growth could be considerable (Figure 4). In addition, even modest herbivory by weevils causes notching of leaves by adults and reduces the attractiveness of plants, making them ultimately less valuable.

Figure 4. Growth of roots and shoots as affected by low (open bars; 0-2 weevils) and high (black bars; > 10 weevils) levels of weevil infestation.

Overall Aim of the project

Previous work at JHI and research by other workers suggested that vine weevils often aggregate and this aggregation is mediated by volatile signals (i.e. not by visual or taste cues). The identity of volatile compound(s) is not presently known. The aim was to identify potential sources of attraction for vine weevils and assess which showed most potential for inclusion in control measures.

Materials and methods

Insect cultures

Adult vine weevils were recovered from soft fruit plantations (comprising raspberry, blackcurrant and strawberry) at the James Hutton Institute and maintained alongside existing insect cultures (Clark *et al.*, 2011; Coyle *et al.*, 2011). Weevils were kept in Petri dishes containing a thin layer of paper towels on the bottom (cut into disks to provide an absorbent "floor"), 1-2 *Fragaria x ananassa* leaves, and a small volume of tap water which

was sprayed directly on to the paper towels. The weevils were transferred to clean Petri dishes with fresh paper towels, leaves and water twice a week as necessary. The Petri dishes were kept in a controlled temperature room at 18°C with a photoperiod of L16:D8.

Source of the cues: olfactometer experiments

A still air olfactometer (similar to that used by Van Tol & Visser, 2002 and Van Tol *et al.*, 2002) was constructed from inert plastic to provide an arena for testing vine weevil attraction to conspecific insects, to strawberry leaves, and to weevil frass (Figure 5). The olfactometer consisted of a central release chamber and two treatment chambers, the latter divided into a lower and upper compartment. The central release chamber consisted of a Petri dish (14.3 cm diameter and 1.5 cm deep) with two holes, 1.4 cm diameter and 4.5 cm apart, in the dish lid. Small tubes (1.6 cm diameter and 2 cm high) were placed on top of these holes to provide access to the treatment chambers, but to prevent insects from returning to the central release chamber. The treatment chambers were placed over the access tubes and comprised two plastic pots (4.1 cm diameter and 4.8 cm high, with a 2.6 cm diameter hole in the bottom) stacked together and separated by a piece of muslin gauze; the upper compartment was held in place over the lower compartment using a cylinder of thin card. Insects placed in the central chamber (Petri dish) could, therefore, enter the lower compartments of either of the treatment chambers, but were prevented from entering the upper compartment by the muslin gauze barrier. Five still air (SA) olfactometers were assembled in a dark controlled-temperature room (20°C) for each experimental replicate. Five weevils that had been starved for 24 h prior to the experiments were placed in the central release chamber of each olfactometer, and sealed in by rapidly assembling the rest of the olfactometer apparatus. Weevils were allowed to explore the apparatus for 2 hours and the number of weevils in each chamber was recorded hourly. For each replicate, a control (empty chamber) and a treatment (strawberry leaf material, live insects or insect frass) were assigned randomly to each of the treatment chambers. The treatment combinations were:

- control vs control;
- control vs five starved weevils;
- control vs 20 frass pellets;
- control vs torn strawberry leaves.

The orientation of each Petri dish was turned through 90º with each experiment, and the apparatus was cleaned thoroughly with methanol between experiments to minimize the influence of contaminant volatiles on insect behaviour.

Figure 5. Still Air Olfactometer. Five starved weevils were placed in the central release chamber and the treatment chambers were assembled on top. Weevils were allowed to explore the apparatus for two hours and the number of weevils in each chamber was recorded hourly. Muslin gauze prevented the weevils from direct contact with the treatments.

A moving air (MA) olfactometer was constructed, from inert plastic and silicone rubber, in order to investigate the attractiveness of conspecific insects, strawberry leaves, and insect frass to vine weevils. The apparatus consisted of five polypropylene screw-cap containers linked together in a U-shape by 4 lengths (2 x 10 cm and 2 x 15 cm) of PTFE tubing (Figure 6). Silicone rubber sheaths were used to seal the joints between PTFE tubes and the polypropylene chambers. The apparatus was set up in a dark controlled-temperature room (20°C). Air was pumped into the olfactometer through a molecular sieve and an activated charcoal filter, entered each arm of the olfactometer (maintained at a flow rate of 800 ml min^{-1}), and passed through the choice chambers and finally the central release chamber. This set-up ensured that air flowed over the treatments assigned to each treatment chamber and into the two choice chambers and exited through a valve in the central release chamber. Insects that moved out of the central release chamber were prevented from contacting the treatment material by muslin gauze barriers (Figure 6).

Treatments were assigned randomly to each of the two treatment chambers. After the air flow had been established, ten mature adult weevils that had been starved for 24 h prior to the experiment were placed in the central release chamber and were left to explore the apparatus for three hours. The number of weevils in each chamber was recorded hourly. Weevils could choose to remain in the central release chamber or travel through the PTFE

tubes into the choice chambers. The experiments were conducted in the dark, beginning one and five hours after the end of the photoperiod in the insect culture room. The treatments were:

- control vs five starved weevils;
- control vs 20 frass pellets; control vs three torn strawberry leaves.

The apparatus was cleaned thoroughly with methanol between experiments to minimise the influence of contaminant volatiles on insect behaviour.

Figure 6. Moving Air Olfactometer. Treatments were placed in the Treatment Chambers and an air flow was passed through these chambers towards the Central release Chamber. Ten starved weevils were released into the Central Chamber and allowed to explore for three hours; the number of weevils in each chamber was recorded hourly. Muslin gauze prevented the weevils from direct contact with the treatments.

8

Range of signal detection: Linear olfactometer

A linear olfactometer, of total length 3 m, was assembled from two 1,500 mm length glass tubes with removable ends and drill holes to accommodate an insect release hole and vent holes for air inflow (Figure 7). Air was pumped into the apparatus through a molecular sieve and an activated charcoal filter, entering each end of the olfactometer at a flow rate of 800 ml min^{-1}, regulated by flow meters, with air outflow through the insect release hole. Experiments were performed in the dark at 20°C. The treatment comprised crushed strawberry leaves, which appeared the most attractive material in moving air olfactometer experiments. The treatment was assigned randomly to either end of the olfactometer tube, positioned at 450 mm or 1,500 mm from the insect release hole. An insect that had been starved for 24 h prior to the experiment was released through the central hole and the position of the insect was recorded over a period of an hour. After an hour, insects that had not moved towards either end of the olfactometer were recorded as making no choice. Forty-three experiments were performed.

Figure 7. Apparatus for testing the range of signal detection by adult weevils.

Volatile Entrainment

In total, 38 volatile samples were collected across seven different sample types. For some insect samples, a muslin gauze barrier was inserted to prevent insects making direct contact with the SPME fibre. The muslin gauze was clipped on to the vial using inert copper wire and the vial was sealed within a copper frame supporting an inert plastic (polyethyleneterephthalate, PET) film to isolate the headspace and minimize external contaminants. The remaining insect samples were collected from insects sealed into the vial, which prevented external contamination but required constant monitoring throughout the volatile entrainment to ensure that the insects did not touch the fibre. Thus, the sample types were controls (sealed empty vials, n=5), weevil frass (n=5), crushed strawberry leaves (n=5), five pre-reproductive adult weevils (n=6), five egg-laying adult weevils (n=4), muslin

9

controls (empty vials with muslin gauze, n=5) and five mixed-age weevils (vials with muslin barrier, n=8).

In each sample, volatiles were entrained using a Supelco solid phase micro extraction (SPME) fibre with polydimethylsiloxane and divinylbenzene (PDMS/DVB) fibre chemistry. Samples were contained in clean 10 ml glass vials sealed with either an airtight septum or an airtight plastic film covering. The SPME fibres were exposed to samples by piercing the seal, and volatiles were entrained for 15 minutes (for septum sealed vials) or for 30 minutes (for plastic-sealed vials containing a muslin barrier). Frass (*c.* 30 droppings for each sample) was collected from the weevil cultures using sterilised forceps and was transferred to a 10 ml glass vial, which was sealed immediately to prevent volatile loss. For leaf samples, one-half of a strawberry leaf was crushed into a pulp using a sterilised metal rod on a chopping board, then transferred to a 10 ml glass vial and sealed immediately to prevent volatile loss. Both frass and leaf samples were incubated at 50°C for two minutes prior to, and during volatile entrainment. All insects were starved for at least 24 h before volatile sampling to reduce the likelihood of frass production during volatile entrainment. Different weevils were used for each insect sample replicate.

After entrainment, the fibre was withdrawn and attached to the gas chromatography mass spectrometry (GC-MS) machine autosampler for analysis. Fibres were conditioned for 30 minutes at 270°C and stored in a sealed glass tube which had been flushed with nitrogen gas prior to volatile sampling. Volatile analysis was carried out as in Shepherd *et al.* (2007) with some minor alterations. Samples were analysed using a ThermoFisher (UK) DSQII quadrupole mass spectrometer with a Trace gas chromatograph and a CombiPal autosampler (CTC Analytics, Switzerland) for automated sampling with SPME fibres. Volatiles were desorbed from the SPME fibre at 250°C for two minutes in a programmable temperature vaporising (PTV) injector operating in splitless mode. Helium carrier gas at a flow rate of 1.5 ml min^{-1} was used to separate the volatiles on a DB 1701 GC column (30 m x 0.25 mm i.d x 0.25 µm film thickness; Agilent Technologies, UK). The GC temperature programme consisted of an initial hold at 40°C for two minutes followed by a 10°C min^{-1} temperature increase up to 240°C, with a further 20 minutes hold at that temperature. The GC-MS interface temperature was 250°C and the MS was used in electron impact mode at 70 eV over a mass range of 25–400 amu with a source temperature of 200°C. Data was acquired at 4 scans sec^{-1} and analysed using the Xcalibur™ V. 2.07 software package.

Selected raw data files obtained from the GC-MS analysis were used to identify the individual volatile compounds present in these samples by comparing information from the

mass spectrum with the characteristic retention times and MS fragmentation ions of a pre-existing library of compounds. These key characteristics were then used to create a processing method in Xcalibur™ which allowed volatile abundances to be calculated automatically from their MS peak areas. For each compound, a time window was defined based around the retention time of the appropriate chromatographic peak and a summed selected ion chromatogram (SIC) was generated for the chosen characteristic fragmentation ions to give the peak area. Appropriate ions for compound identification were chosen according to their relative abundance (the higher the better) and their uniqueness relative to other compounds near the peak's retention time. Corrections were made to the automatically processed data if peak areas were misaligned. Any impurities detected, such as those present in the chemistry lab atmosphere, were excluded from the processing method and therefore excluded from further analysis. The peak areas for each compound and the total volatile abundance and percentage of each compound present in the volatile blend was calculated.

Electroantennogram analysis

Analysis by Gas Chromatography linked to Electroantennography (GC-EAG)

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 (220°C), helium carrier gas (2.4 ml min^{-1}) and the oven temperature programmed from 50°C for two minutes, then at 10°C min^{-1} to 250°C. The GC column effluent was split (1:1) with a push-fit Y-piece between the FID (250°C) and a silanized glass T-piece in the column. Air (200 ml min^{-1}) was blown continuously through the T-piece delivering the sample to the EAG preparation. For the EAG preparation, the vine weevil was anaesthetised using carbon dioxide before excising the weevil's head. 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). The circuit was completed by one antenna being inserted into the recording glass electrode attached to the EAG device. 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)

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 (220°C) and oven temperature programmed from 40°C for 2 min then at 10°C min^{-1} to 240°C.

Synthetic compounds

Candidate compounds that might be responsible for eliciting vine weevil responses were identified from volatile analysis, EAG-GC-MS experiments and the literature and tested in the form of purified synthetic compounds. The tested compounds included (Z)-2-pentenol, (Z)-3-hexenol, (E)-2-hexenol, linalool, (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), methyl eugenol and 1-octen-3-ol, which are green leaf volatiles that dominated the plant volatile samples, and have been shown to elicit insect responses (van Tol *et al.*, 2012). In addition, the compounds β-caryophyllene, germacrene-D and α-farnesene were tested as potential compounds of interest, as these compounds were elevated in volatiles collected from weevil frass, and are known to elicit insect responses (respectively, Burguiere *et al.*, 2001; Innocenzi *et al.*, 2001; and Yan *et al.*, 2003). The latter three compounds were present in ylang ylang oil and were used as such. DMNT was synthesised at NRI, 1-octen-3-ol was obtained from International Flavours and Fragrances, Haverhill, Suffolk, UK, and the remaining compounds were obtained from Sigma Aldrich, Gillingham, Dorset, UK.

Collection of volatiles

Plants of *Euonymus fortunei* were purchased from Homebase and maintained in a greenhouse at NRI. For volatile collections the plant was encased in a polyethyleneterephthalate bag (Sainsburys oven bag; Stewart-Jones and Poppy, 2006). Charcoal-filtered air was blown into the bag to maintain positive pressure and air was withdrawn 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 from intact plants and plants in which 20 leaves had been cut with scissors. Volatiles were collected from intact and damaged strawberry plants similarly.

Testing candidate compounds: MA olfactometer

Three candidate compounds that elicited a strong and consistent EAG response (1-hexanol, (Z)-3-hexenol and (E)-2-hexenol; Sigma-Aldrich, Gillingham, Dorset, UK) were tested for their ability to act as insect attractants or repellents in the MA olfactometer. Each compound was prepared at three dilutions using liquid paraffin: 10, 100 and 500 mg ml^{-1}. Aliquots (10

µl) of the test substance were placed on a glass fibre filter, which was placed in the test chamber; the control consisted of an aliquot (10 µl) of paraffin oil on glass fibre filter. Ten adult weevils were placed into the central release chamber and their location noted at 20, 40, 60 and 80 minutes after release. For all compounds, weevil responses were assessed at 100 mg ml^{-1}, and compounds eliciting responses at this concentration were tested further at 10 and/or 500 mg ml^{-1}.

Statistical analysis

All statistical analyses were performed using Genstat (14th edition; VSN International Ltd, 2011). Analysis of variance (ANOVA), Generalised Linear Model (GLM) analysis and Goodness-of-fit (chi-squared or G-tests) were applied to test weevil movement to olfactometer treatments. Composition of insect, leaf and frass volatiles was assessed graphically using principal component analysis.

Results

Source of potential cues: SA Olfactometer

A general ANOVA showed that weevils tended to stay in the central chamber more than either of the choice chambers ($P < 0.001$). Because most weevils stayed in the central chamber their preferences were not judged to be statistically reliable and no further analysis was attempted (data not shown).

Source of potential cues: MA Olfactometer

Weevils were more responsive in the MA olfactometer. There was a significant interaction between chamber and treatment type ($P < 0.001$) due to more weevils being attracted to treatment chambers containing leaves and slight repellence of weevils from treatment chambers containing conspecific weevils.

Data from the MA olfactometer trials were analysed by Generalised Linear Model (GLM) with binomial distribution and logit link, with the different treatments as independent variables. The GLM showed that there was a significant difference in average weevil distribution between the choice chambers of the olfactometer, depending on the treatment choice, at one and three hour observation intervals ($P = 0.002$, and $P = 0.045$ respectively). Predictions from the regression model confirmed that the responding vine weevils were

attracted to leaf odours and apparently repelled by odour from other vine weevils, whereas frass was neither attractive nor repellent (see Table 1).

Table 1. Responses of weevils in a MA olfactometer to the odour of three *Fragaria x ananassa* leaves, 20 pellets of vine weevil frass, and five vine weevils compared to an empty control. Asterisks indicate significant differences from an even distribution between Choice Chambers at P = 0.05 (*), P = 0.01 (**), or P = 0.001 (***).

Observation interval	Treatment	Responding Weevils Choosing Treatment Chamber (%)	Standard Error
1 Hour	Leaves	88.6**	7.6
	Frass	53.1	12.5
	Weevils	15.4*	10.0
2 Hours	Leaves	68.1	10.7
	Frass	52.0	11.1
	Weevils	24.4*	10.5
3 Hours	Leaves	69.2*	10.8
	Frass	53.9	10.4
	Weevils	32.1	10.8

Chi-square tests were also performed to analyse the choices made by weevils which left the central chamber of the olfactometer apparatus. Strawberry leaves (see Figure 8a) elicited the strongest attractive response from the active weevils, as significantly more weevils moved towards the treatment chamber than the control chamber at one and three hour observation intervals (89%, P = 0.001, and 69%, P = 0.05 respectively). Weevils tended to prefer the leaf treatment at the two hour interval (68%) compared to the control, but this result was not significant at the 95% confidence interval ($P = 0.079$). When weevils had the choice between fresh frass and an empty control chamber, they showed no preferences (see Figure 8b). By contrast, conspecific weevils appeared to repel the responding weevils (Figure 8c), causing most of them to move to the Control Chamber within the first two hours of the experiment (85%, $P = 0.013$ after one hour and 76%, $P = 0.020$ after two hours), although after three hours the distribution was not significant ($P = 0.065$). In each experiment, the number of weevils leaving the central chamber increased as the experiment progressed. Despite the weevils showing no attraction to frass, it was this treatment that caused the most weevils to become active and enter one of the choice chambers.

Range of signal detection: Linear olfactometer

Insects responded positively to crushed strawberry leaves in all trials when the material was placed at 450 mm from the insect release hole. When leaf material was placed at 1,500 mm from the insect release hole, 40% of insects did not make a choice; insects that made a choice selected the olfactometer arm containing crushed leaf material or the empty control arm in almost identical proportions (51% and 49%, respectively; data not shown).

Figure 8. Responses of weevils in a MA olfactometer to odour of (a) three *Fragaria x ananassa* leaves, (b) 20 pellets of vine weevil frass, and (c) five vine weevils compared to an empty control. Asterisks indicate significant differences from an even distribution between Choice Chambers at $P = 0.05$ (*), $P = 0.01$ (**), or $P = 0.001$ (***).

Volatile entrainment

Principal component analysis, a method of visually inspecting large multivariate datasets, indicated large differences in volatile composition between samples of strawberry leaf,

weevils and frass (Figure 9a; Appendix 1). Only a small number of low abundance volatiles were collected from control samples. Strawberry leaf samples were dominated by a large number of green leaf volatile compounds, while frass samples were dominated by sesquiterpene compounds, including α-farnesene, β-caryophyllene and germacrene-D (Figure 9b). Insect volatiles were relatively low in abundance, and comprised a mixture of aromatic aldehydes, branched alkanes and isoprenoids.

Figure 9a. Plot of sample scores on the second and third principal components showing separation of volatile samples by sample type. Controls were sealed empty vials (n=5) or empty vials with muslin gauze (n=5). Samples were crushed strawberry leaves (n=5), weevil frass (n=5), pre-reproductive adult weevils (n=6), egg-laying adult weevils (n=4) or mixed-age weevils (in vials with a muslin barrier, n=8).

Figure 9b. Plot of the loadings of individual volatile compounds on the second and third principal components; regions dominated by sesquiterpene and green leaf volatile groups are indicated.

Electroantennogram analysis

EAG using synthetic compounds

EAG responses to synthetic compounds were measured using GC-EAG so that the insect antenna was exposed to a measured amount of fully volatilised material. Generally 20 ng of material was injected, giving 10 ng to the insect. The frequency of EAG responses to each compound is shown in Table 2.

Reproducible EAG responses were recorded to (*E*)-2-pentenol and (*Z*)-2-pentenol, (*E*)-2-hexenol, (*Z*)-3-hexenol, 1-octen-3-ol, linalool and methyl salicylate. An occasional response to α-farnesene and to methyl eugenol was observed.   No responses were observed to germacrene-D or β-caryophyllene. Interestingly the response to 1-hexanol was detected as a response to a trace impurity in the (*E*)-2-hexenol. Similarly a response to the linalool in the ylang ylang oil was detected, although this is a very minor component. Representative GC-EAG traces are shown in Figures 10–13.

17

Table 2. EAG responses of vine weevil to *c.* 10 ng of each synthetic compound. The retention time (RT) and retention index (RI) of each compound is shown, and the number of times an EAG response was observed out of the total number of runs.

Compound	RT (min)	RI	Frequency of EAG responses
DMNT	6.82	1310	0/2
(*E*)-2-pentenol	6.88	1315	3/4
(*Z*)-2-pentenol	6.98	1323	4/4
hexanol	7.41	1356	16/16
(*Z*)-3-hexenol	7.85	1389	4/4
(*E*)-2-hexenol	8.11	1410	18/18
1-octen-3-ol	8.67	1455	2/2
Linalool[1]	9.87	1552	4/4
β-caryophyllene	10.59	1612	0/16
Germacrene-D	11.88	1727	0/16
α-farnesene	12.22	1757	5/16
methyl salicylate	12.56	1788	4/4
methyl eugenol	14.91	2018	1/2

[1]Linalool was also detected as a very minor component in ylang ylang in 12/18 runs

Figure 10.. GC-EAG analysis of ylang ylang oil with (*E*)-2-hexenol added (approx 10 ng; polar GC column: hexanol at 7.41 min; (*E*)-2-hexenol at 8.11 min; linalool at 9.87 min; caryophyllene at 10.57 min; Germacrene-D at 11.86 min; α-farnesene at 12.19 min; methyl salicylate at 12.36 min; * denotes EAG response).

Figure 11. GC-EAG analysis of synthetic compounds (approx 10 ng; polar GC column: (*Z*)-2-pentenol 6.96 min; (*Z*)-3-hexenol at 7.84 min; linalool at 9.86 min; methyl salicylate at 12.56 min; * denotes EAG response).

Figure 12. GC-EAG analysis of synthetic compounds (approx 10 ng; polar GC column: DMNT 6.82 min; hexanol 7.41 min; (*E*)-2-hexenol 8.12 min; 1-octen-3-ol at 8.67 min; methyl eugenol at 14.91 min; * denotes EAG response).

Figure 13. GC-EAG analysis of synthetic compounds (approx 10 ng; polar GC column: hexanol 6.40 min; (*E*)-2-hexenol 6.77 min; * denotes EAG response).

GC-EAG analysis of volatiles from *Euonymus fortunei*

Volatiles were collected on Porapak from an intact plant of *Euonymus fortunei* after the leaves had been cut with scissors. These were analysed by GC-EAG with a vine weevil EAG preparation. A slightly different GC programme was used from that used in the above studies, so retention times are different. A GC trace of volatiles from damaged *E. fortunei* is shown in Figure 14 with two EAG runs superimposed showing the reproducibility of the results. These are summarised in Table 3 with compounds identified according to their Retention Index, mass spectrum and co-chromatography with authentic material.

Figure 14. GC-EAG analysis of volatiles from *Euonymus fortunei* (polar GC column).

EAG responses were observed to 1-hexanol, (*Z*)-3-hexenol, (*E*)-2-hexenol and methyl salicylate which were tested as synthetics. Additionally EAG responses were obtained to the aldehydes heptanal, octanal and decanal, but these are found generally in collections on Porapak and elicit EAG responses from many insects.

Table 3. GC-EAG analysis of volatiles from *Euonymus fortunei*

RT (min)	RI	Euonymus 2012/054/006		Strawberry 2012/054/08		Compound[1]
		EAG	AREA %	EAG	AREA %	
4.79	1074	+	1.2	+	0.8	*hexanal?*
5.55	1136	++	2.2	+	0.9	*ethyl benzene*
6.15	1185	+	1.5	+	1.0	*heptanal*
6.61	1221	+	0.7		0.0	*methyl ethyl benzene*
7.57	1293		1.0	++	0.6	*octanal*
7.97	1323	+	1.4	(+)	46.4	Z-3-hexenyl acetate
8.21	1341	+	2.3	(+)	2.0	6-methyl-5-hepten-2-one
8.41	1356	+	0.0	++	0.8	1-hexanol
8.84	1389	++	5.0	+++	10.6	Z-3-hexenol
8.98	1399	+	4.5	+	4.4	*nonanal*
9.11	1410		0.0	+	0.5	E-2-hexenol
9.50	1441	+	0.0		0.4	
9.66	1454	+	0.0		0.0	1-octen-3-ol
10.31	1507	+	4.7		3.3	*decanal*
10.61	1531		0.0	+	2.7	benzaldehyde
10.86	1551	+	0.9		0.0	linalool
12.80	1720	+	2.8		0.0	*3-ethylbenzaldehyde*
13.00	1738	+	0.5		0.0	
13.14	1750	++	2.4		0.0	γ-elemene
13.19	1754	+	16.7		0.0	α-farnesene
13.54	1786		2.8	+	3.0	methyl salicylate
13.70	1800		0.9	+	1.9	
13.93	1823	+	1.1		0.4	*Silicon impurity*
14.54	1883		1.8	+	2.9	
14.91	1919		0.0	+	2.2	2-phenylethanol
15.28	1956	++	0.0		0.0	
16.05	2034		0.0	+	0.7	
17.81	2224		0.5	+	0.5	
17.99	2245	++	1.4		0.0	α-cadinol
18.92	2353	+	0.0		0.0	
20.94	2588	+	0.0	0	0.0	

[1]compounds in italics are impurities from Porapak (aromatics) or aldehydes which seem to be in most collections from plants and insects and elicit good EAG responses.

Testing candidate compounds: MA olfactometer

The three tested compounds, 1-hexanol, (*E*)-2-hexenol and (*Z*)-3-hexenol, elicited very different behaviours. On average, approximately half of the experimental insects responded to the treatments. The compounds 1-hexanol (at 100 mg ml^{-1}) and (*Z*)-3-hexenol (at 500 and 100 mg l^{-1}) were repellent to vine weevils (Figure 15a, e and f). The compound (*E*)-2-hexenol had no effect at 500 mg ml^{-1} (Figure 15b), but was attractive at 100 mg ml^{-1} (Figure 15c) and elicited mixed responses at 10 mg ml^{-1} (Figure 15d). Significant differences

between choice chambers were tested with a G-test apart from (c) and (e) where the difference (100 vs 0%) could not be analysed, but was very clear.

Figure 15. Movement of weevils into choice chambers with control (open symbols) and test compounds (closed symbols) at different concentrations. Numbers in parentheses indicate the percentage of responsive weevils in all tests and statistically significant differences are indicated *$P < 0.05$, ** $P < 0.001$ and *** $P < 0.001$; c) and (e) are marked up similarly for consistency although statistical testing was not possible,

Discussion

The primary aim of the study, to characterise the volatiles eliciting weevil movement and aggregation, has been achieved using a range of research techniques to examine insect behavioural responses to volatile cues. The study initially hypothesised that pheromones released from conspecific insects were the source of the volatile cue. However, extensive examination of insect behaviour in olfactometer experiments confirmed that plant material, rather than vine weevils or their frass, was a source of insect attraction (Objective 2). The range over which insects responded (Objective 1) was relatively short (<0.5 m), with consistent attraction to leaf material being detected at 450 mm in the linear olfactometer and the two-arm moving air olfactometer. Insects did not respond to leaf material over long distances (>1 m).

Analysis of volatiles released from leaf and insect material (Objective 2) highlighted substantial differences in volatile composition between leaf material, weevils and weevil frass. Weevil frass stimulated insect activity, possibly due to the high relative abundance of sesquiterpenes such as α-farnesene, β-caryophyllene and germacrene-D, which are known to elicit responses in other weevil species or in other insects (Burguiere *et al.,* 2001; Innocenzi *et al.*, 2001; Yan *et al.*, 2003), but was neither attractive nor repellent to weevils in the present study. By contrast, conspecific insects were slightly deterrent to insects, although the volatile compounds underlying this response were ambiguous. The attractiveness of strawberry leaf material to vine weevils was potentially linked to the high relative abundance of many green leaf volatile compounds.

Electroantennogram experiments to examine vine weevil antennal response to plant material and purified synthetic compounds (Objective 3) identified a number of plant volatiles that elicited consistent signals. In particular, the green leaf volatiles 1-hexanol, (*Z*)-3-hexenol, and (*E*)-2-hexenol gave very good EAG responses, providing further evidence that this group of compounds might play a role in weevil attraction. Other compounds that have been reported to influence vine weevil behaviour [(*Z*)-2-pentenol and methyl eugenol: van Tol *et al.*, 2012] also gave good antennal responses when supplied as synthetic compounds, but these volatiles were not detected in GC-EAG analysis of leaf samples, and only detected at relatively low abundance by volatile entrainment of leaf samples.

In further olfactometer experiments, insect movement was assessed in response to synthetic sources of the green leaf volatiles eliciting good EAG responses, i.e. 1-hexanol,

(*Z*)-3-hexenol and (*E*)-2-hexenol (Objective 4). The compounds provoked a range of insect behaviours. The proportion of insects responding to the compounds 1-hexanol and (*Z*)-3-hexenol was small, and insects that responded were repelled by moderate to high concentrations (100–500 mg ml^{-1}) of the compounds. The compound (*E*)-2-hexenol was attractive to vine weevils at moderate concentrations (100 mg ml^{-1}), but had variable or no effects at higher or lower concentrations (10 and 500 mg ml^{-1}).

One of the constraining factors in studying and trapping insect pests is low levels of insect activity, due to a number of biotic and abiotic factors, which can result in a small proportion of insects responding to a treatment. Recent work by others has indicated the difficulties of motivating vine weevils to move into traps that contain compounds that otherwise appear attractive to the insects (van Tol *et al.*, 2012). Thus, an approach that combines the use of attractants (placed within the crop), perhaps in conjunction with an activity stimulant (e.g. frass volatiles) and repellents (placed on the crop periphery) might be preferable to minimise the number of insects feeding and laying eggs in the crop. This study has identified several plant-derived compounds that might be exploited in such a 'push–pull' approach to optimise vine weevil pest management.

Conclusions

- Leaf-derived volatiles, rather than insect-derived compounds, were attractive to adult vine weevils.
- Specific green leaf volatiles emitted from strawberry leaves were shown to elicit strong and consistent electrical responses in vine weevil antennae.
- Synthetic sources of specific green leaf volatiles were identified to attract ((*E*)-2-hexenol) or repel (1-hexanol and (*Z*)-3-hexenol) vine weevils.
- Insect frass, which released relatively large amounts of the volatiles α-farnesene, β-caryophyllene and germacrene-D, stimulated insect activity but was neither attractive nor repellent to vine weevils.

Knowledge and Technology Transfer

Knowledge transfer activities for the project (Objective 5) are detailed below:

- Fruit for the Future (July 2011): the background and aims of the research were discussed at a grower event held at the James Hutton Institute in Dundee and attended by approximately 100 delegates.
- EMRA/HDC Soft Fruit meeting at East Malling Research, Kent (23 November 2011): The initial research findings of the project were reported in an oral presentation:

‘Aggregation pheromone for the control of vine weevil’. Mitchell C, McLaren R, Karley AJ, Shepherd, T, Johnson, SN, Gregson, R

- LEAF Open Farm Sunday (June 2011): The project was represented as part of an exhibit at an open day for growers and the general public held at the James Hutton Institute, Dundee
- Public communication events: the project will be included as part of the exhibits at upcoming events aimed at school and undergraduate student groups (Techfest, 2012; Animal–Plant Interactions event at JHI, November 2012).
- Articles: an article for HDC News, describing the main research findings, will be produced late in 2012, along with a non-technical article for a relevant trade publication (e.g. Horticulture Week), with the permission of HDC.

Glossary

Electroantennogram is a technique for measuring the electrical output of the antenna to the brain for a given odour, commonly used for studying insect olfactory cues.

Olfactometer is a device used to study insect behaviour in the presence of an olfactory stimulus.

Pheromone is a secreted or excreted chemical that triggers a social response in individuals of the same species.

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Appendix 1

Relative intensities of volatile compounds detected by GC-MS from strawberry leaves, adult vine weevils and insect frass and blank control samples.

Sample	4.37_ethyl_hexane	5.75_hexanal	5.86_2-hexanal	6.08_1-nonene	6.90_terpene	6.94_2-hexanal	7.04_styrene	7.12_2-hexanal	7.15_UK	7.17_2-hexanal	7.23_cis-3-hexen-1-ol	7.28_terpene	7.37_1-hexanol	7.39_cyclohexanol	7.47_4-hydroxy-4-methyl-2-pentanone	7.54_heptanal	7.71_cyclohexanol	7.99_7,_7-dimethyl-1,_3,_5-cycloheptatriene	8.10_1-ethyl_3-methyl-benzene	8.25_2-ethyl-butanal	8.31_terpene	8.41_2,_4-hexadienal	8.59_UK_aromatic	8.71_limonene	9.07_1-octen-3-ol	9.10_3_or_4-hexen-1-ol,_acetate_(z)	9.15_benzaldehyde	9.16_6-methyl-5-hepten-2-one	9.21_trans-2-hexenyl-acetate	9.25_octanal
Control1	0.18	0.61	0.01	0.21	2.48	0.01	1.75	0.03	0.13	0.01	0.00	0.50	0.03	0.01	0.08	0.29	0.01	0.94	0.31	0.17	0.59	0.01	0.46	0.57	0.00	0.01	5.34	0.11	0.00	0.47
Control2	0.32	0.40	0.00	0.13	1.33	0.00	1.75	0.04	0.09	0.00	0.11	0.12	0.01	0.01	0.07	0.30	0.05	0.57	0.15	0.27	0.16	0.00	0.30	0.11	0.01	0.00	2.94	0.65	0.01	0.39
Control3	0.15	0.55	0.01	0.27	0.30	0.01	26.27	0.06	1.20	0.00	0.03	0.34	0.01	0.00	0.05	0.54	0.00	0.09	0.01	0.06	0.07	0.00	0.02	0.08	0.01	0.00	2.80	0.35	0.00	0.53
Control4	0.66	8.44	5.14	0.61	4.28	0.45	3.90	13.09	0.36	0.05	1.09	0.38	0.24	0.20	0.23	1.30	0.06	0.11	0.07	1.04	0.28	0.10	0.10	0.13	0.03	0.10	4.65	1.11	0.01	2.41
Control5	0.50	4.43	11.47	0.72	1.89	0.39	8.44	4.25	0.95	0.05	0.29	0.21	0.05	0.01	0.23	0.88	0.07	0.23	0.10	0.83	0.23	0.08	0.13	0.08	0.03	0.03	5.13	0.51	0.02	1.31
Leaf1	0.01	4.40	3.60	0.00	0.01	1.23	0.01	0.05	5.23	0.04	12.27	0.04	1.01	0.21	0.07	0.24	0.07	0.01	0.04	0.01	0.01	3.13	0.01	0.01	0.85	6.44	1.26	0.32	0.01	0.28
Leaf2	0.02	2.02	1.33	0.01	0.01	0.42	0.00	0.18	3.70	0.02	12.57	0.06	6.39	5.84	0.07	0.33	0.05	0.01	0.04	0.00	0.00	1.70	0.01	0.03	0.98	9.43	1.29	0.23	1.57	0.12
Leaf3	0.01	7.36	6.91	0.13	0.10	3.14	0.92	0.04	2.91	24.42	14.53	0.25	3.45	2.01	0.03	0.29	0.08	0.01	0.02	0.01	0.00	2.27	0.00	0.02	0.23	6.23	0.88	0.47	0.23	0.13
Leaf4	0.01	5.51	5.65	0.09	0.01	0.87	0.00	0.02	2.19	22.97	16.82	0.22	5.49	4.22	0.17	0.28	0.12	0.00	0.04	0.00	0.00	1.88	0.00	0.04	0.20	10.57	0.66	0.38	1.07	0.06
Leaf5	0.01	8.21	23.66	0.04	0.01	3.26	0.14	0.07	1.15	20.14	11.22	0.18	1.62	0.81	0.08	0.12	0.05	0.00	0.01	0.01	0.00	1.98	0.00	0.01	0.18	6.44	0.55	0.09	0.10	0.07
Frass1	0.25	1.11	0.07	0.06	0.82	0.01	0.46	0.10	0.08	0.00	1.16	0.17	0.14	0.06	0.37	0.30	0.39	0.64	0.13	0.02	0.25	0.02	1.05	0.24	1.72	0.02	3.88	0.55	0.00	0.48
Frass2	0.24	1.17	0.24	0.06	0.86	0.02	0.64	0.52	0.15	0.00	8.63	0.17	0.37	0.01	0.33	0.53	0.35	0.55	0.11	0.04	0.24	0.11	0.66	0.22	0.60	0.02	4.45	0.76	0.01	0.46
Frass3	0.37	1.63	0.08	0.08	0.60	0.01	1.44	0.24	0.06	0.00	0.33	0.13	0.05	0.04	0.20	0.38	0.10	0.36	0.10	0.05	0.09	0.02	0.20	0.07	0.19	0.01	2.62	0.92	0.00	0.64
Frass4	0.89	1.40	0.10	0.10	1.26	0.01	1.03	0.23	0.09	0.01	0.40	0.09	0.10	0.02	0.13	0.44	0.39	0.57	0.19	0.07	0.20	0.02	0.27	0.14	0.13	0.00	1.52	0.71	0.00	0.74
Frass5	0.61	0.32	0.02	0.08	0.49	0.00	0.93	0.03	0.08	0.00	0.54	0.50	0.09	0.01	0.05	0.21	2.29	0.15	0.25	0.06	0.07	0.01	0.10	0.09	1.56	0.01	0.95	0.87	0.00	0.45
InsectSeal1	0.18	0.85	0.84	0.19	1.93	0.08	5.78	0.88	0.21	0.01	0.13	0.10	0.05	0.00	0.39	0.22	0.12	0.79	0.24	0.11	0.35	0.03	0.38	0.24	0.09	0.01	5.35	1.96	0.01	0.41
InsectSeal2	0.07	0.58	0.53	0.13	0.78	0.03	3.51	0.48	0.13	0.01	0.03	0.04	0.04	0.00	0.25	0.19	0.05	0.06	0.11	0.09	0.15	0.02	0.02	0.05	0.04	0.02	10.74	0.36	0.01	0.26
InsectSeal3	0.09	0.32	0.00	0.14	0.95	0.00	16.98	0.13	0.53	0.00	0.03	0.09	0.01	0.00	0.07	0.28	0.03	0.57	0.14	0.17	0.30	0.00	0.43	0.31	0.02	0.04	4.92	0.76	0.01	0.58
InsectSeal4	0.10	0.17	0.01	0.01	1.75	0.00	1.00	0.33	0.04	0.01	0.08	0.04	0.13	0.01	0.30	0.08	0.24	0.12	0.10	0.15	0.39	0.03	0.06	0.20	0.05	0.01	5.10	0.06	0.04	0.10
InsectSeal5	0.10	0.16	0.02	0.14	4.00	0.01	3.21	0.08	0.08	0.02	0.01	0.19	0.02	0.00	0.25	0.11	0.02	1.93	0.51	0.03	1.06	0.02	1.20	3.57	0.04	0.00	6.85	0.22	0.01	0.22
InsectSeal6	0.11	0.15	0.00	0.14	1.18	0.02	6.12	0.23	0.13	0.01	0.00	0.04	0.08	0.01	0.18	0.14	0.13	0.12	0.15	0.04	0.30	0.00	0.03	0.20	0.06	0.01	12.26	0.21	0.02	0.25
InsectSeal7	0.15	0.38	0.02	0.10	2.65	0.02	1.19	0.05	0.17	0.01	0.12	0.07	0.06	0.00	0.17	0.19	0.26	2.83	0.72	0.05	0.72	0.02	2.18	0.46	0.69	0.01	9.62	1.83	0.05	0.36
InsectSeal8	0.13	0.21	0.01	0.01	1.02	0.00	1.05	0.15	0.04	0.01	0.04	0.01	0.06	0.00	0.18	0.15	0.31	0.12	0.04	0.04	0.25	0.02	0.03	0.33	0.19	0.01	5.63	0.29	0.02	0.22
InsectSeal9	0.18	0.30	0.02	0.24	2.06	0.02	2.31	0.04	0.17	0.01	0.16	0.02	0.06	0.00	0.17	0.21	0.23	1.78	0.49	0.05	0.63	0.01	0.87	0.83	0.28	0.11	10.27	2.42	0.03	0.32
InsectSeal10	0.14	0.27	0.03	0.11	0.67	0.01	1.76	0.09	0.17	0.02	0.06	0.04	0.06	0.01	0.13	0.33	0.12	0.09	0.08	0.02	0.14	0.02	0.03	0.21	0.13	0.02	8.94	0.75	0.02	0.35
MusCont1	0.15	1.17	0.07	0.59	0.83	0.01	0.24	0.01	0.29	0.00	0.00	0.15	0.18	0.11	39.99	1.58	0.00	0.04	0.06	2.83	0.12	0.00	0.01	0.04	1.04	0.05	1.50	17.09	0.03	2.66
MusCont2	0.22	1.19	0.06	0.60	1.53	0.00	0.54	0.01	0.47	0.00	0.00	0.18	0.02	0.00	6.54	1.42	0.02	0.06	0.08	2.65	0.17	0.00	0.02	0.28	0.08	0.05	2.30	26.20	0.04	3.12
MusCont3	0.14	0.69	0.01	0.41	1.11	0.00	6.49	0.06	0.52	0.01	0.00	0.09	0.27	0.41	40.72	0.85	0.02	0.13	0.05	1.72	0.12	0.00	0.05	0.04	2.42	0.02	3.27	4.79	0.02	0.89
MusCont4	0.44	1.13	0.01	1.07	2.34	0.00	8.02	0.01	0.91	0.00	0.00	0.05	0.03	0.02	4.81	1.30	0.02	0.67	0.17	2.02	0.16	0.00	0.39	0.10	0.06	0.01	5.37	15.65	0.04	2.73
MusCont5	0.44	1.26	0.03	1.40	1.49	0.00	1.55	0.01	0.92	0.01	0.00	0.06	0.03	0.01	5.25	1.50	0.02	0.29	0.12	5.23	0.13	0.00	0.12	0.06	0.05	0.06	4.18	13.83	0.05	3.74
MusInsect1	0.15	0.75	0.01	0.41	0.34	0.00	0.21	0.01	1.87	0.00	0.01	0.97	0.05	0.04	3.45	1.04	0.01	0.05	0.58	1.25	0.11	0.00	0.03	0.35	0.04	0.01	5.11	2.19	0.01	1.17
MusInsect2	0.10	0.70	0.02	0.49	1.17	0.00	0.30	0.05	0.28	0.00	0.01	0.13	0.10	0.08	29.38	1.04	0.05	0.05	0.02	1.16	0.11	0.00	0.02	0.05	0.70	0.04	2.66	22.24	0.01	1.68
MusInsect3	0.20	1.12	0.01	0.58	2.29	0.00	1.94	0.04	0.52	0.02	0.00	0.11	0.14	0.15	28.29	1.06	0.02	0.20	0.10	1.86	0.31	0.00	0.09	0.14	0.52	0.01	5.10	16.04	0.02	1.26
MusInsect4	0.10	0.57	0.02	0.35	1.08	0.00	0.80	0.02	0.25	0.01	0.00	0.06	0.23	0.24	33.56	0.65	0.04	0.09	0.04	1.72	0.12	0.00	0.05	0.05	2.96	0.02	3.50	8.36	0.03	0.63
MusInsect5	0.23	0.75	0.01	0.74	1.02	0.00	7.11	0.00	0.53	0.01	0.00	0.09	0.05	0.04	11.35	0.90	0.02	0.11	0.06	1.41	0.13	0.00	0.05	0.11	0.26	0.03	4.49	25.30	0.05	1.77
MusInsect6	0.49	1.47	0.02	1.51	1.81	0.01	1.71	0.01	0.62	0.01	0.13	0.04	0.03	0.03	8.67	1.75	0.02	0.22	0.09	2.61	0.17	0.00	0.08	0.23	0.06	0.03	4.13	27.84	0.04	3.37
MusInsect7	0.33	0.77	0.00	0.96	0.99	0.00	5.94	0.00	0.59	0.00	0.02	0.07	0.03	0.02	5.82	1.05	0.26	0.18	0.07	1.15	0.08	0.00	0.08	0.07	0.05	0.05	5.18	27.34	0.02	2.37
MusInsect8	0.42	1.41	0.01	1.37	1.43	0.00	0.81	0.01	0.40	0.00	0.00	0.06	0.03	0.01	2.99	1.58	0.10	0.18	0.07	2.24	0.13	0.00	0.07	0.11	0.06	0.02	2.81	10.11	0.02	3.58

Appendix 1 (cont.)

Sample	9.30_6-methyl-5-hepten-2-ol	9.41_3,3-dimethylhexane	9.43_methyl_2-ethylhexanoate	9.57_hexylene_glycol	9.62_oxime-_methoxy-_phenyl-	9.78_4-methyl-3-octanone	9.81_2,_4-heptadienal	9.87_butyrolactone	9.90_2-ethyl-1-hexanol	9.96_5-Ethyl-2(5H)-furanone	10.16_hexanoic_acid	10.30_2-nonen-1-ol	10.59_UK	10.85_nonanal	10.87_L-linalool	11.02_benzenemethanol	11.13_phenol	11.63_2-(4-methylcyclohexyl)ethanol	11.69_5-Ethyl-2(5H)-furanone	11.91_trans-nonen-3-ol	12.08_phenylethyl_alcohol	12.12_L-(-)-Menthol	12.34_decanal	12.39_UK	12.41_tetrahydropyran-2-carbinol	12.44_UK	12.73_UK	12.79_UK	12.86_bicyclohexyl	13.08_p-menth-3-ene	13.17_heptylcyclohaxene
Control1	0.01	0.23	0.03	0.01	0.00	0.01	0.01	11.76	5.63	0.10	1.73	0.11	0.01	1.52	0.02	28.79	5.65	0.01	0.19	0.08	0.01	0.02	0.66	2.58	0.01	0.04	0.02	0.03	0.18	0.14	0.54
Control2	0.01	0.12	0.01	0.02	0.00	0.00	0.00	32.67	8.30	0.12	0.96	0.10	0.00	1.77	0.01	17.14	12.64	0.04	0.07	0.03	0.01	0.01	0.97	1.38	0.01	0.02	0.02	0.00	0.09	0.07	0.21
Control3	0.01	0.05	0.00	0.01	31.10	0.00	0.01	14.51	8.31	0.02	0.79	0.16	0.00	0.83	0.00	0.10	8.15	0.06	0.01	0.01	0.00	0.01	0.45	0.11	0.00	0.00	0.00	0.02	0.00	0.01	0.00
Control4	0.04	0.70	0.06	0.03	8.41	0.02	0.04	0.36	6.34	0.15	0.44	0.36	0.01	7.96	0.03	0.31	1.94	0.13	0.03	0.03	0.05	0.26	6.43	2.02	0.05	0.01	0.02	0.03	0.02	0.11	0.10
Control5	0.04	0.60	0.03	0.04	27.41	0.06	0.00	0.38	5.22	0.05	0.55	0.37	0.00	3.55	0.03	0.24	1.94	0.03	0.10	0.06	0.02	0.16	3.96	1.18	0.04	0.01	0.02	0.03	0.03	0.12	0.05
Leaf1	1.04	0.02	0.00	0.00	2.39	0.24	0.82	0.17	0.69	2.50	0.75	0.04	0.01	1.11	0.23	3.45	0.25	3.84	0.69	0.15	33.87	0.17	0.70	0.05	1.99	0.02	0.01	0.00	0.02	0.03	0.01
Leaf2	1.13	0.02	0.03	0.00	0.63	0.06	0.40	0.02	0.47	0.19	1.38	0.02	0.01	1.04	1.23	4.88	0.13	2.01	0.15	0.15	32.11	0.20	0.16	0.02	0.34	0.01	0.00	0.00	0.01	0.02	0.01
Leaf3	0.79	0.01	0.00	0.00	3.53	0.28	0.48	0.14	0.41	1.33	0.24	0.02	0.00	0.64	1.00	1.33	0.46	1.53	0.28	0.16	8.08	0.03	0.24	0.01	0.93	0.00	0.00	0.01	0.00	0.00	0.00
Leaf4	0.69	0.00	0.00	0.00	0.22	0.12	0.33	0.06	0.25	0.42	0.31	0.03	0.01	1.03	1.76	1.81	0.05	1.75	0.15	0.25	8.98	0.01	0.15	0.01	0.38	0.00	0.00	0.01	0.00	0.00	0.00
Leaf5	0.51	0.01	0.00	0.00	0.71	0.70	0.44	0.04	0.25	5.10	0.12	0.01	0.00	0.32	0.27	1.01	0.07	1.28	1.18	0.08	4.34	0.01	0.16	0.03	2.68	0.00	0.00	0.00	0.00	0.00	0.00
Frass1	0.01	0.09	0.01	0.00	0.00	0.00	0.16	2.48	6.36	0.07	0.56	0.13	0.70	4.43	0.17	1.38	2.37	0.03	0.19	0.05	0.10	0.03	0.31	1.58	0.00	0.21	0.16	0.01	0.78	0.53	0.90
Frass2	0.04	0.09	0.01	0.01	0.00	0.00	0.50	0.38	7.87	0.09	1.43	0.21	0.41	3.08	0.11	2.45	1.88	0.04	0.32	0.12	0.18	0.06	0.53	2.05	0.01	0.30	0.13	0.02	0.72	0.51	1.06
Frass3	0.04	0.06	0.00	0.00	0.04	0.00	0.14	18.43	8.24	0.16	0.86	0.16	0.10	6.74	0.08	3.21	3.49	0.10	0.23	0.20	0.09	0.20	0.51	1.53	0.01	0.21	0.16	0.04	0.75	0.46	0.98
Frass4	0.05	0.14	0.01	0.02	0.00	0.00	0.10	0.23	7.00	0.15	1.54	0.21	0.08	6.40	0.19	1.90	1.74	0.14	0.28	0.24	0.92	0.10	0.80	1.70	0.02	0.18	0.11	0.01	0.80	0.43	0.98
Frass5	0.24	0.12	0.01	0.00	0.00	0.00	0.09	9.07	6.58	0.06	0.90	0.13	0.14	2.26	0.32	1.80	0.97	0.06	0.16	0.11	5.01	0.05	0.52	1.24	0.01	0.20	0.11	0.03	0.48	0.39	0.86
InsectSeal1	0.04	0.60	0.03	0.05	31.27	0.05	0.01	0.06	7.93	0.06	2.49	0.44	0.29	1.08	0.01	0.44	1.44	0.21	0.24	0.05	0.18	0.17	0.85	0.84	0.02	0.32	0.18	0.17	0.80	0.50	0.84
InsectSeal2	0.02	0.15	0.08	0.11	56.01	0.01	0.02	0.10	2.45	0.04	2.35	0.22	0.05	0.71	0.01	0.13	1.13	0.06	0.13	0.02	0.07	0.05	0.85	0.41	0.01	0.12	0.07	0.08	0.35	0.24	0.39
InsectSeal3	0.02	0.09	0.01	0.01	51.11	0.01	0.01	0.81	3.89	0.07	0.25	0.13	0.02	1.16	0.03	0.15	4.75	0.04	0.05	0.08	0.01	0.75	1.02	0.27	0.01	0.06	0.04	0.03	0.24	0.13	0.31
InsectSeal4	0.02	0.52	1.88	0.07	60.90	0.02	0.03	0.03	2.27	0.03	1.49	0.25	0.06	0.14	0.03	0.19	0.62	0.10	0.17	0.17	0.13	1.26	0.15	0.38	0.00	0.27	0.13	0.30	0.73	0.50	0.36
InsectSeal5	0.03	0.34	0.94	0.03	31.82	0.01	0.02	0.05	5.16	0.03	0.80	0.40	0.10	0.56	0.01	0.13	0.76	0.32	0.34	0.19	0.02	4.30	0.37	0.66	0.00	0.41	0.35	0.13	1.17	0.84	0.66
InsectSeal6	0.02	0.28	0.98	0.01	49.10	0.01	0.01	0.08	2.94	0.02	0.64	0.24	0.03	0.55	0.01	0.20	0.70	0.11	0.17	0.07	0.03	2.05	0.49	0.32	0.01	0.18	0.29	0.14	0.58	0.38	0.42
InsectSeal7	0.06	0.48	0.03	0.03	18.95	0.02	0.03	0.04	6.44	0.04	0.52	0.36	0.06	0.80	0.03	0.20	0.74	0.21	0.45	0.32	0.21	3.48	0.10	1.25	0.01	0.41	0.29	0.07	1.29	0.72	1.42
InsectSeal8	0.04	0.29	0.06	0.01	60.85	0.02	0.02	0.05	2.35	0.03	1.61	0.36	0.02	0.43	0.01	0.05	0.46	0.07	0.18	0.18	0.04	1.85	0.17	0.51	0.01	0.18	0.11	0.07	0.45	0.35	0.64
InsectSeal9	0.06	0.42	0.14	0.51	9.11	0.02	0.02	0.02	9.75	0.11	1.30	0.78	0.09	0.69	0.05	0.22	1.14	0.22	0.45	0.28	0.38	3.34	0.22	1.36	0.02	0.59	0.41	0.08	1.15	0.79	1.42
InsectSeal10	0.06	0.37	0.03	0.12	38.23	0.01	0.02	0.04	2.92	0.03	0.47	0.45	0.02	0.83	0.01	0.20	1.06	0.09	0.22	0.05	0.13	2.78	0.39	0.82	0.01	0.30	0.29	0.09	0.63	0.50	1.02
MusCont1	0.02	0.04	0.00	8.51	0.69	0.01	0.00	0.32	0.41	0.01	0.17	0.45	0.00	8.78	0.07	0.31	0.27	0.01	0.01	0.05	0.00	0.00	6.74	0.06	0.00	0.00	0.00	0.01	0.01	0.01	0.01
MusCont2	0.02	0.04	0.01	0.61	1.30	0.00	0.01	0.20	0.76	0.03	0.92	0.54	0.00	11.44	0.14	0.10	0.38	0.01	0.00	0.43	0.08	16.41	14.03	0.17	0.01	0.01	0.06	0.02	0.01	0.04	0.01
MusCont3	0.01	0.08	0.02	11.84	9.71	0.01	0.01	0.22	1.56	0.03	0.29	0.18	0.00	2.55	0.03	0.29	1.95	0.01	0.01	0.01	0.02	0.02	3.30	0.27	0.01	0.00	0.01	0.02	0.00	0.02	0.01
MusCont4	0.02	0.12	0.00	0.72	14.81	0.02	0.01	0.20	1.95	0.05	0.43	0.60	0.00	9.77	0.08	0.60	2.11	0.03	0.01	0.07	0.02	0.19	14.02	0.12	0.01	0.02	0.03	0.05	0.00	0.06	0.01
MusCont5	0.02	0.14	0.03	0.44	7.58	0.02	0.01	0.14	1.62	0.02	0.19	0.87	0.00	15.39	0.14	0.36	0.70	0.08	0.01	0.05	0.02	1.58	23.17	0.26	0.01	0.02	0.04	0.07	0.02	0.04	0.01
MusInsect1	0.01	0.07	0.02	5.64	34.06	0.00	0.02	18.17	10.06	0.14	0.71	0.18	0.00	2.30	0.03	0.62	2.53	0.00	0.04	0.15	0.00	0.00	1.73	0.08	0.01	0.01	0.00	0.02	0.02	0.01	0.01
MusInsect2	0.02	0.04	0.01	10.91	1.60	0.00	0.01	0.09	0.77	0.02	0.11	0.42	0.04	5.29	0.06	1.97	0.39	0.01	0.01	0.05	0.02	3.69	6.00	0.16	0.01	0.03	0.01	0.01	0.05	0.03	0.04
MusInsect3	0.02	0.14	0.01	4.70	4.17	0.01	0.01	0.22	2.37	0.05	0.38	0.24	0.00	3.09	0.03	4.78	2.06	0.06	0.08	0.03	0.03	0.01	4.63	0.43	0.02	0.03	0.04	0.00	0.16	0.12	0.19
MusInsect4	0.01	0.06	0.01	23.37	4.67	0.01	0.00	0.06	1.06	0.03	0.11	0.16	0.00	2.08	0.02	3.00	1.00	0.02	0.03	0.01	0.01	0.00	3.53	0.17	0.01	0.02	0.01	0.03	0.04	0.03	0.04
MusInsect5	0.01	0.07	0.03	2.03	8.18	0.00	0.00	0.31	1.69	0.04	0.26	0.45	0.01	5.10	0.06	3.75	3.75	0.03	0.03	0.02	0.00	0.02	7.80	0.27	0.01	0.05	0.01	0.02	0.09	0.11	0.08
MusInsect6	0.02	0.12	0.04	0.70	2.33	0.01	0.01	0.12	1.53	0.06	0.22	0.73	0.01	10.30	0.10	1.43	1.00	0.05	0.03	0.16	0.03	0.87	14.01	0.20	0.01	0.00	0.01	0.04	0.03	0.10	0.02
MusInsect7	0.05	0.07	0.02	0.53	18.83	0.01	0.00	0.31	1.71	0.02	0.32	0.48	0.01	7.48	0.07	1.13	2.68	0.02	0.02	0.01	0.00	0.00	7.81	0.14	0.01	0.03	0.02	0.02	0.05	0.07	0.04
MusInsect8	0.07	0.10	0.03	0.14	1.18	0.01	0.00	0.07	1.28	0.01	0.15	0.82	0.01	12.37	0.12	0.34	0.52	0.02	0.01	0.36	0.05	27.96	17.86	0.19	0.02	0.01	0.03	0.05	0.07	0.06	0.04

Sample	13.41_farnesan	13.48_endobornyl_acetate	13.54_1,_1,-1-bicyclohexyl,_2-methyl,_cis-	13.68_UK	13.74_UK	13.82_2-phenoxy-ethanol	13.84_sesquiterpene	13.93_4-phenylcyclohexene	14.24_nonanoic_acid	14.33_UK	14.51_UK	14.72_caryophyllene	14.85_benzosuberan-9-carboxaldehyde	15.01_UK	15.16_eugenol	15.17_UK	15.28_propanoic_acid_ester	15.42_sesquiterpene	15.45_UK	15.57_germacrene-D	15.64_a-farnesene	15.83_UK	16.22_UK	16.38_2H-Benzimidazol-2-one,_1,_3-dihydro-5-methyl	16.47_2H-Benzimidazol-2-one,_1,_3-dihydro-5-methyl	16.57_Indan-1,_3-diol_monopropionate	17.15_7-ethyl-1,_3,_5-cycloheptatriene	17.40_2,_6,_10-trimethylpentadecane	17.48_UK	18.79_amantidine	18.87_2,_6-diisopropylnaphthalene	18.99_2,_6-Bis(1,_1-dimethylethyl)-4-(1-oxopropyl)_phenol
Control1	1.17	0.02	0.39	0.04	2.66	0.01	0.03	0.18	0.88	0.16	1.31	0.01	0.00	1.45	0.01	0.02	5.89	0.01	0.20	0.02	0.01	0.83	1.26	0.02	0.00	3.34	0.24	1.91	1.66	0.07	0.27	0.53
Control2	0.46	0.01	0.07	0.02	1.25	0.01	0.08	0.09	0.41	0.03	0.75	0.00	0.00	1.11	0.02	0.01	3.64	0.02	0.15	0.00	0.01	0.36	0.72	0.01	0.00	1.20	0.10	1.19	0.81	0.02	0.10	0.25
Control3	0.00	0.00	0.00	0.00	0.07	0.06	0.02	0.00	0.29	0.00	0.02	0.00	0.00	0.07	0.00	0.00	0.68	0.00	0.00	0.00	0.00	0.01	0.04	0.00	0.00	0.01	0.00	0.07	0.09	0.00	0.00	0.03
Control4	0.04	0.19	0.02	0.07	0.76	0.16	0.14	0.04	0.04	0.03	0.43	0.03	0.00	1.97	0.03	0.02	5.06	0.00	0.25	0.00	0.02	0.18	0.69	0.01	0.01	0.32	0.08	1.04	0.94	0.02	0.15	0.13
Control5	0.04	0.15	0.02	0.06	0.47	0.12	0.03	0.01	0.18	0.02	0.26	0.01	0.00	1.51	0.01	0.02	4.16	0.10	0.18	0.01	0.01	0.10	0.39	0.00	0.00	0.17	0.04	0.76	0.92	0.04	0.17	0.13
Leaf1	0.01	0.05	0.02	0.00	0.06	0.00	0.02	0.00	0.54	0.00	0.02	0.00	0.00	0.09	1.71	0.28	0.66	0.02	0.02	0.00	0.00	0.06	0.05	0.02	0.05	0.05	0.00	0.06	0.07	0.00	0.01	0.01
Leaf2	0.01	0.02	0.02	0.00	0.04	0.00	0.02	0.00	0.10	0.00	0.01	0.00	0.00	0.08	3.30	0.48	0.41	0.01	0.01	0.00	0.00	0.02	0.02	0.00	0.00	0.13	0.00	0.03	0.03	0.00	0.00	0.00
Leaf3	0.00	0.03	0.00	0.00	0.01	0.00	0.01	0.00	0.03	0.00	0.00	0.00	0.00	0.01	0.65	0.12	0.06	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.01	0.01	0.00	0.00	0.00
Leaf4	0.00	0.01	0.00	0.00	0.01	0.00	0.04	0.00	0.02	0.01	0.00	0.00	0.00	0.01	1.22	0.20	0.06	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Leaf5	0.00	0.02	0.00	0.00	0.01	0.00	0.02	0.00	0.02	0.00	0.00	0.00	0.00	0.01	0.19	0.04	0.07	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00
Frass1	2.04	0.04	1.28	0.53	2.12	0.34	1.01	1.63	1.97	0.92	3.10	6.84	0.01	1.18	0.96	0.42	5.84	2.76	0.14	4.47	11.75	2.92	1.50	0.12	0.00	1.32	0.50	2.92	2.22	0.05	0.47	0.29
Frass2	2.21	0.07	1.91	0.83	3.59	0.80	2.48	2.15	3.09	2.09	4.47	5.93	0.00	2.03	0.38	0.27	5.42	1.12	0.14	1.31	3.61	0.95	1.42	0.07	0.00	0.41	0.44	3.56	1.31	0.04	0.48	0.35
Frass3	2.05	0.08	1.53	0.75	2.34	0.32	0.77	1.30	2.24	1.47	4.21	2.69	0.00	2.16	0.41	0.49	8.36	0.85	0.06	0.56	1.11	1.31	1.30	0.08	0.00	0.62	0.40	2.95	1.39	0.04	0.47	0.21
Frass4	2.05	0.07	1.54	0.69	1.96	0.22	1.32	1.11	1.10	0.77	3.53	4.83	0.04	2.22	0.59	0.30	12.17	0.85	0.09	4.53	5.26	2.34	2.11	0.18	0.01	1.18	0.69	6.15	2.72	0.05	0.72	0.91
Frass5	1.67	0.05	1.05	0.46	2.78	0.27	0.19	0.97	1.41	0.73	4.06	1.17	2.06	1.98	1.13	0.33	5.49	0.77	0.10	1.45	2.68	1.30	1.76	5.89	7.27	0.22	0.53	6.70	1.65	0.04	0.46	0.60
InsectSeal1	1.85	0.60	1.63	0.86	1.33	7.02	0.51	0.88	0.17	1.14	2.54	0.08	0.00	1.26	0.04	0.18	1.96	0.54	0.18	0.02	0.19	0.38	0.44	0.01	0.00	0.13	0.44	1.14	0.54	0.06	0.15	0.09
InsectSeal2	0.77	0.13	0.66	0.37	0.76	3.54	0.17	0.24	0.04	0.61	1.19	0.09	0.00	1.09	0.01	0.07	1.67	0.05	0.04	0.00	0.11	0.35	0.40	0.02	0.00	0.05	0.40	0.74	0.53	0.26	0.31	0.10
InsectSeal3	0.48	1.62	0.52	0.32	0.21	0.06	0.09	0.38	0.04	0.42	0.54	0.03	0.00	0.10	0.02	0.02	0.44	0.02	0.20	0.04	0.01	0.21	0.26	0.00	0.02	0.10	0.07	0.15	0.07	0.00	0.06	0.04
InsectSeal4	1.29	0.89	1.58	1.06	0.26	0.52	0.30	0.39	0.05	1.64	1.68	0.11	0.00	1.33	0.04	0.16	1.90	0.15	0.07	0.03	0.09	0.33	0.37	0.00	0.00	0.04	0.57	0.69	0.35	0.04	0.11	0.03
InsectSeal5	1.85	2.78	2.68	1.28	0.52	0.90	0.30	1.22	0.05	2.81	2.15	0.37	0.00	0.52	0.22	0.50	1.40	0.34	0.05	0.02	0.24	0.62	0.73	0.00	0.01	0.08	0.86	1.66	0.43	0.10	0.20	0.06
InsectSeal6	1.16	0.46	1.35	0.65	0.34	0.41	0.06	0.22	0.05	1.19	1.41	0.28	0.00	1.52	0.04	0.29	2.31	0.16	0.05	0.02	0.12	0.44	0.69	0.02	0.02	0.03	0.95	1.28	0.59	0.17	0.27	0.12
InsectSeal7	2.84	0.27	2.55	1.88	2.72	4.75	0.82	1.23	0.15	2.18	3.96	0.51	0.00	2.01	0.08	0.21	4.15	0.05	0.06	0.20	0.22	0.68	0.55	0.08	0.02	0.09	0.65	1.12	0.66	0.62	0.42	0.04
InsectSeal8	1.38	0.18	1.21	0.45	0.49	0.79	0.26	0.32	0.06	1.16	1.70	0.32	0.00	2.06	0.01	0.17	4.16	0.05	0.08	0.13	0.12	0.45	0.29	0.02	0.01	0.04	0.51	0.63	0.58	0.09	0.24	0.01
InsectSeal9	2.90	0.49	3.10	1.91	1.87	2.47	0.81	2.34	0.09	3.03	4.95	0.69	0.00	2.20	0.13	0.95	4.00	0.17	0.42	1.33	0.17	1.51	0.79	0.56	0.13	0.11	1.06	1.12	0.58	0.12	0.48	0.10
InsectSeal10	1.86	0.15	1.63	0.59	0.82	1.10	0.66	0.84	0.04	2.12	3.04	0.16	0.02	2.92	0.04	0.36	5.07	0.28	0.26	1.64	0.26	0.63	1.10	0.43	0.09	0.42	1.28	1.38	0.78	0.65	2.26	0.12
MusCont1	0.01	0.02	0.01	0.00	0.13	0.11	0.02	0.00	0.01	0.00	0.03	0.00	0.04	0.14	0.00	0.00	0.29	0.00	0.01	0.00	0.00	0.20	0.04	0.30	0.68	0.04	0.00	0.06	0.02	0.00	0.00	0.01
MusCont2	0.02	0.09	0.01	0.00	0.31	0.03	0.06	0.01	0.03	0.00	0.02	0.00	0.02	0.28	0.00	0.00	0.43	0.00	0.06	0.01	0.01	1.41	0.11	0.34	0.63	0.16	0.01	0.15	0.07	0.00	0.01	0.02
MusCont3	0.02	0.05	0.01	0.00	0.22	0.03	0.03	0.00	0.02	0.00	0.08	0.00	0.00	0.26	0.00	0.00	0.68	0.01	0.08	0.00	0.00	0.25	0.11	0.00	0.00	0.06	0.02	0.11	0.07	0.00	0.01	0.02
MusCont4	0.05	0.61	0.01	0.04	0.41	0.04	0.05	0.01	0.04	0.00	0.11	0.00	0.00	0.45	0.01	0.00	1.27	0.01	0.87	0.01	0.00	1.31	0.14	0.00	0.00	0.45	0.02	0.20	0.11	0.00	0.00	0.02
MusCont5	0.03	0.12	0.01	0.02	0.61	0.05	0.08	0.00	0.01	0.00	0.05	0.00	0.00	0.54	0.00	0.02	1.05	0.00	0.25	0.00	0.00	1.29	0.20	0.00	0.00	0.13	0.04	0.37	0.17	0.01	0.01	0.02
MusInsect1	0.03	0.31	0.04	0.00	0.13	0.00	0.02	0.01	0.11	0.01	0.05	0.17	0.03	0.09	0.02	0.01	0.43	0.02	0.01	0.44	0.07	0.24	0.08	0.10	0.48	0.02	0.01	0.08	0.14	0.00	0.00	0.02
MusInsect2	0.12	0.05	0.06	0.04	0.56	1.43	0.14	0.04	0.02	0.05	0.20	0.01	0.00	0.20	0.00	0.02	0.24	0.01	0.03	0.01	0.01	0.75	0.10	0.28	0.52	0.10	0.06	0.28	0.07	0.01	0.02	0.01
MusInsect3	0.49	0.16	0.29	0.14	0.60	0.51	0.14	0.06	0.04	0.25	0.82	0.12	0.00	0.75	0.01	0.04	1.67	0.02	0.21	0.01	0.01	2.02	0.48	0.01	0.00	0.17	0.12	0.26	0.25	0.01	0.05	0.04
MusInsect4	0.11	0.04	0.08	0.04	0.23	0.61	0.12	0.02	0.01	0.06	0.17	0.01	0.00	0.34	0.00	0.00	0.98	0.00	0.05	0.01	0.01	1.38	0.10	0.00	0.00	0.07	0.05	0.20	0.11	0.00	0.00	0.02
MusInsect5	0.30	1.08	0.18	0.10	0.77	0.27	0.14	0.05	0.03	0.15	0.56	0.03	0.00	0.37	0.01	0.03	0.84	0.01	0.57	0.01	0.01	2.09	0.22	0.01	0.00	0.60	0.10	0.28	0.15	0.02	0.06	0.02
MusInsect6	0.11	1.60	0.05	0.08	0.45	0.18	0.10	0.01	0.02	0.01	0.27	0.03	0.00	0.60	0.00	0.02	1.88	0.01	0.83	0.00	0.00	1.16	0.13	0.00	0.00	0.39	0.06	0.35	0.16	0.01	0.03	0.01
MusInsect7	0.13	0.33	0.08	0.04	0.27	0.13	0.07	0.04	0.03	0.06	0.17	0.01	0.00	0.24	0.00	0.02	0.51	0.01	0.12	0.01	0.01	1.37	0.16	0.01	0.01	0.11	0.09	0.21	0.16	0.02	0.04	0.02
MusInsect8	0.21	0.46	0.13	0.08	0.60	0.13	0.02	0.02	0.01	0.09	0.28	0.02	0.00	0.41	0.00	0.02	1.11	0.00	0.48	0.01	0.02	0.74	0.17	0.00	0.00	0.30	0.12	0.28	0.17	0.02	0.03	0.02