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An Apple a Day Does Not Keep the Weevils Away: Enhancing Vine Weevil Monitoring With Fruit-Based Volatiles

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ABSTRACT

Vine weevil (*Otiorhynchus sulcatus* Fabricius; Coleoptera: Curculionidae) is an economically important pest of soft fruit and ornamental crops worldwide. Despite extensive research over three decades, the development of an effective semiochemical lure to improve monitoring for this pest remains a challenge. This study investigated the behavioural and electrophysiological responses of adult vine weevils to apple sauce volatiles under laboratory conditions, using Y-tube olfactometer bioassays and headspace analysis using gas chromatography coupled to mass spectrometry (GC–MS) and GC coupled to electroantennographic detection (GC-EAD). In Y-tube bioassays, more adults selected the olfactometer arm containing apple sauce volatiles compared to the control arm at lower doses (0.1, 1 and 10 g) but not at higher doses (20 g). Thirteen compounds were identified in volatiles collected from apple sauce, with the major components being furfural and sorbic acid. Consistent electrophysiological responses were recorded to (E)-2-heptenal, 1-hexanol, (Z)-3-hexenol and (E)-2-hexenol. The behavioural response of vine weevil adults to refuges baited with semiochemical lures was also tested under glasshouse conditions. In this scenario, a greater proportion of individuals were recorded in refuges baited with apple sauce compared to unbaited refuges. A similar behavioural response was also recorded when refuges were baited with a combination of apple sauce and Fortune's spindle (*Euonymus fortunei* (Turcz.) Hand.-Maz.; Celastrales: Celastraceae) compared to those that were unbaited or individually baited with Fortune's spindle or apple sauce. This study indicates that apple sauce positively influences positively vine weevil behaviour and could serve as a basis for developing a novel lure for improved monitoring.

1 | Introduction

Vine weevil (*Otiorhynchus sulcatus* Fabricius; Coleoptera: Curculionidae) is an economically important pest of soft fruit and ornamental crops worldwide (Pope and Roberts 2022). Adult weevils feed on leaves, resulting in characteristic 'notching' along the edge of the leaf (Bennison et al. 2018), which is considered mainly cosmetic damage with limited impact on plant health (Pope and Roberts 2022). By contrast, the larvae feed on subterranean plant tissues, which reduces vigour and can kill the plant if damage is severe (Smith 1932; Moorhouse et al. 1992; Bennison et al. 2018).

A challenge in managing vine weevil is the timely application of control measures (van Tol et al. 2012). This difficulty partly arises because the adults are nocturnal and the larvae live underground, making it hard to detect the pest within crops (van Tol et al. 2012). Growers currently monitor vine weevil presence using manual night-time crop inspections or by assessing the leaf notching caused by adult feeding (Bennison et al. 2016, 2018). However, these monitoring techniques are often time-consuming and unreliable in identifying infested areas within a crop. To implement effective integrated management programmes for vine weevil, it is necessary to develop a sensitive and reliable monitoring system that considers a mixture of both visual and olfactory cues (van Tol et al. 2012; Roberts et al. 2020; Fezza et al. 2022, 2023). Like many other insect pests, vine weevils use visual cues for orientation (Roberts et al. 2020; Fezza et al. 2022) and depend on olfactory cues to differentiate between host and non-host plants (van Tol et al. 2004; Roberts et al. 2019). Therefore, integrating these sensory modalities into monitoring tools could significantly enhance detection sensitivity and improve pest management effectiveness.

Fruit-based volatiles have been widely reported to enhance pest monitoring (Natale et al. 2004; Bartelt and Hossain 2006; Knight 2010; Stuhl 2021). Volatile organic compounds (VOCs) derived from apple (*Malus domestica* Borkh; Rosales: Rosaceae) flowers and fruit have been used as lures to enhance pest monitoring efficacy (Bengtsson et al. 2001; Coracini et al. 2004). Apple-derived volatiles have previously been identified to evoke positive behavioural responses in a variety of weevil (Curculionidae) pests (Smith 1932). For example, Leskey and Prokopy (2000) reported that the plum curculio (*Conotrachelus nenuphar* Herbst; Coleoptera: Curculionidae) preferentially moves towards volatiles from the McIntosh red apple variety (*Malus domestica McIntosh*), while Melander and Spuler (1926) used apple pomace, the residue from juice and cider production, as a bait to coat inorganic insecticides (e.g., zinc arsenite, magnesium arsenate or calcium arsenate) to control strawberry root weevil (*Otiorhynchus ovatus* L.; Coleoptera: Curculionidae) adults. Similarly, Mote and Wilcox (1927) reported the use of an apple-based bait to increase the efficacy of a killing agent used against the strawberry root weevil and the rough strawberry weevil (*O. rugosostriatus* Goeze; Coleoptera: Curculionidae). Li et al. (1995) tested the use of apple pomace to increase the efficacy of grooved board traps to capture vine weevil adults in raspberries (*Rubus idaeus* L.; Rosales: Rosaceae), but the addition of the apple pomace did not increase monitoring tool efficacy.

Although the use of apple-based products as lures has been investigated for some weevil species, direct evidence for these influencing vine weevil behaviour remains limited. For the purposes of this study, apple sauce was selected as the test material because it is commercially available, has a consistent composition and is easily quantifiable, unlike fresh fruit and apple pomace, which are of variable quality and deteriorate rapidly under field conditions.

Using apple sauce as a fruit-based lure, a series of laboratory and glasshouse experiments were carried out to: (i) investigate the behavioural responses of vine weevil adults to apple sauce under laboratory conditions; (ii) evaluate attractiveness using refuges baited with apple sauce under glasshouse conditions and (iii) identify VOCs using gas chromatography coupled to mass spectrometry (GC–MS) and detect electrophysiologically active compounds using gas chromatography coupled to electroantennographic detection (GC-EAD).

2 | Materials and Methods

2.1 | Insects

Adult vine weevils were collected from commercial strawberry (*Fragaria × ananassa cv. Duchesne*) crops in Norfolk (UK) during summer 2023. Adults were maintained in plastic terraria (30×19.3×20.6 cm; Exo Terra, Castleford, UK). Terraria contained three components that were replaced weekly: Fortune's spindle (*Euonymus fortunei* (Turcz.) Hand.-Maz.; Celastrales: Celastraceae) foliage as a food source, a moist paper towel as a water source and a cotton wool ball as an oviposition site. These terraria were housed in a controlled environment room maintained at 20°C and 60% relative humidity with a 16:8 light:dark photoperiod (Fitotron, Weiss Technik, Ebbw Vale, Wales, UK). Eggs were collected every 3 days from the cotton wool balls in each terrarium using a fine 000 paintbrush. Collected eggs were maintained in plastic containers (17×10.2×3 cm, GP Globe Packaging, UK) filled with compost (SylvaGrow John Innes Number 2, Tetbury, UK) housed within a controlled environment room (20°C, 60% relative humidity; Fitotron) under complete darkness to promote egg hatch and larval development. Carrot slices were placed on the surface of the growing media each week as a food source for emerging larvae. Larvae that completed their development were maintained as adults in terraria under the environmental conditions previously described. All adult weevils used in the following experiments were at least 3 months old and reproductively active.

2.2 | Behavioural Bioassays

2.2.1 | Y-Tube Olfactometer Bioassay

Behavioural responses of adult vine weevils to chemical stimuli were tested using the glass Y-tube olfactometer setup previously described by Roberts et al. (2019, 2023). The olfactometer consisted of a release chamber (Ø = 100 mm) connected to a 120-mm-long glass tube that branched into two 190 mm arms with an internal diameter of 18 mm (Sci-Glass Consultancy, Bere Alston, UK). Airflow through the olfactometer was purified by first passing it through a charcoal filter, then humidified by passing it through tap water before being pumped through 500 mL Drechsel bottles containing the chemical stimuli being tested. The airflow was set to 1200 mL min^{-1}, with odour sources held in the Drechsel bottles for at least 1 h before an experiment began. An empty Drechsel bottle supplying clean, humidified air was used for the control. The olfactory stimuli tested in the olfactometer were Bramley apple sauce (Tesco Stores Ltd., UK) at four doses (0.1, 1, 10, 20 g) or small branches of Fortune's spindle (10 or 20 g; Ø ~1 cm and length ~10 cm) as this was previously identified as eliciting a positive behavioural response in vine weevil adults (Roberts et al. 2019). Freshly cut branches of Fortune's spindle were collected from the Harper

Adams University grounds and used in the experiments within 4 h of collection.

All olfactometer bioassays were carried out in a controlled environment room (20°C and 60% relative humidity; Fitotron) between 07:00 and 11:00 a.m. The controlled environment room was illuminated to stimulate light levels at dawn as this is a known period of adult vine weevil activity (Philips Master TL-D 70 W/840, UK; 138 Lux).

Vine weevils were starved for 48 h prior to use in olfactometer bioassay. Single vine weevil adults were introduced into the release chamber of the olfactometer using a 000 brush. A choice was recorded when an individual reached the end of an olfactometer arm within the 20 m observation period. Non-responding adults were recorded as individuals that remained stationary in the release chamber or failed to reach the collecting points at the end of the branched arms within the observation period. Each pair of odour sources was tested 20 times using fresh individuals. To minimise any potential directional bias, the position of the odour sources was alternated between replicates. After each replicate, all glassware was thoroughly cleaned by rinsing with warm water followed by HPLC-grade acetone (Sigma Aldrich now Merck, Gillingham, Dorset, UK) and then baked in a glassware oven at 120°C for 15 m (Roberts et al. 2019, 2023).

2.2.2 | Glasshouse Bioassay

Bioassays testing vine weevil behaviour towards refuges baited with apple sauce and/or Fortune's spindle were carried out in a semi-field environment. This semi-field environment was created using strawberry plants (cv. Elsanta; RW Walpole, King's Lynn, UK) in pots (Ø = 13 cm; Teku VCH13, Pöppelmann, Lohne, Germany) placed in a fine mesh tent cage (145 × 145 × 152 cm) (Insectopia, Austrey, UK) situated within a glasshouse (mean temperature = 19.2 ± 0.8°C; mean humidity = 52.2 ± 1.6%) (Figure 1). Four potted strawberry plants were positioned equidistant from one another along the perimeter of a 110 cm × 110 cm square centrally positioned within the tent cage (Figure 1), providing both a food source and alternative shelters. Refuges were created from paper cups (height = 11.3 cm, Ø = base 5.8 cm, Ø rim = 8.9 cm) (Comfy Package, New York, US), externally and internally painted black using poster paint (Galeria Acrylic, Windsor and Newton, London, UK) (Fezza et al. 2022, 2023). Paper cups were inverted so that the rim became the refuge base, and four equally spaced entrances were made by cutting 1 cm^2 openings around the cup rim. A roll of corrugated card (length 30 cm, width 3 cm) was inserted into each refuge to provide shelter by exploiting the thigmotactic behaviour exhibited by this species.

The behavioural responses of adult vine weevils to baited refuges were tested in five binary-choice bioassays as outlined in Table 1. Small branches of Fortune's spindle (5 g; Ø ~1 cm, length ~4 cm) were placed in white organza bags (7 × 9 cm; OWill, UK). Apple sauce (5 g) was contained in plastic Petri dishes (35 × 10 mm; Sarstedt AG and Co. KG, Germany). A drill (Dremel 3000, Eternal Tools, UK) was used to make a 1 cm hole in the lid of these Petri dishes, and a fine mesh was glued over this hole to prevent direct contact between the adults and the treatments. For the control, empty organza bags and Petri dishes were used. Bait position and the tent cage ($n = 2$) to which they were allocated were re-randomised each day to exclude the effect of position. Vine weevil populations, along with the refuges and odour sources, were replaced between each replicate. Forty adult vine weevils were collected from the laboratory culture, placed in a plastic box (Ø = 12 cm) and then released into the centre of the experimental arena between 17:00 and 20:00. This period coincides with dusk, when vine weevils naturally begin

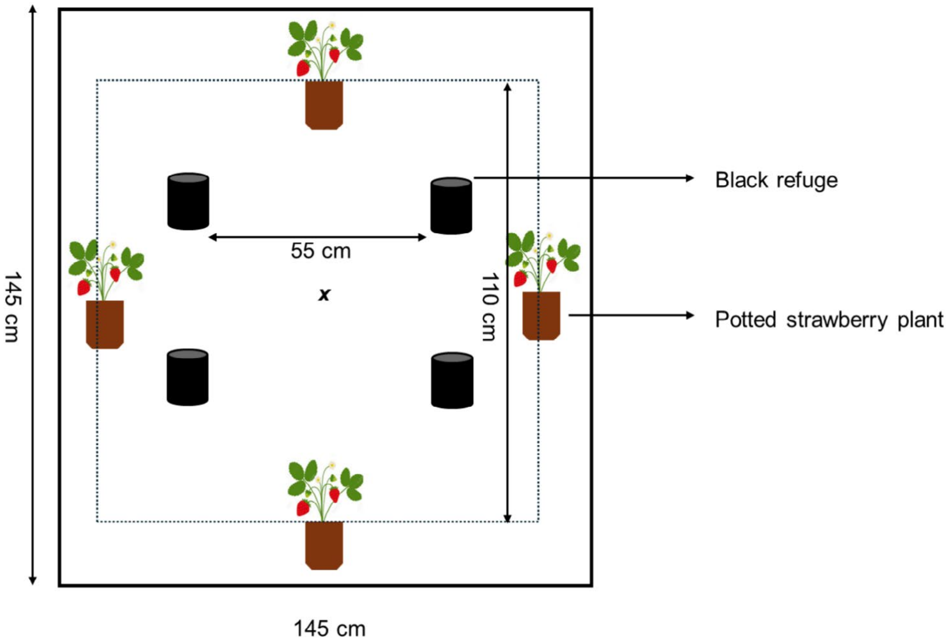

FIGURE 1 | Schematic diagram showing the arrangement within each tent cage for the glasshouse bioassays. The vine weevil release point is shown by ×. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 | Experiments testing the effect of different olfactory cues on vine weevil behaviour under glasshouse conditions.

Experiment	Refuge 1	Refuge 2	No. replicates
1	Apple sauce[a]	Control	5
2	Apple sauce	Fortune's spindle[b]	5
3	Apple sauce + Fortune's spindle	Control	5
4	Apple sauce + Fortune's spindle	Fortune's spindle	5
5	Apple sauce + Fortune's spindle	Apple sauce	5

[a]Apple sauce (5 g) was used for this experiment.
[b]Small branches of Fortune's spindle (*Euonymus fortunei*) (5 g, Ø ≈ 1 cm and length ≈ 4 cm) were used for the experiments with Fortune's spindle.

their nocturnal activity. The location of each vine weevil was recorded between 08:00 and 09:00 the following day, allowing a full overnight response period and for vine weevils to select a refuge following sunrise. Each binary-choice experiment tested a total of 400 individuals over the course of two replicates per day for 5 days.

2.3 | Volatile Analysis

2.3.1 | Apple Sauce Volatile Collection

Headspace samples were collected from Bramley apple sauce (5 g; Tesco Stores Ltd., UK) spread on a plastic weighing dish (5 cm × 2.5 cm). This was placed in a glass chamber (12 cm × 5 cm) and air was drawn at 2 L min^{-1} through a filter containing activated charcoal (20 cm × 1.5 cm; 6–10 mesh) and out through a filter containing Porapak Q (200 mg; 50–80 mesh; Supelco, Gillingham, Dorset, UK) held between two silanised glass wool plugs in a Pasteur pipette (4 mm i.d.). The Porapak Q was purified by Soxhlet extraction with chloroform for 4 h and washing with dichloromethane (Pesticide Residue Grade, Sigma Aldrich, now Merck, Gillingham, Dorset, UK) immediately before use. Volatile collections were carried out at 20°C for 1 h to minimise breakthrough of highly volatile compounds. Trapped volatiles were eluted using dichloromethane (1.2 mL) and stored at 4°C until analysis. Four collections were made and analysed by GC–MS without concentration. One sample was concentrated to approximately 100 μL under a gentle stream of purified nitrogen for analysis by GC-EAD.

2.3.2 | Analysis of Apple Sauce Volatile Collections by GC–MS and GC-FID

Apple sauce volatile collections were analysed using a HP6890N gas chromatograph (GC) coupled to a 5973 Mass Selective Detector (Agilent Technologies, Cheadle, UK). The GC was fitted with a fused silica capillary column (30 mm × 0.25 mm × 0.25 μm film thickness) coated with DBWax (Supelco). Manual injections of 1 μL were made in splitless mode at 240°C. The column oven temperature was held at 40°C for 2 m and then programmed at 10°C min^{-1} to 250°C and held for 3 m. Compounds were identified from their mass spectrum, retention index compared to *n*-alkanes and co-chromatography with authentic compounds. Relative amounts of the compounds present were calculated by comparison of their peak areas.

Amounts of compounds present in the collections were quantified by adding an internal standard (decyl acetate; 5 μg) to two of the collections and analysing these by GC with flame ionisation detection (FID; 250°C) using a HP6850 GC (Agilent) with column and running conditions as above. Approximate amounts of the compounds present were calculated from their peak area relative to that of the internal standard.

2.3.3 | Analysis of Apple Sauce Volatile Collections and Synthetic Compounds by GC-EAD

Analyses were carried out using a HP6890 gas chromatograph (GC) (Agilent Technologies) fitted with a flame ionisation detector (FID) and fused silica capillary columns (30 m × 0.25 mm × 0.32 μm film thickness) coated with DBWax and DB5 (Supelco). Injections onto the DBWax column were made in splitless mode at 220°C, with the column oven temperature held at 40°C for 2 m and then programmed at 20°C min^{-1} to 250°C and held for 3 m. The effluents from both columns were combined using a glass push-fit Y-tube connector (Agilent Technologies), linked to another Y-tube connector via deactivated fused silica tubing (10 cm × 0.32 mm i.d.). One arm of this connector was connected to the FID (at 250°C) using 50 cm of fused silica tubing (0.32 mm i.d.), while the other arm led through a heated transfer line (250°C; Syntech, Hilversum, The Netherlands, now Kirchzarten, Germany) into a glass tube (4 mm i.d.), where air (500 mL/min) passed over the EAG preparation. Electroantennographic detection (EAD) recordings were carried out using a portable INR-02 device (Syntech) connected as a second detector of the GC for analog-to-digital (A/D) conversion. Glass electrodes containing an electrolyte solution (0.1 M potassium chloride with 1% polyvinylpyrrolidone) were mounted onto silver wires held in micromanipulators. Vine weevils were anaesthetised using carbon dioxide before excising an antenna using a scalpel. The base of the antenna was inserted into the reference electrode while the circuit was completed by placing the recording electrode in contact with the tip of the antenna. Both the flame ionisation detector (FID) and EAD signals were recorded and analysed using EZChrom software (Elite v3.0; Agilent Technologies). For analysis of apple sauce volatile collections (N = 3), 2 μL of the concentrated solution was injected. Synthetic 1-hexanol and furfural (98%; Merck), two components of the apple sauce volatile collections, were also analysed ($N=2$), injecting 1 μL of a hexane solution containing 10 ng/μL of each compound.

2.4 | Statistical Analyses

All statistical analyses were carried out using R (Version 4.2.2) (R Core Team 2022). Y-tube olfactometer bioassay data were analysed using an exact binomial test against the null hypothesis that the number of vine weevils reaching the end of the olfactometer arm had a 50:50 distribution (Roberts et al. 2019, 2023). For binary-choice experiments carried out under glasshouse conditions, the number of individuals within a refuge (i.e., refuge performance) was also analysed using an exact binomial test against the null hypothesis that the number of vine weevils seeking refuge had a 50:50 distribution (Roberts et al. 2020). Replicates from each experiment were pooled prior to analysis, and non-responding individuals were excluded.

3 | Results

3.1 | Behavioural Bioassays

3.1.1 | Dose–Response to Apple Sauce

In a series of binary-choice experiments, vine weevil adults were presented with a choice between a clean-air control and apple sauce at different doses (0.1, 1, 10 and 20 g). Vine weevils exhibited strong preferences for apple sauce over the clean-air control at doses of 0.1 g (80% of responding individuals; binomial exact test: no. of successes = 16, no. of trials = 20, $p < 0.05$; Figure 2A), 1 g (75% of responding individuals; binomial exact test: no. of successes = 15, no. of trials = 20, $p < 0.05$; Figure 2B) and 10 g (75% of responding individuals; binomial exact test: no. of successes = 15, no. of trials = 20, $p < 0.05$; Figure 2C). However, at the highest dose of 20 g, no significant preference was observed, with 70% of responding individuals choosing the clean-air control arm over the olfactometer arm containing apple sauce (binomial exact test: no. of successes = 14, no. of trials = 20, $p > 0.05$) (Figure 2D).

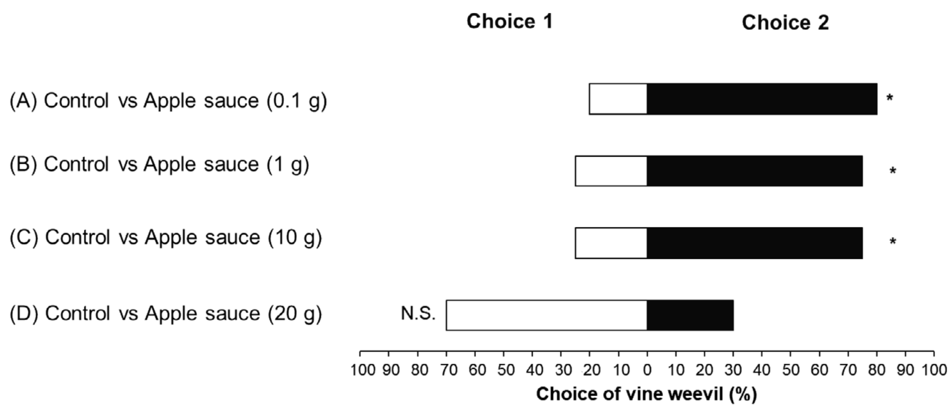

FIGURE 2 | Behavioural responses of vine weevil adults towards volatiles from apple sauce at different doses of 0.1 g (A), 1 g (B), 10 g (C) and 20 g (D) compared with clean air (control) in a Y-tube olfactometer. For each experimental treatment 20 vine weevil adults were tested (binomial exact test: *$p < 0.05$; N.S. = not significant).

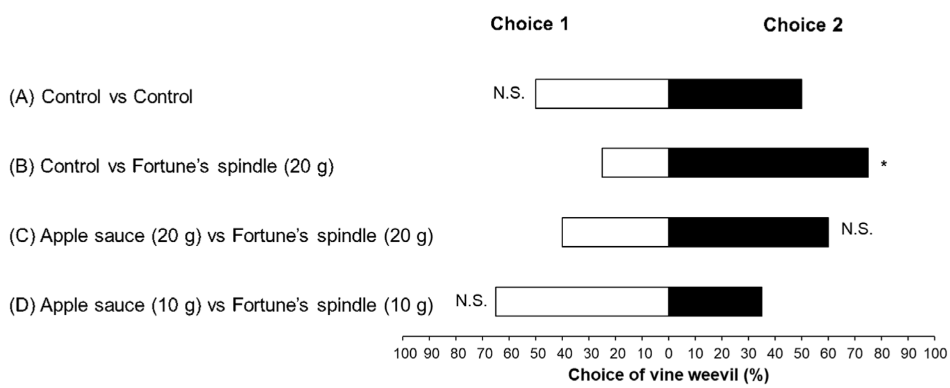

FIGURE 3 | Behavioural responses of vine weevil adults in a Y-tube olfactometer in four experimental comparisons: (A) control vs. control, (B) control vs. Fortune's spindle (20 g), (C) apple sauce (20 g) vs. Fortune's spindle (20 g) and (D) apple sauce (10 g) vs. Fortune's spindle (10 g). For each experimental treatment, 20 vine weevil adults were tested (binomial exact test: *$p < 0.05$; N.S. = not significant).

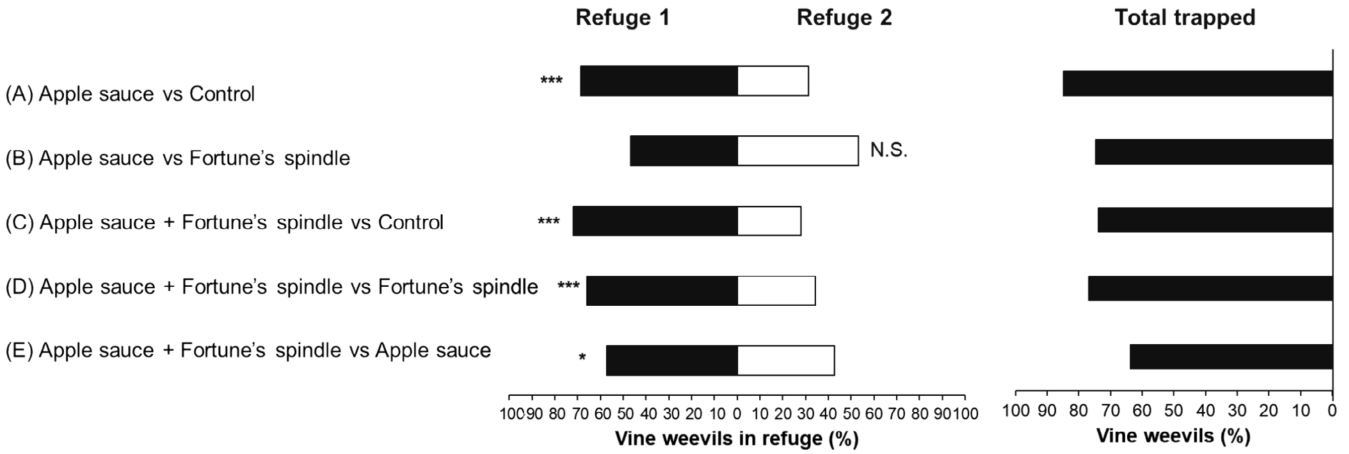

FIGURE 4 | Percentage of vine weevil adults recorded in refuges under five experimental treatments: (A) apple sauce vs. control, (B) apple sauce vs. Fortune's spindle, (C) apple sauce plus Fortune's spindle vs. control, (D) apple sauce plus Fortune's spindle vs. Fortune's spindle and (E) apple sauce plus Fortune's spindle vs. apple sauce. For each experimental treatment, vine weevil adults were released as a group of 40 individuals (binomial exact test: *$p<0.05$, **$p<0.01$, ***$p<0.001$; N.S. = not significant).

3.1.2 | Olfactory Responses to Fortune's Spindle and Apple Sauce

In a series of binary-choice experiments, vine weevil adults were presented with a choice between control (empty Y-tube olfactometer arm) and treatments (Y-tube olfactometer arm with Fortune's spindle or apple sauce volatiles). The first experiment confirmed no inherent bias in the setup, with no preference observed between the two Y-tube olfactometer clean-air control arms (binomial exact test: no. of successes = 10, no. of trials = 20, $p>0.05$; Figure 3A). When testing plant material, vine weevils exhibited a significant preference for the Y-tube olfactometer arm containing 20 g of Fortune's spindle over the clean-air control arm (75% of responding individuals; binomial exact test: no. of successes = 15, no. of trials = 20, $p<0.05$; Figure 3B). In direct comparisons between plant material and apple sauce, no significant preferences were observed: 60% of responding individuals chose the arm containing 20 g of Fortune's spindle over the arm with 20 g of apple sauce (binomial exact test: no. of successes = 12, no. of trials = 20, $p>0.05$; Figure 3C), while 65% chose the arm with 10 g of apple sauce over the arm with 10 g of Fortune's spindle (binomial exact test: no. of successes = 13, no. of trials = 20, $p>0.05$; Figure 3D).

3.1.3 | Evaluation of Single and Combined Baits

In a series of binary-choice experiments, adult vine weevils were presented with refuges baited with various combinations of apple sauce, Fortune's spindle or left unbaited. When comparing baited versus unbaited refuges, more weevils were recorded in refuges baited with apple sauce alone (68.73% vs. 31.27%; binomial exact test: no. of successes = 233, no. of trials = 339, $p<0.001$; Figure 4A) and in refuges baited with the combination of apple sauce plus Fortune's spindle (71.86% vs. 28.14%; binomial exact test: no. of successes = 212, no. of trials = 295, $p<0.001$; Figure 3C). When directly comparing different bait types, there was no significant difference between apple sauce and Fortune's spindle (46.82% vs. 53.17%; binomial exact test: no. of successes = 140, no. of trials = 299, $p>0.05$; Figure 4B). However, the combined bait (apple sauce plus Fortune's spindle) performed significantly better than Fortune's spindle alone (65.79% vs. 34.20%; binomial exact test: no. of successes = 202, no. of trials = 307, $p<0.001$; Figure 4D) and marginally better than apple sauce alone (57.25% vs. 42.74%; binomial exact test: no. of successes = 146, no. of trials = 255, $p<0.05$; Figure 4E).

TABLE 2 | Compounds detected in GC–MS analyses of collections of volatiles from apple sauce.

Compound	Retention time (min)	RI[a]	Relative amount (%) ± SE[b]
3-hexanol	6.79	1199	5.0 ± 1.4
2-methylbutanol	6.91	1208	16.3 ± 1.6
2-hexanol	7.12	1223	3.0 ± 0.7
(*E*)-2-heptenal	8.65	1332	1.8 ± 0.3
1-hexanol	9.02	1358	8.9 ± 0.9
(*Z*)-3-hexenol	9.45	1389	0.9 ± 0.0
Nonanal	9.64	1402	3.3 ± 2.0
(*E*)-2-hexenol	9.76	1411	2.6 ± 0.4
Furfural	10.60	1476	38.1 ± 0.6
Decanal	11.05	1511	1.8 ± 1.2
1-octanol	11.77	1566	1.1 ± 0.6
1-decanol	14.17	1767	1.2 ± 0.7
Sorbic acid	18.14	2146	16.0 ± 8.2

[a]RI is retention index relative to retention times of *n*-alkanes on a DBWax GC column.
[b]Relative amounts are means of four replicates.

3.2 | Analyses of Apple Sauce Volatile Collections by GC–MS, GC-FID and GC-EAD

GC–MS analysis of apple sauce volatile collections identified 13 compounds in measurable amounts in all four collections (Table 2). The most abundant were 2-methylbutanol, 1-hexanol, furfural and sorbic acid. Approximate rates of release from 5 g of apple sauce at 20°C were 0.62, 0.30, 1.13 and 1.22 $\mu g\,h^{-1}$, respectively, as determined by GC-FID analysis.

GC-EAD analysis of a concentrated sample detected consistent EAD responses to (E)-2-heptenal, 1-hexanol, (Z)-3-hexenol and (E)-2-hexenol (Figure 5). A possible response to furfural was observed (Figure 5), but in GC-EAD analyses of equal amounts of 1-hexanol and furfural, a strong response was observed to the 1-hexanol but not to furfural (Figure 6).

4 | Discussion

Phytophagous insects rely on olfaction as the principal sensory modality for detecting their external environment and mediating behavioural changes such as mate selection or food choice (Hansson 1999). Attraction of insects to fruit-derived volatiles, involving detection of specific blends or single compounds, has been successfully exploited for both monitoring and direct control of insect pest populations (Abd El-Ghany 2019).

This study investigated the potential of apple sauce as an effective lure for improving vine weevil monitoring by examining adult behavioural responses under both laboratory and glasshouse conditions. Results consistently demonstrated positive chemotaxis towards apple sauce across multiple experimental settings. In Y-tube olfactometer assays, vine weevil adults exhibited

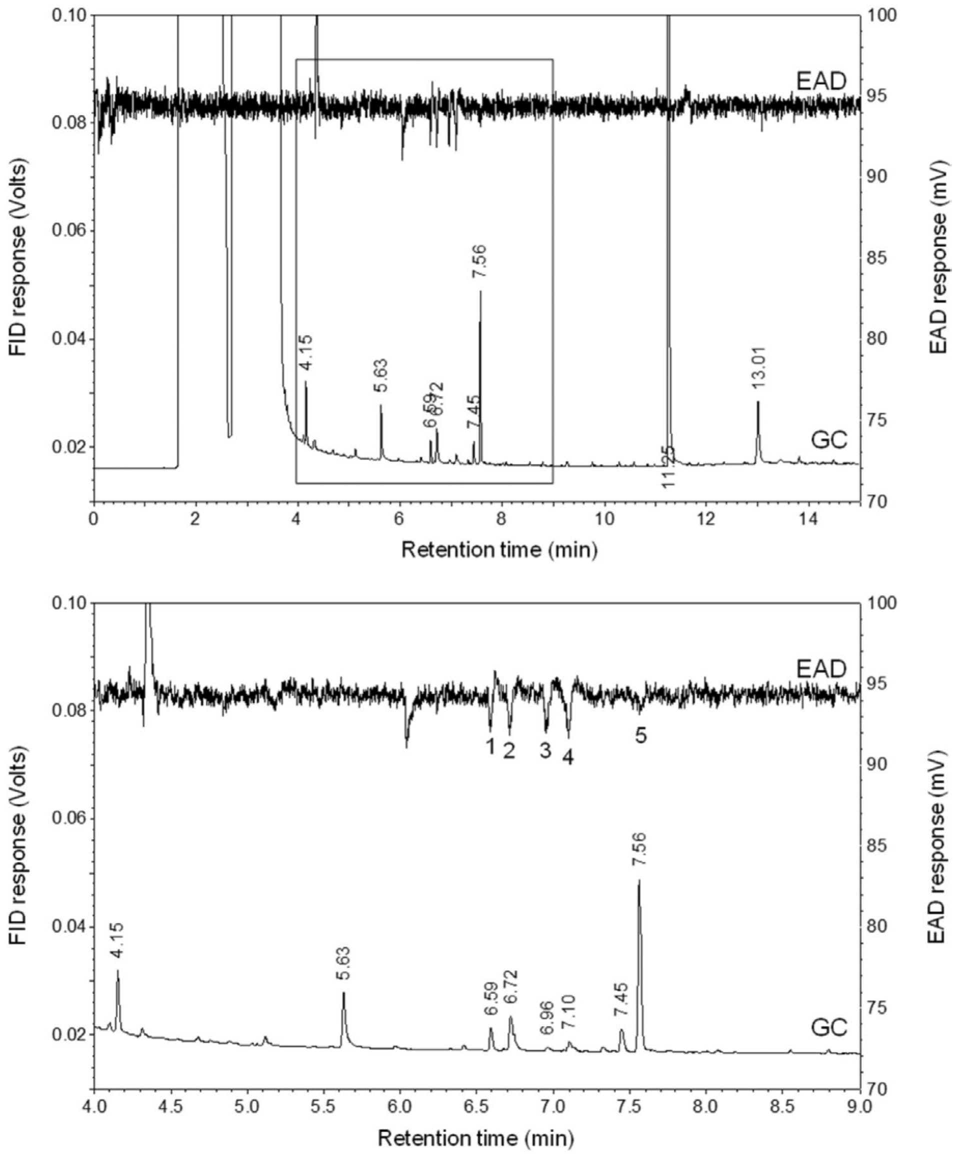

FIGURE 5 | Representative GC-EAD analysis of volatile collections from apple sauce with vine weevil antenna (lower figure is an expansion of the window in the upper trace) (N=3). EAD responses (1) (E)-2-heptenal 6.59 min; (2) 1-hexanol 6.72 min; (3) (Z)-3-hexenol 6.95 min; (4) (E)-2-hexenol 7.10 min and possibly (5) furfural 7.56 min. The response at 6.05 min was not observed in other EAD analyses and is considered an artefact. Other compounds 2-methylbutanol 5.63 min; sorbic acid 11.25 min.

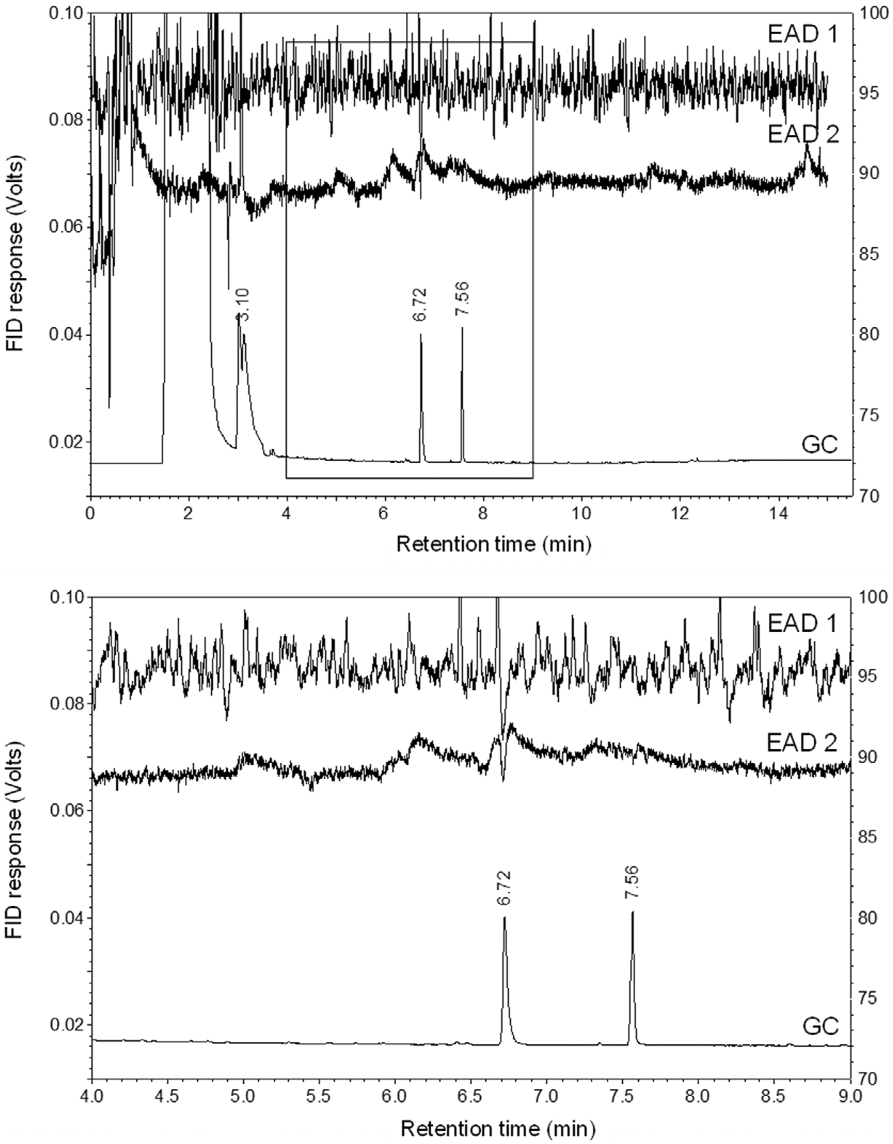

FIGURE 6 | GC-EAD analysis of two synthetic compounds 1-hexanol (6.72 min) and furfural (7.56 min) (10 ng) showing consistent response to 1-hexanol but not furfural ($N = 2$). Lower trace is expansion of window in upper trace.

significant preferences for arms containing apple sauce at doses of 0.1 g, 1 g and 10 g compared to clean-air control arms. These laboratory findings were corroborated in glasshouse trials, where significantly more individuals were recorded in refuges baited with apple sauce than were recorded in unbaited controls. Other weevil species have also shown positive behavioural responses to apple volatiles. For example, the heaven root weevil (Eucryptorrhynchus scrobiculatus Motschulsky; Coleoptera: Curculionidae) has been reported to respond positively to a mixture composed of apple juice, vinegar and ethanol (Yang et al. 2019) in laboratory choice tests.

GC–MS analyses identified 13 compounds in apple sauce volatile collections, with furfural and sorbic acid as the main components. The former is formed during sugar cooking processes and the latter is a common preservative. Short-chain esters typically found in fresh fruit volatiles, such as ethyl butyrate, were notably absent, presumably lost during the cooking process. The volatile profile contained relatively small amounts of short-chain alcohols and aldehydes, compounds commonly found in various fruits and plants that are often associated with green, fruity and floral odours known to influence insect behaviour (van Tol and Visser 2002; Wei and Kang 2011). Consistent EAD responses were observed to (E)-2-heptenal, 1-hexanol, (Z)-3-hexenol and (E)-2-hexenol, suggesting their potential relevance in vine weevil olfactory perception. These findings align with previous research by van Tol et al. (2012), who reported strong EAD responses to (Z)-2-pentenol, (Z)-3-hexenol and (E)-2-hexenol from Fortune's spindle, and with Roberts et al. (2019), who similarly found strong EAD responses to 1-hexanol, (Z)-3-hexenol and (E)-2-hexenol. The consistent EAD activity of these compounds across different studies strengthens the evidence for their importance in vine weevil chemical ecology.

Insect behavioural responses to olfactory cues are influenced by multiple factors, including biotic and abiotic environmental factors, the chemical and physical properties of host plants (Binyameen et al. 2021), and the physiological and motivational state of the insect (Gadenne et al. 2016). The specific ratios and the combinations of compounds in plant volatile blends are considered particularly important in mediating plant–insect interactions (Najar-Rodriguez et al. 2010). In this study, dose–response experiments demonstrated that vine weevil behavioural responses are concentration-dependent, with adults showing a preference for apple sauce presented at lower and medium (0.1, 1 and 10 g) doses compared to the highest dose tested (20 g). This dose-dependent pattern is consistent with other insect studies. For example, Webster et al. (2010) found that the black bean aphid (*Aphis fabae* Scopoli, Hemiptera: Aphididae) responded positively to 1-hexanol and (*Z*)-3-hexen-1-ol only at the highest dose tested (100 ng). Similarly, the Fuller's rose weevil (*Pantomorus cervinus* Boheman; Coleoptera: Curculionidae) exhibited a positive response to medium dosages of synthetic lemon leaf volatiles (50.0 mg/100 mL) but was repelled at the highest concentration (500.0 mg/100 mL) (Wee et al. 2008). The observed reduction in attractiveness at the highest apple sauce dose (20 g) tested could be attributed to sensory adaptation, receptor saturation or the emergence of repellent effects at high concentrations. This finding highlights the importance of optimising odour stimulus concentration when developing effective lures for pest monitoring systems.

To enhance knowledge of the interaction between behaviourally effective constituents in complex odour blends, this study investigated the effect of apple sauce on vine weevil adults when presented alongside or in the absence of other host volatiles such as Fortune's spindle. When presented with a choice between Fortune's spindle and apple sauce individually, no significant differences were found in both laboratory and glasshouse tests. However, refuges baited with the combination of Fortune's spindle plus apple sauce recorded higher numbers of weevils compared to those baited with only apple sauce, and substantially higher numbers compared to refuges baited with only Fortune's spindle or completely unbaited controls. This enhanced attractiveness of the combined lure could be attributed to either different attractive compounds present in the two odour sources or increased amounts of the same attractive compounds. Roberts et al. (2019) recorded EAD responses to 22 compounds in volatiles from Fortune's spindle, including some of the same compounds found in apple sauce (1-hexanol, (Z)-3-hexenol and (E)-2-hexenol). However, other EAD-active compounds unique to Fortune's spindle may have contributed to the increased attractiveness of the combination. The synergistic effect observed with the combined lure suggests that vine weevil attraction may be optimised through strategic combinations of complementary volatile blends rather than single odour sources.

Several studies have reported the use of monitoring tools baited with natural lures (e.g., food-based) or fermented products, vinegars, wines, yeast-sugar solutions and fruit juices to improve monitoring tool efficacy (Cha et al. 2013). For instance, cut orange peels and orange juice increased catches of the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann; Diptera: Tephritidae), with varying effectiveness depending on the specific tissues and preparations tested (Katsoyannos et al. 1997). These natural baits have been widely recommended for various pest species (e.g., for the control of Tephritidae and Drosophilidae spp. as well as stored-product insects) due to their ubiquity, simplicity and cost effectiveness (Rosa et al. 2017). Despite these advantages, natural lures present several limitations, including variability due to lack of standardisation of manufacture (Rosa et al. 2017), reduced efficacy over time, sex-biased captures, frequent re-baiting requirements (Candia et al. 2019), and potential to attract non-target and beneficial insects (Cha et al. 2013), including predatory wasps, bees and lacewings (Candia et al. 2019). In this study, commercially available apple sauce was selected as a candidate bait due to its ability to release volatiles consistently over time, unlike fresh fruit, which varies in composition and deteriorates rapidly. Although the commercial apple sauce proved to be an effective attractant for vine weevil, the cooking process may have degraded many of the fruity esters characteristic of the fresh fruit. Therefore, further research should investigate whether uncooked apple sauce, potentially containing a more diverse volatile profile, would elicit stronger behavioural responses in vine weevil. Additionally, the EAD-active compounds identified in this study should be evaluated in synthetic blends under field conditions to develop standardised, long-lasting lures for practical implementation in vine weevil monitoring programmes.

5 | Conclusions

This study provides insights into the potential use of apple sauce as attractants for the development of semiochemical-based IPM approaches for monitoring and controlling vine weevil. The results show that apple sauce elicits positive behavioural responses in vine weevil adults under both laboratory and glasshouse settings. Similarly, higher numbers of vine weevil adults were recorded in refuges baited with both apple sauce and Fortune's spindle than in refuges baited with only apple sauce or Fortune's spindle. However, further work is needed to optimise apple sauce as a lure suitable for use within vine weevil IPM programmes.

Author Contributions

Eugenia Fezza: conceptualisation (equal), data curation (lead), formal analysis (lead), investigation (lead), methodology (equal), validation (lead), visualisation (lead), writing original draft preparation (lead), writing reviewing and editing (lead); Joe M. Roberts: conceptualisation (equal), data curation (equal), methodology (equal), writing original draft preparation (equal), writing reviewing and editing (equal), supervision; David R. Hall: data curation (equal), formal analysis (equal), methodology (equal), validation (equal), writing reviewing and editing (equal); steven j. harte: data curation (equal), formal analysis (equal), methodology (equal), validation (equal), writing reviewing and editing (equal); Daniel P. Bray: data curation (equal), formal analysis (equal), methodology (equal), validation (equal), writing reviewing and editing (equal); Toby J. A. bruce: reviewing and editing (equal); Lael E. Walsh: reviewing and editing (equal); Michael T. Gaffney: conceptualisation (equal), writing reviewing and editing (equal), funding acquisition (equal), supervision; Tom W. Pope: conceptualisation (equal), methodology (equal), project administration (lead), resources (lead), writing original draft preparation (equal), writing reviewing and editing (equal), funding acquisition (lead), supervision. "catch me if you can" improving monitoring and control of black vine weevil (*Otiorhynchus sulcatus*) in soft fruit and ornamental crops. e.f.'s contribution formed part of a phd study (director of studies t.w.p.).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in "figshare" at https://doi.org/10.6084/m9.figshare.29149427.v2.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1** Supporting Information