# Decoding attraction: improving vine weevil monitoring by exploiting key sensory cues

by Fezza, E., Roberts, J.M., Bruce, T.J., Walsh, L.E., Gaffney, M.T. and Pope, T.W.

**Copyright, publisher and additional information:** Publishers' version distributed under the terms of the <u>Creative Commons Attribution NonCommerical NoDerivatives License</u>

DOI link to the version of record on the publisher's site



Fezza, E., Roberts, J.M., Bruce, T.J., Walsh, L.E., Gaffney, M.T. and Pope, T.W. (2023) 'Decoding attraction: improving vine weevil monitoring by exploiting key sensory cues', *Pest Management Science*.

Received: 8 May 2023

Revised: 3 July 2023

(wileyonlinelibrary.com) DOI 10.1002/ps.7665

# Decoding attraction: Improving vine weevil monitoring by exploiting key sensory cues

Eugenia Fezza,<sup>a,b\*</sup> <sup>©</sup> Joe M. Roberts,<sup>a</sup> <sup>©</sup> Toby J. A. Bruce,<sup>c</sup> <sup>©</sup> Lael E. Walsh,<sup>b</sup> <sup>©</sup> Michael T. Gaffney<sup>b</sup> <sup>©</sup> and Tom W. Pope<sup>a</sup> <sup>©</sup>

#### Abstract

BACKGROUND: Monitoring is an integral component of integrated pest management (IPM) programmes used to inform crop management decisions. Vine weevil, *Otiorhynchus sulcatus* F. (Coleoptera: Curculionidae), continues to cause economically significant losses in horticultural crops due to an inability to reliably detect the presence of this species before crop damage occurs. To improve vine weevil monitoring we investigated the behavioural responses of adult vine weevils to visual (monitoring tool shade/colour, height and diameter as well as the effect of monitoring tool and plant density) and olfactory (host plant and conspecifics) cues under glasshouse conditions.

RESULTS: Monitoring tool shade, height and diameter all influenced monitoring tool efficacy, with individuals exhibiting a preference for black, tall and wide monitoring tools. The total number of individuals recorded in monitoring tools increased with monitoring tool density. By contrast, plant density did not influence the number of individuals recorded in monitoring tools. Yew-baited monitoring tools retained a larger number of individuals compared to unbaited ones. Similarly, more vine weevils were recorded in monitoring tools baited with yew and conspecifics than in unbaited monitoring tools or those baited with only yew. Baiting monitoring tools with conspecifics alone did not enhance the number of vine weevils recorded in monitoring tools.

CONCLUSIONS: Our study confirms that visual and olfactory cues influence vine weevil behaviour. This provides information on key factors that influence vine weevil monitoring tool efficacy and can be used to inform the development of a new monitoring tool for this pest.

© 2023 The Authors. Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Keywords: integrated pest management; traps; weevil pest; visual cues; olfactory cues; semiochemicals

#### 1 INTRODUCTION

Effective integrated pest management (IPM) programmes require reliable pest population monitoring techniques<sup>1</sup> to determine when economic thresholds have been exceeded and then implement appropriate control measures. Various methods are used to detect the presence of pests and monitor their population growth within crops, but many approaches use monitoring tools designed for specific pests.<sup>2</sup> A wide range of monitoring tools have been used to detect insect pests as a result,<sup>3</sup> but the efficacy of each tool design is determined by an ability to exploit knowledge of the target pest's ecology and behavioural response to visual and olfactory stimuli.

Vine weevil, *Otiorhynchus sulcatus* Fabricius (Coleoptera: Curculionidae), remains one of the most important pests of horticultural and ornamental crops globally.<sup>4,5</sup> This is largely due to the fact that monitoring is challenging as the larvae feed below ground while the adults are nocturnal, therefore controls are often applied too late to prevent economically significant losses.<sup>5</sup> The importance of correctly timing control applications has increased as vine weevil management has shifted from a reliance on persistent broad-spectrum insecticides to entomopathogenic nematodes (EPNs) and fungi (EPFs) used to target vine weevil larvae.<sup>6,7</sup> Indeed, despite widespread use of such controls, growers continue to report significant crop losses associated with vine weevil infestation and it is likely that this is in part due to the application of controls being incorrectly timed without use of reliable pest monitoring.<sup>5</sup> Use of monitoring tools for vine weevil adults is not considered reliable, largely due to gaps in knowledge of the biology and ecology of this species<sup>4</sup> as well as the unreliability of commercially available monitoring tools.<sup>5</sup>

Visual appearance is important in the design of effective monitoring tools for many insect pests.<sup>8,9</sup> Colour is often an important design element and the preference for a particular colour is typically species specific and, in some cases, habitat dependent.<sup>10</sup>

- a Centre for Crop and Environmental Science, Agriculture and Environment Department, Harper Adams University, Newport, UK
- b Horticulture Development Department, Teagasc, Ashtown Research Centre, Dublin, Ireland
- c Centre for Applied Entomology and Parasitology, School of Life Sciences, Huxley Building, Keele University, Keele, UK

© 2023 The Authors. *Pest Management Science* published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

<sup>\*</sup> Correspondence to: E Fezza, Centre for Crop and Environmental Science, Agriculture and Environment Department, Harper Adams University, Newport, Shropshire TF10 8NB, UK. E-mail: efezza@live.harper.ac.uk

monitoring tool choices of vine weevil adults under glasshouse conditions. Specifically, the study sought to answer the following questions: (i) do shade, height and diameter influence the efficacy of vine weevil monitoring tools under glasshouse conditions, (ii) is the efficacy of vine weevil monitoring tools influenced by abiotic factors such as monitoring tool or plant density and (iii) do vine weevil adults show a preference for volatiles released from host plant material and/or conspecific baited monitoring tools? 2 MATERIALS AND METHODS 2.1 Insects Adult female vine weevils were collected from commercial strawberry (Fragaria × ananassa) crops grown in Staffordshire, UK (52° 48'10.79 N, 2°08'35.4 W), during May 2022. These individuals were maintained on yew (Taxus baccata Linnaeus) sprigs in plastic terrariums  $(30 \times 19.3 \times 20.6 \text{ cm}; \text{Exo Terra, Castleford, UK})$  and moist paper towels that were replaced weekly.<sup>4</sup> Thirty-five to 40 weevils were placed in each terrarium and maintained in a controlled environment room (20 °C, 60% relative humidity, 16:8 h light:dark photoperiod; Fitotron, Weiss Technik, Ebbw Vale, Wales, UK) until their use in experiments. 2.2 Effect of vine weevil monitoring tool characteristics on performance 2.2.1 Experimental set-up

Experiments testing vine weevil behaviour towards different monitoring tool characteristics were carried out in a glasshouse representing a semifield environment. This glasshouse environment was created using potted ( $\emptyset = 13$  cm; Teku VCH13, Pöppelmann, Lohne, Germany) strawberry plants (cv. Elsanta; RW Walpole, King's Lynn, UK) placed in a fine mesh tent cage ( $145 \times 145 \times 152$  cm) (Insectopia, Austrey, UK) situated within a glasshouse (mean temperature 19.2  $\pm$  0.8 °C, mean humidity 52.2  $\pm$  1.6%). Four potted strawberry plants were positioned equidistant from one another along the perimeter of a 110 cm<sup>2</sup> square centrally positioned within the tent cage (Fig. 1), providing both a food source and alternative refuges. Unless otherwise stated monitoring tools 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 & Newton, London, UK). Paper cups were inverted so that the rim became the monitoring tool base and four equally distanced entrances were made in the monitoring tool by cutting 1 cm<sup>2</sup> 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.

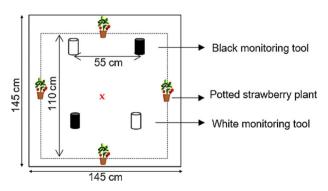
The present study investigated the role of vision and olfaction in

#### 2.2.2 Shade

The behavioural response of adult vine weevils to monitoring tool colour was tested in a binary-choice experiment under the semi-field conditions described in Section 2.2.1. Alongside the standard black monitoring tools, white ones were created by painting paper cups white using poster paint (Galeria Acrylic). Experimental arenas were completed by placing four monitoring tools (two black and two white) at the corners of a 55 cm<sup>2</sup> square centrally positioned within the tent cage (Fig. 1). Monitoring tools and the tent cage (n = 2), to which monitoring tools were allocated, were re-randomised each day to exclude the effect of position. Vine weevil populations, along with the monitoring tools, were replaced between each replicate. Forty adult vine weevils

Even for nocturnal species, such as the adult banana root borer (Cosmopolites sordidus Germar), colour preference has been reported, in this case to mahogany-brown monitoring tools.<sup>11</sup> Similarly, vine weevil adults have a preference for black as well as darker coloured rather than white or lighter coloured monitoring tools.<sup>4</sup> Other visual cues used by insects for orientation include size and shape.<sup>12,13</sup> As a result, monitoring tool size, shape and entrance number/location have been suggested to be key factors in determining the efficacy of monitoring tools.<sup>14</sup> For example, large monitoring tools have been reported to be more effective than smaller ones in some weevil species, including the banana root borer.<sup>11</sup> For vine weevil, while diameter has not previously been investigated, monitoring tool height and entrance position have been shown to be important for vine weevil monitoring design.<sup>4</sup> Monitoring tool position and density in relation to plants found in the same environment may also influence the number of individuals interacting with it.<sup>15</sup>

The addition of olfactory cues such as pheromones and/or host plant volatiles has been shown to improve the efficacy of monitoring tools for Curculionidae pest species.<sup>11,16</sup> For example, ramp traps baited with the aggregation pheromone sordidin (Cosmolure+) caught significantly more banana root borer adults than those without pheromone lures.<sup>17</sup> Similarly, monitoring tools baited with host plant volatiles (e.g., α-pinene and ethanol) caught more pine bark beetles (Hylastes ater Paykull) and red-haired pine bark beetles (Hylurgus ligniperda Fabricius) than unbaited traps.<sup>18</sup> Pheromones associated with the pest species may also be used together with host plant volatiles to enhance monitoring reliability. For example, Evenden et al.<sup>19</sup> showed that the addition of host plant volatiles from faba beans (Vicia faba Linnaeus) to an aggregation pheromone lure enhances lure effectiveness for trapping the pea leaf weevil (Sitona lineatus Linnaeus). The use of semiochemicals, such as pheromones, that could be used as a lure to enhance vine weevil monitoring tool efficacy is limited by the fact that adults, which are all female, reproduce parthenogenetically and are not thought to produce a sex pheromone.<sup>20</sup> Vine weevil adults do, however, display a strong aggregation behaviour<sup>21</sup> and adults prefer refuges previously occupied by their conspecifics.<sup>22</sup> It is not known what mediates this aggregation behaviour. For this reason, most work investigating vine weevil lures has focused on identifying volatiles produced by their host plants. Behavioural studies have shown that adult vine weevils respond to a wide range of plant odours.<sup>20,22-24</sup> However, to date, no effective and reliable synthetic lure has been identified<sup>5</sup> for use in a monitoring tool system.





N



were collected from the laboratory culture, placed in a plastic box  $(\emptyset = 12 \text{ cm})$  and then released into the centre of the experimental arena between 17:00 and 20:00. The location of each vine weevil was recorded between 08:00 and 09:00 the following day. A total of 400 individuals were tested (two replicates per day for 5 days) during this experiment.

#### 2.2.3 Heiaht

The behavioural response of adult vine weevils to monitoring tool height was tested in a binary-choice experiment under the semifield conditions described in Section 2.2.1. Monitoring tools were created as described in Section 2.2.1, but modified to two heights: 11.3 and 3 cm. Experimental arenas were completed by placing four monitoring tools (two of each height) at the corner of a 55 cm<sup>2</sup> square centrally positioned within the tent cage. Monitoring tools and the tent cage (n = 2) to which monitoring tools were allocated were re-randomised each day to exclude the effect of position. Vine weevil populations, along with the monitoring tools, were replaced between each replicate. Forty adult vine weevils were collected from the laboratory culture, placed in a plastic box ( $\emptyset = 12$  cm) and then released into the centre of the experimental arena between 17:00 and 20:00. The location of each vine weevil was recorded between 08:00 and 09:00 the following day. A total of 400 individuals were tested (two replicates per day for 5 days) during this experiment.

#### 2.2.4 Diameter

The behavioural response of adult vine weevils to monitoring tool diameter was tested in a binary-choice experiment under the semifield conditions described in Section 2.2.1. Monitoring tools were created as described in Section 2.2.1 and Kraft bowls (height 6.9 cm, Ø base 16.4 cm, Ø rim 18.4 cm; GoCoPack, UK). To ensure heights were consistently 6.9 cm between treatments, paper cups were modified by removing the base and sides to create monitoring tools with the same height as the Kraft bowls but with different diameters: 8.9 and 18.4 cm. For Kraft bowls, four equally distanced entrances were made in the monitoring tool by cutting  $2 \text{ cm}^2$ openings around the Kraft bowl rim. Experimental arenas were completed by placing four monitoring tools (two of each diameter) at the corner of a 55 cm<sup>2</sup> square centrally positioned within the tent cage. Monitoring tools and the tent cage (n = 2) to which monitoring tools were allocated were re-randomised each day to exclude the effect of position. Vine weevil populations, along with the monitoring tools, were replaced between each replicate. Forty adult vine weevils were collected from the laboratory culture, placed in a plastic box ( $\emptyset = 12$  cm) and then released into the centre of the experimental arena between 17:00 and 20:00. The location of each vine weevil was recorded between 08:00 and 09:00 the following day. A total of 400 individuals were tested (two replicates per day for 5 days) during this experiment.

#### 2.2.5 Density

The behavioural response of adult vine weevils to monitoring tool density was tested in a three-choice experiment under the semifield conditions described in Section 2.2.1. Four potted strawberry plants were positioned at the corners of a 110 cm<sup>2</sup> square centrally positioned within the cage (Fig. 2(A)-(C)), providing both a food source and a range of alternative refuges. Monitoring tools were created as described in Section 2.2.1. Experimental arenas were completed by placing different numbers of monitoring tools inside the tent cage: one monitoring tool (~0.5 monitoring tool/m<sup>2</sup>) (Fig. 2(A)) positioned in the middle of a centrally

positioned 110 cm<sup>2</sup> square within the tent cage, two monitoring tools (~0.95 monitoring tool/m<sup>2</sup>) (Fig. 2(B)) positioned opposite one another along all four sides of a 110 cm<sup>2</sup> square, and four monitoring tools ( $\sim$ 1.9 monitoring tool/m<sup>2</sup>) (Fig. 2(C)) positioned equidistant from one another along the perimeter of a 110 cm<sup>2</sup> square. Monitoring tools and the tent cage (n = 3) to which monitoring tools were allocated were re-randomised each day to exclude the effect of position. Vine weevil populations, along with the monitoring tools, were replaced between each replicate. Forty adult vine weevils were collected from the laboratory culture, placed in a plastic box ( $\emptyset = 12$  cm) and then released into the centre of the experimental arena between 17:00 and 20:00. The location of each vine weevil was recorded between 08:00 and 09:00 the following day. A total of 1200 individuals were tested (three replicates for 10 days) during this experiment.

#### 2.3 Effect of plant density on vine weevil monitoring tool performance

The behavioural response of adult vine weevils to plant density was tested in a three-choice experiment under the semifield conditions described in Section 2.2.1. One, four or eight potted strawberry plants were positioned within a tent cage (Fig. 2(D)-(F)). Monitoring tools were created as described in Section 2.2.1. Experimental arenas were completed by placing four monitoring tools equidistant from one another along the perimeter of a 110 cm<sup>2</sup> square within the tent cage (Fig. 2(D)–(F)). Potted strawberry plants were positioned within the tent cages: one plant  $(\sim 0.5 \text{ plants/m}^2)$  positioned in the middle of a 110 cm<sup>2</sup> square (Fig. 2(D)) centrally positioned within the tent cage, four plants  $(\sim 1.9 \text{ plants/m}^2)$  positioned at the corners of a 110 cm<sup>2</sup> square (Fig. 2(E)), and eight plants ( $\sim$ 3.8 plants/m<sup>2</sup>) positioned at the corners of two squares (55 cm<sup>2</sup> and 110 cm<sup>2</sup>; Fig. 2(F)). Monitoring tools and the tent cage (n = 3) to which monitoring tools were allocated were re-randomised each day to exclude the effect of position. Vine weevil populations, along with the monitoring tools, were replaced between each replicate. Forty adult vine weevils were collected from the laboratory culture, placed in a plastic box ( $\emptyset = 12$  cm) and then released into the centre of the experimental arena between 17:00 and 20:00. The location of each vine weevil was recorded between 08:00 and 09:00 the following day. A total of 1200 individuals were tested (three replicates for 10 days) during this experiment.

#### 2.4 Effect of olfactory cues on vine weevil monitoring tool performance

The behavioural response of adult vine weevils to olfactory cues was tested in four binary-choice experiments under the semifield conditions described in Section 2.2.1. Monitoring tools were modified as described in Section 2.2.1. Experimental arenas were completed by placing four monitoring tools at the corner of a 55 cm<sup>2</sup> square centrally positioned within the tent cage. Small branches of yew (T. baccata) ( $\emptyset \sim 1$  cm and length  $\sim 4$  cm) and/or 20 adult vine weevils were placed in a white organza bag ( $7 \times 9$  cm; OWill, UK), which was then placed inside a monitoring tool. For experiment 1 (Table 1) two monitoring tools were baited with 3 g of yew and two contained an empty organza bag. For experiment 2 (Table 1) two monitoring tools were baited with 20 weevils and two contained an empty organza bag. For experiment 3 (Table 1) two monitoring tools were baited with 3 g of yew plus 20 weevils and two contained an empty organza bag. In experiment 4 (Table 1) two monitoring tools were baited with 3 g of yew plus 20 weevils and two monitoring tools were baited with

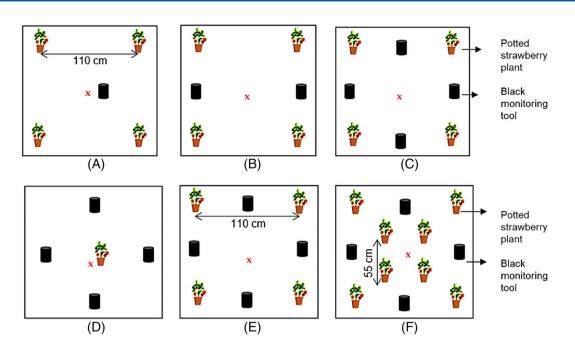


Figure 2. Schematic diagram showing the arrangement within each tent cage for the monitoring tool (A, B, C) and plant (D, E, F) density experiment. The vine weevil release point is shown by x.

Experiment	Monitoring tool 1	Monitoring tool 2	No. of replicate
1	Unbaited	Yew <sup>a</sup>	5
2	Unbaited	Conspecifics <sup>b</sup>	5
3	Unbaited	Yew + conspecific	5
4	Yew	Yew + conspecific	5

<sup>a</sup> Small branches of yew (*Taxus baccata*, Ø ~1 cm and length ~4 cm) were used for the experiments with yew. <sup>b</sup> Twenty vine weevil adults were used for the experiments with conspecifics.

3 g of yew. Monitoring tools and the tent cage (n = 2) to which monitoring tools were allocated were re-randomised each day to exclude the effect of position. Vine weevil populations, along with the monitoring tools and odour sources, were replaced between each replicate. Forty adult vine weevils were collected from the laboratory culture, placed in a plastic box ( $\emptyset = 12$  cm) and then released into the centre of the experimental arena between 17:00 and 20:00. The location of each vine weevil was recorded between 08:00 and 09:00 the following day. For each binary choice experiment (Table 1), a total of 400 individuals were tested (two replicates per day for 5 days).

#### 2.5 Statistical analyses

-

All statistical analyses were carried out using R (Version 4.2.2).<sup>25</sup> For binary-choice experiments the number of individuals within a monitoring tool (*i.e.*, monitoring tool performance) was analysed using an exact binomial test against the null hypothesis that the number of vine weevils seeking refuge had a 50:50 distribution.<sup>26</sup> Replicated results from each of the choice experiment tested, prior to carrying out statistical analyses, were pooled. For monitoring tool and plant density experiments the number of individuals within a monitoring tool was analysed using generalised linear models (GLMs) fitted with Poisson probability distributions. Tukey's HSD tests were then used to evaluate multiple comparisons for the GLM analyses. Individuals not recorded in the monitoring tools were excluded from statistical analysis.

## **3 RESULTS**

# 3.1 Effect of vine weevil monitoring tool characteristics on performance

#### 3.1.1 Shade

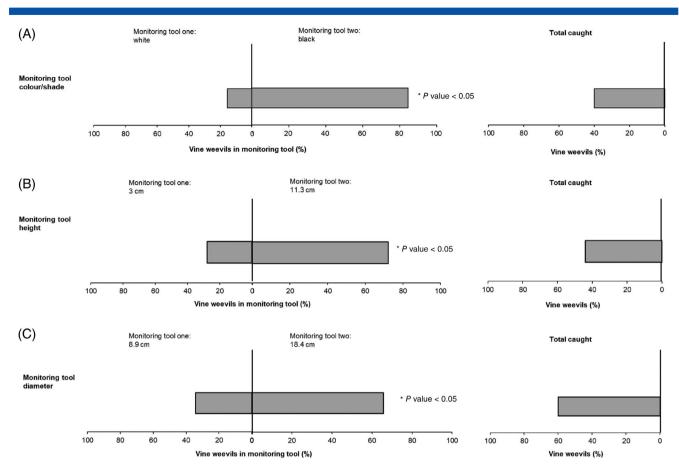
In this binary-choice experiment presenting adult vine weevils with black and white monitoring tools, 84% of individuals were recorded in black monitoring tools compared to 16% in white ones (binomial exact test: no. successes = 135, no. trials = 160, P < 0.001). Total catch was 40% of the introduced weevil population (Fig. 3(A)).

#### 3.1.2 Height

In this binary-choice experiment presenting adult vine weevils with monitoring tools of varying heights, 72% of individuals were recorded in taller monitoring tools compared to 28% in shorter ones (binomial exact test: no. successes = 127, no. trials = 176, P < 0.001). Total catch was 44% of the introduced weevil population (Fig. 3(B)).

ίĝη

www.soci.org



**Figure 3.** Percentage of vine weevil adults recorded in monitoring tools when testing (A) monitoring tool colour/shade, (B) monitoring tool height and (C) monitoring tool diameter. Vine weevil adults were released as a group of 40 individuals (number of replicate days = 5). Asterisks indicate significant differences between tested monitoring tools (binomial exact test: P < 0.05).

#### 3.1.3 Diameter

In this binary-choice experiment presenting adult vine weevils with monitoring tools of varying diameters, 65.7% of individuals were recorded in large-diameter monitoring tools compared to 34.3% in smaller-diameter ones (binomial exact test: no. successes = 159, no. trials = 242, P < 0.001). Total catch was 60.5% of the introduced weevil population (Fig. 3(C)).

#### 3.1.4 Density

Monitoring tool density significantly influenced the total number of individuals recorded in the monitoring tools (generalised linear model:  $F^2 = 121.15$ , df = 27, P < 0.001). Experimental arenas with four monitoring tools had the highest vine weevil retention (49%) (Tukey's HSD test: P < 0.05) while in arenas with two or one monitoring tools the retention rate was only 30% and 21% of the introduced vine weevils, respectively (Fig. 4(A)).

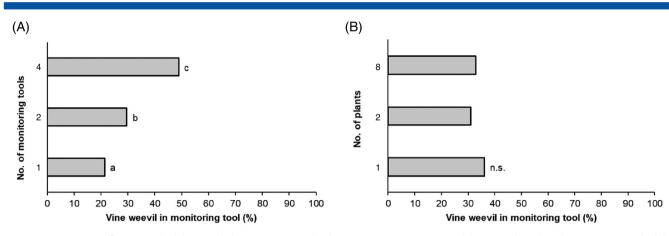
# 3.2 Effect of plant density on vine weevil monitoring tool performance

Plant density did not significantly influence the number of individuals recorded in monitoring tools (generalised linear model:  $F^2 = 143.53$ , df = 117, P = 0.1). Monitoring tools in experimental arenas containing one plant retained 36.1% of the introduced vine weevils while in arenas with four plants the retention rate was 31.1% and in arenas with eight plants it was 32.8% (Fig. 4(B)).

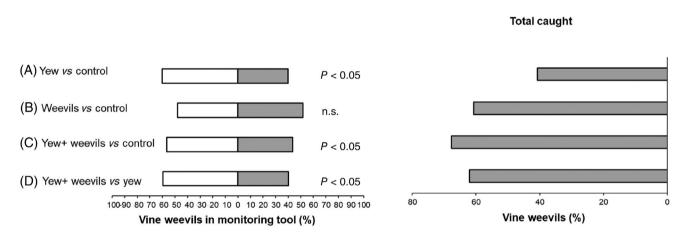
## **3.3** Effect of olfactory cues on vine weevil monitoring tool performance

In a series of four binary-choice experiments adult vine weevils were presented with monitoring tools baited with combinations of the following: yew, conspecifics, yew + conspecifics or left unbaited. In the first experiment, 60% of the individuals were recorded in monitoring tools baited with yew compared to 40% in the ones left unbaited (binomial exact test: no. of successes = 98, no. of trials = 163, P = 0.01). Total catch in this experiment was 40.75% of the introduced weevil population (Fig. 5(A)). In the second experiment, there was no significant difference between the number of weevils recorded in monitoring tools with 48.1% retained in those baited with 20 conspecifics compared to 51.9% in those left unbaited (binomial exact test: no. of successes = 117, no. of trials = 43, P = 0.6). Total catch in this experiment was 60.7% of the introduced weevil population (Fig. 5(B)). In the third experiment, 56.5% of individuals were recorded in monitoring tools baited with yew plus 20 conspecifics compared to 43.5% in those left unbaited (binomial exact test: no. of successes = 153, no. of trials = 271, P = 0.03). Total catch rate in this experiment was 67.8% of the introduced weevil population (Fig. 5(C)). In the final experiment, 59.7% of individuals were recorded in monitoring tools baited with yew plus conspecifics compared to 40.3% of those baited with only yew (binomial exact test: no. of successes = 148, no. of trials = 248, P = 0.008). Total catch in this experiment was 62% of the introduced weevil population (Fig. 5(D)).

www.soci.org



**Figure 4.** Percentage of vine weevil adults recorded in monitoring tools when testing (A) monitoring tool density and (B) plant density. Vine weevil adults were released as a group of 40 individuals (number of replicate days = 10). Different letters indicate significant differences between tested monitoring tools (Tukey's HSD test: P < 0.05).



**Figure 5.** Percentage of vine weevil adults recorded in monitoring tools under four experimental treatments: (A) yew and control, (B) weevils and control, (C) yew plus weevils and control, and (D) yew plus weevils and yew. For each experimental treatment vine weevil adults were released as a group of 40 individuals (number of replicate days = 5). Asterisks indicate significant differences between experimental treatments (binomial exact test: P < 0.05); n.s., not significant.

#### 4 DISCUSSION

Visual and olfactory cues play an important role in host plant location for insect pests.<sup>14,27</sup> Such cues have been shown to be key components to consider when developing monitoring tools for curculionid species.<sup>28</sup> Monitoring tool design,<sup>29</sup> colour,<sup>30</sup> density<sup>31</sup> and position<sup>12,32</sup> as well as olfactory components, such as pheromones<sup>33</sup> and plant volatiles,<sup>34</sup> may be used to augment monitoring tool efficacy. Hence, an understanding as to how these factors influence monitoring tool–insect interactions can inform their development for improved efficacy and reliability.

Monitoring tool colour is a key factor that influences monitoring tool–insect interactions.<sup>9</sup> The importance of using dark colours to increase monitoring tool efficacy for weevils has been extensively reported.<sup>35</sup> For example, the New Guinea sugarcane weevil (*Rhab-doscelus obscurus* Boisduval) shows a preference for russet-brown colours in field conditions,<sup>12</sup> whereas the apple blossom weevil (*Anthonomus pomorum* Linnaeus) exhibits a preference for blue-coloured monitoring tools.<sup>36</sup> Hallet *et al.*<sup>29</sup> reported that more red palm weevils (*Rhynchophorus ferrugineus* Olivier) were captured in black inverted bucket monitoring tools than in white ones. For vine weevil, laboratory bioassays have demonstrated that small groups of vine weevil adults preferred dark monitoring

tools over white or light-coloured ones.<sup>4</sup> To further explore monitoring tool colour preference and confirm whether black monitoring tools may be used as an effective method to detect this species, this study investigated the efficacy of black monitoring tools under glasshouse conditions. Our results confirm that black monitoring tools are more effective than white ones under field representative environmental conditions.

Monitoring tool height, as previously reported,<sup>4</sup> influenced the number of vine weevils retained within the monitoring tools. Larger numbers of weevils were recorded in taller monitoring tools, suggesting that taller silhouettes may be an important cue for this species. Weevils have been shown to respond positively to visual stimuli such as two-dimensional silhouettes. For example, white pine weevil (*Pissodes strobi Peck*)<sup>37</sup> adults preferentially orientate toward silhouettes that are vertical, tall and large. Similarly, monitoring tool diameter has been reported to significantly affect trapping efficiency in some species of moth<sup>38,39</sup> and typically the efficacy of a monitoring tool increases with the size of the tool used.<sup>11</sup> The situation for vine weevil adults appears to be the same, with larger-diameter monitoring tools being more effective than monitoring tools with a smaller diameter.

www.soci.org

In addition, other visual characteristics, including design, shape, texture and location, may all affect monitoring tool efficacy. For example, Reddy et al.40 showed that Pherocon unitraps caught higher numbers of the sweet potato weevil, Cylas formicarius (Fabricius, Coleoptera: Brentidae), than ground, funnel water or delta traps. Previous studies have also reported that the responses of insect pests to the visual characteristics (e.g., colour, height, design) of monitoring tools may be affected by environmental factors, including soil type, temperature, humidity and light intensity,<sup>32</sup> which may vary through the day and the seasons. Increasing knowledge on how vine weevil adults respond to environmental change may provide insights into the development of a more effective monitoring tool for this species.

Monitoring tool density determines the system efficacy in terms of detecting insect pests and the likely cost effectiveness of the approach. Our study demonstrates that monitoring tool density positively correlates with the proportion of the vine weevil population recorded within monitoring tools. These results are in line with previous studies showing that monitoring efficacy can be improved by increasing the density of monitoring tools.<sup>31,32</sup> Vidyasagar et al.<sup>41</sup> showed that a mean of 61.5 red palm weevils were captured when eight monitoring tools were deployed for every 4 ha compared to a mean of 10.0 weevils captured when one monitoring tool was deployed for every 4 ha. In addition to monitoring tool density, it is necessary to consider the number of plants in an area<sup>42</sup> as well as the area covered.<sup>31</sup> In this study the effect of plant density on monitoring tool efficacy was evaluated. The results showed that the number of plants within a constant area and with a known vine weevil population did not influence the number of weevils recorded within monitoring tools. In a previous study the number of saddled prominent moths (Heterocampa guttivitta Walker, Lepidoptera: Notodontidae) recorded in monitoring tools increased when the abundance and density of the host plant (sugar maple, Acer saccharum Marshall) also increased.<sup>42</sup> By contrast, Westerberg et al.<sup>43</sup> showed that the number of pollinating insects in pan traps decreased as the abundance of surrounding flowers increased. However, this pattern may differ between taxa and often varies depending on flower colours, spatial scale considered and timing of the monitoring activity.

While vine weevil adults have been shown to respond positively to visual stimuli,<sup>4</sup> it is important to also consider how olfactory cues may enhance monitoring tool efficacy. It has been shown that when both olfactory and visual stimuli are provided more weevils (e.g., pine weevil) are caught than when olfactory and visual stimuli are provided separately.<sup>28</sup>

Semiochemicals are often used in monitoring tools<sup>44–46</sup> and significantly improve their sensitivity by enabling detection of the pest species even at low densities.<sup>47</sup> Typically, volatile organic compounds isolated from either host plants or conspecifics are used.<sup>48,49</sup> Plant volatiles are characterised by different active compounds that can be used to target more than one pest species and may be more practical and economical for both monitoring and mass trapping than those for single species. For example, Sweeney et al.<sup>50</sup> reported that a monitoring tool baited with a blend of monoterpenes, isolated from spruce (Picea spp.), and ethanol increased the probability of detecting not only the brown spruce longhorn beetle (Tetropium fuscum Fabricius) but also other longhorn beetles.

Host plant volatiles impact insect pests in different ways<sup>45</sup> and may also play a role in modifying the behaviour of weevils<sup>51</sup> as attractants, oviposition stimulants or as potential synergists for

an aggregation pheromone.<sup>52,53</sup> The results from this study show that monitoring tools baited with yew were preferred by vine weevil adults to unbaited monitoring tools. The response of vine weevil to host plant volatiles has been the focus of several studies.<sup>20,24,54,55</sup> For example, Roberts et al.<sup>26</sup> showed that vine weevil adults preferentially used refuges baited with Fortune's spindle (Euonymus fortune Turcz) or yew (T. baccata) foliage compared to unbaited refuges, but that this was influenced by previous feeding experience of the adults. Although this species has a wide host range, it discriminates between the odours of potential host plants.<sup>20,24</sup> Understanding which plant volatiles elicit vine weevil responses and the correct ratio when using blends of plant volatiles is important in developing an effective semiochemical lure for monitoring this pest. The behavioural response of insects to plant volatiles is influenced by many factors, including sex, development stage, physiological state, degree of ecological specialization and feeding experience.<sup>56</sup> Therefore, to develop an effective semiochemical-based pest management strategy using host plant volatiles requires further studies to address each of these factors.

Pheromone-based pest management strategies have been used as a tool for monitoring and mass trapping insect pests, including weevils.<sup>46</sup> Sex and aggregation pheromones have been identified in several insect pest species,<sup>46</sup> and attraction to a pheromone-based lure is typically species specific. While vine weevil adults exhibit a strong tendency to aggregate, the mechanism underlying this behaviour has not, to date, been identified. Van Tol et al.<sup>23</sup> and Nakamuta et al.<sup>57</sup> reported positive behavioural responses between conspecifics, while Pickett et al.<sup>22</sup> suggested that previously occupied refuges stimulate aggregation behaviour. In this study the presence of weevils in monitoring tools alone did not significantly affect the response of conspecifics. Pope and Roberts<sup>5</sup> suggested that aggregation and conspecific attraction may be mediated by frass or other nonchemical cues rather than by pheromones. Indeed, here we show that higher numbers of vine weevil adults were recorded in monitoring tools with yew and conspecifics than in monitoring tools left unbaited or containing only yew. This suggests that vine weevil adults may use volatiles associated with frass or induced by conspecifics during feeding as olfactory cues. Similar results have been reported for other weevil species.<sup>58-60</sup> For example, the tea weevil (Myllocerinus aurolineatus Voss) responds positively to tea plants infested by conspecifics but not to undamaged tea plants.<sup>58</sup> Similarly, Addesso et al.<sup>61</sup> showed that the pepper weevil (Anthonomus eugenii Cano) preferred damaged flowering and fruiting plants over undamaged plants. In addition, the odour from flowering and fruiting plants with actively feeding weevils was preferred to the odour from plants with older feeding damage. The results presented here combined with knowledge of the olfactory responses of other species of weevils highlight the importance of identifying and exploiting herbivore-induced host plant volatiles for monitoring purposes.

#### CONCLUSIONS 5

This study investigated factors that may enhance monitoring tool efficacy by comparing the effects of visual and olfactory cues on vine weevil retention. Based on the experimental results generated in this study, the most effective monitoring tool design is black and tall with a large diameter. By increasing the number of monitoring tools per unit area, the proportion of vine weevil population recorded could be increased. By contrast, within the range tested, crop density was found to have no effect on monitoring tool efficacy. To use monitoring tools under field conditions their position and distribution pattern should be carefully considered as well as the duration of the monitoring period. Each of these considerations should be further investigated in subsequent research.

Olfactory cues may improve the efficacy of the monitoring tool. Although efforts are being made to identify an effective semiochemical lure for vine weevil, further studies need to be completed to identify and include these olfactory cues in monitoring tools. For an effective monitoring system to be developed for vine weevil adults, knowledge of the mechanisms involved in the attraction of this insect to its host and conspecifics is crucial. The results from this study suggest that induced host plant volatiles and/or volatiles associated with weevil frass or adult weevils themselves may have potential application in the development of improved monitoring systems.

### **AUTHOR CONTRIBUTIONS**

Experimental design: Eugenia Fezza, Joe M. Roberts, Michael T. Gaffney and Tom W. Pope. Data acquisition: Eugenia Fezza. Data analysis: Eugenia Fezza and Joe M. Roberts. Data interpretation: Eugenia Fezza, Joe M. Roberts, Michael T. Gaffney and Tom W. Pope. Manuscript preparation: Eugenia Fezza, Joe M. Roberts and Tom W. Pope. Manuscript editing and reviewing: Eugenia Fezza, Joe M. Roberts, Toby J. A. Bruce, Lael E. Walsh, Michael T. Gaffney and Tom W. Pope. All authors have read and agreed to the published version of the manuscript.

#### **FUNDING INFORMATION**

This research was funded by the Teagasc Walsh Scholarships Programme, n: 2020034, 'Catch me if you can' – improving monitoring and control of black vine weevil (*Otiorhynchus sulcatus*) in soft fruit and ornamental crops.

## **CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript or in the decision to publish the results.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

#### REFERENCES

- Barzman M, Bàrberi P, Birch ANE, Boonekamp P, Dachbrodt-Saaydeh S, Graf B et al., Eight principles of integrated pest management. Agron Sustain Dev 35:1199–1215 (2015).
- 2 Preti M, Verheggen F and Angeli S, Insect pest monitoring with camera-equipped traps: strengths and limitations. *J Pest Sci* **94**: 203–217 (2021).
- 3 Cardim Ferreira Lima M, de Almeida D, Leandro ME, Valero C, Pereira Coronel LC and Gonçalves Bazzo CO, Automatic detection and monitoring of insect pests—a review. Agri 10:161 (2020).
- 4 Fezza E, Roberts JM, Bruce TJA, Walsh LE, Gaffney MT and Pope TW, Optimising vine weevil, Otiorhynchus sulcatus F (Coleoptera: Curculionidae), monitoring tool design. Insects 13:80 (2022).
- 5 Pope TW and Roberts JM, Vine weevil, Otiorhynchus sulcatus (Coleoptera: Curculionidae), management: current state and future perspectives. Annu Rev Entomol 67:221–238 (2022).

- 6 Ansari MA, Shah FA and Butt TM, Combined use of entomopathogenic nematodes and *Metarhizium anisopliae* as a new approach for black vine weevil, *Otiorhynchus sulcatus*, control. *Entomol Exp Appl* **129**: 340–347 (2008).
- 7 Haukeland S and Lola-Luz T, Efficacy of the entomopathogenic nematodes Steinernema kraussei and Heterorhabditis megidis against the black vine weevil Otiorhynchus sulcatus in open field-grown strawberry plants. Agric For Entomol 12:363–369 (2010).
- 8 Lee JC, Burrack HJ, Barrantes LD, Beers EH, Dreves AJ, Hamby KA et al., Evaluation of monitoring traps for Drosophila suzukii (Diptera: Drosophilidae) in North America. J Econ Entomol **105**: 1350–1357 (2012).
- 9 Mahot HC, Mahob JR, Hall DR, Arnold SEJ, Fotso AK, Membang G et al., Visual cues from different trap colours affect catches of Sahlbergella singularis (Hemiptera: Miridae) in sex pheromone traps in Cameroon cocoa plantations. Crop Prot 127:104959 (2020).
- 10 Mizell RF and Tedders WL, Evaluation of trap type and color for monitoring *Hylobius pales* and *Pachylobius picivorus* in Florida. *Fla Entomol* **82**:615 (1999).
- 11 Reddy GVP, Cruz ZT and Guerrero A, Development of an efficient pheromone-based trapping method for the banana root borer *Cosmopolites sordidus. J Chem Ecol* **35**:111–117 (2009).
- 12 Reddy GVP, Balakrishnan S, Remolona JE, Kikuchi R and Bamba JP, Influence of trap type, size, color, and trapping location on capture of *Rhabdoscelus obscurus* (Coleoptera: Curculionidae). *Ann Entomol Soc Am* **104**:594–603 (2011).
- 13 Wang X, Ji YC, Wen C, Zhang GY and Wen JB, Effects of trap color and shape on the capture of *Eucryptorrhynchus scrobiculatus* (Coleoptera: Curculionidae). J Econ Entomol **112**:2744–2750 (2019).
- 14 Prokopy RJ and Owens ED, Visual detection of plants by herbivorous insects. Annu Rev Entomol **28**:337–364 (1983).
- 15 Lessio F and Alma A, Dispersal patterns and chromatic response of *Scaphoideus titanus* Ball (Homoptera Cicadellidae), vector of the phytoplasma agent of grapevine *Flavescence doree. Agric For Entomol* **6**:121–128 (2004).
- 16 Oehlschlager AC, Gonzalez L, Gomez M, Rodriguez C and Andrade R, Pheromone-based trapping of west indian sugarcane weevil in a sugarcane plantation. J Chem Ecol 28:1653–1664 (2002).
- 17 Reddy G, Cruz Z, Naz F and Muniappan R, A pheromone-based trapping system for monitoring the population of *Cosmopolites Sordidus* (Germar) (Coleoptera: Curculionidae). J Plant Prot Res 48:515–527 (2008).
- 18 Kerr JL and Kelly D, Bader MK-F and Brockerhoff EG, Olfactory cues, visual cues, and semiochemical diversity interact during host location by invasive forest beetles. J Chem Ecol 43:17–25 (2017).
- 19 Evenden ML, Whitehouse CM, St-Onge A, Vanderark L, Lafontaine JP, Meers S et al., Potential for semiochemical-based monitoring of the pea leaf weevil (Coleoptera: Curculionidae) on field pea (Fabaceae) in the Canadian Prairie Provinces. Can Entomol 148: 595–602 (2016).
- 20 van Tol RWHM, Visser JH and Sabelis MW, Olfactory responses of the vine weevil, *Otiorhynchus sulcatus*, to tree odours. *Physiol Entomol* **27**:213–222 (2002).
- 21 Kakizaki M, Aggregation behavior of black vine weevil female adults (Otiorhynchus sulcatus (Fabricius)) (Coleoptera: Curculionidae) occurring in Japan. Soc Plant Prot North Jpn 52:201–203 (2001).
- 22 Pickett JA, Bartlett E, Buxton JH, Wadhams LJ and Woodcock CM, Chemical ecology of adult vine weevil. *Mitteilungen Biol Bundesanst* 316:41–45 (1996).
- 23 van Tol RWHM, Visser JH and Sabelis MW, Behavioural responses of the vine weevil, Otiorhynchus sulcatus, to semiochemicals from conspecifics, Otiorhynchus salicicola, and host plants. Entomol Exp Appl 110: 145–150 (2004).
- 24 Roberts JM, Kundun J, Rowley C, Hall DR, Douglas P and Pope TW, Electrophysiological and behavioral responses of adult vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae), to host plant odors. J Chem Ecol **45**:858–868 (2019).
- 25 R Core Team, *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria (2022). Available online). https://www.r-project.org/ Accessed on 21 February 2022.
- 26 Roberts JM, Jahir A, Graham J and Pope TW, Catch me if you can: the influence of refuge/trap design, previous feeding experience, and semiochemical lures on vine weevil (Coleoptera: Curculionidae) monitoring success. *Pest Manag Sci* **76**:553–560 (2020).



- 27 Johnston N and Martini X, The influence of visual and olfactory cues in host selection for *Bemisia tabaci* Biotype B in the presence or absence of Tomato Yellow Leaf Curl Virus. *Insects* **11**:115 (2020).
- 28 Bjorklund N, Nordlander G and Bylund H, Olfactory and visual stimuli used in orientation to conifer seedlings by the pine weevil, Hylobius abietis. *Physiol Entomol* **30**:225–231 (2005).
- 29 Hallett RH, Oehlschlager AC and Borden JH, Pheromone trapping protocols for the Asian palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). *Int J Pest Manag* 45:231–237 (1999).
- 30 Silva D, Salamanca J, Kyryczenko-Roth V, Alborn HT and Rodriguez-Saona C, Comparison of trap types, placement, and colors for monitoring Anthonomus musculus (Coleoptera: Curculionidae) adults in highbush blueberries. J Insect Sci 18:1–9 (2018).
- 31 Faleiro JR, El-Saad MA and Al-Abbad AH, Pheromone trap density to mass trap *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae/Rhynchophoridae/Dryophthoridae) in date plantations of Saudi Arabia. *Int J Trop Insect Sci* **31**:75–77 (2011).
- 32 Tinzaara W, Gold CS, Dicke M, Van Huis A and Ragama PE, Factors influencing pheromone trap effectiveness in attracting the banana weevil, Cosmopolites sordidus. Int J Pest Manag 51:281–288 (2005).
- 33 Borges M, Moraes MCB, Peixoto MF, Pires CSS, Sujii ER and Laumann RA, Monitoring the Neotropical brown stink bug *Euschistus heros* (F.) (Hemiptera: Pentatomidae) with pheromone-baited traps in soybean fields: Pheromone traps to stink bug monitoring. *J Appl Entomol* **135**:68–80 (2011).
- 34 Xiu CL, Pan HS, Liu B, Luo ZX, Williams L, Yang YZ *et al.*, Perception of and behavioral responses to host plant volatiles for three Adelphocoris species. *J Chem Ecol* **45**:779–788 (2019).
- 35 Abuagla AM and Al-Deeb MA, Effect of bait quantity and trap color on the trapping efficacy of the pheromone trap for the red palm weevil, Rhynchophorus ferrugineus. *J Insect Sci* **12**:1–6 (2012).
- 36 Hausmann C, Samietz J and Dorn S, Visual orientation of overwintered Anthonomus pomorum (Coleoptera: Curculionidae). Environ Entomol 33:1410–1415 (2004).
- 37 Vandersar TJD and Borden JH, Visual orientation of *Pissodes strobi* Peck (Coleoptera: Curculionidae) in relation to host selection behaviour. *Can J Zool* **55**:2042–2049 (1977).
- 38 Yongmo W, Feng G, Xianghui L, Feng F and Lijun W, Evaluation of mass-trapping for control of tea tussock moth *Euproctis pseudocon-spersa* (Strand) (Lepidoptera: Lymantriidae) with synthetic sex pheromone in south China. *Int J Pest Manag* **51**:289–295 (2005).
- 39 Zhao Z, Rong E, Zhang J and Ma R, Determination of optimal diameter of sex pheromone trap for oriental fruit moth. *Afr J Agric RES* 7:1192– 1197 (2012).
- 40 Reddy GVP, Gadi N and Taianao AJ, Efficient sex pheromone trapping: catching the sweetpotato weevil, *Cylas formicarius. J Chem Ecol* **38**: 846–853 (2012).
- 41 Vidyasagar P, Aldosari SA, Sultan EM, Al Saihati A and Mumtaz Khan R, Efficiency of optimal pheromone trap density in management of red palm weevil, *Rhynchophorus ferrugineus* Olivier. *Afr J Agric Res* **11**: 1071–1078 (2016).
- 42 Spear-O'mara J and Allen DC, Monitoring populations of saddled prominent (Lepidoptera: Notodontidae) with pheromone-baited traps. *J Econ Entomol* **100**:335–342 (2007).
- 43 Westerberg L, Berglund H, Jonason D and Milberg P, Color pan traps often catch less when there are more flowers around. *Ecol Evol* **11**: 3830–3840 (2021).
- 44 El-Shafie HAF and Faleiro JR, Semiochemicals and their potential use in pest management, in *Shields VDC, Biological control of pest and vector insects*, IntechOpenScience, pp. 3–22 (2017).

- 45 Abd El-Ghany NM, Semiochemicals for controlling insect pests. J Plant Prot Res, Committee of Plant Protection PAS (2019).
- 46 Bandeira PT, Fávaro CF, Francke W, Bergmann J and Zarbin PHG, Aggregation pheromones of weevils (Coleoptera: Curculionidae): advances in the identification and potential uses in semiochemical-based pest management strategies. J Chem Ecol 47:968–986 (2021).
- 47 Mahroof RM and Phillips TW, Responses of stored-product Anobiidae to pheromone lures and plant-derived volatiles. J Appl Entomol 132:161–167 (2008).
- 48 Cano-Ramírez C, Armendáriz-Toledano F, Macías-Sámano JE, Sullivan BT and Zúñiga G, Electrophysiological and behavioral responses of the bark beetle *Dendroctonus rhizophagus* to volatiles from host pines and conspecifics. J Chem Ecol **38**:512–524 (2012).
- 49 García-Díaz É, Tafoya F and Elizalde-González MP, Behavioral and electroantennographic responses of adults of guava weevil, *Conotrachelus dimidiatus* (Coleoptera: Curculionidae), to synthetic hostassociated and conspecific volatiles. *Environ Entomol* **49**:810–814 (2020).
- 50 Sweeney J, Gutowski JM, Price J and De Groot P, Effect of Semiochemical release rate, killing agent, and trap design on detection of *Tetropium fuscum* (F.) and other longhorn beetles (Coleoptera: Cerambycidae). *Environ Entomol* **35**:645–654 (2006).
- 51 Reddy GVP and Guerrero A, Interactions of insect pheromones and plant semiochemicals. *Trends Plant Sci* **9**:253–261 (2004).
- 52 Tilles DA, SjÖDin K, Gör N and Eidmann HH, Synergism between ethanol and conifer host volatiles as attractants for the pine weevil, *Hylobius abietis* (L.) (Coleoptera: Curculionidae). *J Econ Entomol* **79**:970– 973 (1986).
- 53 Tinzaara W, Gold CS, Dicke M, Van Huis A and Ragama PE, Host plant odours enhance the responses of adult banana weevil to the synthetic aggregation pheromone Cosmolure+\*. Int J Pest Manag 53: 127–137 (2007).
- 54 van Tol RWHM, van Dijk N and Sabelis MW, Host plant preference and performance of the vine weevil Otiorhynchus sulcatus. Agric For Entomol 6:267–278 (2004).
- 55 van Tol RWHM and Visser JH, Olfactory antennal responses of the vine weevil Otiorhynchus sulcatus to plant volatiles. Entomol Exp Appl 102:49–64 (2002).
- 56 Anderson P and Anton S, Experience-based modulation of behavioural responses to plant volatiles and other sensory cues in insect herbivores: Experience modulates herbivore behaviour. *Plant Cell Environ* 37:1826–1835 (2014).
- 57 Nakamuta K, van Tol RWHM and Visser JH, An olfactometer for analyzing olfactory responses of death-feigning insects. *Appl Entomol Zool* 40:173–175 (2005).
- 58 Sun XL, Wang GC, Cai XM, Jin S, Gao Y and Chen ZM, The tea weevil, Myllocerinus aurolineatus, is attracted to volatiles induced by conspecifics. J Chem Ecol 36:388–395 (2010).
- 59 Magalhães DM, Borges M, Laumann RA, Sujii ER, Mayon P, Caulfield JC et al., Semiochemicals from herbivory induced cotton plants enhance the foraging behavior of the cotton boll weevil, Anthonomus grandis. J Chem Ecol 38:1528–1538 (2012).
- 60 Barnes BN and Capatos D, Evidence for an aggregation pheromone in adult frass of banded fruit weevil, *Phlyctinus callosus* (Schoenherr) (Col, Curculionidae). J Appl Entomol **108**:512–518 (1989).
- 61 Addesso KM, McAuslane HJ and Alborn HT, Attraction of pepper weevil to volatiles from damaged pepper plants: pepper weevil attraction to damaged pepper plants. *Entomol Exp Appl* **138**: 1–11 (2011).