

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <https://orca.cardiff.ac.uk/id/eprint/122692/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Vuts, József, Woodcock, Christine M, Caulfield, John C., Powers, Stephen J, Pickett, John A. and Birkett, Michael A 2018. Isolation and identification of floral attractants from a nectar plant for the dried bean beetle, *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae, Bruchinae). *Pest Management Science* 74 (9) , pp. 2069-2075. 10.1002/ps.4903

Publishers page: <http://dx.doi.org/10.1002/ps.4903>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



# Isolation and identification of floral attractants from a nectar plant for the dried bean beetle, *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae, Bruchinae)

József Vuts,<sup>\*</sup> Christine M Woodcock, John C Caulfield, Stephen J Powers, John A Pickett and Michael A Birkett



## Abstract

**BACKGROUND:** The response of virgin females of the legume pest *Acanthoscelides obtectus* (Coleoptera: Bruchidae) to headspace extracts of volatiles collected from flowers of a nectar plant, *Daucus carota*, was investigated using behaviour (four-arm olfactometry) and coupled gas chromatography–electroantennography (GC-EAG).

**RESULTS:** Odours from inflorescences were significantly more attractive to virgin female beetles than clean air. Similarly, a sample of volatile organic compounds (VOCs) collected by air entrainment (dynamic headspace collection) was more attractive to beetles than a solvent control. In coupled GC-EAG experiments with beetle antennae and the VOC extract, six components showed EAG activity. Using coupled GC–mass spectrometry (GC–MS) and GC peak enhancement with authentic standards, the components were identified as -pinene (S:R 16:1), sabinene, myrcene, limonene (S:R 1:3), terpinolene and (S)-bornyl acetate. Females preferred the synthetic blend of *D. carota* EAG-active volatiles to the solvent control in bioassays. When compared directly, odours of *D. carota* inflorescences elicited stronger positive behaviour than the synthetic blend.

**CONCLUSION:** This is the first report of behaviourally active volatiles linked to pollen location for *A. obtectus*, and development of the six-component blend is being pursued, which could underpin the design of semiochemical-based field management approaches against this major pest of stored products.

**Keywords:** *Acanthoscelides obtectus*; plant volatiles; gas chromatography – electroantennography (GC-EAG); attractant; chemical ecology; behavioural assays

## 1 INTRODUCTION

The dried bean beetle, *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae, Bruchinae), is a major global pest of dry beans, *Phaseolus vulgaris* L. (Fabaceae),<sup>1</sup> especially on smallholder farms,<sup>2–5</sup> causing 7–13% losses in Latin America<sup>6</sup>. Karel and Autrique<sup>7</sup> reported that, in Africa, farm storage of beans for 6 months was accompanied by about 40% loss in weight with as much as 80% of the seed being infested and unfit for human consumption, and with 23–73% yield losses. Originating in the Neotropics, *A. obtectus* has become cosmopolitan through human-mediated migrations since the domestication and distribution of beans.<sup>8</sup> It can have several generations a year and can multiply both in the field and in granaries.<sup>9,10</sup> The control of *A. obtectus* using various chemical, biological, mechanical and cultural methods has met with varied success,<sup>4,11–18</sup> and is still lacking a sensitive tool for the detection and monitoring of infestations. Also, the recent ban of methyl bromide, a broad-spectrum fumigant,<sup>19</sup> facilitated the search for alternatives in stored product pest management.<sup>20,21</sup> The use of attractive semiochemicals

(pheromones and other semiochemicals, e.g. plant volatiles that act as kairomones)<sup>22</sup> to track the spatial and temporal population dynamics of *A. obtectus* represents a promising approach for surveillance programmes both in the field and in store houses. Surveillance in the field is crucial because harvested seeds that are already infested are a primary cause of the build-up of infestations in granaries.<sup>10</sup> In our earlier studies, we identified the composition of the male-produced sex pheromone of *A. obtectus*.<sup>23,24</sup>

Adult beetles are known to visit a range of flowering plants primarily for their pollen,<sup>25–27</sup> and laboratory feeding experiments with females have demonstrated that pollen consumption stimulates ovarian production.<sup>28</sup> Bruchid beetles, including *Acanthoscelides* and *Bruchus* spp.,<sup>29,30</sup> can be seen on members of the Apiaceae.<sup>26,27</sup> Of these, we chose *Daucus carota* L.<sup>31</sup> as the

\* Correspondence to: J Vuts, Rothamsted Research, Harpenden AL5 2JQ, UK. E-mail: jozsef.vuts@rothamsted.ac.uk

Rothamsted Research, Harpenden, UK

model plant to study floral odour detection in *A. obtectus* and hypothesized that females utilize volatile compounds to find inflorescences. We used behavioural (four-arm olfactometer) bioassays and coupled gas chromatography – electroantennography (GC-EAG) to confirm that *D. carota* was a suitable source of plant-based attractants, the identification of which would under-pin the development of kairomones for use in low-cost bean beetle management strategies that are affordable for smallholder bean farmers.

## 2 MATERIALS AND METHODS

### 2.1 Live material

*Daucus carota* inflorescences were collected from plants growing in the verge of a crop field near Rothamsted Research, Harpenden, UK. Flowers were used for experimental purposes immediately after a short transportation period between the field and the laboratory. Bruchid cultures, originating from a field infestation in Hungary, were kept on dry *P. vulgaris* ‘Cannellini’ beans in plastic containers, and maintained under artificial lighting at 20 °C/60% relative humidity (RH) with a 16:8 h light:dark photoperiod. To obtain virgin females, seeds were kept individually in wells of a plastic rack until emergence of adults, when sexes were separated immediately.

### 2.2 Collection of volatiles

Dynamic headspace collection (air entrainment)<sup>32</sup> was used to collect volatile compounds from *D. carota* flowers. Twenty inflorescences (umbels) were placed within a 2-L glass chamber (Biochem Glass Apparatus Ltd, Milton Keynes, UK). Air was pumped through activated charcoal filters at 600 mL min<sup>-1</sup> into the chamber to provide a positive pressure of clean air. At another outlet was placed a glass tube [8 cm × 0.3 cm internal diameter (ID)] containing Porapak Q (50 mg) (Sigma-Aldrich, Gillingham, UK) sandwiched between glass wool plugs. Air was drawn from the chamber through the tube under negative pressure at a flow rate of 500 mL min<sup>-1</sup>. Volatile organic compounds (VOCs) were collected on Porapak Q traps for 6 h and were eluted with 750 L of freshly distilled diethyl ether. The extract was concentrated to 100 L under a gentle stream of nitrogen and kept at -20 °C until use.

### 2.3 Electrophysiology

Electroantennogram recordings from the antennae of adult female *A. obtectus* were made using Ag –AgCl glass electrodes filled with saline solution composed as in Maddrell,<sup>33</sup> but without glucose. An antenna was excised and suspended between the two electrodes. The tip of the terminal process of the antenna was removed to ensure a good contact. The signals were passed through a UN-06 high-impedance amplifier (Ockenfels Syntech GmbH, Kirchzarten, Germany). The coupled GC-EAG system has been described previously.<sup>34</sup> Separation of the collected *D. carota* volatiles was achieved on a high-resolution gas chromatograph (Agilent 6890 N; Agilent Technologies, Santa Clara, CA, USA), equipped with a cool on-column injector and a flame ionization detector (FID), using a 50 m × 0.32 mm ID HP-1 column (J & W Scientific, Folsom, CA, USA). The oven temperature was maintained at 30 °C for 2 min and then programmed at 15 °C/min to 250 °C. The carrier gas was helium. The outputs from the EAG amplifier and the FID were monitored simultaneously and analysed using a customized software package (Ockenfels Syntech GmbH). A compound was defined as EAG-active if it evoked an

antennal response, distinguishable from background noise, in three or more of five coupled runs (i.e. five independent antennal preparations).

### 2.4 Gas chromatography analysis

The collected volatile extract was analysed by high-resolution GC using an Agilent 6890A gas chromatograph equipped with a cool on-column injector, an FID and a 50 m × 0.32 mm ID HP-1 column (J & W Scientific). The oven temperature was maintained at 30 °C for 1 min, then programmed at 5 °C/min to 150 °C and held for 0.1 min, then programmed at 10 °C/min to 250 °C and held for 20 min. The carrier gas was hydrogen. The extract was also analysed by enantioselective GC using an Agilent 6890 N gas chromatograph equipped with a cool on-column injector, an FID and a 30 m × 0.25 mm ID × 0.25 m film thickness SUPELCO® Beta DEX™ (Sigma-Aldrich, Gillingham, UK) 120 fused silica capillary column. The oven temperature was maintained at 30 °C for 1 min and then programmed at 5 °C/min to 150 °C, then at 10 °C/min to 230 °C and held for 22 min. The carrier gas was hydrogen. Quantification of compounds was achieved using the single-point external standard method with a series of C7 – C22 alkanes.

### 2.5 Coupled gas chromatography–mass spectrometry (GC–MS)

Electrophysiologically active GC peaks were analysed using a 50 m × 0.32 mm ID HP-1 column (J & W Scientific), equipped with a cool on-column injector, that was coupled to a Waters Autospec Ultima magnetic sector mass spectrometer (Waters Corp., Manchester, UK). Ionization was by electron impact at 70 eV and 220 °C. The GC oven temperature was maintained at 30 °C for 1 min, then programmed at 5 °C/min to 150 °C and held for 0.1 min, then programmed at 10 °C/min to 250 °C and held for 34.9 min. Tentative identifications were made by comparison of mass spectra to those contained in a library database,<sup>35</sup> and confirmed by GC peak enhancement using authentic samples of compounds purchased from commercial suppliers.

### 2.6 Chemicals

Synthetic standards required for confirmation of identity and behavioural bioassays were purchased from Sigma-Aldrich, Gillingham, UK [(*R*)-pinene 98%, (*S*)-pinene 97%, (*R/S*)-sabinene 75%, myrcene ≥90%, (*R*)-limonene 97% and (*S*)-limonene 96%] and Fluka (Loughborough, UK) [terpinolene ≥90% and (*S*)-bornyl acetate 99%].

### 2.7 Olfactometer bioassays

To determine the behavioural responses of virgin *A. obtectus* females to *D. carota* flower headspace, headspace extract and a synthetic blend of electrophysiologically active compounds, a Perspex four-arm olfactometer was used.<sup>36</sup> The olfactometer rested on a stand (a rubber cork; 5 cm high and 4 cm wide) and was connected to glass chambers enclosing one, fully opened, white flower head on a live *D. carota* or left empty as controls (experiment 1; see below). The connections were made using Teflon tubing, through a 3-mm-diameter hole at the end of each of the four arms. When testing the headspace extract or synthetic blend (experiments 2 and 3), glass arms (narrow part: 50 mm in length × 2.5 mm in diameter; wide part: 90 mm in length × 20 mm in diameter) were directly attached to the olfactometer. Test solutions were each applied to a piece of filter paper (ca.



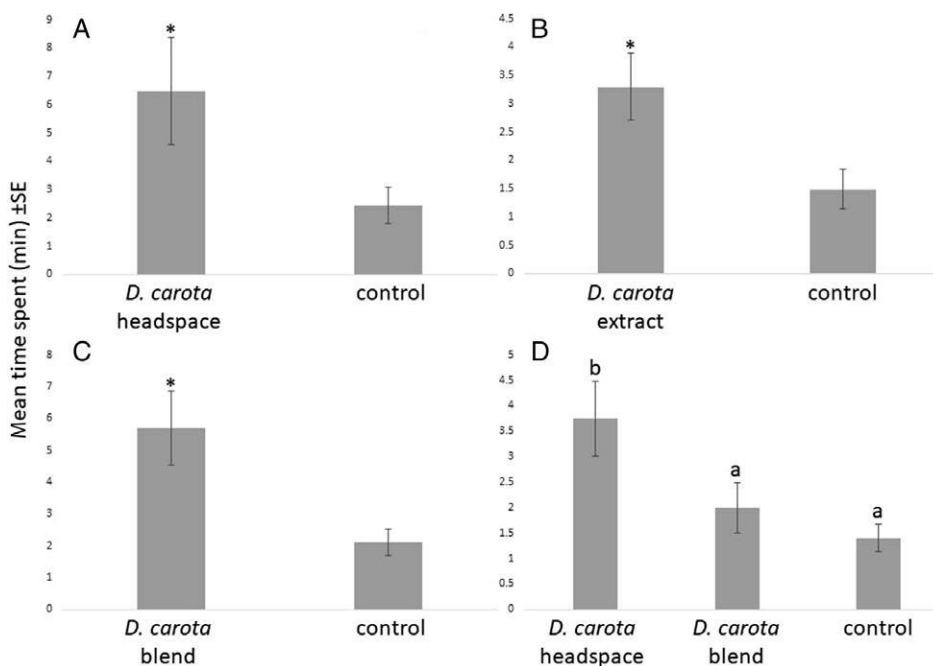
2 cm<sup>2</sup>), which was then placed into one of the glass arms of the olfactometer and tested against three control arms, except for Experiment 4, testing synthetic blend versus flower headspace, where two control arms were used. Prior to each experiment, all glassware was washed with Teepol detergent (Teepol, Orpington, UK), rinsed with acetone and distilled water and baked in an oven at 130 °C for 2 h. Perspex components were washed with Teepol solution, rinsed with 80% ethanol solution and distilled water and left to air-dry. The olfactometer was illuminated from above by diffuse uniform lighting from two 18 W/35 white fluorescent light bulbs screened with red acetate<sup>37</sup>. It was surrounded by black paper to remove any external visual stimuli. Charcoal-filtered air was pumped into the glass chambers at a rate of 100 mL min<sup>-1</sup>, then drawn through the central hole of the olfactometer by another vacuum pump (220–240 V AC; Charles Austen Pumps Ltd, Byfleet, UK) and thereby pulled through each of the four side arms (75 mL min<sup>-1</sup>/arm), and subsequently exhausted from the room. A single beetle was introduced into the olfactometer at each test period. Each beetle ( $n = 10$ ) was given 2 min to acclimatize in the olfactometer, after which the experiment was run for 16 min by rotating it 90° on the stand every 2 min to control for any directional bias (temperature 22 °C). The olfactometer was divided into five regions that corresponded to each of the four glass arms and the central compartment, and the time spent in each area was recorded using specialist software (OLFA, Udine, Italy). In order to account for the replication and areas within each replication as variance components in a split-plot design, the method of residual

maximum likelihood (REML) was used to fit a linear mixed model to the time spent data, nesting the areas within each replication and testing the treatment effect using an approximate  $F$ -test. The data were analysed on the square root scale to account for some heterogeneity of variance over the treatments. Means are presented with standard error of the difference (SED) values for their comparison, and the least significant difference (LSD) at the 5% ( $P = 0.05$ ) level of significance was used for separation of means when there were three treatments (experiment 4). Genstat (18th edition; VSN International Ltd, Hemel Hempstead, UK) was used for the analysis.

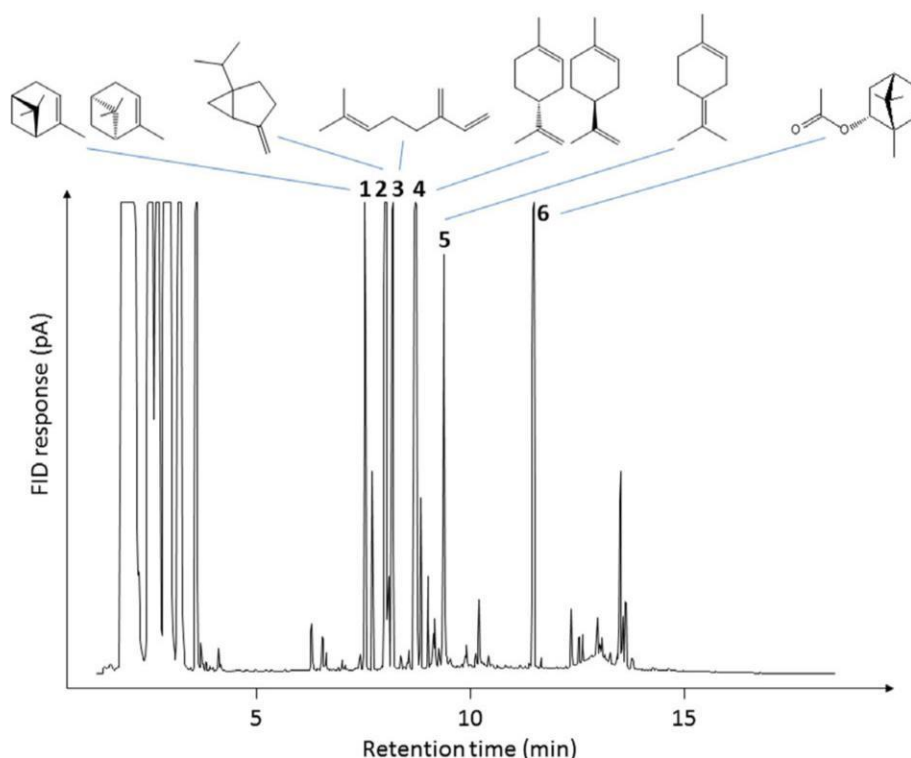
Experiments comprised (1) the headspace of one *D. carota* flower versus blank air; (2) one *D. carota* inflorescence-equivalent amount of headspace extract (released over ca. 20 min) versus diethyl ether (10 L); (3) a synthetic blend containing identified EAG-active compounds (ng/L) in a 10-L hexane solution at similar concentration and ratio as released by one *D. carota* inflorescence/20 min [calculated from headspace samples, i.e. (S)- $\alpha$ -pinene 37.6 ng, (R)- $\alpha$ -pinene 2.4 ng, (RS)-sabinene 120 ng, myrcene 50 ng, (S)-limonene 32.5 ng, (R)-limonene 97.5 ng, terpinolene 10 ng and (S)-bornyl acetate 90 ng], versus hexane (10 L); (4) the headspace of one *D. carota* flower versus the synthetic blend (10 L) versus hexane (10 L).

### 3 RESULTS

In behavioural (four-arm olfactometer) assays, adult virgin female *A. obtectus* spent more time in the arm containing the odour



**Figure 1.** Behavioural response of virgin female *Acanthoscelides obtectus* to *Daucus carota* floral headspace [A; predicted means on square root scale: control = 1.199 ( $n = 33$ ); floral headspace = 2.086 ( $n = 11$ ); SED = 0.4038; df = 42], to a headspace extract prepared from umbels [B; predicted means on square root scale: control = 0.991 ( $n = 30$ ); extract = 1.567 ( $n = 10$ ); SED = 0.2880; df = 38], to a synthetic blend of electrophysiologically active compounds identified from *D. carota* headspace extracts [C; predicted means on square root scale: control = 1.226 ( $n = 30$ ); synthetic blend = 2.249 ( $n = 10$ ); SED = 0.2939; df = 38], and to floral headspace versus a synthetic blend [D; predicted means on square root scale: control = 0.925 ( $n = 32$ ); floral headspace = 1.762 ( $n = 16$ ); synthetic blend = 1.138 ( $n = 16$ ); SED = 0.2827 (df = 61) for comparison to control and 0.2448 for comparison of floral headspace to synthetic blend]. The response was measured as the mean ( $\pm$  standard error (SE)) time spent in the arms of the olfactometer. Controls were clean air for floral headspace, diethyl ether for the air entrainment extract and hexane for the synthetic blend. \*Significantly different ( $P \leq 0.053$ ;  $F$ -test; see main text of Results for exact  $P$ -values) by the method of residual maximum likelihood (REML), used to fit a linear mixed model to the time spent data, nesting the areas within each replication and testing the treatment effect using an approximate  $F$ -test. The data were analysed on the square root scale to account for some heterogeneity of variance over the two treatments. Columns with the same letters are not significantly different ( $P < 0.05$ ; LSD).



**Figure 2.** Gas chromatography analysis of *Daucus carota* headspace extract (50 m × 0.32 mm ID HP-1 column), highlighting EAG-active peaks. Numbers correspond to identified compounds in Table 1.

of *D. carota* inflorescences compared with control arms containing clean air ( $F$ -test:  $F = 4.83$ ;  $df = 1, 42$ ;  $P = 0.034$ ) (experiment 1; Fig. 1A). There was evidence of attraction to the extract of VOCs collected from *D. carota* inflorescences compared with the control arms ( $F$ -test:  $F = 4.00$ ;  $df = 1, 38$ ;  $P = 0.053$ ) (experiment 2; Fig. 1B). Using coupled GC-EAG with the antennae of female *A. obtectus*, six peaks were located in the volatile extract with EAG activity (Fig. 2, Table 1 and Supporting Information Fig. S1). These were identified by coupled GC-MS and GC peak enhancement, including enantioselective (chiral) GC using authentic standards, as -pinene ( $R:S = 1:16$ ), sabinene (stereochemistry not determined), myrcene, limonene ( $R:S = 3:1$ ), terpinolene and (*S*)-bornyl acetate, and were the most abundant constituents of the *D. carota* flower headspace, comprising 71.2% of all compounds. The ratio of compounds, based on GC peak areas, was 4:12:5:13:1:9, respectively. Females preferred the synthetic blend of *D. carota* EAG-active volatiles to the solvent control in bioassays ( $F$ -test:  $F = 12.10$ ;  $df = 1, 38$ ;  $P = 0.001$ ) (experiment 3; Fig. 1C). In the final experiment, there was a significant ( $F$ -test:  $F = 6.89$ ;  $df = 1, 61$ ;  $P = 0.011$ ) difference between time spent in the arms with the natural and synthetic flower scents as a whole, compared with the control. Having accounted for this overall difference, there was also a difference between the two flower scent treatments ( $F$ -test:  $F = 4.88$ ;  $df = 1, 61$ ;  $P = 0.031$ ), the beetles spending more time in the area of the olfactometer flushed with live flower headspace, compared with that with the synthetic blend (experiment 4; Fig. 1D).

## 4 DISCUSSION

This is the first report on the activity of flower-derived semiochemicals for the adult dried bean beetle, *A. obtectus*, a member of the

**Table 1.** Electrophysiologically active compounds identified in headspace extracts of *Daucus carota* inflorescences, using virgin female *Acanthoscelides obtectus* antennae ( $n = 5$ ). Tentative identifications were made by GC-MS and confirmed by GC peak enhancement with authentic standards (see Materials and Methods)

| Number | Compound                    | Retention index <sup>a</sup> (non-polar) | Retention index <sup>b</sup> (chiral) | Concentration (ng/L) |
|--------|-----------------------------|--|---------------------------------------|----------------------|
| 1      | ( <i>S</i> )-Pinene         | 936                                      | 1026                                  | 37.6                 |
|        | ( <i>R</i> )-Pinene         |  | 1033                                  | 2.4                  |
| 2      | Sabinene <sup>c</sup>       | 972                                      | 1054                                  | 120                  |
| 3      | Myrcene                     | 987                                      | 1038                                  | 50                   |
| 4      | ( <i>S</i> )-Limonene       | 1031                                     | 1104                                  | 32.5                 |
|        | ( <i>R</i> )-Limonene       |  | 1108                                  | 97.5                 |
| 5      | Terpinolene                 | 1086                                     | 1161                                  | 10                   |
| 6      | ( <i>S</i> )-Bornyl acetate | 1286                                     | 1401                                  | 90                   |

<sup>a</sup> On an Agilent HP-1 GC column.  
<sup>b</sup> On a SUPELCO<sup>®</sup> Beta DEX<sup>™</sup> 120 fused silica GC column. <sup>c</sup> Stereochemistry not determined.

Bruchinae subfamily of Chrysomelidae. The volatiles identified as EAG-active from *D. carota* flowers in this study had also been identified in headspace extracts<sup>38</sup> and essential oils extracted from the umbels.<sup>39</sup> Similarly, the essential oils and headspace extracts of the closely related *D. muricatus* L. flowers contained all six compounds identified here, but with no reference to their enantiomeric composition.<sup>40</sup> In Zachariae,<sup>31</sup> flowers of *Ranunculus arvensis* L., *Ranunculus repens* L. (Ranunculaceae), *Fragaria grandiflora* Ehrh., *Potentilla anserina* L., *Potentilla reptans* L. (Rosaceae), *Petroselinum sativum* Hoffm., *Torilis anthriscus* Gm. and *Aethusa*

*cynapium* L. (Apiaceae) are reported to be visited for nectar by adult *A. obtectus*, in addition to *D. carota*. Analysis of available data on the distribution of volatiles identified in floral bouquets across the above six genera (Table S1) revealed that limonene and (*E*)-ocimene are the most widespread constituents found in *Ranunculus*, *Fragaria*, *Potentilla* and *Daucus*, including *D. carota*. (*Z*)-Ocimene, myrcene, linalool (unknown chirality), germacrene D, (*E*)-caryophyllene, methyl salicylate, (*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate each occur in three genera, but the latter three compounds do not occur in *Daucus*. These ubiquitous volatiles have been identified from a large number of plant species<sup>41</sup> with various roles in plant–arthropod interactions, including attractant properties for insects in many cases.<sup>42–48</sup> Of the 39 mostly terpenoid compounds reported in the present study for *D. carota* (Table S1), 20 of them (51%) are also found in other *Daucus* species. The six EAG-active *D. carota* volatiles appear to be a unique (and perhaps characteristic) combination in the floral headspace of *Daucus* spp. -Pinene, myrcene, limonene and terpinolene are attractants of bark beetles,<sup>44</sup> -pinene and sabinene are suggested to contribute to the attractiveness of certain *D. carota* cultivars to *Trioza apicalis* Förster (Homoptera: Psylloidea),<sup>38</sup> whereas -pinene, limonene and bornyl acetate are constituents of an attractive blend for *Smicronyx fulvus* Le Conte (Coleoptera: Curculionidae).<sup>49</sup>

Prior to this study, the few chemical ecology studies on host plant – Bruchinae relationships have primarily focused on lar-val host plants.<sup>50–52</sup> Pouzat<sup>53</sup> demonstrated the EAG activity of the vapour of bean pods, as well as synthetic amyl acetate, in *A. obtectus*. Female bruchids, including *A. obtectus*,<sup>26,28,31</sup> however, often visit flowers to obtain pollen for their eggs to mature.<sup>11</sup> Examples are *Bruchus pisorum* L. on *Pisum sativum* L. (Fabaceae), where obligatory pre-copulation feeding on pollen was reported,<sup>54</sup> as well as nectar feeding to obtain a readily available source of energy to sustain flight,<sup>55</sup> and *Spermophagus sericeus* Geoffroy on *Daucus* spp.<sup>11</sup> Pollen has a high protein content plus sugar, starch, fat, and traces of vitamins and inorganic salts, while nectar primarily consists of a solution of sugars, especially glucose, fructose and sucrose.<sup>56</sup> *Bruchus rufimanus* Boheman is often found in flowers of *Vicia faba* L. and is attracted to a synthetic mixture of *V. faba* floral scent constituents, (*R*)-linalool, cinnamyl alcohol and cinnamaldehyde, identified from headspace extracts.<sup>57</sup> Ceballos *et al.*<sup>58</sup> identified a range of volatiles from *P. sativum* leaves, flowers and pods, and demonstrated that headspace extracts evoked positive responses from *B. pisorum* in behavioural assays. The compounds responsible for the bioactivity of the extracts are unknown. Our results provide supporting evidence that floral volatiles play a part in the interactions between *A. obtectus* and *D. carota*, aiding location of nectar plants. It is appreciated, however, that under our experimental circumstances, the bouquet of the natural floral headspace was behaviourally preferred to its synthetic mimic, indicating that there are further compounds in the floral headspace that could not be located by GC-EAG and which would increase the attractiveness of the synthetic blend, or that the blend composition, i.e. ratios and doses of constituents, requires further refinement. Optimization studies for practical uses will be carried out via trapping trials in bean fields and granaries. These will assess blend composition, dose and dispenser type to achieve maximum attractiveness.

Future blend development may also include compounds from other listed nectar plants,<sup>31</sup> as well as those from ripening bean pods. This approach could be particularly important for females, which directly determine the size of future generations.<sup>57</sup> In

this way, semiochemical-focused management strategies would directly reduce future population densities locally by reducing the number of females via trapping or the recruitment of natural enemies.<sup>59</sup> Such research efforts should focus on field-testing synthetic blends of compounds, using trap designs developed for bruchids<sup>57</sup> and other chrysomelids.<sup>60</sup> Trap development and design for coleopteran pests need to consider the specific behaviour of each species. Many chrysomelid species start climbing upwards after landing on a vertical surface. Traps for *Diabrotica v. virgifera* LeConte or *Phyllotreta* spp. take advantage of this behaviour and direct crawling insects upwards into a catch container.<sup>60</sup> The vertical landing surface also takes advantage of the preference of these species for a hue of yellow, the attractive effect of which is synergized by semiochemicals. Bruce *et al.*<sup>57</sup> used a light green-coloured cone trap to monitor *B. rufimanus* in the field, which affected the orientation behaviour of *B. rufimanus* differently from that of *A. obtectus*. The latter species typically moves upwards on vertical surfaces, suggesting that a trap design described in Tóth *et al.*<sup>60</sup> might be more suitable. Other floral cues may certainly be important to elicit this behaviour, including the colour of inflorescences. Attraction of flower-visiting insects can be enhanced by the combination of odour and colour stimuli,<sup>61</sup> whereas in other cases, colour does not synergize the effect of odour.<sup>62</sup> Little is known about colour preference in *A. obtectus*; Zachariae<sup>31</sup> lists nectar plants that all appear white or light yellow to the human eye, and traps coloured in white or yellow were found to be more attractive than other colours. Thus, trapping trials will need to assess a range of colours, including those mimicking the reflectance spectra of common inflorescences visited by the species.

The interactions between intra- and interspecific chemical signals are regulated at the olfactory periphery and in the central nervous system,<sup>63</sup> and can often result in behavioural synergism.<sup>64</sup> Thus, the efficiency of a plant volatile-based lure for *A. obtectus* may also be enhanced by its co-application with the male-produced sex pheromone.<sup>23</sup> In conclusion, development of the six-component blend is being pursued, which could underpin the design of semiochemical-based field management approaches against this major pest of stored products.

## ACKNOWLEDGEMENTS

The authors thank Zafirah Hussain for help with the olfactometer assays. This work was in part supported by the Hungarian Scientific Research Fund (OTKA) (grant K81494), and by the Research and Technology Innovation Fund (grant OMF0609/2010). Rothamsted receives grant-aided support from the Biotechnology and Biological Sciences Research Council (BBSRC) of the UK.

## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

## REFERENCES

- Alvarez N, Hossaert-McKey M, Rasplus J-Y, McKey D, Mercier L, Soldati L, Aebi A, Shani T and Benrey B. Sibling species of bean bruchids: a morphological and phylogenetic study of *Acanthoscelides obtectus* Say and *Acanthoscelides obvelatus* Bridwell. *J Zool Syst Evol Res* **43**:29–37 (2005a).
- Mishili F, Temu A, Fulton J and Lowenberg-DeBoer J. Consumer preferences as drivers of the common bean trade in Tanzania: a marketing perspective. *J Int Food Agribus Marketing* **23**:110–127 (2011).



- 3 Thakur DR, Taxonomy, distribution and pest status of Indian biotypes of *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae: Bruchinae) - a new record. *Pakistan J Zool* **44**:189 – 195 (2012).
- 4 Mutungi C, Affognon HD, Njoroge AW, Manono J, Baributsa D and Murdock LL, Triple-layer plastic bags protect dry common beans (*Phaseolus vulgaris*) against damage by *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae) during storage. *J Econ Entomol* **108**:2479 – 2488 (2015).
- 5 Soares MA, Quintela ED, Mascarín GM and Arthurs SP, Effect of temperature on the development and feeding behavior of *Acanthoscelides obtectus* (Chrysomelidae: Bruchinae) on dry bean (*Phaseolus vulgaris* L.). *J Stored Prod Res* **61**:90 – 96 (2015).
- 6 Cardona C, Insects and Other Invertebrate Bean Pests, in *Bean Production Problems in the Tropics*, ed. by Schwartz HF and Pastor-Corrales MA, Colombia, CIAT, Cali, pp. 505 – 570 (1989).
- 7 Karel AK and Autrique A, Insects and other pests in Africa, in *Bean Production Problems in the Tropics*, ed. by Schwartz HF and Pastor-Corrales MA, Colombia, CIAT, Cali, pp. 455 – 504 (1989).
- 8 Alvarez N, McKey D, Hossaert-McKey M, Born C, Mercier L and Benrey B, Ancient and recent evolutionary history of the bruchid beetle, *Acanthoscelides obtectus* Say, a cosmopolitan pest of beans. *Mol Ecol* **14**:1015 – 1024 (2005b).
- 9 Zachariae G, Kann sich der Speisebohnenkäfers *Acanthoscelides obtectus* Say als Freilandsschädling in Norddeutschland einbürgern? *Z ang Entomol* **45**:225 – 267 (1960).
- 10 Paul UV, Hilbeck A and Edwards PJ, Pre-harvest infestation of beans (*Phaseolus vulgaris* L.) by *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) in relation to bean pod maturity and pod aperture. *Int J Pest Manag* **56**:41 – 50 (2010).
- 11 Southgate BJ, Biology of the Bruchidae. *Annu Rev Entomol* **24**:449 – 473 (1979).
- 12 Abate T and Ampofo JKO, Insect pests of beans in Africa: their ecology and management. *Annu Rev Entomol* **41**:45 – 73 (1996).
- 13 Papachristos DP and Stamopoulos DC, Repellent, toxic and reproduction inhibitory effects of essential oil vapours on *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). *J Stored Prod Res* **38**:117 – 128 (2002).
- 14 Papachristos DP and Stamopoulos DC, Selection of *Acanthoscelides obtectus* (Say) for resistance to lavender essential oil vapour. *J Stored Prod Res* **39**:433 – 441 (2003).
- 15 Velten G, Rott AS, Conde Petit BJ, Cardona C and Dorn S, Improved bruchid management through favorable host plant traits and natural enemies. *Biol Cont* **47**:133 – 140 (2008).
- 16 Kamanula J, Sileshi GW, Belmain SR, Sola P, Mvumi BM, Nyirenda GKC, Nyirenda SP and Stevenson PC, Farmers' insect pest management practices and pesticidal plant use in the protection of stored maize and beans in Southern Africa. *Int J Pest Manag* **57**:41 – 49 (2011).
- 17 Boyer S, Zhang H and Lempérière G, A review of control methods and resistance mechanisms in stored-product insects. *Bull Entomol Res* **102**:213 – 229 (2012).
- 18 Freitas RS, Faroni LRA and Sousa AH, Hermetic storage for control of common bean weevil, *Acanthoscelides obtectus* (Say). *J Stored Prod Res* **66**:1 – 5 (2016).
- 19 Mouttet R, Escobar-Gutiérrez A, Esquibet M, Gentzmittel L, Mugniéry D, Reignault P, Sarniguet C and Castagnone-Sereno P, Banning of methyl bromide for seed treatment: could *Ditylenchus dipsaci* again become a major threat to alfalfa production in Europe? *Pest Manag Sci* **70**:1017 – 1022 (2014).
- 20 Shaaya E and Kostyukovsky M, Alternative fumigants to methyl bromide for the control of pest infestation in grain and dry food products. 10th International Working Conference on Stored Product Protection, 27 June – 2 July 2010, Lisbon, Portugal (2010).
- 21 Njoroge AW, Affognon H, Mutungi C, Richter U, Hensel O, Rohde B and Mankin RW, Bioacoustics of *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae: Bruchinae) on *Phaseolus vulgaris* (Fabaceae). *Florida Entomol* **100**:109 – 115 (2017).
- 22 Pickett JA, Aradottir GI, Birkett MA, Bruce TJA, Chamberlain K, Khan ZR, Midega CAO, Smart LE and Woodcock CM, Aspects of insect chemical ecology: exploitation of reception and detection as tools for deception of pests and beneficial insects. *Physiol Entomol* **37**:2 – 9 (2012).
- 23 Vuts J, Francke W, Mori K, Zarbin PHG, Hooper AM, Millar JG, Pickett JA, Tóth M, Chamberlain K, Caulfield JC, Woodcock CM, Tröger AG, Bálintné Csonka É and Birkett MA, Pheromone bouquet of the dried bean beetle, *Acanthoscelides obtectus* (Col.: Chrysomelidae), now complete. *Eur J Org Chem* **2015**:4843 – 4846 (2015a).
- 24 Vuts J, Powers SJ, Caulfield JC, Pickett JA and Birkett MA, Multiple roles of a male-specific compound in the sexual behaviour of the dried bean beetle, *Acanthoscelides obtectus*. *J Chem Ecol* **41**:287 – 293 (2015b).
- 25 Leroi B, Feeding, longevity and reproduction of adults of *Acanthoscelides obtectus* Say in laboratory conditions, in *The ecology of bruchids attacking legumes (pulses)*, ed. by Labeyrie V, Series Entomol **19**:101 – 111 (1981).
- 26 Jarry M, Diet of the adults of *Acanthoscelides obtectus* and its effect on the spatial pattern of the attacks in the fields of *Phaseolus vulgaris*, in *Insects-Plants*, ed. by Labeyrie V, Fabres G and Lachaise D, W Junk, Dordrecht, The Netherlands, pp. 71 – 75 (1987).
- 27 Szentesi Á, Bruchidae, in *Handbook of plant protection zoology 3/B*, ed. by Jermy T and Balázs K, Akadémiai Press, Budapest, Hungary, pp. 339 – 363 (1990). (in Hungarian)
- 28 Huignard J and Leroi B, Influence of adult food on the reproduction of virgin females of an *Acanthoscelides obtectus* strain originating from Colombian altiplanos. *Experientia* **37**:831 – 833 (1981).
- 29 Lago PK and Mann MO, Survey of Coleoptera associated with flowers of wild carrot (*Daucus carota* L.) (Apiaceae) in Northern Mississippi. *Coleopt Bull* **41**:1 – 8 (1987).
- 30 Evans AV, Beetles of Eastern North America. Princeton University Press, USA, p 560 (2014).
- 31 Zachariae G, Das Verhalten des Speisebohnenkäfers *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) im Freien in Norddeutschland. *Z ang Entomol* **43**:345 – 365 (1958).
- 32 Birkett MA, The chemistry of plant signalling, in *Plant communication from an ecological perspective*, ed. by Baluška F and Ninkovic V, Springer, Berlin-Heidelberg, pp. 21 – 42 (2010).
- 33 Maddrell SHP, Secretion by the Malpighian tubules of *Rhodnius*. The movement of ions and water. *J Exp Biol* **51**:71 – 97 (1969).
- 34 Wadhams LJ, The use of coupled gas chromatography: electrophysiological techniques in the identification of insect pheromones, in *Chromatography and isolation of insect hormones and pheromones*, ed. by McCaffery AR and Wilson ID, Plenum Press, New York/London, pp. 289 – 298 (1990).
- 35 NIST (2008) Software NIST/EPA/NIH Mass Spectral Library 2008
- 36 Pettersson J, An aphid sex attractant I. Biological studies. *Insect Syst Evol* **1**:63 – 73 (1970).
- 37 Shields EJ, Artificial light: experimental problems with insects. *Bull Entomol Soc Am* **35**:40 – 44 (1989).
- 38 Nehlin G, Valtrová I and Borg-Karlson A-K, Monoterpenes released from Apiaceae and the egg-laying preferences of the carrot psyllid, *Trioza apicalis*. *Entomol Exp Appl* **80**:83 – 86 (1996).
- 39 Maxia A, Marongiu B, Piras A, Porcedda S, Tuveri E, Gonçalves MJ, Cavaleiro C and Salgueiro L, Chemical characterization and biological activity of essential oils from *Daucus carota* L. subsp. *carota* growing wild on the Mediterranean coast and on the Atlantic coast. *Fitoterapia* **80**:57 – 61 (2009).
- 40 Bendiabdellah A, Amine Dib M El, Djabou N, Allali H, Tabti B, Muselli A and Costa J, Biological activities and volatile constituents of *Daucus muricatus* L. from Algeria. *Chem Cent J* **6**:48 (2012).
- 41 Knudsen JT, Eriksson R, Gershenzon J and Stahl B, Diversity and distribution of floral scent. *Bot Rev* **72**:1 – 120 (2006).
- 42 Miller DR, Limonene: attractant kairomone for white pine cone beetles (Coleoptera: Scolytidae) in an eastern white pine seed orchard in western North Carolina. *J Econ Entomol* **100**:815 – 22 (2007).
- 43 Farré-Armengol G, Filella I, Llusà J and Peñuelas J, -Ocimene, a key floral and foliar volatile involved in multiple interactions between plants and other organisms. *Molecules* **22**:1148 (2017).
- 44 Gitau CW, Bashford R, Carnegie AJ and Gurr GM, A review of semio-chemicals associated with bark beetle (Coleoptera: Curculionidae: Scolytinae) pests of coniferous trees: A focus on beetle interactions with other pests and their associates. *Forest Ecol Manag* **297**:1 – 14 (2013).
- 45 Mozuraitis R, Stranden M, Ramirez MI, Borg-Karlson A-K and Mustaparta H, (-)-Germacrene D increases attraction and oviposition by the tobacco budworm moth *Heliothis virescens*. *Chem Sense* **27**:505 – 509
- 46 Flint HM, Salter SS and Walter S, Caryophyllene, an attractant for the green lacewing *Chrysopa carnea*. *Environ Entomol* **8**:1123 – 1126 (1979).
- 47 Mallinger RE, Hogg DB and Gratton C, Methyl Salicylate attracts natural enemies and reduces populations of soybean aphids (Hemiptera: Aphididae) in soybean agroecosystems. *J Econ Entomol* **104**:115 – 124 (2011).

- 48 Vuts J, Woodcock CM, Sumner ME, Caulfield JC, Reed K, Inward DJG, Leather SR, Pickett JA, Birkett MA, Denman S, Responses of the two-spotted oak buprestid, *Agilus biguttatus* (Coleoptera: Buprestidae), to host tree volatiles. *Pest Manag Sci* **72**:845 – 851 (2016).
- 49 Roseland CR, Bates MB, Carlson RB and Oseto CY, Discrimination of sunflower volatiles by the red sunflower seed weevil. *Entomol Exp Appl* **62**:99 – 106 (1992).
- 50 Babu A, Hern A and Dorn S, Sources of semiochemicals mediating host finding in *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Bull Entomol Res* **93**:187 – 192 (2003).
- 51 Khelfane-Goucem K, Medjdoub-Bensaad F, Leppik E and Frerot B, Dry bean volatile organic compounds mediating host choice in *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae: Bruchinae). *Ann Soc Entomol France* **50**:167 – 176 (2014).
- 52 Nazzi F, Vidoni F and Frilli F, Semiochemicals affecting the host-related behaviour of the dry bean beetle *Acanthoscelides obtectus* (Say). *J Stored Prod Res* **44**:108 – 114 (2008).
- 53 Pouzat J, The role of sense organs in the relations between bruchids and their host plants, in The ecology of bruchids attacking legumes (pulses), ed. by Labeyrie V, *Series Entomol* **19**:61 – 72 (1981).
- 54 Pajni HR, Trophic relations and ecological status of the adults of *Bruchus Pisorum* L. and allied field species of Bruchidae (Coleoptera). *Series Entomol* **19**:125 – 129 (1981).
- 55 Clement SL, On the function of pea flower feeding by *Bruchus pisorum*. *Entomol Exp Appl* **63**:115 – 121 (1992).
- 56 Wäckers FL, Romeis J and van Rijn P, Nectar and pollen feeding by insect herbivores and implications for multitrophic interactions. *Annu Rev Entomol* **52**:301 – 323 (2007).
- 57 Bruce TJA, Martin JL, Smart LE and Pickett JA, Development of semio-chemical attractants for monitoring bean seed beetle, *Bruchus rufi-manus*. *Pest Manag Sci* **67**:1303 – 1308 (2011).
- 58 Ceballos R, Fernandez N, Zuniga S and Zapata N, Electrophysiological and behavioral responses of pea weevil *Bruchus pisorum* L. (Coleoptera: Bruchidae) to volatiles collected from its host *Pisum sativum* L. *Chilean J Agric Res* **75**:202 – 209 (2015).
- 59 Pickett JA and Khan ZR, Plant volatile-mediated signalling and its application in agriculture: successes and challenges. *New Phytol* **212**:856 – 870 (2016).
- 60 Tóth M, Bálintné Csonka É, Szarukán I, Vörös G, Furlan L, Imrei Z and Vuts J, The KLP+ (“hat”) trap, a non-sticky, attractant-baited trap of novel design for catching the western corn rootworm (*Diabrotica v. virgifera*) and cabbage flea beetles (*Phyllotreta* spp.) (Coleoptera: Chrysomelidae). *Int J Hort Sci* **12**:57 – 62 (2006).
- 61 Vuts J, Kaydan MB, Yarimbatman A and Tóth M, Field catches of *Oxythyrea cinctella* using visual and olfactory cues. *Physiol Entomol* **37**:92 – 96 (2012).
- 62 Tóth M, Furlan L, Szarukán I and Vuts J, Development of a female-targeted attractant for the click beetle *Agriotes ustulatus* Schwarz. *Acta Phytopath Entomol Hung* **46**:237 – 248 (2011).
- 63 Dupuy F, Rouyar A, Deisig N, Bourgeois T, Limousin D, Wycke M-A, Anton S and Renou M, A background of a volatile plant compound alters neural and behavioral responses to the sex pheromone blend in a moth. *Front Physiol* **8**:79 (2017).
- 64 Reddy GVP and Guerrero A, Interactions of insect pheromones and plant semiochemicals. *Trends Plant Sci* **9**:253 – 261 (2004).