Parasitoids select plants more heavily infested with their caterpillar hosts: a new approach to aid interpretation of plant headspace volatiles

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Plants produce volatile organic compounds (VOCs) in response to herbivore attack, and these VOCs can be used by parasitoids of the herbivore as host location cues. We investigated the behavioural responses of the parasitoid Cotesia vestalis to VOCs from a plant–herbivore complex consisting of cabbage plants (Brassica oleracea) and the parasitoid host caterpillar, Plutella xylostella. A Y-tube olfactometer was used to compare the parasitoids’ responses to VOCs produced as a result of different levels of attack by the caterpillar and equivalent levels of mechanical damage. Headspace VOC production by these plant treatments was examined using gas chromatography–mass spectrometry. Cotesia vestalis were able to exploit quantitative and qualitative differences in volatile emissions, from the plant–herbivore complex, produced as a result of different numbers of herbivores feeding. Cotesia vestalis showed a preference for plants with more herbivores and herbivore damage, but did not distinguish between different levels of mechanical damage. Volatile profiles of plants with different levels of herbivores/herbivore damage could also be separated by canonical discriminant analyses. Analyses revealed a number of compounds whose emission increased significantly with herbivore load, and these VOCs may be particularly good indicators of herbivore number, as the parasitoid processes cues from its external environment.

Keywords: herbivore-induced plant volatiles; Hymenoptera; Lepidoptera; parasitoid host location; tritrophic; volatile organic compounds

1. INTRODUCTION

Vet & Dicke [1] hypothesized that parasitoids may experience a ‘reliability–detectability problem’ while locating their hosts and their hosts’ habitats, whereby chemical cues released directly from their hosts are likely to be highly reliable and taxonomically specific, but, may have low detectability because natural selection has reduced host conspicuousness. They hypothesized that volatile organic compounds (VOCs) produced by plants provide alternate cues. Plant VOCs are produced in greater volume than host cues, and so are more detectable, but may be less reliable because they might convey information that is less taxonomically specific [1]. However, feeding by a herbivore can cause quantitative and qualitative changes in plant VOC emissions [2,3]. Considerable evidence shows that parasitoids exploit herbivore-induced plant VOCs in a wide variety of tritrophic systems consisting of many different plant, herbivore and parasitoid species (e.g. [2,4–10]).

Despite recent advances in understanding of the genetics and molecular biology of the production of herbivore-induced plant VOCs (e.g. [11–14]), little is known regarding host-density effects on VOC production, although host density significantly influences parasitoid foraging [15–17]. Parasitoids use their ability to differentiate between complex volatile blends to forage optimally for herbivore hosts [18]. Guerrieri et al. [19] demonstrated that broad-bean plants require a minimum infestation level of 40 Acyrthosiphon pisum aphids for at least 48–72 h to produce VOCs detectable to their parasitoid Aphidius ervi. If a parasitoid could use VOCs from the plant–herbivore complex to judge the density of a herbivore population on a plant, it could forage more efficiently, by concentrating its close-range search behaviour and allocating more foraging effort to patches with more herbivores.

Herbivore-induced plant VOCs are often extremely complex blends, differing in composition quantitatively and qualitatively between plant species [3] and qualitatively within a plant species, when it is attacked by different herbivore species [2,3,20,21]. Parasitoids show preferences for VOCs from plants infested with their host herbivore over a non-host herbivore [5], but not if both herbivore species are suitable hosts [22].
produce different VOCs after attack by different instars of a herbivore, which are differentially attractive to parasitoids [9,20,23,24]. Herbivore-induced VOCs also change temporally in relation to damage [19,25].

The responses of parasitoids and other natural enemies to VOCs are unlikely to be determined by individual compounds in the VOC profiles but more likely by a combination of compounds and their ratios in the VOC blend [26–28]. The use of multivariate analyses for the examination of VOC profiles has recently increased [29–33] because they allow analysis of the VOC profile as a whole, and this form of integrated analysis more closely resembles information processing in insects. Van Dam & Poppy [34] proposed that multivariate analysis techniques be applied more innovatively to help interpret changes in patterns of VOC blends.

We investigated whether a plant–herbivore complex emits different headspace VOC blends as a result of different degrees of herbivore infestation as well as equivalent mechanical damage, and, whether parasitoids respond behaviourally to any of these differences. We used a trirophic system consisting of cabbage plants (*Brassica oleracea*), the diamondback moth *Plutella xylostella* and its specialist parasitoid *Cotesia vestalis* (formerly known as *plutellae*), which responds to plant VOCs produced resulting from *P. xylostella* infestation [8].

### 2. MATERIAL AND METHODS

#### (a) Plants

*Brassica oleracea* var. *capitata* cv. Derby day seeds (Tozer Seeds, Surrey, UK) were sown in John Innes no. 2 compost (Minster Brand Products, Dorchester, UK) in a greenhouse with a minimum day-time temperature of 20°C (16 h) and a minimum night-time temperature of 14°C (8 h). Overhead lighting (mercury halide and sodium bulbs) was provided during the day to ensure a minimum light intensity of 300 W m$^{-2}$. Seedlings were transplanted after two weeks into individual 13 cm plastic plant pots and grown for another four weeks.

#### (b) Insects

Colonies of both *P. xylostella* and *C. vestalis* were established from long-term UK laboratory strains kept by Rothamsted Research, and reared on six-week-old Chinese cabbage (*Brassica rapa* L. ssp. *pekinesis* (Lour.) var. Wong Bok) (Kings Seeds, Surrey, UK) grown in John Innes no. 2 compost. Both colonies were kept in 1 × 1 m Perspex cages in controlled temperature rooms, at 20 ± 2°C under a 16 L: 8 D light regime. *Cotesia vestalis* pupae were continuously collected from host–parasitoid cultures and isolated in empty cages. When they emerged, they were allowed to mate and were provided with a 10 per cent honey solution as a food source. All parasitoids used in experiments were naive, excluding the conditioning they receive upon emergence, to ensure that their responses to the different VOC profiles were innate and not altered by habituation to an alternate profile [35].

#### (c) Olfactometry

A glass Y-tube olfactometer (e.g. [5]) was used to record behavioural responses of *C. vestalis* to pairs of different volatile sources. The olfactometer had an internal diameter of 2 cm, an 18 cm stem and 16 cm arms at a 60° angle. Air was pumped through Teflon tubing, purified by passage through activated charcoal and humidified by bubbling through distilled water. Airflow was divided by a brass T-junction (Swagelok, OH, USA) and then passed through two separate flow meters, which regulated the flow rate to 0.61 min$^{-1}$. The air passed through two Plexiglas chambers (500 mm high × 300 mm diameter), containing the volatile sources to be tested, and into the arms of the olfactometer. All tests were performed in a constant-temperature room at 23 ± 2°C. Visual cues were excluded by placing a white cardboard screen around the Y-tube. The Y-tube was lit from above by two 15 W fluorescent lamps, producing a light intensity of 1.7 klx and fitted with a prismatic filter to ensure an even distribution of light. Both parasitoids and volatile sources were placed under the lamp at least 1 h before any experiments were conducted to allow them to acclimatize to light and temperature levels. Individual naive female *C. vestalis* that were 2–5 days old were introduced to the Y-tube and given 5 min to make a choice. When a parasitoid moved 8 cm up an arm of the olfactometer, it was defined as having selected an odour. The position of the treatments was swapped between the arms of the Y-tube every five replicates. Between trials, all glassware was washed in Decon90, rinsed with acetone and distilled water and then baked at 200°C for 3–4 h to remove any volatile chemicals adhering to the glass. Airflow was continuously maintained through the apparatus and tubing when not conducting an experiment.

#### (d) Dual-choice experiments

Two experiments were conducted. In the first, *B. oleracea* plants were either undamaged or infested with 5, 10 or 20 third-instar *P. xylostella* larvae for 72 h prior to the trial. To investigate whether *C. vestalis* responds differently to different levels of *P. xylostella* damage, the responses of female *C. vestalis* were tested to each of following pairs of *B. oleracea* treatments: (i) undamaged versus infested with five larvae, (ii) infested with five larvae versus infested with 10 larvae, and (iii) infested with 10 larvae versus infested with 20 larvae. After testing, the plants were photographed and the leaf area and the area consumed by *P. xylostella* calculated using IMAGEJ (v. 1.37 National Institutes of Health: [36]).

In the second experiment, plants used were either undamaged or mechanically damaged with a total of 10, 20 or 40 holes, using a hole punch cumulatively, once a day, over 3 days. The final damage was done a minimum of 2 h before the start of bioassays (ca 11.00). One hole produced 0.29 cm$^2$ of damage, and the total number of holes punched removed an equivalent area of the leaf that would have been removed by 5, 10 and 20 *P. xylostella* outlined in the first experiment above. To investigate whether *C. vestalis* responds differently to different levels of mechanical damage, the responses of naive female *C. vestalis* were tested to each of following pairs of *B. oleracea* treatments: (i) undamaged versus damaged with 10 holes, (ii) damaged with 10 holes versus damaged with 20 holes, and (iii) damaged with 20 holes versus damaged with 40 holes.

The six different dual-choice experiments were conducted over a 5 day period; on each of the 5 days, separate replicates for every dual-choice experiment were conducted. On each day for each dual choice, the responses of 10 different naive *C. vestalis* to a pair of plants were tested; different plants were used every day and each *C. vestalis* was only ever used once. Therefore, for each dual-choice experiment, the responses of a total of 50 individuals were tested to a total...
of five plants of each treatment, as is standard for these types of experiments (e.g. [2,37]).

(c) Headspace volatile entrainments
Volatile entrainments were collected for both mechanically damaged and P. xylostella-infested B. oleracea plants. In the first experiment, plants were either undamaged or infested with 5, 10 or 20 third-instar P. xylostella larvae for 72 h prior to the trial. Insects were left on the plants during volatile collections. In the second experiment, plants were either undamaged or damaged with 10, 20 or 40 holes, as in the dual-choice experiments above. Entrainments were completed using a method similar to that described by Stewart-Jones & Poppy [38]. Plants were enclosed in a poly(ethylene terephthalate) (PET) tube (22 cm diameter, 60 cm length, 25 μm thickness; Kalle UK Ltd, Witham, UK), which had previously been purged overnight by purified air (0.91 min⁻¹; activated charcoal, BDH, 10–14 mesh, 50 g). The top of the plant pots was wrapped in three pieces of aluminium sheet to isolate soil from vegetative parts, but the roots remained aerated. The PET tube enclosed the whole plant and was fixed to the rim of the pot using a loop of wire and was closed loosely at the top with some wire. Air was filtered through activated charcoal and blown in (0.91 min⁻¹) near the base of the plant and drawn out at the top through a Tenax TA trap (0.61 min⁻¹). This created a positive pressure within the bag, which ensured unfiltered air did not enter. Plants were enclosed between 10.00 and 11.00 and before sampling was started the system was purged for 30 min by blowing air in only. VOCs were collected on Tenax TA (50 mg, mesh 60–80, Supelco, Bellefonte, PA, USA) held in thermal desorption sampling tubes (Anatune Optic PTV liner, Cambridgeshire, UK) by plugs of silanized wool. Tenax TA filters were first washed thoroughly with redistilled diethyl ether (5 ml) and baked under a slow flow of purified nitrogen (225°C, 2 h). All entrainments were carried out between 11.00 and 15.30, and each entrainment lasted 4 h, after which liners were sealed in glass tubes and stored in the freezer (−22°C) until analysis. After sampling, the plants were cut, photographed and the leaf area calculated. There were no significant differences between total leaf areas across treatments (ANOVA, F₃,₁₆ = 0.64, p = 0.599).

(f) Coupled gas chromatography–mass spectrometry
Samples were thermally desorbed using an Optic 2 programmable injector (Anatune, Cambridgeshire, UK) fitted to an Agilent 6890 GC coupled to a Hewlett-Packard 5972 Quadrupole Mass Selective Detector that ionized by electron ionization (−70 eV). VOCs collected and trapped on the Tenax TA were released directly onto the column by thermal desorption. The whole liner was inserted into the inlet. Injector conditions were equilibrated (30 s) and then ramped from 30°C to 220°C at 16°C s⁻¹, the carrier gas was helium (8 psi constant) and the injector was operated in a split mode (1:2) for rapid transfer of analytes to the column. The capillary column was a BPX5MS (SGE, Australia; 30 m × 0.25 mm i.d., 0.5 μm film), and oven temperature was held at 30°C for 2 min, then programmed at 4°C min⁻¹ to 200°C, then 10°C min⁻¹ to 250°C and held for 10 min. The mass spectrometer (172°C) scanned from mass 330 to 33 at a rate of 2.5 times s⁻¹ and data were captured and analysed by ENHANCED CHEMSTATION software (v. B.01.00). VOCs were initially tentatively identified by comparing spectra with those in Wiley 275 and Nist 98 analytical databases and our own database, and their peak areas recorded. Confirmation was achieved for a number of the VOCs by comparison of spectra and retention times with those of authenticated standards. For some of the compounds for which standards were unavailable, tentative identifications were determined from Kovat's indices and database matches. To avoid coelution problems and improve limits of detection, total ion current was not quantified. A target ion was quantified for each compound and validated using two qualifier ions for which a relative response was determined.

(g) Statistical analyses
Behavioural preferences of C. vestalis in dual-choice tests were analysed using generalized linear mixed models (GLMM) for both the P. xylostella and the mechanical damage experiments. Each GLMM used a Poisson distribution and logit link function. Within an experiment, replicates for all three dual-choice tests were conducted over a number of days; for each replicate, the preference of 10 wasps was determined to a fresh pair of plants on the same day. The total preferences of 10 wasps per plant pair were used as the replicate to avoid pseudoreplication in GLMM analyses, rather than the responses of individual wasps. Plant pair was nested within dual-choice test type as a random effect. To assess whether parasitoids displayed a significant preference between plant treatments in each dual-choice test, the mean logit for each dual-choice test type was compared with zero. In addition, comparisons were made to investigate whether parasitoid preferences changed between dual-choice tests. GLMM tests were conducted using R (v. 2.11.1).

Air entrainment data were analysed to test for differences in VOC emissions between plant treatments. To compare the differences in recorded peak areas between plant treatments for each individual VOC, peak areas were first tested for univariate normality. A series of Shapiro–Wilk tests and residual plots demonstrated that peak areas and their residuals were non-normal and could not be normalized by transformation for a number of VOCs. Therefore, a series of randomized ANOVAs were conducted, using RESAMPLING PROCEDURES software (v. 1.3), to investigate whether the distribution of observed peak areas to each treatment was significantly different from that expected from a random assignment. For each randomized ANOVA the data were randomized 10,000 times.

Canonical discriminant analysis (CDA) by the forward stepwise method was completed on the peak areas of the different VOCs for both entrainment experiments. CDA allows for the identification of those VOCs that discriminate most clearly between treatments. For both experiments, emissions of VOCs varied qualitatively between plants, meaning that for many of the VOCs, only a few observations were recorded. In addition, some VOCs only contributed a very minor proportion to total emissions. Therefore, to focus analyses on VOCs that contributed a consistent and substantial proportion to VOC emissions, only those VOCs whose peak area contributed more than 0.5 per cent of the total peak area of all VOCs were included in CDAAs; Degen et al. [30] used a similar cut-off point. Consequently, for the P. xylostella and mechanical damage experiments, 29 and 22 VOCs, respectively, were selected for inclusion in each CDA. This method avoids the inclusion of VOCs based upon a priori biases resulting from previous research.
and instead bases selection purely on their contribution to the
overall profile. Omnibus tests for multivariate normality
on these data demonstrated that they were not normally
distributed. Therefore, a randomization CDA test was con-
ducted for each experiment, in which observed peak areas
for each VOC were randomly assigned to treatments and
the $\chi^2$ statistic for all canonical functions calculated.
This procedure was repeated 1000 times. $p$-values were calculated
as the proportion of randomized $\chi^2$ statistics equal to or
greater than the observed $\chi^2$. CDAs were conducted using
SPSS (v. 15.0).

3. RESULTS

(a) Olfactometry

In all experiments conducted, 99 per cent of C. vestalis
chose between odour sources, indicating that the volatile
sources were very attractive. Overall, naïve female
C. vestalis significantly preferred cabbage plants infested
with more hosts (Wald $Z_{2.6} = 5.049$, $p < 0.001$). In
dual-choice tests, 64 per cent of 50 females preferred
plants infested with five larvae for 3 days over undamaged
plants. Furthermore, 70 per cent of females significantly
preferred plants infested with 10 larvae over those
infested with five larvae and 80 per cent preferred plants
infested with 20 larvae over 10 larvae. Although these
results suggest that increasing P. xylostella infestation
may enhance the attractiveness of cabbage, this increase
was not quite significant (Wald $Z_{2.0} = 1.762$, $p =
0.078$). In the comparative experiments, naïve female
C. vestalis exhibited no preference based on the severity
of mechanical damage (Wald $Z_{2.0} = 0.164$, $p = 0.87$).

(b) Headspace volatile entrainment

Qualitative differences in VOC emissions occurred
between undamaged and P. xylostella-infested plants;
infested plants produced 125 different VOCs, 16 more
than undamaged control plants. Plants infested with five
larvae emitted 120 VOCs, those with 10 and 20 larvae
both emitted 125 VOCs. Randomized ANOVAs indicated
that the distribution of observed peak areas to each plant
treatment was significantly different from random for
44 VOCs; in all cases, emissions increased after infesta-
tion. Twelve of these compounds were significantly
different from random at $p < 0.001$, 18 at $p < 0.01$ and
14 at $p < 0.05$. The four most significant compounds
were dimethyl trisulphide, 3-methyl-2-pentanol (tentative
ID); indole and methyl salicylate (table 1). Of these
44 VOCs, 34 were released by both undamaged and
infested plants, demonstrating quantitative differences in
emission rates between treatments.

The randomized CDA for P. xylostella-infested plants
demonstrated that the discrimination ability of the cano-
nical functions, for the observed data, was significantly
different from functions obtained when peak areas were
randomly assigned to treatments ($p = 0.012$). The first
canonical function explained 99.9 per cent of the variance
and the second only 0.1 per cent (figure 1). There was
good separation on the first axis between plant treat-
ments, with width of separation increasing at higher
infestation levels, and group means differed significantly
(Wilks’ $\lambda_{2,16} = 0.02$, $p < 0.001$). Two chemicals were
included in the stepwise classification of VOCs; dimethyl
trisulphide and 6-methyl-5-hepten-2-ol (tentative ID; figure 2).

By contrast, mechanically damaged plants showed few
differences between treatments. These plant treatments
emitted 106 different VOCs, with 94, 93 and 96 VOCs
emitted from plants with 10, 20 or 40 holes, respectively.
Undamaged control plants emitted 100 VOCs. Few
qualitative differences in VOC profiles occurred, with
only five VOCs extra being emitted by at least one of the
mechanical damage treatments, but not the unda-
maged controls. Randomized ANOVAs indicated that
the distribution of observed peak areas to each plant treat-
ment was significantly different from random for only five
VOCs (electronic supplementary material, table S2).
Only benzyl nitrile (tentative ID) emissions increased
with damage. The randomized CDA for mechanically
damaged plants showed that the discrimination ability
of the canonical functions was not different from func-
tions obtained from a random assignment of peak areas
to treatments ($p = 0.396$).

4. DISCUSSION

To identify a suitable host patch, a parasitoid requires
unambiguous and reliable cues that indicate the location,
quality and density of its host. It is well established that
VOCs produced from a plant–herbivore complex provide
both reliable and detectable cues that parasitoids can use
to locate their herbivore hosts [2,5,7,10]. Parasitoids can
use these herbivore-induced VOCs when in a patch, to
assess its profitability and adapt their patch residence
time, in order to forage more optimally [18]. Parasitoids
are unable, at a distance, to visually assess the density
of host herbivores on a plant and require other forms of
information. Our findings, that naïve C. vestalis signifi-
cantly prefers VOCs from B. oleracea plants infested with
more P. xylostella in a density-dependant fashion, provide
evidence that a parasitoid is capable of responding
innately to VOC cues from a plant–herbivore complex
distinctly between plants of the same species with
different levels of herbivore infestation/damage. These
experiments used walking assays, which may produce
different responses to flying assays; therefore, caution
must be taken in extrapolating the conclusions of this
study to insects locating patches in-flight.

The results of the VOC analyses complemented these
behavioural data, with qualitative increases and greater
differences in emissions between plant treatments as
herbivore loads rose. As figure 2 illustrates, emission of
some VOCs exhibits an accelerating positive relation
with herbivore density. If parasitoids perceive VOCs in
proportion to their concentration, they should therefore
exhibit a stronger preference for plants with more larvae
when confronted with 20 versus 10 larvae than with
10 versus 5 larvae, which in turn should elicit a stronger
preference than a comparison between 5 versus 0 larvae.
This increasing preference appears apparent from the
behavioural data but was not quite statistically significant.

The fact that parasitoids preferentially select plants
infested with more herbivores could have implications
for how they regulate host populations and therefore
their effectiveness as biocontrol agents. An ideal biocon-
trol agent suppresses a pest population but does not
eliminate it [39]. When foraging in an agricultural

Proc. R. Soc. B
Table 1. Volatile compounds collected from the headspace of *B. oleracea* plants either undamaged or infested with 5, 10 or 20 *P. xylostella* larvae for 72 h prior to volatile collection. (Data are expressed as the mean peak areas (× 1000) of five plants for each treatment ± s.e. U followed by a number represents an unidentified compound. *F*<sub>obs</sub> are *F*-values observed from one-way ANOVA analyses. *p*-values were calculated as the proportion of *F*-values (obtained from 10,000 ANOVAs conducted on randomizations of the data) greater than *F*<sub>obs</sub> only those compounds significant at *p* < 0.01 are shown (for all other significant VOCs, see electronic supplementary material, table S1).)

<table>
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<th>volatile compound</th>
<th>number of larvae on plant</th>
<th>proportion of randomized</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th><em>F</em>&lt;sub&gt;obs&lt;/sub&gt;</th>
<th><em>F</em> &gt; <em>F</em>&lt;sub&gt;obs&lt;/sub&gt;</th>
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<td>49±2</td>
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<td>290±38</td>
<td>857±39</td>
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<td></td>
<td>70±12</td>
<td>184±41</td>
<td>294±46</td>
<td>662±98</td>
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<td>indole</td>
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<td>81±29</td>
<td>602±201</td>
<td>574±153</td>
<td>1509±142</td>
<td>16.714</td>
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<td></td>
<td>27±14</td>
<td>203±41</td>
<td>129±35</td>
<td>465±74</td>
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<td>29±8</td>
<td>205±53</td>
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<td>(E)-10-tenecicosene&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>1±1</td>
<td>2±1</td>
<td>8±2</td>
<td>13±3</td>
<td>7.372</td>
<td>0.002</td>
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<tr>
<td>benzyl nitrile&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>159±42</td>
<td>616±160</td>
<td>1465±345</td>
<td>2590±735</td>
<td>6.652</td>
<td>0.003</td>
</tr>
<tr>
<td>C20 hydrocarbon&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>0±0</td>
<td>1±1</td>
<td>6±2</td>
<td>11±3</td>
<td>8.341</td>
<td>0.004</td>
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<tr>
<td>2-ethyl-3-methyl-maleimide&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>26±5</td>
<td>104±32</td>
<td>89±24</td>
<td>180±24</td>
<td>7.195</td>
<td>0.004</td>
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<tr>
<td>(Z)-2-penten-1-ol, acetate&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>44±14</td>
<td>137±46</td>
<td>202±28</td>
<td>323±75</td>
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<td>0.004</td>
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<tr>
<td>U9</td>
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<td></td>
<td>2±3</td>
<td>81±12</td>
<td>84±17</td>
<td>440±736</td>
<td>2.593</td>
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<tr>
<td>α,α-dimethyl-benzene ethanol&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
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<td>2±1</td>
<td>5±3</td>
<td>30±10</td>
<td>6.551</td>
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<tr>
<td>U10</td>
<td></td>
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<td>2±1</td>
<td>25±11</td>
<td>22±6</td>
<td>61±16</td>
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<tr>
<td>β-phenyl butyrate&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<td>2±2</td>
<td>48±18</td>
<td>57±16</td>
<td>212±75</td>
<td>5.433</td>
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<td></td>
<td>0±0</td>
<td>2±1</td>
<td>11±4</td>
<td>37±15</td>
<td>4.609</td>
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<tr>
<td>U12</td>
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<td>4±4</td>
<td>23±9</td>
<td>29±7</td>
<td>64±20</td>
<td>4.51</td>
<td>0.008</td>
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<td>amyl acetate&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td>2±1</td>
<td>7±3</td>
<td>8±1</td>
<td>26±9</td>
<td>4.667</td>
<td>0.009</td>
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<tr>
<td>U13</td>
<td></td>
<td></td>
<td>16±6</td>
<td>15±3</td>
<td>14±2</td>
<td>54±18</td>
<td>4.176</td>
<td>0.009</td>
</tr>
</tbody>
</table>

<sup>a</sup>Tentative identifications.

Monoculture, where there are likely to be only short distances between hosts, a parasitoid that selects plants producing a strong signal, and therefore with more hosts, should reduce the likelihood that it will eliminate a host population but may still suppress outbreaks. The responses here are from naive parasitoids, suggesting that they are innate and that parasitoids may prefer a volatile source based on the strength of a signal without prior experience of how volatile emissions relate to herbivore density. Therefore, assuming that the response is owing to an overall increase in certain VOCs and not how their relative concentrations vary within the blend, a foraging parasitoid may prefer the strongest signal whether that is the result of low damage on a plant spatially close or high damage on one further away. In this scenario, it may be advantageous for a plant to produce as strong a signal as possible. Shiojiri et al. [40] recently proposed the concept of "cry wolf" signalling, where plants 'dishonestly' produce strong VOC emissions in the presence of only minor damage. They demonstrated that *C. vestalis* were unable to distinguish between different damage levels on *B. oleracea* var. *capitata* cv. Shikidori, but did show density-dependent responses on *B. oleracea* var. *acephala*. They also demonstrate that both plants signal 'honestly' in response to attack by the cabbage white larvae, *P. rapae*. Our data suggest that *B. oleracea* var. *capitata* cv. Derby day infested with *P. xylostella* produce 'honest' signals, which may be more common than 'dishonest' signals.

An ability to use VOCs from the plant—herbivore complex to locate more profitable patches may not be restricted to parasitoids; Gols et al. [41] demonstrated that a predatory mite responds with increasing strength to plants infested with increasing numbers of its spider mite prey. Horiuchi et al. [42] found that plants with higher infestations of spider mites produced greater emissions of certain volatiles and to a limited extent, these differences correlated with the plants' attractiveness to a predator of the mite. However, only 10 VOCs were analysed, based on a priori knowledge of this system from previous investigation, half of which did not increase as a result of infestation.

Van Dam & Poppy [34] highlighted that current methodologies often struggle to identify causal links between an insects' behaviour and the exact VOCs that actually elicit that behaviour. Currently, this issue is only
addressed by the use of coupled gas chromatography–electroantennography (e.g. [44,45]) and neurophysiology techniques (e.g. [44,45]) to identify those volatiles to which the insect is physiologically responsive. However, this does not always translate to behavioural responsiveness. Here, we demonstrate that by using CDA and ANOVAs, it is possible to identify those VOCs that vary positively with herbivore density and which could therefore be reliable indicators of increasing herbivore numbers, assuming that parasitoid sensitivity increases linearly with concentration and equally among VOCs.

In the current analyses, the VOCs that these analyses intimated could be key compounds included dimethyl trisulphide, methyl salicylate, indole and dimethyl disulphide. The next step would be to investigate the behavioural responsiveness of C. vestalis to these VOCs, to confirm their suitability as VOCs that may be used to gauge herbivore number on a plant. However, it has already been shown that C. vestalis responds significantly to dimethyl trisulphide in Y-tube olfactometry tests [46], and both indole and methyl salicylate are attractive to other closely related Cotesia spp. [47,48]. Dimethyl disulphide and dimethyl trisulphide are given off by frass of P. xylostella [49] and were emitted in significantly greater quantities from plants infested with higher herbivore numbers (table 1). These two compounds may have been derived from frass still on the plant, as would be found in real foraging situations, rather than from the plants themselves. Whatever their derivation, their presence and concentration may still be good indicators of herbivore presence and load. As a result of natural selection, parasitoids are likely to weight the importance of certain compounds during neural processing based on their reliability, and, therefore, these analyses could be used as a powerful technique to identify such putative key compounds in parasitoid host location.

Behavioural and chemical analyses did not distinguish between plant treatments in the mechanical damage experiment (figure 2). By contrast, C. vestalis has previously been shown to prefer VOCs from plants with similar mechanical damage over undamaged plants [8]. Different methods of administering mechanical damage result in different VOC emissions and chemical elicitors present in herbivores saliva also alter plant VOC emissions. Controversy exists in the literature as to how best mimic caterpillar damage [29,50–53].

Insect parasitoids that exploit insect herbivores as hosts comprise a vast and varied group, which employ a huge range of different foraging strategies based upon their life-history characteristics. Many of these parasitoid species are able to use VOCs from the plant–herbivore complex to increase their host foraging efficiency. If, as our findings suggest, a parasitoid can further increase its patch-finding ability by using VOC emissions to select more rewarding host patches, then this strategy has the potential to be pervasive among other species of insect parasitoids that possess similar life histories.

We thank Dr Simon Leather for his comments on the research and Prof. Lawrence Harder for his advice on statistical analyses. Funding was provided by BBSRC grant BB/D01154x/1.

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