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Item Type	Article
Authors	Goelen, Tim;Sobhy, Islam S.;Vanderaa, Christophe;de Boer, Jetske G.;Delvigne, Frank;Francis, Frédéric;Wäckers, Felix;Rediers, Hans;Verstrepen, Kevin J.;Wenseleers, Tom;Jacquemyn, Hans;Lievens, Bart
Citation	Goelen, T., Sobhy, I. S., Vanderaa, C., de Boer, J. G., Delvigne, F., Francis, F., Wäckers, F., Rediers, H., Verstrepen, K. J., Wenseleers, T., Jacquemyn, H., & Lievens, B. (2020). Volatiles of bacteria associated with parasitoid habitats elicit distinct olfactory responses in an aphid parasitoid and its hyperparasitoid. <i>Functional Ecology</i> , 34(2), 507-520. https://doi.org/10.1111/1365-2435.13503
DOI	10.1111/1365-2435.13503
Publisher	Wiley;British Ecological Society
Journal	Functional Ecology
Download date	2026-05-19 16:53:07
Item License	https://creativecommons.org/licenses/by-nc-nd/4.0/
Link to Item	http://hdl.handle.net/10034/629727

Volatiles of bacteria associated with parasitoid habitats elicit distinct olfactory responses in an aphid parasitoid and its hyperparasitoid

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1 **ABSTRACT**

- 2 1. To locate mating partners and essential resources such as food, oviposition sites and
3 shelter, insects rely to a large extent on chemical cues. While most research has
4 focused on cues derived from plants and insects, there is mounting evidence that
5 indicates that microorganisms emit volatile compounds that may play an important
6 role in insect behaviour.
- 7 2. In this study, we assessed how volatile compounds emitted by phylogenetically
8 diverse bacteria affected the olfactory response of the primary parasitoid *Aphidius*
9 *colemani* and one of its secondary parasitoids, *Dendrocerus aphidum*. Olfactory
10 responses were evaluated for volatile blends emitted by bacteria isolated from diverse
11 sources from the parasitoid's habitat, including aphids, aphid mummies and
12 honeydew, and from the parasitoids themselves.
- 13 3. Results revealed that *A. colemani* showed wide variation in response to bacterial
14 volatiles, ranging from significant attraction over no response to significant
15 repellence. Our results further showed that the olfactory response of *A. colemani* to
16 bacterial volatile emissions was different from that of *D. aphidum*. Gas
17 chromatography-mass spectrometry (GC-MS) analysis of the volatile blends
18 revealed that bacterial strains repellent to *A. colemani* produced significantly higher
19 amounts of esters, organic acids, aromatics and cycloalkanes than attractive strains.
20 Strains repellent to *D. aphidum* produced significantly higher amounts of alcohols
21 and ketones, whereas the strains attractive to *D. aphidum* produced higher amounts
22 of the monoterpenes limonene, linalool and geraniol.
- 23 4. Overall, our results indicate that bacterial volatiles can have an important impact on
24 insect olfactory responses, and should therefore be considered as an additional, so far

25 often overlooked factor in studying multitrophic interactions between plants and
26 insects.

27

28 **Keywords:**

29 *Aphidius colemani*, chemical communication, *Dendrocerus aphidum*, microbial odour,
30 multitrophic interactions, natural enemy, semiochemical, VOCs

31 **Introduction**

32 Insects rely to a large extent on the detection of olfactory cues facilitating their most basic
33 survival functions, including feeding, mating and oviposition (de Bruyne & Baker, 2008).
34 While gustatory information may indicate the quality of food, mates or egg deposition
35 sites after contact, both visual and olfactory cues provide information about where to
36 search in the first place (Wäckers & Lewis, 1994). Likewise, several insects use olfaction
37 to avoid threats and risks associated with toxic environments, pathogens or predators
38 (Stensmyr et al., 2012; Yanagawa, Imai, Akino, Toh, & Yoshimura, 2015).

39 Previous studies have revealed that insect behaviour is strongly mediated by
40 volatile organic compounds (VOCs) emitted by plants and insects when foraging for food,
41 hosts or conspecifics (Meiners, 2015; Vet & Dicke, 1992). Volatiles from both aerial and
42 underground plant parts play an important role in the communication between plants and
43 insects (Meiners, 2015; Soler, Bezemer, & Harvey, 2013), while insect-derived volatiles
44 have been intensively studied to better understand critical processes in insects such as
45 mating, reproduction, aggregation and alarming conspecifics about danger (Wyatt, 2014).
46 More recently, increasing evidence indicates that microorganisms like bacteria and fungi
47 also emit volatile compounds that affect insect behaviour (Davis, Crippen, Hofstetter, &
48 Tomberlin, 2013; Dzialo, Park, Steensels, Lievens, & Verstrepen, 2017; Leroy et al.,
49 2011b). Although production of microbial volatile organic compounds (mVOCs) is
50 commonplace (Piechulla & Degenhardt, 2013), little is still known about their ecological
51 role and how they interfere with volatile-mediated insect foraging behaviour. In spite of
52 this, a growing body of literature suggests that responding to mVOCs benefits insects in
53 various ways. Many insects employ mVOCs to locate appropriate resources such as

54 suitable food sources or oviposition sites (Becher et al., 2012; Leroy et al., 2011a; Sobhy
55 et al., 2018; 2019; Rering, Beck, Hall, McCartney, & Vannette, 2018). By contrast, some
56 mVOCs have also been found to repel insects, e.g. when signalling unsuitable food
57 sources, unsuitable hosts or hostile environments (Azeem, Rajarao, Nordenhem,
58 Nordlander, & Borg-Karlson, 2013; Huang, Miller, Chen, Vulule, & Walker, 2006;
59 Stensmyr et al., 2012). The advantage for the microorganisms is less clear, but it is
60 reasonable to assume that they may benefit from being vectored to new habitats or get
61 protection in the insects during unfavourable conditions (Christiaens et al., 2014; Pozo et
62 al., 2018).

63 Recent research suggests that chemical signalling and insect attraction is a
64 conserved trait in yeasts. Bioassays using the vinegar fly *Drosophila melanogaster* to
65 assess odours of nine phylogenetically and ecologically distinct yeast species revealed
66 that the flies were attracted to all yeast species studied (Becher et al., 2018). So far, only
67 very little is known whether these results are also representative for bacteria, which often
68 produce different mVOCs or use other biochemical synthesis pathways than yeasts
69 (Dzialo et al., 2017; Schulz & Dickschat, 2007). However, there is already some evidence
70 that insects are attracted to bacteria that live on or near hosts or preys (Leroy et al., 2012a;
71 Leroy et al., 2012b; Mazzetto et al., 2016) and that the mVOCs produced by these bacteria
72 can be exploited by natural enemies to locate their hosts or preys (Boone, Six, Zheng &
73 Raffa, 2008; Dillon, Vennard, & Charnley, 2000; Leroy et al., 2011a). Furthermore, little
74 is known about whether and how mVOCs mediate insect behaviour across trophic levels.
75 Previous studies on plant-insect interactions have shown that herbivore-induced plant
76 volatiles (HIPVs) are an important source of information mediating multitrophic
77 interactions (Dicke & Baldwin, 2010; van Oudenhove, Mailleret, & Fauvergue, 2017),

78 not only attracting primary parasitoids but also mediating the behavioural response of
79 secondary parasitoids (also referred to as “hyperparasitoids” having primary parasitoids
80 as their host) (Cusumano, Harvey, Dicke, & Poelman, 2019; Poelman et al., 2012).
81 Virtually nothing is known so far on the role of microbial volatiles in the chemical
82 ecology of hyperparasitoids.

83 Here, we asked the question whether mVOCs emitted by bacteria affect insect
84 olfactory response, particularly parasitoids. Parasitoids constitute a very important group
85 of natural enemies in the context of biological pest control, whose adult females lay eggs
86 in or on other insects. The parasitoid larvae develop by feeding on the host bodies,
87 eventually killing the host. Female parasitoids have to complete several foraging tasks
88 during their adult lifetime to maximize reproductive success, including searching for
89 suitable food sources, for a mating partner and for suitable hosts (Aartsma, Bianchi, van
90 der Werf, Poelman, & Dicke, 2017; de Rijk, Dicke, & Poelman, 2013). Therefore, the
91 olfactory response of female parasitoids and their efficiency in localizing and parasitizing
92 hosts will have direct consequences on host-parasitoid population dynamics, and are
93 hence key determinants of their effectiveness as biological control agents (Lewis, Vet,
94 Tumilson, van Lenteren, & Papai, 1990; Mills, & Wajnberg, 2008). In previous research
95 we have shown that mVOCs produced by nectar-inhabiting yeasts had a marked effect on
96 the olfactory response of *Aphidius ervi* (Hymenoptera: Braconidae), a generalist primary
97 parasitoid (Sobhy et al., 2018, 2019), but so far it is unclear whether bacterial odours elicit
98 similar responses in parasitoids. Further, we asked whether mVOCs emitted by bacteria
99 have similar effects on olfactory responses across trophic levels. Experiments were
100 performed using the primary aphid parasitoid *Aphidius colemani* Viereck (Hymenoptera:
101 Braconidae) and one of its secondary parasitoids, *Dendrocerus aphidum* (Hymenoptera:

102 Megaspilidae). Olfactory response was evaluated for mVOC blends emitted by bacteria
103 isolated from diverse sources from the parasitoid's habitat, including hosts and host
104 products (honeydew) and from the parasitoids themselves. The composition of the
105 volatile blends produced by the bacteria was analysed using gas chromatography-mass
106 spectrometry (GC-MS) to find out whether there were differences in mVOC profiles
107 between attractive, neutral and repellent strains.

108

109 **Materials and Methods**

110 *Study organisms*

111 *Insects*

112 Experiments were performed using adult females of the primary parasitoid *A. colemani*
113 and one of its hyperparasitoids, *D. aphidum*. *Aphidius colemani* is a generalist aphid
114 parasitoid. *D. aphidum* is a generalist, secondary idiobiont ectoparasitoid attacking pre-
115 pupal and pupal stages of hymenopteran primary parasitoids such as *Aphidius* spp. inside
116 aphid mummies (Walker & Cameron, 1981). Both species preferentially feed on nectar
117 and honeydew as a main source of sugars in their adult stage. *Aphidius colemani* was
118 obtained in the form of parasitized aphid mummies from Biobest (Westerlo, Belgium)
119 (*Aphidius*-system[®]). *Dendrocerus aphidum* was reared in the laboratory on fresh (1 day
120 old) *Acyrtosiphon pisum* mummies parasitized by *A. ervi*. For both species, mummies
121 were placed inside a nylon insect cage (20 cm × 20 cm × 20 cm, BugDorm, MegaView
122 Science Co., Ltd., Taichung, Taiwan) and kept under controlled conditions (22°C, 70%
123 relative humidity and a 16:8-h light:dark photoperiod) until parasitoid emergence. All
124 experiments were performed with <24-h-old, food and water-starved females.

125

126 Bacteria

127 In total, 38 bacterial strains were used in this study (Table S1, Supporting Information).
128 Strains were isolated from diverse sources sampled in greenhouses or a laboratory
129 environment, including unparasitized aphids, aphid mummies, honeydew and *Aphidius*
130 and *Dendrocerus* female adults. Studied strains represented a phylogenetically diverse
131 collection of bacteria belonging to Actinobacteria, Firmicutes and Proteobacteria, which
132 are typically associated with insects and insect-derived products (Engel & Moran, 2013,
133 Grigorescu et al., 2018; Leroy et al., 2011a; Luna et al., 2018). Further details on the
134 isolation source (e.g. insect species or origin of honeydew) are given in Table S1
135 (Supporting Information). Honeydew was collected according to the procedure outlined
136 by Leroy et al. (2011a). For the isolations from insect specimens, whole insects were
137 used. Previous research has shown that insects can be attracted to volatiles produced by
138 both symbiotic gut microorganisms and microbes on the exterior of the insects (Davis et
139 al., 2013; Dillon et al., 2000; Leroy et al., 2011b; Mazzetto et al., 2016; Scheidler, Liu,
140 Hamby, Zalom, & Syed, 2015). Insect specimens were homogenized with a motorized
141 homogenizer (Precellys 24, Bertin Instruments, Montigny-le-Bretonneux, France) in 250
142 μ L sterile physiological water (0.9% NaCl) with 0.01% Tween80 using 2 mm diameter
143 glass beads, and then plated on tryptic soy agar (TSA; Oxoid, Hampshire, UK)
144 supplemented with 0.3 g/L cycloheximide to prevent fungal growth. It has to be noted
145 that this method not only samples bacteria that come in contact with the insect's
146 environment, but may also yield endosymbionts living in specific host cells or
147 compartments. Nevertheless, as such symbionts are generally not isolated through
148 classical microbiological methods (Dale, Beeton, Harbison, Jones & Pontes, 2006), there

149 is only a small chance that they were taken into account. For the isolations from
150 honeydew, a 10-fold dilution series was plated on the same medium. Plates were
151 incubated at 25°C for five days, and the most abundant morphotypes were purified and
152 used for the study. Bacterial isolates were identified by amplifying and sequencing the
153 16S ribosomal RNA (rRNA) gene and comparison with the EzBiocloud 16S rRNA gene
154 and whole-genome assembly database (Yoon et al., 2017). All isolates were kept in tryptic
155 soy broth (TSB; Oxoid, Hampshire, UK) containing 25% glycerol at -80°C until further
156 use.

157

158 *Production of mVOCs*

159 For production of mVOCs, the procedure of Sobhy et al. (2018) was followed with a few
160 minor adjustments for bacteria. Briefly, bacterial stock cultures were plated on TSA and
161 incubated at 25°C for 24h, followed by a re-streak on the same medium and incubation
162 at 25°C for another 24h. Subsequently, single colonies were inoculated in 10 mL TSB
163 and incubated overnight at 25°C in a rotary shaker at 120 rpm. Next, cells were washed
164 twice in sterile physiological water (0.9% NaCl) and diluted in sterile physiological water
165 until an optical density (OD 600 nm) of 1 was reached. Next, 1.5 mL of this cell
166 suspension was used to inoculate a 250 mL Erlenmeyer flask containing 150 mL GYP25
167 medium prepared by filter-sterilizing (pore size 0.22 µm; Rapid-Flow™, Thermo
168 Scientific, Waltham, USA) a medium of 5% w/v glucose (Sigma-Aldrich, Saint Louis,
169 USA), 0.5% w/v peptone (Bacto™ Peptone, BD Biosciences, San Jose, USA) and 0.25%
170 w/v yeast extract (Sigma-Aldrich, Saint Louis, USA). Erlenmeyer flasks were sealed with
171 sterile silicone plugs and incubated at 25°C in a rotary shaker at 120 rpm for 48h. Each
172 strain was cultivated in triplicate, and non-inoculated, blank medium was included as a

173 negative control (also in triplicate). The GYP25 medium was selected to ensure abundant
174 bacterial growth and mVOC production, while the medium itself had no significant effect
175 on the parasitoid olfactory response. After incubation, the media were centrifuged for 15
176 min at 10,000 g, and subsequently filter-sterilized to obtain cell-free supernatants
177 containing the produced mVOCs. The cell-free samples were then stored in small aliquots
178 in sterile, amber glass vials at -20°C until further use.

179

180 *Olfactometer bioassays*

181 Insect olfactory response was evaluated using the Y-tube olfactometer bioassay described
182 by Sobhy et al. (2018) (for details see Fig. S1, Supporting Information). The glass Y-tube
183 olfactometer was placed on a table that was homogeneously illuminated by four high
184 frequency 24W T5 TL-fluorescent tubes (16 x 549 mm, 1350 Lumen, 5500K; True-
185 Light®, Naturalite Benelux, Ansen, The Netherlands) with a 96% colour representation
186 of true daylight at a height of 0.45 m. To eliminate visual cues that could affect parasitoid
187 responses, the olfactometer was fully enclosed with white curtains. Further, to improve
188 parasitoid responsiveness, the olfactometer was positioned at a 20° incline to stimulate
189 movement of the insects towards the bifurcation.

190 To test a given bacterial strain, 150 µL of the cell-free cultivation medium was
191 loaded on a 37 mm-diameter filter paper (Macherey-Nagel, Düren, Germany) which was
192 subsequently placed in one of the odour chambers, whereas in the second chamber
193 another filter paper was placed on which 150 µL blank medium was added as a control.
194 The bioassay was performed by releasing twelve consecutive cohorts of five adult females
195 at the base of the olfactometer and evaluating their response 10 min after parasitoid
196 release. Individuals that passed a set line at the end of one of the olfactometer arms (1 cm

197 from the Y-junction) and remained there at the time of evaluation were considered to have
198 chosen the odour source presented by that olfactometer arm. Parasitoids that did not make
199 a choice at the time of evaluation were considered non-responding individuals and were
200 excluded from the statistical analysis. New parasitoids were used for every release, and
201 after every two releases the filter papers inside the odour chambers were renewed. To
202 avoid positional bias, the odour chambers were rotated after every six cohorts. At the
203 same time, the Y-tube glassware was also renewed by cleaned glassware. At the end of
204 the assay, all olfactometer parts were thoroughly cleaned with tap water, distilled water,
205 acetone and finally pentane, after which the parts were placed overnight in an oven at
206 150°C. All bioassays were conducted at $21 \pm 2^\circ\text{C}$, $60 \pm 5\%$ RH and performed between
207 09:00 and 16:00 h.

208 In a first experiment, bioassays were performed for *A. colemani* using one of the
209 three medium replicates for all 38 bacterial strains investigated in this study. Further, to
210 determine whether bacterial mVOC blends elicit the same response in primary and
211 secondary parasitoids, a second experiment was performed for a subset of seven strains
212 (see below). In this experiment, bioassays were performed using all three biological
213 replicates with *A. colemani* and *D. aphidum*.

214

215 *Chemical analysis of mVOCs*

216 To determine the chemical composition of the mVOC blends, the cell-free cultivation
217 medium of each biological replicate ($n = 3$) for the seven strains selected for the second
218 experiment was analysed by headspace solid phase micro extraction gas chromatography
219 followed by mass spectrometry detection (HS-SPME-GC-MS). The non-inoculated,
220 sterile medium ($n = 3$) was used as a reference to find out how volatile composition

221 changed by bacterial inoculation. GC-MS analyses were performed with a Thermo Trace
222 1300 system (Thermo Fisher Scientific, Waltham, USA) fitted with a MXT-5 column (30
223 m length \times 0.18 mm inner diameter \times 0.18 μ m film thickness; Restek, Bellefonte, USA)
224 and a ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, USA). 5 mL of each
225 sample was supplemented with 1.75 g of NaCl and was kept at 60°C under constant
226 agitation in a TriPlus RSH SMPE auto sampler (Thermo Fisher Scientific, Waltham,
227 USA). The HS-SPME volatile collection was conducted using an 50/30 μ m
228 DVB/CAR/PDMS coating fibre (Supelco, Bellefonte, USA). Splitless injection was used
229 with an inlet temperature of 320 °C, a split flow of 9 mL/min, a purge flow of 5 mL/min
230 and an open valve time of 3 min. To obtain a pulsed injection, a programmed gas flow
231 was used whereby the helium gas flow was set at 2.7 mL/min for 0.1 min, followed by a
232 decrease in flow of 20 mL/min² to the normal 0.9 mL/min. The GC oven was programmed
233 as follows: the temperature was initiated at 30°C, held for 3 min and then raised to 80°C
234 at 7°C/min. Next, the temperature was raised to 125°C at 2°C/min, and finally the
235 temperature was raised to 270°C at 8°C/min. Mass spectra were recorded in centroid
236 mode using a mass acquisition range of 33 to 550 atomic mass units, a scan rate of 5
237 scans/s and an electron impact ionization energy of 70 eV. A mix of linear n-alkanes
238 (from C7 to C40, Supelco, Bellefonte, USA) were injected into the GC-MS under
239 identical conditions to serve as external retention index markers.

240 Volatile compounds were identified and quantified as in Reher et al. (2019).
241 Briefly, chromatograms were analysed with AMDIS v2.71 (Stein, 1999) to deconvolute
242 overlapping peaks, and obtained spectra were manually annotated using the NIST MS
243 Search v2.0g software, using the NIST2011, FFNSC and Adams libraries, taking into
244 account the expected retention time. This resulted in a list of 245 tentatively identified

245 target compounds that were present in the samples. To extract and integrate the compound
246 elution profiles, a file was used with all our target compounds containing the expected
247 retention times and spectrum profiles. Extraction was performed for every compound in
248 every chromatogram over a time restricted window using weighted non-negative least
249 square analysis (Lawson & Hanson, 1995). Finally, the peak areas were computed from
250 the extracted profiles and summarized in a table. For all chemical compounds, the mean
251 and standard error (SE) were calculated for every bacterial strain ($n = 3$). A univariate
252 ANOVA was performed on the peak areas of the individual compounds to test for
253 differences in compound concentration between bacterial strains and the blank medium
254 followed by a Tukey's HSD test with adjusted P -values as calculated after correcting for
255 multiple comparisons. A Kruskal-Wallis test was used when the data did not conform to
256 the criteria of normality and homogeneity of variance required for a parametric statistical
257 test. Compounds that did not show a significant difference in relative concentration
258 compared to the blank medium in at least one bacterial mVOC profile were considered
259 not to be related to bacterial activity, and were removed from the table. This resulted in a
260 total of 97 different compounds that were retained in the dataset (Table S2, Supporting
261 Information).

262

263 *Data analysis*

264 *Olfactometer bioassays*

265 For each bacterial strain, parasitoid olfactory response was analysed using a Generalized
266 Linear Mixed Model (GLMM) based on a binomial distribution (binary: choice for either
267 control side or treatment side) with a logit link function (logistic regression) using
268 bacterial treatment as fixed factor (performed in R with the glmer function from the lme4

269 package). Each release of one cohort of five individuals served as a replicate. To adjust
270 for overdispersion and to prevent pseudoreplication, the release of each cohort ($n = 12$)
271 was included in the model as a random factor. For the second experiment, in which all
272 three biological replicates of the strains were tested, biological replicate was included as
273 a random factor as well. The number of parasitoids choosing for the control or treatment
274 side in each cohort was entered as response variable. Parasitoid response in each treatment
275 was compared to a control in which parasitoids were provided a blank medium in both
276 arms of the olfactometer, using analysis of variance Type III Wald chi-square tests in the
277 GLMM. Results were presented by calculating the Preference Index (PI) by dividing the
278 difference between the number of parasitoids choosing for the bacterial odours and the
279 parasitoids choosing for the control by the total number of responding insects.
280 Additionally, a GLMM was used to determine whether the source of isolation of the
281 bacterial strains (i.e. aphid, parasitoid or honeydew) had a significant influence on the
282 olfactory response of *A. colemani*, by using the number of parasitoids in each cohort
283 choosing for either the control or the treatment side of the Y-tube as a dependent variable,
284 and source of isolation as fixed factor. The release of each cohort ($n = 12$) was again
285 included in the model as a random factor. Strains originating from hyperparasitoids and
286 aphid mummies were excluded from the analysis, due to the low numbers of strains
287 representing these habitats.

288

289 Chemical analysis

290 To visualize the differences in the mVOC composition, a heat map was constructed from
291 strain*volatile peak area matrix of the mean-centered, log transformed data, using the
292 comprehensive online tool suite MetaboAnalyst 4.0 (Chong et al., 2018). Additionally, a

293 non-metric multidimensional scaling (NMDS) was performed on the strain*volatile peak
294 area matrix by using a Bray-Curtis distance matrix (Vegan package V2.4-6 in R). A
295 permutational multivariate analysis of variance (perMANOVA) was carried out on the
296 strain*volatile peak area matrix to test for significant differences in chemical composition
297 of mVOCs produced by the tested strains, based on 1000 permutations. The analysis was
298 performed by using the adonis function (Vegan package V2.4-6) in R. To further elucidate
299 differences in mVOC composition at the level of compound classes, a univariate ANOVA
300 followed by a Tukey's HSD test was performed on the summed peak areas of the
301 compounds belonging to the same chemical class when strains were grouped according
302 to olfactory response. Specifically, data were combined for strains evoking parasitoid
303 attraction, repellence or a neutral response. Chemical classes generally induce similar
304 responses in insects (Dzialo et al., 2017). However, caution should be taken when
305 interpreting results as this is not always the case, e.g. for terpenes (Raffa, 2014). All
306 statistical analyses and evaluation of normality and homoscedasticity of the data were
307 performed in R 3.3.2 (R Core Team, 2014).

308

309 **Results**

310 *Olfactory response of A. colemani to bacterial volatile emissions*

311 Olfactory response of *A. colemani* varied significantly between the volatile emissions of
312 the 38 bacterial strains tested ($\chi^2_{(38)} = 74.71$, $P < 0.001$; Fig. 1). One bacterial strain
313 (ST18.16/150) was found to significantly attract *A. colemani* (PI = 0.36, $P = 0.048$), while
314 one other strain (ST18.17/002) was significantly deterrent (PI = -0.41, $P = 0.039$).
315 Volatile blends emitted by the other strains and the blank medium had no statistically

316 significant effect on the olfactory response of *A. colemani* (Fig. 1). Most strains having
317 high PI-values belonged to the genus *Bacillus*, while strains belonging to the genus
318 *Staphylococcus* showed relatively low PI-values. When evaluating the effect of origin,
319 only honeydew had a significant influence on the olfactory response of *A. colemani* (χ^2 (2)
320 = 17.9, $P < 0.001$). *Aphidius colemani* showed significantly lower PI-values when
321 exposed to mVOCs produced by bacteria originating from honeydew.

322

323 *Differences in olfactory response between A. colemani and D. aphidum*

324 In order to test whether bacterial mVOC emissions elicited the same response in the
325 primary parasitoid and one of its secondary parasitoids, olfactory responses of *A.*
326 *colemani* and *D. aphidum* were compared for a selection of strains using three
327 independent biological replicates for each strain. Selected strains included three strains
328 having the highest PI-value when tested against *A. colemani* (ST18.16/150, ST18.16/043
329 and ST18.16/133), three strains with the lowest PI-value (ST18.17/002, ST18.17/028 and
330 ST18.16/160) and one strain with a PI-value close to zero (ST18.16/085) (Fig. 1). Results
331 confirmed that the strains with the highest PI-values were significantly attractive to *A.*
332 *colemani* ($P \leq 0.005$), while the strains with the lowest PI-values significantly repelled *A.*
333 *colemani* ($P \leq 0.017$) (Fig. 2). Results also showed that insect response differed between
334 the tested insect species (Fig. 2). Volatile emissions from the strains that were
335 significantly attractive to *A. colemani* had no significant effect on the olfactory response
336 of *D. aphidum*. Further, the volatile emissions of two strains that were repellent to *A.*
337 *colemani* were also significantly repellent to *D. aphidum* (ST18.17/002: PI = -0.37, $P =$
338 0.003; ST18.17/028: PI = -0.25, $P = 0.043$). By contrast, the mVOC mixture emitted by

339 ST18.16/085, which was neutral to *A. colemani*, was significantly attractive to *D.*
340 *aphidum* (PI = 0.31, $P = 0.006$). Additionally, strain ST18.16/160 which was repellent to
341 *A. colemani*, tended to attract *D. aphidum*, albeit not significantly (PI = 0.18, $P = 0.102$)
342 (Fig. 2).

343

344 *mVOC composition*

345 The mVOC composition differed significantly between the seven bacterial treatments and
346 the blank medium (perMANOVA: $F = 38.6$, $P < 0.001$). Overall, volatiles produced in
347 the highest amounts belonged to alcohols, esters, ketones and organic acids (Table S2,
348 Supporting Information; Fig. S2, Supporting Information). For a few compounds,
349 concentrations were significantly higher in the blank medium compared to the bacterial
350 treatments (i.e. phenylacetaldehyde, nonane, methyl pyrazine and 2-propyl-1,3-
351 dioxolane), indicating that some compounds were partly consumed or converted during
352 cultivation (Table S2, Supporting Information; Fig. S2, Supporting Information). NMDS
353 ordination of the mVOC composition (Fig. 3) separated strain ST18.16/133 and to a lesser
354 extent strain ST18.17/002 from the rest of the bacterial strains along the first NMDS axis.
355 The second NMDS axis led to further separation of the strains, particularly separating the
356 three *Bacillus* strains (ST18.16/133, ST18.16/043 and ST18.16/150) from the rest of the
357 strains (Fig. 3). Notably, these three strains elicited significant attraction in *A. colemani*
358 (Fig. 2). Additionally, the NMDS showed that the composition of the volatile blends of
359 these strains (especially ST18.16/043 and ST18.16/150) was more closely related to the
360 blank medium in comparison with the other strains (Fig. 3).

361

362 *Differences in mVOC composition between attractive, neutral and repellent strains*

363 Grouping the strains based on the effect of their mVOC blends on the olfactory response
364 of the parasitoids (attraction, repellence or neutral response) showed that the strains
365 repellent to *A. colemani* (ST18.16/160, ST18.17/028 and ST18.17/002) produced
366 significantly higher amounts of esters, organic acids, aromatics and cycloalkanes, when
367 compared to the attractive strains (ST18.16/150, ST18.16/133 and ST18.16/043) (Fig. 4;
368 Table S2, Supporting Information; Fig. S2, Supporting Information). The strains repellent
369 to the hyperparasitoid *D. aphidum* (ST18.17/028 and ST18.17/002) produced
370 significantly higher amounts of alcohols and ketones, whereas the strain significantly
371 attractive to *D. aphidum* (ST18.16/085) produced higher amounts of alkenes (especially
372 9-methyl-1-decene) and the three monoterpenes limonene, linalool and geraniol (Fig. 5;
373 Table S2, Supporting Information; Fig. S2, Supporting Information). These terpenes were
374 also produced in high amounts by ST18.16/160 which yielded a relatively high PI-value
375 as well (PI = 0.18), although not significantly attractive.

376

377 **Discussion**

378 In this study, we assessed the olfactory response of a generalist aphid parasitoid and one
379 of its secondary parasitoids to mVOCs produced by phylogenetically diverse bacteria
380 isolated from the habitat of the parasitoids. Further, we investigated whether the chemical
381 composition of the mVOC blends differed between attractive, neutral and repellent
382 strains. It has to be noted that the bacterial strains used here originated from samples
383 collected from greenhouses and laboratory environments. Given the fact that insect
384 microbiomes are partly acquired from their host's environment (Hannula, Zhu, Heinen,

385 & Bezemer, 2019; Jones et al., 2018), it cannot be excluded that the strains investigated
386 may not be representative for what the insects would carry in more natural situations.
387 However, most of the bacteria investigated here were previously found in association with
388 aphids, parasitoids and their environment (Grigorescu et al., 2018; Leroy et al., 2011a;
389 Luna et al., 2018), reinforcing the robustness of our results.

390

391 *Olfactory response of A. colemani to bacterial VOCs depends on bacterial strain*

392 Our results show that *A. colemani* females responded differently to the various bacteria
393 occurring in the parasitoid's habitat. Based on the experiments performed in this study,
394 three significantly attractive and three significantly repellent strains to *A. colemani* were
395 identified, while the majority of strains did not have a significant effect. Strikingly, all
396 three attractive strains (as well as other strains with relatively high PI-values) belonged
397 to the genus *Bacillus*. *Bacillus* species are ubiquitous in nature and are known to produce
398 a wide array of volatiles (Kai et al., 2009), some of which can promote plant growth
399 without physical contact (Ping & Boland, 2004) or have antimicrobial activity (Gao,
400 Zhang, Liu, Han & Zhang, 2017). Additionally, a number of studies have shown that
401 *Bacillus* volatiles may also affect insect behaviour. For example, both Rockett (1987) and
402 Poonam and colleagues (Poonam, Paily, & Balaraman, 2002) showed that volatiles
403 produced by *Bacillus* species exhibited oviposition stimulation in *Culex* females.
404 Furthermore, the melon fruit fly *Bactrocera cucurbitae* was attracted to broth cultures of
405 *Bacillus cereus* (Mishra, Sharma, & Subramanian, 2018). Strikingly, one of the attractive
406 *Bacillus* strains (ST18.16/043) was isolated from the hyperparasitoid *D. aphidum*, which
407 complicates predictions regarding the adaptive value of responding to microbial volatiles.
408 Given these observations, our results seem to suggest that mVOC-mediated insect

409 responses may be correlated with bacterial phylogeny. Recent studies indicate that the
410 phylogeny of microorganisms may reflect functional traits and ecological characteristics,
411 pointing towards phylogenetic conservatism in phenotypic traits (Martiny, Jones, Lennon,
412 & Martiny, 2015; Martiny, Treseder, & Pusch, 2013). However, it is unclear so far
413 whether there are also phylogenetic signals in mVOC composition and insect response.

414

415 *Olfactory response to bacterial VOCs differs between primary and secondary parasitoids*

416 Primary parasitoids and their secondary parasitoids often forage for similar resources in
417 the same habitat, and share part of their decision-making strategy in host finding (Aartsma
418 et al., 2019). Therefore, it may be expected that generalist species such as *A. colemani*
419 and *D. aphidum* respond similarly to olfactory cues occurring in their habitat. Our
420 findings showed that responses of *A. colemani* were different from the responses of *D.*
421 *aphidum*. Particularly, the *Bacillus* strains attractive to *A. colemani* did not elicit a
422 significant olfactory response in its hyperparasitoid. Furthermore, it was found that one
423 of the three strains that was significantly repellent to *A. colemani* (ST18.16/160;
424 putatively identified as *Staphylococcus saprophyticus*) yielded a relatively high PI-value
425 for *D. aphidum*, pointing towards attraction, albeit without statistical support.
426 Additionally, the strain that was neutral to *A. colemani* (ST18.16/085; *Curtobacterium*
427 sp.) elicited a significant attractive response in the hyperparasitoid. Hence, this suggests
428 that the olfactory response of primary and secondary parasitoids towards mVOCs is
429 different, as has also been found for HIPVs (Cusumano et al., 2019; Poelman et al., 2012).

430

431 *Bacterial VOCs resemble plant and insect volatiles*

432 The bacterial VOC blends comprised typical microbial fermentation products, such as
433 methylated, low molecular weight alcohols and corresponding aldehydes and organic
434 acids (Dzialo et al., 2017; Schmidt, Cordovez, de Boer, Raaijmakers, & Garbeva, 2015).
435 However, some compounds like geraniol, linalool, limonene, 2-phenylethanol,
436 phenylacetaldehyde and acetophenone are also commonly reported as typical plant
437 volatiles (Bruce & Pickett, 2011; Dudareva, Klempien, Muhlemann, & Kaplan, 2013).
438 Moreover, certain compounds have been reported as insect pheromones, e.g. acetoin, 2,3-
439 butanediol, 2-(3,3-dimethylcyclohexylidene)-ethanol, linalool and nonan-2-ol (Borg-
440 Karlson et al., 2003; Löfstedt et al., 2008; Rochat et al., 2002). Nevertheless, it has to be
441 noted that so-called “insect pheromones” are not necessarily produced by the insects
442 themselves, but may also be derived from the gut bacteria of insects (Dillon et al., 2000).
443 This could also suggest that volatiles detected from plants are not necessarily (only)
444 produced by the plants themselves, which may also explain the considerable variation in
445 plant volatiles, even when exposed to similar conditions (Takabayashi, Dicke, &
446 Posthumus, 1994; Webster, Gezan, Bruce, Hardie & Pickett, 2010).

447 Compared to plant and insect volatiles, still very little is known about the
448 ecological role and biological function of mVOCs in the foraging behaviour of insects.
449 However, there is increasing evidence that mVOCs signal important aspects of habitat or
450 food suitability for foraging insects. For example, Leroy et al. (2011a) showed that aphid
451 honeydew is particularly attractive to aphid natural enemies when it is contaminated with
452 an aphid-associated bacterium like *Staphylococcus sciuri* producing mVOCs that act as
453 effective attractants and ovipositional stimulants. However, in contrast with this study,
454 our results suggest that *A. colemani* parasitoids are not attracted to, and can even be
455 repelled by mVOCs produced by bacteria originating from aphid honeydew. Further

456 research is needed to better understand the biological role of microbial volatiles in
457 volatile-mediated foraging behaviour.

458

459 *Differences in mVOC profiles between attractive, neutral and repellent strains*

460 In general, tested strains emitted a similar set of volatile compounds, and most mVOCs
461 produced by the strains that were attractive to *A. colemani* were also produced by the
462 neutral and repellent strains, but often in lower concentrations and in significantly
463 different ratios. This suggests that mVOCs may elicit a different response in insects
464 depending on the concentration of the compounds and the composition of the blend, most
465 probably determined by the presence of particular bioactive compounds or specific ratios
466 of ubiquitous compounds (Bruce, Wadhams, & Woodcock, 2005; Bruce, Webster,
467 Pickett, & Hardie, 2010; Mumm & Hilker, 2005; Takemoto & Takabayashi, 2015). More
468 specifically, the mVOC blends of the strains attractive to *A. colemani* had lower
469 concentrations of esters, aromatics, organic acids and cycloalkanes when compared to the
470 composition of the mVOC mixtures emitted by the repellent strains. This might indicate
471 that *Aphidius* parasitoids require lower concentrations of these compounds to become
472 attractive or that the concentrations in the repellent mixture were too high and masked
473 otherwise attractive compounds (Aartsma et al., 2017).

474 As was found for *A. colemani*, the chemical composition of the mVOC blends also
475 differed between attractive and repellent strains for *D. aphidum*. In particular, strains
476 attractive to *D. aphidum* produced significantly greater amounts of monoterpenes, while
477 repellent strains emitted significantly greater amounts of alcohols and ketones. The
478 monoterpenes produced included limonene, geraniol and linalool, which are known as
479 typical plant volatiles, many of which have been shown to be attractive to several insect

480 species, including natural enemies (Koschier, De Kogel, & Visser, 2000; McCormick,
481 Unsicker, & Gershenzon, 2012).

482 Surprisingly, the mVOC composition of strain ST18.16/133, which was attractive
483 to *A. colemani*, and the repellent strain ST18.17/002 were quite similar, yet they elicited
484 opposite olfactory responses. This suggests that changes in ratios of a select number of
485 compounds can reverse the behavioural response of insects. Indeed, it has previously been
486 shown that changing the concentration of certain compounds in an attractive blend of
487 ubiquitous plant volatiles can disrupt attraction of herbivorous insects (Bruce & Pickett,
488 2011). Moreover, we have to take into account that often just a fraction of the volatile
489 compounds present in the environment can be detected and subsequently cause a
490 behavioural response in insects (Bruce & Pickett, 2011). Therefore, insect behaviour does
491 not always reflect complete mVOC profiles, but rather the concentration and ratio of a
492 select number of compounds that are detected by the insects (Conchou et al., 2019).

493

494 *Concluding remarks*

495 Although our study has greatly contributed to our understanding of the role of mVOCs in
496 insect behavioural ecology, the next challenge is to study their ecological role and
497 influence under more natural conditions. In this study, experiments were performed under
498 controlled conditions in a clean environment using laboratory bioassays. However, in
499 their natural environment, insects encounter numerous volatile signals, from different
500 sources and in different concentrations, from which they need to derive reliable
501 information for accurate behavioural decisions (Aartsma, et al., 2017). It has been shown
502 that background odours can have different effects on volatile-mediated foraging
503 behaviour. Background odour can be irrelevant and not interact with foraging behaviour,

504 or may mask resource-indicating target cues, thereby reducing the response of insects to
505 attractants. Additionally, there is some evidence that background odours may also
506 enhance insect response to cues indicating the presence or suitability of resources
507 (Schröder & Hilker, 2008). Emission of mVOCs, including their chemical composition,
508 is also dependent on a variety of factors, including growth stage of the microbes, nutrient
509 availability, temperature, oxygen availability, pH, etc. (Tyc, Song, Dickschat, Vos, &
510 Garbeva, 2017). Future experiments should therefore be performed to investigate to what
511 extent the mVOCs measured here mimic those that are emitted under more natural
512 conditions, and how parasitoids will experience mVOCs in more natural settings, in
513 combination with food, host or habitat odours, like HIPVs.

514 Altogether, we have shown that insect responses to bacterial volatile emissions
515 depend on the bacterial strain. Further, we have shown that the olfactory response of an
516 aphid parasitoid and one of its hyperparasitoids to bacterial VOCs is different, and that
517 mVOC composition differed between attractive, neutral and repellent strains. Future
518 research should focus on how these mVOCs influence insect behaviour when perceived
519 with other cues related to food and hosts.

520

521 **Authors' contributions**

522 TG, ISS, FW, HR, HJ and BL conceived the ideas and designed methodology. TG and
523 CV collected the data. FD, FF, KJV and TW contributed to equipment and reagents, and
524 contributed to the mVOC analysis. JDB and FW provided insects required for
525 experiments. TG, CV, TW, HJ and BL analysed the data. TG, HJ and BL led the writing
526 of the manuscript. All authors contributed critically to the drafts and gave final approval
527 for publication. The authors have declared that no competing interests exist.

528

529 **Acknowledgements**

530 We are grateful to the Flemish Research Foundation (FWO) for supporting this research
531 (project 1S15116316N). Additionally, we would like to thank all students who helped
532 during the course of the experiments.

533

534 **Data accessibility**

535 16S rRNA gene sequences of the studied bacteria have been deposited in GenBank under
536 the Accession Numbers MK875098– MK875135. Further, underlying experimental data
537 can be found at the Dryad Digital Repository <https://doi.org/10.5061/dryad.fj6q573q9>
538 (Goelen et al., 2019).

539

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786

787 **Supporting information**

788 Additional supporting information may be found in the online version of this article.

789

790 Table S1 Bacterial isolates used in this study

791 Table S2 Microbial volatile organic compound (mVOC) composition of the cell-free
792 cultivation media used in this study

793 Figure S1 Schematic representation of the two-choice Y-tube olfactometer used in the
794 bioassays

795 Figure S2 Heat map of the mVOC composition of seven bacterial strains investigated in
796 this study

797

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801

802 **Figure captions**

803

804 **Figure 1.** Olfactory response of adult *Aphidius colemani* females when given a choice
805 between the odour of a test bacterium ($n = 38$) grown in GYP25 medium and the odour
806 of the blank GYP25 medium in a Y-tube olfactometer. Insect response is expressed as the
807 Preference Index (PI). In total, 60 individuals were tested (12 releases of 5 females). Non-
808 responders were excluded from the statistical analysis. Grey bars indicate non-significant
809 olfactory responses ($P > 0.05$), green bars indicate significant attractive responses ($P \leq$
810 0.05) and red bars indicate significant repellent responses ($P \leq 0.05$) when compared to
811 the control in which parasitoids were offered GYP25 medium in both arms of the Y-tube
812 olfactometer. Error bars represent standard error of the mean. Overall parasitoid
813 responsiveness was higher than 80%. Coloured symbols indicate the source of isolation.
814 Strains that were selected for the remainder of the study are indicated in bold

815

816 **Figure 2.** Different olfactory response of adult *Aphidius colemani* females (A) and adult
817 *Dendrocerus aphidum* females (B) when given a choice between the odour of a test
818 bacterium grown in GYP25 medium and the odour of the blank GYP25 medium in a Y-
819 tube olfactometer. Insect response is expressed as the mean Preference Index (PI)
820 obtained for three biological replicates ($n = 3$; per replicate, 60 individuals were tested in
821 12 releases of 5 females). Non-responders were excluded from the statistical analysis.
822 Grey bars indicate non-significant olfactory responses ($P > 0.05$), green bars indicate
823 significant attractive responses ($P \leq 0.05$) and red bars indicate significant repellent
824 responses ($P \leq 0.05$) when compared to the control in which parasitoids were offered
825 blank GYP25 medium in both arms of the Y-tube olfactometer. Error bars represent
826 standard error of the mean. Overall parasitoid responsiveness was higher than 70%

827

828 **Figure 3.** Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis
829 dissimilarities of the mVOC composition of seven bacterial strains investigated in this
830 study ($n = 3$) (stress value = 0.158). The mVOC composition differed significantly
831 between the bacterial strains and the blank medium (perMANOVA: $F = 38.6$, $P < 0.001$).
832 Symbol colours indicate the effect of the mVOC blends on the olfactory response of the
833 primary parasitoid *Aphidius colemani*, i.e. green = attractive, grey = neutral, and red =
834 repellent. Blue refers to the blank medium. Symbol shapes indicate the effect of the
835 mVOC blends on the olfactory response of the hyperparasitoid *Dendrocerus aphidum*,
836 i.e. circle = attractive, square = neutral and cross = repellent

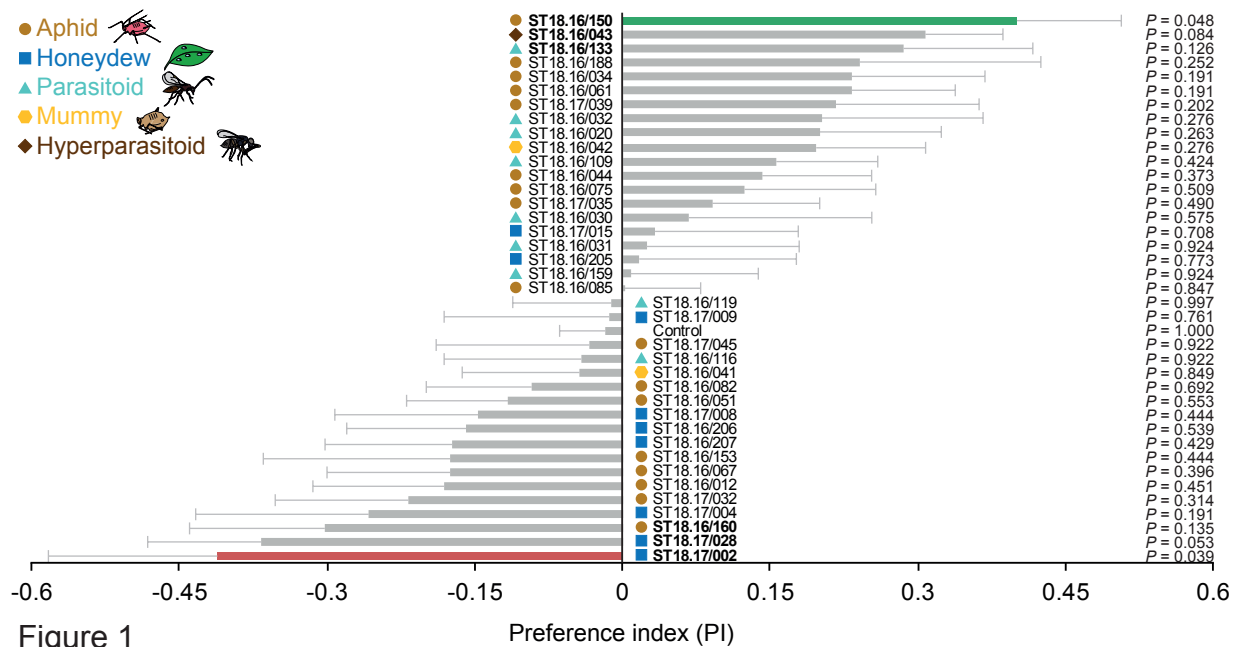
837

838 **Figure 4.** Linkage between the volatile composition of the cell-free bacterial cultivation
839 media and the olfactory response of *Aphidius colemani*. Results are shown for alcohols
840 (A), aldehydes (B), esters (C), ketones (D), alkanes (E), cycloalkanes (F), alkenes (G),
841 aromatics (H), organic acids (I), terpenes (J), and miscellaneous (K). Presented values are
842 the sum of peak areas of corresponding compounds per chemical class as detected by the
843 MXT-5 equipped GC-MS, and the result from three biological replicates ($n = 3$). Bacterial
844 strains are grouped by the effect of their mVOCs on the olfactory response of the tested
845 parasitoid: Attractive = ST18.16/150, ST18.16/133 and ST18.16/043; Neutral = blank
846 medium and ST18.16/085; Repellent = ST18.17/002, ST18.17/028 and ST18.16/160.
847 Different letters indicate significant differences ($P \leq 0.05$) between bacterial strains based
848 on an univariate ANOVA or Kruskal-Wallis non-parametric test

849

850 **Figure 5.** Linkage between the volatile composition of the cell-free bacterial cultivation
851 media and the olfactory response of *Dendrocerus aphidum*. Results are shown for

852 alcohols (A), aldehydes (B), esters (C), ketones (D), alkanes (E), cycloalkanes (F),
853 alkenes (G), aromatics (H), organic acids (I), terpenes (J), and miscellaneous (K).
854 Presented values are the sum of peak areas of corresponding compounds per chemical
855 class as detected by the MXT-5 equipped GC-MS, and the result from three biological
856 replicates ($n = 3$). Bacterial strains are grouped by the effect of their mVOCs on the
857 olfactory response of the tested parasitoid: Attractive = ST18.16/085; Neutral = blank
858 medium, ST18.16/150, ST18.16/133, ST18.16/043 and ST18.16/160; Repellent =
859 ST18.17/002 and ST18.17/028. Different letters indicate significant differences ($P \leq$
860 0.05) between bacterial strains based on an univariate ANOVA or Kruskal-Wallis non-
861 parametric test



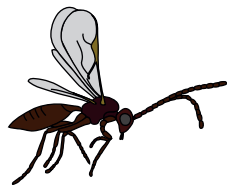
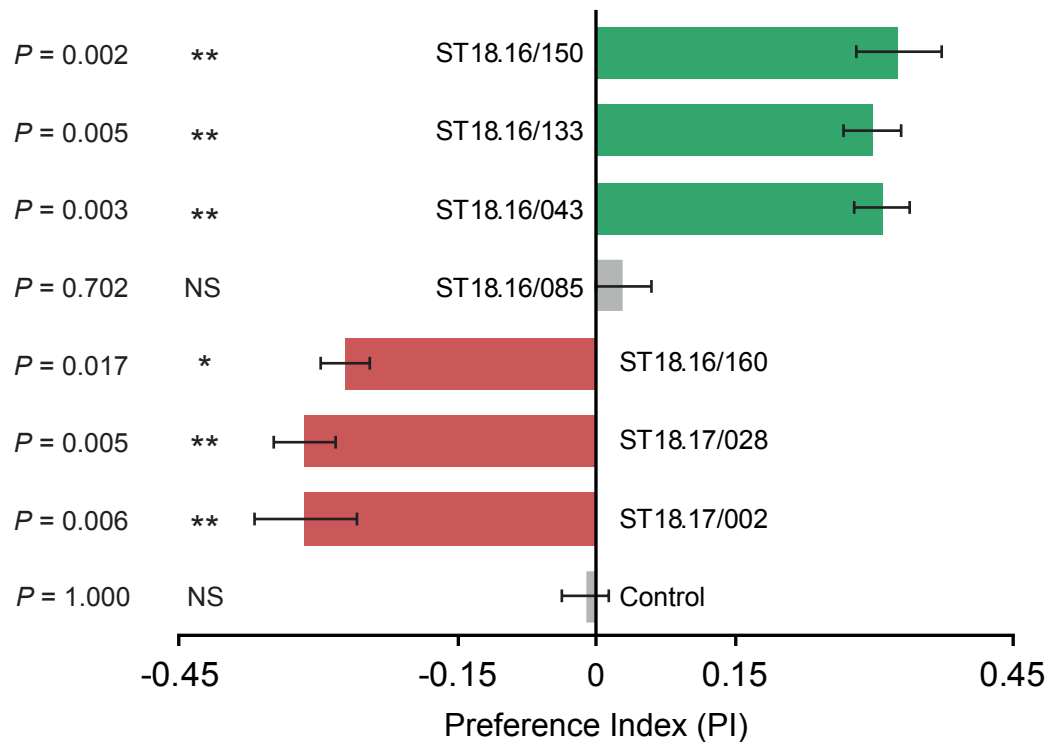
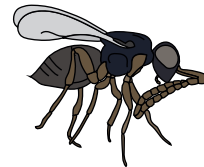
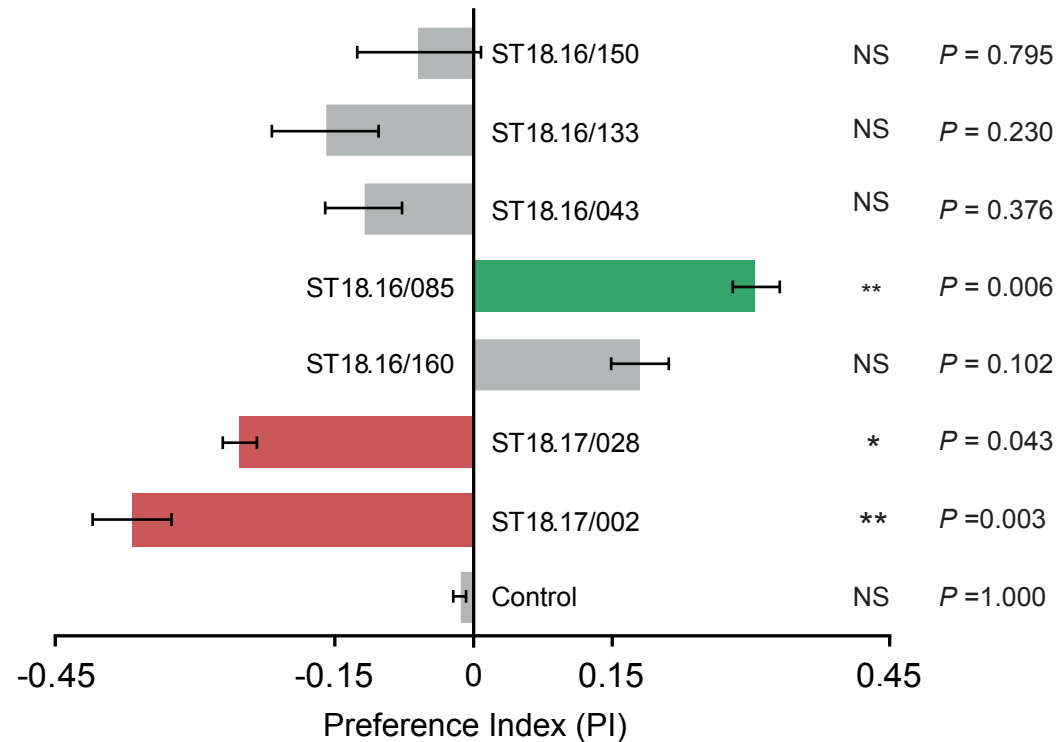
A*Aphidius colemani***B***Dendrocerus aphidum*

Figure 2

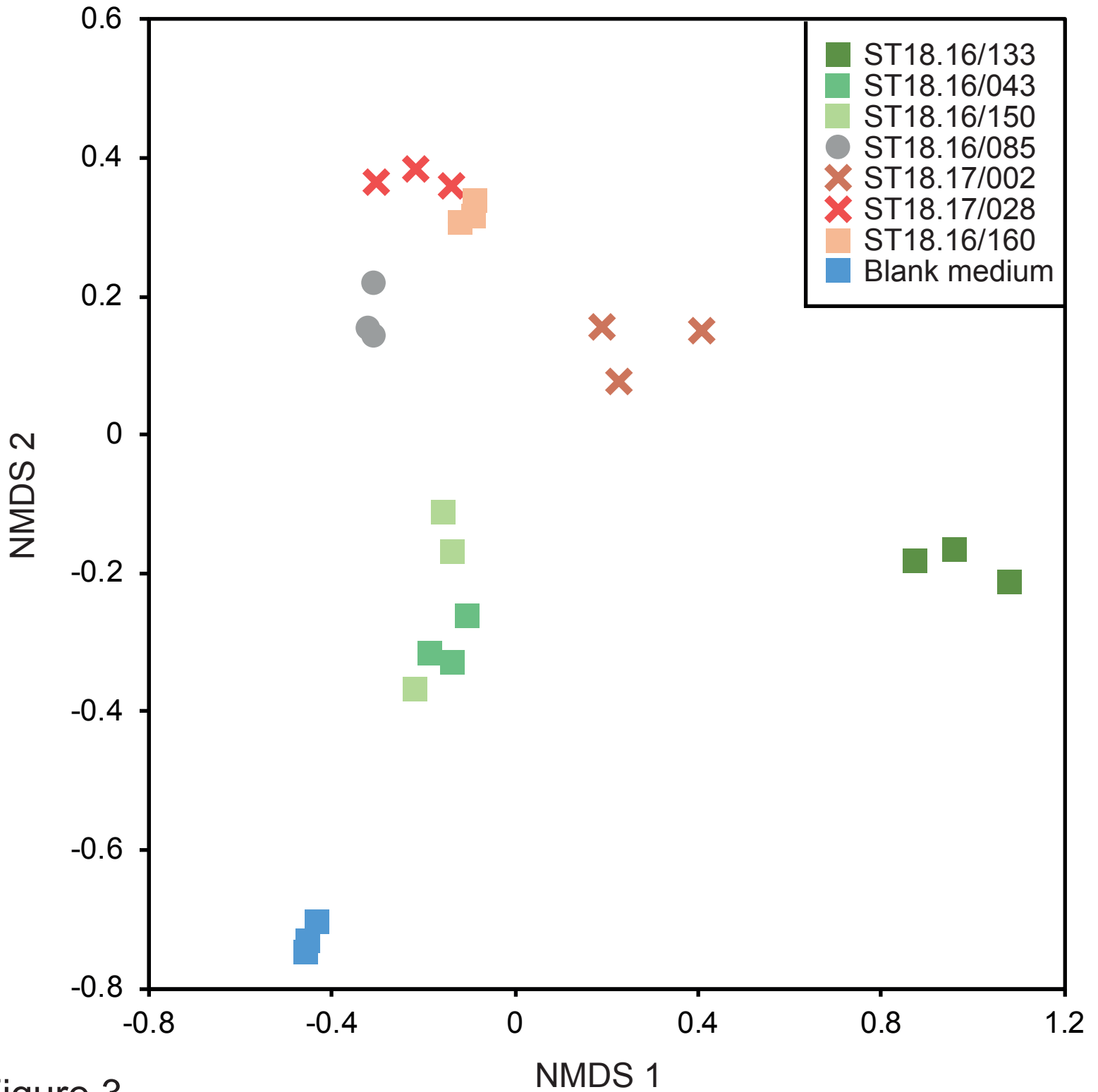


Figure 3

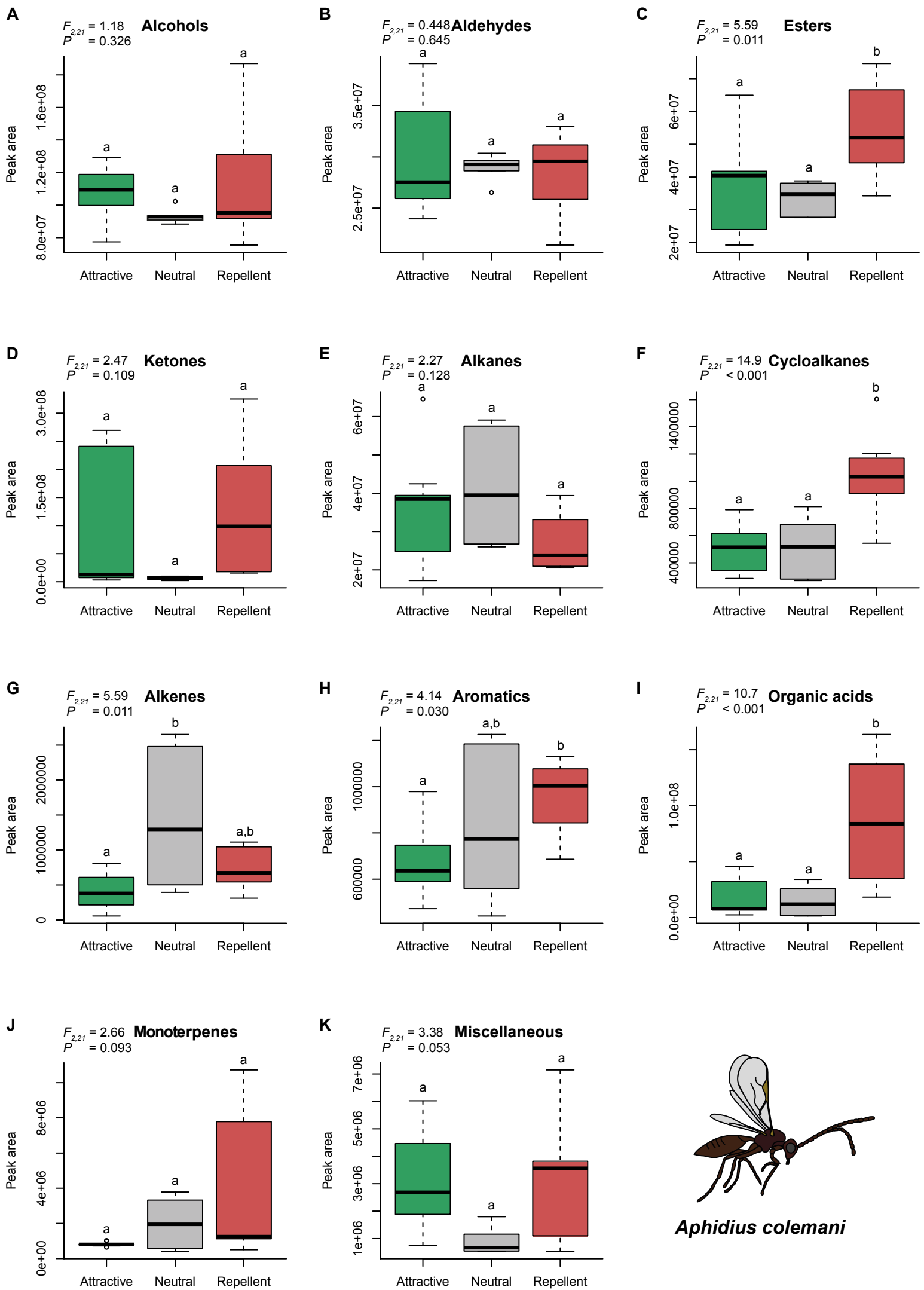


Figure 4

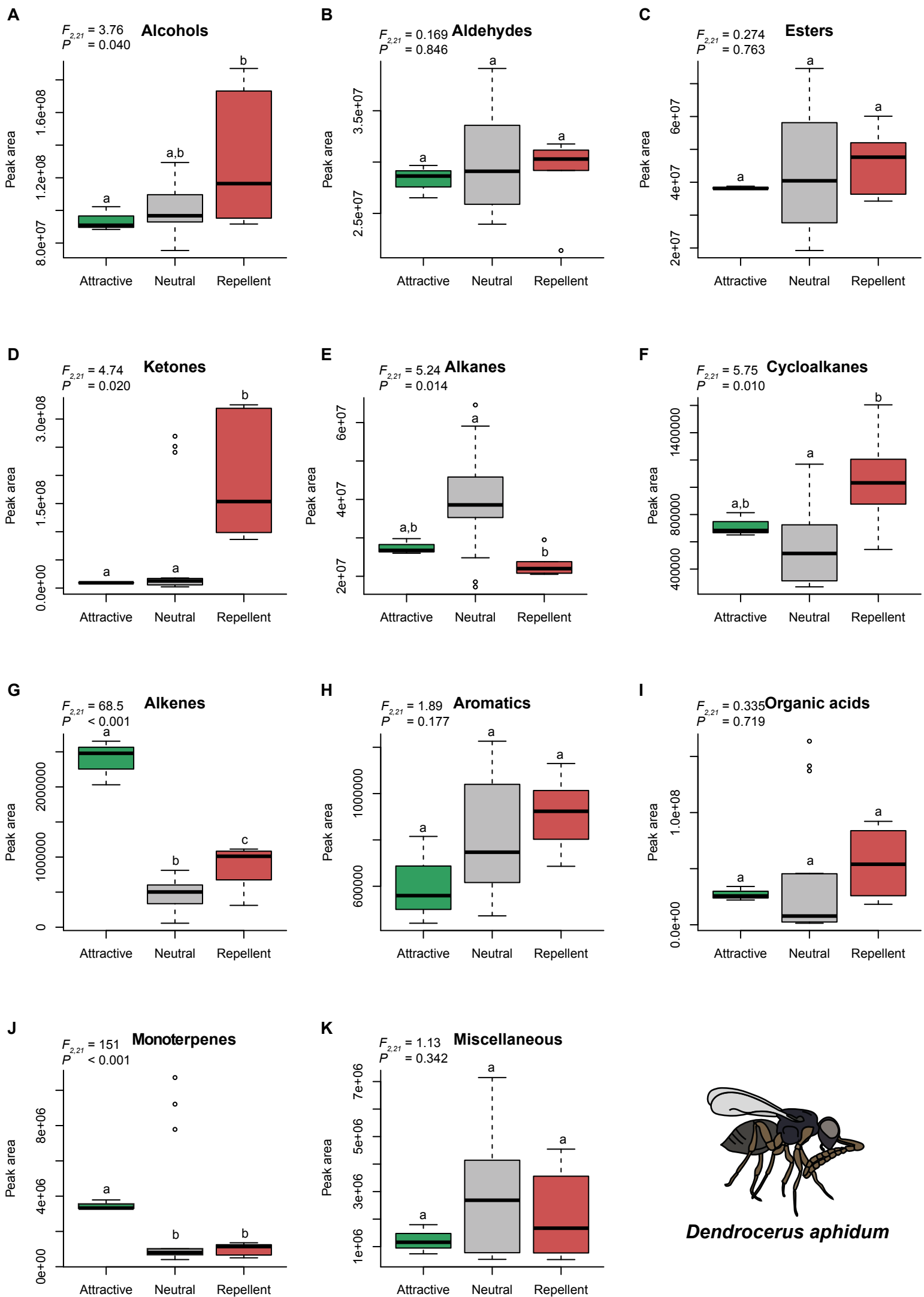


Figure 5

Volatiles of bacteria associated with parasitoid habitats elicit distinct olfactory responses in an aphid parasitoid and its hyperparasitoid

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Table S1. Bacterial isolates used in this study

Isolate identifier (GenBank Accession N°) ^a	Phylogenetic affiliation based on 16S rRNA gene sequence similarity ^b				Isolation origin ^c
	Phylum	Family	Closest match in EZBiocloud to identified species	Similarity (%) ^d	
ST18.17/034 (MK875132)	Actinobacteria	Corynebacteriaceae	<i>Corynebacterium sputi</i>	99.88	<i>Myzus persicae</i> var. <i>nicotianae</i> *
ST18.16/042 (MK875104)	Actinobacteria	Dermabacteraceae	<i>Brachybacterium rhamnosum</i>	99.69	<i>Acyrtosiphon pisum</i> mummy*
ST18.17/039 (MK875134)	Actinobacteria	Microbacteriaceae	<i>Leucobacter tardus</i>	99.24	<i>Myzus persicae</i> var. <i>nicotianae</i> *
ST18.16/109 (MK875113)	Actinobacteria	Microbacteriaceae	<i>Microbacterium paraoxydans</i>	99.93	<i>Aphidius ervi</i> *
ST18.16/085 (MK875112)	Actinobacteria	Micrococcaceae	<i>Curtobacterium</i> sp. (<i>C. flaccumfaciens</i> , <i>C. oceanosedimentum</i>)	99.66	<i>Myzus persicae</i> var. <i>nicotianae</i>
ST18.16/082 (MK875111)	Actinobacteria	Micrococcaceae	<i>Glutamicibacter halophytocola</i>	98.90	<i>Myzus persicae</i> var. <i>nicotianae</i>
ST18.17/002 (MK875125)	Actinobacteria	Micrococcaceae	<i>Glutamicibacter halophytocola</i>	99.40	Honeydew <i>M. persicae</i> / <i>Capsicum annum</i> *
ST18.17/032 (MK875131)	Actinobacteria	Micrococcaceae	<i>Kocuria halotolerans</i>	99.88	<i>Myzus persicae</i> var. <i>nicotianae</i> *
ST18.16/067 (MK875109)	Actinobacteria	Micrococcaceae	<i>Paeniglutamicibacter antarcticus</i>	99.36	<i>Myzus persicae</i> var. <i>nicotianae</i>
ST18.16/153 (MK875118)	Firmicutes	Bacillaceae	<i>Bacillus altitudinis</i>	99.27	<i>Macrosiphum euphorbiae</i>
ST18.16/150 (MK875117)	Firmicutes	Bacillaceae	<i>Bacillus circulans</i>	99.04	<i>Macrosiphum euphorbiae</i>
ST18.16/061 (MK875108)	Firmicutes	Bacillaceae	<i>Bacillus endophyticus</i>	99.93	<i>Myzus persicae</i> var. <i>nicotianae</i>
ST18.16/043 (MK875105)	Firmicutes	Bacillaceae	<i>Bacillus massiliosenegalensis</i>	99.51	<i>Dendrocerus aphidum</i> *
ST18.16/044 (MK875106)	Firmicutes	Bacillaceae	<i>Bacillus megaterium</i>	99.92	<i>Myzus persicae</i> var. <i>nicotianae</i>
ST18.16/075 (MK875110)	Firmicutes	Bacillaceae	<i>Bacillus</i> sp. (<i>B. siamensis</i> , <i>B. velezensis</i>)	99.86	<i>Myzus persicae</i> var. <i>nicotianae</i>
ST18.16/020 (MK875099)	Firmicutes	Bacillaceae	<i>Bacillus</i> sp. (<i>B. subtilis</i> , <i>B. siamensis</i> , <i>B. velezensis</i> , <i>B. amyloliquefaciens</i>)	100	<i>Aphidius colemani</i> *
ST18.16/188 (MK875121)	Firmicutes	Bacillaceae	<i>Bacillus velezensis</i>	99.41	<i>Macrosiphum euphorbiae</i>
ST18.16/133 (MK875116)	Firmicutes	Bacillaceae	<i>Bacillus zhangzhouensis</i>	99.56	<i>Aphidius ervi</i> *

ST18.16/051 (MK875107)	Firmicutes	Leuconostocaceae	<i>Weissella soli</i>	99.69	<i>Myzus persicae</i> var. <i>nicotianae</i>
ST18.16/041 (MK875103)	Firmicutes	Paenibacillaceae	<i>Paenibacillus glucanolyticus</i>	100	<i>Myzus persicae</i> var. <i>nicotianae</i> mummy*
ST18.17/035 (MK875133)	Firmicutes	Staphylococcaceae	<i>Corticococcus populi</i>	100	<i>Myzus persicae</i> var. <i>nicotianae</i> *
ST18.16/119 (MK875115)	Firmicutes	Staphylococcaceae	<i>Staphylococcus hominis</i>	99.65	<i>Aphidius ervi</i> *
ST18.16/160 (MK875120)	Firmicutes	Staphylococcaceae	<i>Staphylococcus saprophyticus</i>	99.50	<i>Macrosiphum euphorbiae</i>
ST18.16/012 (MK875098)	Firmicutes	Staphylococcaceae	<i>Staphylococcus sciuri</i>	99.90	<i>Myzus persicae</i> var. <i>nicotianae</i> *
ST18.16/031 (MK875101)	Firmicutes	Staphylococcaceae	<i>Staphylococcus sciuri</i>	99.71	<i>Aphidius colemani</i> *
ST18.16/206 (MK875123)	Firmicutes	Staphylococcaceae	<i>Staphylococcus xylosus</i>	99.52	Honeydew <i>M. persicae</i> / <i>Capsicum annuum</i>
ST18.16/207 (MK875124)	Firmicutes	Staphylococcaceae	<i>Staphylococcus xylosus</i>	99.55	Honeydew <i>M. persicae</i> / <i>Capsicum annuum</i>
ST18.17/004 (MK875126)	Firmicutes	Staphylococcaceae	<i>Staphylococcus xylosus</i>	99.78	Honeydew <i>M. persicae</i> / <i>Capsicum annuum</i> *
ST18.16/116 (MK875114)	Proteobacteria	Acetobacteraceae	<i>Asaia lannensis</i>	100	<i>Aphidius ervi</i> *
ST18.16/030 (MK875100)	Proteobacteria	Erwiniaceae	<i>Erwinia iniecta</i>	100	<i>Aphidius colemani</i> *
ST18.16/032 (MK875102)	Proteobacteria	Erwiniaceae	<i>Erwinia tasmaniensis</i>	99.89	<i>Aphidius colemani</i> *
ST18.17/028 (MK875130)	Proteobacteria	Erwiniaceae	<i>Pantoea dispersa</i>	99.85	Honeydew <i>M. persicae</i> / <i>Capsicum annuum</i> *
ST18.16/159 (MK875119)	Proteobacteria	Moraxellaceae	<i>Acinetobacter lwoffii</i>	99.84	<i>Aphidius rhopalosiphi</i>
ST18.17/045 (MK875135)	Proteobacteria	Pseudomonadaceae	<i>Pseudomonas hunanensis</i>	99.87	<i>Myzus persicae</i> var. <i>nicotianae</i> *
ST18.17/015 (MK875129)	Proteobacteria	Pseudomonadaceae	<i>Pseudomonas paralactis</i>	100	Honeydew <i>M. persicae</i> / <i>Capsicum annuum</i> *
ST18.17/008 (MK875127)	Proteobacteria	Pseudomonadaceae	<i>Pseudomonas soli</i>	100	Honeydew <i>M. persicae</i> / <i>Capsicum annuum</i> *
ST18.16/205 (MK875122)	Proteobacteria	Pseudomonadaceae	<i>Pseudomonas</i> sp. (<i>P. lurida</i> , <i>P. lactis</i> , <i>P. paralactis</i>)	99.87	Honeydew <i>M. persicae</i> / <i>Capsicum annuum</i>
ST18.17/009 (MK875128)	Proteobacteria	Pseudomonadaceae	<i>Stenotrophomonas maltophilia</i>	99.14	Honeydew <i>M. persicae</i> / <i>Capsicum annuum</i> *

^aIsolates indicated in bold were selected for the second experiment.

^bBased on EZBiocloud identification (November 2018). Only closest matches to type strains are reported.

^cSamples were collected in greenhouses or from a laboratory environment (lab cultures; indicated with an asterisk).

^dAverage fragment length was 1017 bp.

Table S2. Microbial volatile organic compound (mVOC) composition* of the cell-free cultivation media used in this study

Class	Compound	Retention index	Peak area ($\times 10^4$)								
			Blank medium	ST18.16/150	ST18.16/133	ST18.16/043	ST18.16/085	ST18.17/002	ST18.17/028	ST18.16/160	
Alcohol	ethanol	578	8796 \pm 53 ^a	10206 \pm 948 ^a	9129 \pm 369 ^a	8762 \pm 841 ^{a,b}	7776 \pm 431 ^{a,b}	7836 \pm 527 ^{a,b}	8323 \pm 296 ^{a,b}	6943 \pm 597 ^b	
	3-methyl-4-penten-2-ol	686	0.31 \pm 0.06 ^b	0.38 \pm 0.17 ^{a,b}	1.8 \pm 0.51 ^{a,b}	0.30 \pm 0.04 ^{a,b}	1.9 \pm 0.42 ^{a,b}	3.3 \pm 1.5 ^{a,b}	1.9 \pm 0.49 ^{a,b}	3.7 \pm 1.5 ^a	
	isopentyl alcohol	729	0.23 \pm 0.06 ^b	60 \pm 13 ^b	199 \pm 5.2 ^b	58 \pm 25 ^b	310 \pm 6.4 ^b	174 \pm 23 ^b	5030 \pm 914 ^a	914 \pm 85 ^b	
	2-methyl-1-butanol	731	1.3 \pm 0.10 ^b	2.2 \pm 0.98 ^b	0.21 \pm 0.11 ^b	0.69 \pm 0.24 ^b	125 \pm 4.6 ^b	2.3 \pm 1.1 ^b	578 \pm 123 ^a	0.09 \pm 0.01 ^b	
	3-methyl-2-buten-1-ol	750	1.2 \pm 0.12 ^b	3.6 \pm 1.5 ^{a,b}	60 \pm 1.1 ^a	3.7 \pm 1.3 ^{a,b}	8.9 \pm 1.1 ^{a,b}	18 \pm 5.4 ^{a,b}	6.1 \pm 2.69 ^{a,b}	5.6 \pm 2.7 ^{a,b}	
	2,3-butanediol	752	0.15 \pm 0.03 ^b	4.7 \pm 3.3 ^{a,b}	533 \pm 42 ^a	1.2 \pm 0.50 ^{a,b}	4.1 \pm 1.0 ^{a,b}	515 \pm 140 ^a	41 \pm 5.2 ^{a,b}	1.2 \pm 0.16 ^{a,b}	
	2-methyl-mercaptoethanol	819	0.17 \pm 0.04 ^b	1.2 \pm 0.65 ^{a,b}	0.22 \pm 0.04 ^{a,b}	5.0 \pm 4.07 ^{a,b}	0.92 \pm 0.20 ^{a,b}	31 \pm 3.1 ^a	74 \pm 32 ^a	2.5 \pm 0.01 ^{a,b}	
	pinacol	849	21 \pm 5.0 ^c	18 \pm 5.1 ^c	86 \pm 7.9 ^a	16 \pm 6.4 ^c	27 \pm 7.3 ^{b,c}	86 \pm 9.4 ^a	16 \pm 6.9 ^c	58 \pm 11 ^{a,b}	
	n-hexanol	869	18 \pm 1.5 ^d	33 \pm 4.7 ^{b,c,d}	64 \pm 4.1 ^{b,c,d}	28 \pm 12 ^{c,d}	74 \pm 4.9 ^a	60 \pm 16 ^{b,c,d}	74 \pm 14 ^a	45 \pm 0.83 ^{b,c,d}	
	4-heptanol	894	5.6 \pm 0.21 ^b	2.1 \pm 1.2 ^b	9.1 \pm 1.1 ^b	1.8 \pm 0.98 ^b	5.0 \pm 1.3 ^b	502 \pm 160 ^a	27 \pm 5.9 ^b	1.5 \pm 0.70 ^b	
	1-butoxy-2-propanol	951	212 \pm 14 ^a	133 \pm 52 ^{a,b}	37 \pm 37 ^b	29 \pm 28 ^b	8.1 \pm 1.4 ^b	28 \pm 26 ^b	29 \pm 28 ^b	46 \pm 45 ^b	
	3-(methylthio)-1-propanol	989	20 \pm 1.4 ^b	158 \pm 32 ^a	48 \pm 4.0 ^{a,b}	25 \pm 9.8 ^b	42 \pm 1.6 ^{a,b}	3.3 \pm 0.44 ^b	72 \pm 20 ^{a,b}	5.0 \pm 1.5 ^b	
	2-methyl-2-octen-4-ol	1028	17 \pm 0.72 ^b	16 \pm 1.4 ^b	1262 \pm 206 ^a	14 \pm 1.0 ^b	18 \pm 1.5 ^b	22 \pm 3.2 ^b	17 \pm 1.3 ^b	43 \pm 5.1 ^b	
	4-methyl-2-propyl-1-pentanol	1033	37 \pm 3.1 ^b	71 \pm 29 ^b	20 \pm 8.7 ^b	52 \pm 8.7 ^b	246 \pm 9.5 ^a	52 \pm 20 ^b	45 \pm 15 ^b	47 \pm 9.1 ^b	
	2-methyl-6-methylene-2-octanol	1077	71 \pm 9.5 ^b	64 \pm 5.2 ^b	371 \pm 21 ^a	105 \pm 17 ^b	196 \pm 14 ^b	173 \pm 35 ^b	175 \pm 44 ^b	164 \pm 25 ^b	
	n-octanol	1078	61 \pm 10 ^b	125 \pm 22 ^b	29 \pm 5.1 ^b	63 \pm 25 ^b	123 \pm 3.3 ^b	25 \pm 7.9 ^b	948 \pm 219 ^a	70 \pm 22 ^b	
	nonan-2-ol	1111	2.1 \pm 0.46 ^b	7.2 \pm 3.2 ^b	6.0 \pm 0.52 ^b	11 \pm 6.6 ^b	140 \pm 6.9 ^a	2.7 \pm 1.2 ^b	10 \pm 2.8 ^b	1.5 \pm 0.52 ^b	
	2-phenylethanol	1144	0.03 \pm 0.02 ^b	2.9 \pm 1.3 ^{a,b}	20 \pm 5.3 ^{a,b}	1.01 \pm 0.37 ^b	67 \pm 4.1 ^a	16 \pm 4.9 ^{a,b}	28 \pm 9.7 ^{a,b}	1.3 1.1 0.29 ^b	
	isoborneol	1162	11 \pm 0.92 ^b	8.5 \pm 0.09 ^b	12 \pm 2.3 ^b	17 \pm 0.70 ^{a,b}	18 \pm 0.74 ^{a,b}	22 \pm 2.8 ^a	11 \pm 3.5 ^b	16 \pm 1.9 ^{a,b}	
	4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	1183	9.0 \pm 0.54 ^c	20 \pm 3.5 ^{a,b,c}	18 \pm 1.8 ^{a,b,c}	23 \pm 4.1 ^{a,b,c}	43 \pm 3.6 ^a	17 \pm 3.9 ^{a,b,c}	35 \pm 8.1 ^{a,b}	24 \pm 4.2 ^{a,b,c}	
	α -methyl-cyclohexanepropanol	1249	0.95 \pm 0.09 ^b	3.0 \pm 1.8 ^b	0.94 \pm 0.78 ^b	1.5 \pm 1.3 ^b	64 \pm 6.5 ^a	0.41 \pm 0.05 ^b	1.6 \pm 0.11 ^b	2.7 \pm 1.6 ^b	
	9-decen-1-ol	1260	0.01 \pm 0.01 ^b	0.59 \pm 0.19 ^b	1.4 \pm 0.72 ^{a,b}	0.22 \pm 0.12 ^b	1.5 \pm 0.15 ^{a,b}	0.59 \pm 0.59 ^b	1.3 \pm 0.82 ^{a,b}	3.9 \pm 0.96 ^a	
	n-decanol	1280	4.0 \pm 1.0 ^b	107 \pm 58 ^b	17 \pm 1.4 ^b	27 \pm 14 ^b	62 \pm 5.7 ^b	12 \pm 7.3 ^b	561 \pm 98 ^a	73 \pm 30 ^b	
2-(3,3-dimethylcyclohexylidene)-ethanol	1288	11 \pm 0.86 ^b	14 \pm 5.5 ^b	10 \pm 1.5 ^b	9.4 \pm 2.3 ^b	22 \pm 6.8 ^{a,b}	19 \pm 8.3 ^{a,b}	39 \pm 3.7 ^a	37 \pm 4.3 ^a		
n-tetradecanol	1675	3.0 \pm 0.23 ^b	0.85 \pm 0.44 ^b	0.63 \pm 0.10 ^b	0.67 \pm 0.37 ^b	0.34 \pm 0.04 ^b	0.21 \pm 0.18 ^b	233 \pm 29 ^a	3.8 \pm 1.7 ^b		
Aldehyde	acetaldehyde	578	2494 \pm 19.0 ^b	2894 \pm 499 ^a	2457 \pm 115 ^b	2602 \pm 177 ^b	2481 \pm 113 ^b	2309 \pm 223 ^b	2285 \pm 190 ^b	2257 \pm 211 ^b	

	butanal	612	86 ± 7.9 ^b	159 ± 26 ^b	62 ± 5.6 ^b	330 ± 90 ^{a,b}	40 ± 8.9 ^b	610 ± 189 ^a	50 ± 15 ^b	79 ± 15 ^b
	3-methylthio-propionaldehyde	915	44 ± 3.3 ^a	8.8 ± 1.3 ^b	14 ± 6.4 ^b	14 ± 1.4 ^b	36 ± 1.4 ^a	4.7 ± 0.95 ^b	5.0 ± 1.6 ^b	5.9 ± 0.56 ^b
	phenylacetaldehyde	1048	335 ± 29 ^a	276 ± 110 ^{b,c}	40 ± 3.5 ^{b,c}	178 ± 36 ^b	155 ± 8.5 ^{b,c}	47 ± 12 ^{b,c}	105 ± 30 ^{b,c}	91 ± 12 ^{b,c}
	3,5-dimethyl-benzaldehyde	1228	2.9 ± 1.3 ^c	60 ± 19 ^c	9.1 ± 1.9 ^c	11 ± 7.9 ^c	116 ± 20 ^{b,c}	21 ± 11 ^c	365 ± 123 ^a	343 ± 56 ^{a,b}
Ester	isobutyl-formate	670	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.11 ± 0.08 ^a	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.04 ± 0.04 ^{a,b}	0.02 ± 0.02 ^{a,b}	0.00 ± 0.00 ^b
	butyl-formate	672	2571 ± 117 ^{b,c}	1751 ± 157 ^c	2890 ± 216 ^{b,c}	3006 ± 10 ^{b,c}	3178 ± 46 ^{a,b}	2919 ± 82 ^{b,c}	3250 ± 235 ^{a,b}	3634 ± 17 ^a
	ethyl butanoate	757	0.05 ± 0.01 ^b	19 ± 19 ^{a,b}	2.3 ± 0.63 ^{a,b}	0.11 ± 0.11 ^b	0.61 ± 0.14 ^{a,b}	2.7 ± 0.44 ^{a,b}	20 ± 7.1 ^a	1.1 ± 0.06 ^{a,b}
	butyl acetate	780	6.81 ± 0.66 ^d	190 ± 67 ^d	1314 ± 447 ^{b,c}	411 ± 146 ^{c,d}	267 ± 12.6 ^d	2063 ± 202 ^{a,b}	183 ± 49 ^d	2937 ± 234 ^a
	ethyl-3-methyl butanoate	850	0.00 ± 0.00 ^b	0.13 ± 0.09 ^b	65 ± 9.8 ^a	1.8 ± 1.8 ^b	2.1 ± 0.23 ^b	57 ± 6.5 ^a	10 ± 2.6 ^b	100 ± 3.9 ^a
	butyl propanoate	916	0.86 ± 0.12 ^b	1.1 ± 0.16 ^b	331 ± 151 ^a	29 ± 4.8 ^b	9.4 ± 1.3 ^b	43 ± 9.1 ^b	5.9 ± 1.9 ^b	3.7 ± 0.95 ^b
	butyl-isobutyrate	961	51 ± 2.6 ^b	64 ± 1.6 ^b	63 ± 11 ^b	434 ± 71 ^a	56 ± 0.85 ^b	77 ± 4.0 ^b	42 ± 4.6 ^b	42 ± 4.7 ^b
	butyl-butanoate	1000	8.3 ± 0.64 ^e	7.7 ± 1.3 ^e	5.1 ± 2.8 ^e	102 ± 13 ^{a,b}	133 ± 8.2 ^a	70 ± 9.5 ^{b,c}	49 ± 11 ^{c,d}	25 ± 2.3 ^{d,e}
	butyl 2-methyl butanoate	1042	8.2 ± 5.4 ^b	8.5 ± 0.06 ^b	18 ± 2.6 ^b	168 ± 58 ^a	14 ± 1.1 ^b	6.6 ± 1.1 ^b	20 ± 0.78 ^b	13 ± 1.9 ^b
	butyl Isovalerate	1050	1.5 ± 0.03 ^b	2.3 ± 1.1 ^b	15 ± 3.1 ^{a,b}	116 ± 51 ^a	1.3 ± 0.18 ^b	0.38 ± 0.05 ^b	1.5 ± 0.93 ^b	2.1 ± 0.68 ^b
	o-tert-butyl cyclohexyl acetate	1295	29 ± 3.2 ^c	37 ± 17 ^{b,c}	44 ± 2.8 ^{b,c}	48 ± 3.2 ^{b,c}	96 ± 2.6 ^{b,c}	135 ± 55 ^a	133 ± 11 ^a	95 ± 5.9 ^{b,c}
	butyl-isobutyl-phthalate	1886	220 ± 23 ^a	145 ± 15 ^{a,b}	94 ± 14 ^{a,b}	137 ± 13 ^b	67 ± 11 ^b	61 ± 9 ^b	117 ± 7.2 ^{a,b}	135 ± 20 ^{a,b}
Ketone	2,3-butanedione	611	8.8 ± 1.06 ^b	602 ± 60 ^a	585 ± 34 ^a	27 ± 13 ^b	108 ± 16 ^b	361 ± 59 ^a	379 ± 99 ^a	41 ± 4.0 ^b
	2-butanone	612	22 ± 1.7 ^b	20 ± 9.0 ^b	17 ± 1.1 ^b	73 ± 27 ^{a,b}	59 ± 19 ^{a,b}	420 ± 213 ^a	21 ± 11 ^b	34 ± 9.4 ^b
	2,3-pentanedione	679	188 ± 56 ^a	202 ± 129 ^{b,c}	14 ± 7.0 ^{b,c}	75 ± 13 ^{b,c}	20 ± 5.2 ^{b,c}	12 ± 0.69 ^{b,c}	26 ± 7.1 ^{b,c}	81 ± 15 ^{a,b}
	1-hydroxy-2-propanone	684	0.52 ± 0.13 ^b	4.97 ± 0.26 ^b	13 ± 0.76 ^{a,b}	4.0 ± 0.61 ^b	5.6 ± 1.2 ^b	5.5 ± 1.1 ^b	24 ± 7.8 ^a	8.4 ± 0.53 ^b
	acetoin	714	58 ± 3.9 ^b	134 ± 35 ^b	22607 ± 781 ^a	85 ± 9.4 ^b	36 ± 6.1 ^b	8466 ± 710 ^b	27333 ± 3836 ^a	1124 ± 144 ^b
	4-methyl-pentan-2-one	734	0.00 ± 0.00 ^b	0.01 ± 0.01 ^b	98 ± 24 ^a	0.02 ± 0.01 ^b	0.11 ± 0.03 ^b	0.31 ± 0.05 ^b	0.89 ± 0.56 ^b	0.2 ± 0.03 ^b
	2-hexanone	755	2.5 ± 0.26 ^d	21 ± 7.0 ^{c,d}	277 ± 7.0 ^a	12 ± 1.6 ^d	94 ± 2.8 ^b	55 ± 8.7 ^{b,c}	8.9 ± 4.2 ^d	13 ± 1.2 ^d
	4-methyl-2-heptanone	948	8.0 ± 0.32 ^b	146 ± 63 ^{a,b}	136 ± 14 ^a	119 ± 23 ^{a,b}	86 ± 9.8 ^b	35 ± 3.6 ^{a,b}	81 ± 24 ^{a,b}	91 ± 11 ^{a,b}
	5,5-dimethyl-2,4-hexanedione	1020	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	1465 ± 72 ^a	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	4.5 ± 4.5 ^b	0.13 ± 0.03 ^b	0.00 ± 0.00 ^b
	acetophenone	1073	56 ± 7.8 ^b	105 ± 37 ^b	178 ± 76 ^{a,b}	182 ± 68 ^{a,b}	469 ± 3.4 ^a	150 ± 49 ^{a,b}	263 ± 145 ^{a,b}	252 ± 86 ^{a,b}
	4-cyclohexyl-2-butanone	1237	3.7 ± 0.19 ^b	0.69 ± 0.39 ^b	0.00 ± 0.00 ^b	0.50 ± 0.41 ^b	41 ± 1.8 ^a	2.2 ± 0.74 ^b	2.1 ± 1.0 ^b	0.55 ± 0.40 ^b
	undecan-2-one	1298	3.2 ± 0.46 ^b	5.2 ± 0.60 ^b	9.0 ± 1.5 ^{a,b}	4.7 ± 1.9 ^{a,b}	14 ± 5.5 ^b	7.0 ± 2.2 ^{a,b}	223 ± 127 ^a	9.2 ± 0.77 ^{a,b}
	tridecan-2-one	1487	1.2 ± 0.23 ^b	1.1 ± 0.10 ^b	1.9 ± 0.39 ^b	2.4 ± 0.71 ^b	7.9 ± 0.39 ^a	2.2 ± 0.84 ^b	3.0 ± 0.50 ^b	1.7 ± 0.88 ^b
Alkane	n-hexane	611	3.5 ± 1.1 ^b	38 ± 16 ^{a,b}	137 ± 57 ^a	40 ± 24 ^{a,b}	0.81 ± 0.41 ^b	69 ± 10 ^{a,b}	0.00 ± 0.00 ^b	18 ± 14 ^b
	2,2,4-trimethyl-pentane	674	5501 ± 306 ^a	4672 ± 868 ^a	1880 ± 243 ^b	3929 ± 157 ^a	2716 ± 120 ^{a,b}	2095 ± 94 ^b	2440 ± 256 ^b	3629 ± 198 ^{a,b}

	n-octane	760	15 ± 0.8 ^{a,b,c}	11 ± 2.4 ^{b,c}	1.9 ± 0.55 ^c	16 ± 1.6 ^{a,b}	22 ± 1.6 ^a	4.90 ± 2.9 ^{b,c}	8.0 ± 2.3 ^{b,c}	18 ± 3.5 ^{a,b}
	nonane	882	7.0 ± 0.99 ^a	0.50 ± 0.29 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.07 ± 0.07 ^b	0.00 ± 0.00 ^b
	tetradecane	1403	0.01 ± 0.00 ^b	0.11 ± 0.00 ^b	0.01 ± 0.00 ^b	0.05 ± 0.03 ^b	11 ± 5.4 ^a	1.6 ± 0.66 ^b	1.4 ± 0.32 ^b	1.0 ± 0.79 ^b
Cycloalkane	1,3-dimethyl-cyclopentane	687	31 ± 3.6 ^b	43 ± 4.9 ^b	65 ± 5.4 ^{a,b}	32 ± 3.8 ^b	69 ± 5.7 ^{a,b}	86 ± 17 ^a	85 ± 12 ^a	96 ± 8.3 ^a
	1,5-dimethyl-6-oxa-bicyclo[3,1,0]hexane	728	0.00 ± 0.00 ^b	3.4 ± 3.3 ^b	4.3 ± 0.48 ^b	0.06 ± 0.06 ^b	2.9 ± 1.6 ^b	0.90 ± 0.19 ^b	38 ± 11 ^a	8.0 ± 0.96 ^b
Alkene	1-decene	996	2.2 ± 0.42 ^b	0.37 ± 0.13 ^b	0.00 ± 0.00 ^b	6 ± 2.4 ^b	19 ± 2.8 ^a	1.7 ± 1.2 ^b	4.8 ± 0.75 ^b	3.2 ± 1.0 ^b
	9-methyl-1-decene	1033	46 ± 4.6 ^b	51 ± 13 ^b	10 ± 5.2 ^b	48 ± 12 ^b	218 ± 17 ^a	79 ± 25 ^b	56 ± 12 ^b	43 ± 8.2 ^b
	1-tetradecene	1396	0.38 ± 0.14 ^b	8 ± 1.1 ^b	1.1 ± 0.09 ^b	1.5 ± 0.30 ^b	1.43 ± 0.95 ^b	0.86 ± 0.50 ^b	31 ± 9.4 ^a	7.8 ± 0.54 ^b
Aromatic	benzene	679	87 ± 18 ^a	35 ± 12 ^b	9.1 ± 2.1 ^b	42 ± 7.8 ^b	24 ± 6.8 ^b	11 ± 1.1 ^b	22 ± 6.9 ^b	51 ± 5.2 ^{a,b}
	1,3-dimethyl-benzene	860	3.3 ± 0.99 ^c	12 ± 3.5 ^{b,c}	42 ± 9.9 ^{a,b}	6.5 ± 5.9 ^c	21 ± 5.2 ^{b,c}	36 ± 7.7 ^{a,b}	22 ± 6.2 ^{b,c}	42 ± 2.8 ^a
	1-ethyl-2-methyl-benzene	984	1.23 ± 0.18 ^b	12 ± 1.3 ^a	3.1 ± 1.4 ^{a,b}	1.68 ± 0.35 ^{a,b}	4.2 ± 0.22 ^{a,b}	2.8 ± 0.19 ^{a,b}	3.3 ± 0.65 ^{a,b}	2.2 ± 0.07 ^{a,b}
	cyclopropyl-benzene	1028	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	3.3 ± 0.72 ^a	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b
	para-cymene	1060	1.2 ± 0.48 ^d	7.7 ± 0.99 ^d	14 ± 0.88 ^{c,d}	4.02 ± 1.5 ^d	7.6 ± 0.84 ^d	39 ± 3.6 ^a	22 ± 3.1 ^{b,c}	3.6 ± 0.67 ^d
	ortho-cymene	1063	11 ± 1.3 ^a	4.1 ± 0.81 ^{b,c}	0.43 ± 0.02 ^c	4.2 ± 0.74 ^b	1.5 ± 0.03 ^{b,c}	0.89 ± 0.26 ^{b,c}	2.7 ± 0.94 ^{b,c}	1.7 ± 0.34 ^{b,c}
	indole	1290	1.3 ± 0.06 ^b	2.0 ± 1.0 ^b	0.75 ± 0.16 ^b	0.79 ± 0.02 ^b	1.9 ± 1.1 ^b	0.30 ± 0.20 ^b	20 ± 6.0 ^a	4.8 ± 1.3 ^b
Organic acid	acetic acid	647	8.5 ± 2.3 ^b	32 ± 2.5 ^b	44 ± 7.6 ^b	17 ± 2.6 ^b	706 ± 108 ^b	109 ± 32 ^b	619 ± 403 ^b	7598 ± 441 ^a
	2-methyl-propanoic acid	746	102 ± 4.0 ^c	87 ± 24 ^c	200 ± 23 ^{b,c}	140 ± 21 ^{b,c}	262 ± 10 ^{a,b}	149 ± 58 ^{b,c}	165 ± 37 ^{b,c}	334 ± 12 ^a
	butanoic acid	769	0.77 ± 0.32 ^e	65 ± 15 ^{d,e}	170 ± 11 ^c	16 ± 8.9 ^e	280 ± 10 ^b	95 ± 14 ^d	494 ± 20 ^a	433 ± 15 ^a
	3-methyl-pyruvic acid	777	17 ± 0.70 ^b	16 ± 1.9 ^b	462 ± 40 ^a	23 ± 5.5 ^b	13 ± 1.0 ^b	131 ± 18 ^{a,b}	11 ± 5.3 ^b	176 ± 14 ^{a,b}
	3-methyl-butanoic acid	855	1.2 ± 0.57 ^c	17 ± 11 ^c	1488 ± 224 ^b	23 ± 22 ^c	28 ± 2.8 ^c	1035 ± 193 ^b	157 ± 30 ^c	2610 ± 202 ^a
	2-methyl-butanoic acid	868	1.2 ± 0.09 ^b	1.2 ± 0.17 ^b	1155 ± 84 ^a	73 ± 70 ^b	142 ± 14 ^b	846 ± 208 ^a	108 ± 21 ^b	1015 ± 115 ^a
	n-heptanoic acid	1093	3.5 ± 0.04 ^b	14 ± 8.0 ^b	7.6 ± 0.67 ^b	3.2 ± 0.07 ^b	6.7 ± 4.7 ^b	1.7 ± 0.64 ^b	107 ± 31 ^a	41 ± 11 ^{a,b}
	2-ethyl-hexanoic acid	1135	1.0 ± 0.17 ^c	7.6 ± 3.3 ^c	6.41 ± 1.1 ^c	3.4 ± 0.40 ^c	133 ± 55 ^a	23 ± 4.9 ^c	80 ± 18 ^{a,b}	52 ± 6.3 ^{a,b}
	3,3-dimethyl-heptanoic acid	1152	0.63 ± 0.34 ^b	2.4 ± 1.4 ^b	2.9 ± 1.4 ^b	0.12 ± 0.04 ^b	11 ± 0.69 ^b	7.6 ± 3.8 ^b	53 ± 8.8 ^a	36 ± 5.2 ^a
	octanoic acid	1194	9.5 ± 3.6 ^c	117 ± 11 ^c	187.1 ± 25 ^c	42 ± 6.9 ^c	191 ± 92 ^c	68 ± 7.8 ^c	2308 ± 63 ^a	779 ± 142 ^b
	nonanoic acid	1288	21 ± 6.7 ^c	373 ± 31 ^c	379 ± 104 ^c	82 ± 44 ^c	931 ± 429 ^{b,c}	118 ± 43 ^c	4156 ± 308 ^a	1658 ± 402 ^b
	isobornyl acrylate	1376	0.00 ± 0.00 ^b	2.5 ± 1.5 ^b	0.12 ± 0.09 ^b	0.48 ± 0.47 ^b	3.99 ± 2.6 ^{a,b}	34 ± 17 ^a	4.0 ± 2.7 ^{a,b}	0.86 ± 0.74 ^b
	methyl-ethyl-adipate	1389	1.3 ± 0.25 ^b	8.5 ± 0.09 ^b	1.8 ± 0.93 ^b	2.1 ± 0.28 ^b	22 ± 7.6 ^{a,b}	13 ± 8.0 ^{a,b}	45 ± 17 ^a	11 ± 3.3 ^{a,b}
Terpene	limonene	1031	17 ± 1.2 ^b	16 ± 1.0 ^b	0.77 ± 0.32 ^b	13 ± 2.3 ^b	35 ± 1.5 ^a	16 ± 3.7 ^b	18 ± 3.7 ^b	21 ± 2.0 ^b
	linalool	1106	34 ± 4.6 ^c	61 ± 2.8 ^{b,c}	63 ± 3.7 ^{b,c}	59 ± 3.09 ^{b,c}	150 ± 6.6 ^b	65 ± 14 ^{b,c}	79 ± 15 ^{b,c}	784 ± 68 ^a
	geraniol	1262	0.51 ± 0.13 ^c	1.3 ± 0.18 ^c	31 ± 4.2 ^b	1.3 ± 0.75 ^c	163 ± 8.4 ^a	12 ± 3.8 ^b	11 ± 4.8 ^b	119 ± 16 ^a

Misc.	azetidine	610	1.8 ± 0.27 ^b	24 ± 4.2 ^{a,b}	38 ± 11 ^{a,b}	11 ± 6.7 ^{a,b}	3.1 ± 1.0 ^a	66 ± 33 ^a	1.7 ± 1.2 ^b	7.9 ± 3.8 ^{a,b}
	1-butanamine	611	8.1 ± 2.3 ^b	82 ± 12 ^{a,b}	81 ± 12 ^{a,b}	64 ± 32 ^{a,b}	14 ± 5.2 ^b	187 ± 65 ^a	1.4 ± 0.28 ^b	17 ± 7.3 ^b
	ammonium acetate	649	0.00 ± 0.00 ^b	0.03 ± 0.03 ^b	2.7 ± 0.80 ^b	0.00 ± 0.00 ^b	37 ± 26 ^b	13 ± 9.3 ^b	47 ± 37 ^b	404 ± 115 ^a
	methyl pyrazine	808	5.6 ± 0.21 ^a	3.7 ± 0.27 ^{b,c}	2.2 ± 0.06 ^c	4.1 ± 0.19 ^b	3.85 ± 0.12 ^b	1.2 ± 0.15 ^c	1.9 ± 0.30 ^c	2.2 ± 0.08 ^c
	2-propyl-1,3-dioxolane	834	16 ± 0.44 ^a	3.9 ± 1.7 ^b	0.02 ± 0.01 ^c	4.8 ± 1.4 ^b	0.10 ± 0.02 ^c	0.46 ± 0.29 ^c	0.00 ± 0.00 ^c	0.27 ± 0.09 ^c
	dihydro-2-methyl-thiophen-3-one	987	0.28 ± 0.08 ^c	370 ± 42 ^a	75 ± 12 ^b	14 ± 2.5 ^{b,c}	17 ± 1.5 ^{b,c}	6.9 ± 1.7 ^{b,c}	21 ± 6.6 ^{b,c}	15 ± 1.5 ^{b,c}
	2-methyl-5-(1-methylethyl)-pyrazine	1057	17 ± 1.7 ^b	16 ± 2.8 ^b	19 ± 5.0 ^b	12 ± 0.26 ^b	36 ± 1.5 ^a	9.2 ± 1.2 ^b	14 ± 2.2 ^b	14 ± 1.2 ^b
	tetramethyl-pyrazine	1096	3.1 ± 1.1 ^c	6.2 ± 3.6 ^{b,c}	65 ± 6.9 ^a	1.4 ± 0.49 ^c	3.0 ± 0.43 ^c	20 ± 6.6 ^b	1.3 ± 0.40 ^c	0.95 ± 0.16 ^c
	benzothiazole	1226	5.0 ± 0.23 ^b	6.1 ± 2.4 ^b	2.4 ± 0.06 ^b	2.6 ± 0.19 ^b	9.8 ± 2.1 ^b	3.0 ± 1.4 ^b	29 ± 9 ^a	30 ± 4.2 ^a

*Peak areas and retention indices were recovered from a MXT-5 equipped GC-MS. Presented values are means ± SE of three biological replicate fermentations ($n = 3$) with the tested bacterial strains. Different letters in each row indicate statistically significant differences between treatments ($P \leq 0.05$), based on a univariate ANOVA followed by a Tukey's HSD test with adjusted P -values as calculated after correcting for multiple comparisons. A Kruskal-Wallis test was used when the data did not conform to the criteria of normality and homogeneity of variance required for a parametric statistical test.

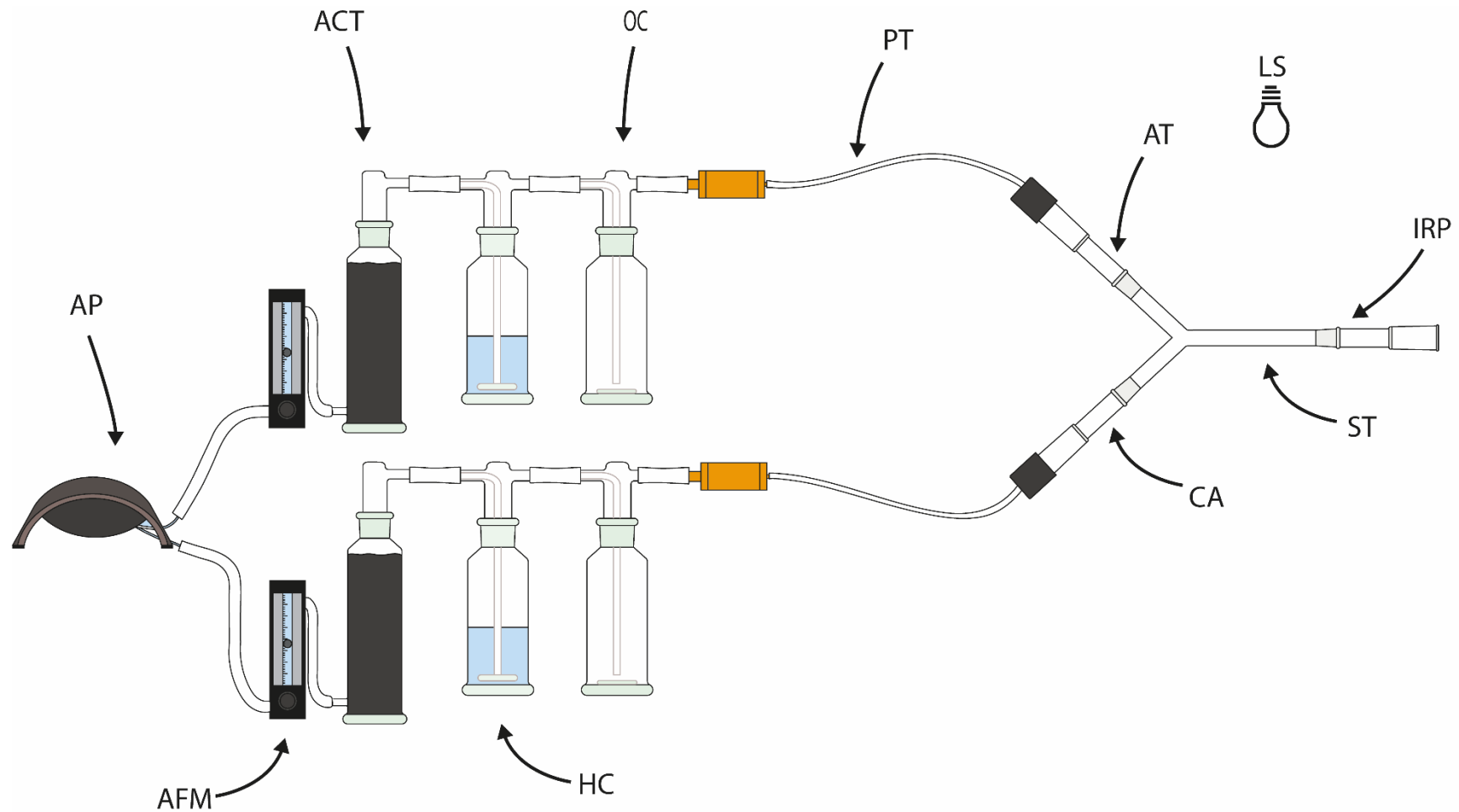


Figure S1. Schematic representation of the two-choice Y-tube olfactometer used in the bioassays. The olfactometer consisted of a 20-cm-long stem tube with 1.5 cm internal diameter and two 12-cm-long lateral arms with a 60° angle at the Y-junction. The detailed depiction of the various parts and connections is as follows: ACT, activated charcoal tower; AFM, air flowmeter (400 mL/min); AP, air pump; AT, arm tube; CA, choice area; HC, humidifier chamber; IRP, insect release point; LS, light source (10 000 lux); OC, odour chamber; ST, stem tube, PT, PTFE tubing

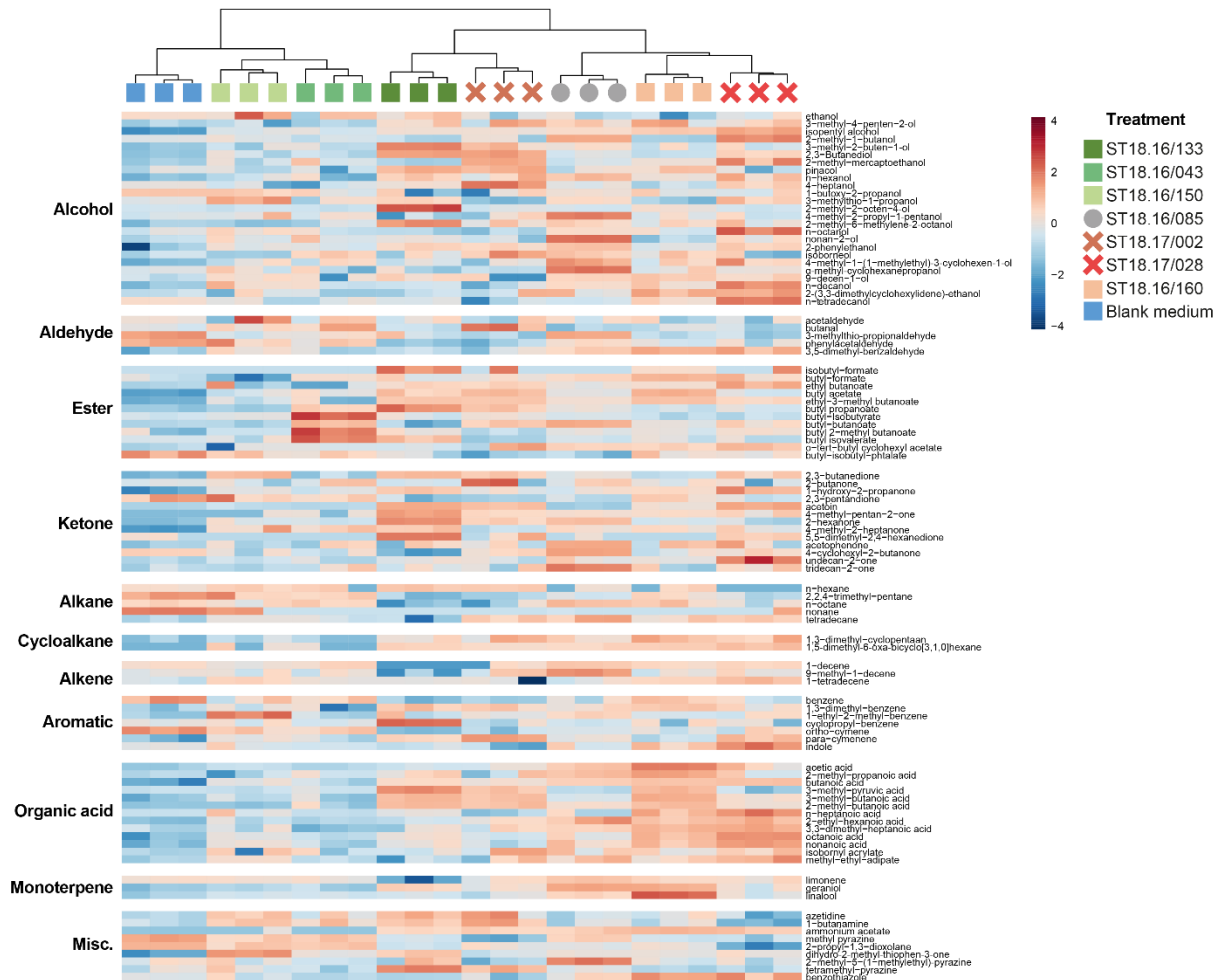


Figure S2. Heat map of the mVOC composition of seven bacterial strains investigated in this study ($n = 3$). Symbol colours indicate the effect of the mVOCs on the olfactory response of the primary parasitoid *Aphidius colemani*, i.e. green = attractive, grey = neutral, and red = repellent. Blue refers to the blank medium. Symbol shapes indicate the effect of the mVOCs on the olfactory response of the hyperparasitoid *Dendrocerus aphidum*, i.e. circle = attractive, square = neutral and cross = repellent