

# *Concurrent anthropogenic air pollutants enhance recruitment of a specialist parasitoid*

Article

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1 **Concurrent anthropogenic air pollutants enhance recruitment of a specialist parasitoid**

2

3 James M.W. Ryalls<sup>1</sup>, Lisa M. Bromfield<sup>1</sup>, Luke Bell<sup>1</sup>, Jake Jasper<sup>2</sup>, Neil J. Mullinger<sup>3</sup>, James D. Blande<sup>4</sup>  
4 and Robbie D. Girling<sup>1</sup>

5

6 <sup>1</sup>School of Agriculture, Policy and Development, University of Reading, Whiteknights, Earley Gate,  
7 Reading, Berkshire, RG6 6EU, UK

8 <sup>2</sup>School of Chemistry, Food and Pharmacy, University of Reading, PO Box 226, Whiteknights,  
9 Reading, Berkshire, RG6 6AP, UK

10 <sup>3</sup>UK Centre for Ecology and Hydrology, Bush Estate, Penicuik, Midlothian, EH26 0QB, UK

11 <sup>4</sup>Department of Environmental and Biological Sciences, University of Eastern Finland, P. O. Box 1672,  
12 70211, Kuopio, Finland

13

14 **Running title:** Air pollutants differentially modify parasitoid recruitment

15

16 **Abstract**

17 Air pollutants, such as nitrogen oxides, emitted in diesel exhaust, and ozone, disrupt interactions  
18 between plants, the insect herbivore pests that feed upon them, and natural enemies of those  
19 herbivores (e.g., parasitoids). Using eight field-based rings that emit regulated quantities of diesel  
20 exhaust and ozone, we investigated how both pollutants, individually and in combination, altered  
21 the attraction and parasitism rate of a specialist parasitoid (*Diaeretiella rapae*) on aphid-infested and  
22 un-infested *Brassica napus* plants. Individual effects of ozone decreased *D. rapae* abundance and  
23 emergence by 37% and 55%, respectively, compared with ambient (control) conditions. When ozone  
24 and diesel exhaust were emitted concomitantly, *D. rapae* abundance and emergence increased by  
25 79% and 181%, respectively, relative to control conditions. This attraction response occurred  
26 regardless of whether plants were infested with aphids and was associated with an increase in  
27 concentration of aliphatic glucosinolates, especially gluconapin (3-butenyl glucosinolate), within *B.*  
28 *napus* leaves. Plant defensive responses and their ability to attract natural aphid enemies may be  
29 beneficially impacted by pollution exposure. These results demonstrate the importance of  
30 incorporating multiple air pollutants when considering the effects of air pollution on plant-insect  
31 interactions.

32

33 **Keywords:** Air pollution; aphid population; diesel exhaust; glucosinolates; ozone; parasitoid  
34 recruitment

35 **Introduction**

36 Insects utilise a variety of stimuli when interacting with their environment but rely heavily upon  
37 olfactory stimuli and particularly volatile organic compounds (VOCs) to perceive and interact with  
38 other organisms. As such, VOCs are used during critical stages of the life cycles of many insects, such  
39 as for locating hosts, food or mates [1]. Common atmospheric pollutants, such as nitrogen oxides  
40 (NO and NO<sub>2</sub>, collectively NO<sub>x</sub>) that are released from diesel vehicle exhausts, and ozone (O<sub>3</sub>), are  
41 capable of chemically altering many of the VOCs that insects use for communication [2]. Disruption  
42 of these VOC cues may have wide ranging impacts on the important ecosystem services that insects  
43 provide (e.g., pest-regulation services or pollination), which are critical for the functioning of  
44 terrestrial ecosystems.

45

46 Parasitic wasps (or parasitoids) are a provider of critical pest-regulation services, both in natural  
47 ecosystems and in many horticultural and arable cropping systems [3]. Their larvae live as parasites  
48 on or in other host insects (which can be crop pests), feeding upon them until the host dies [4].  
49 Parasitoids of insect herbivores locate their herbivore hosts using VOC cues released by: i) the  
50 herbivores and, ii) the herbivore's host plants, which are produced and released in response to  
51 herbivore feeding [5]. Air pollution has been shown, in laboratory assays, to negatively affect the  
52 ability of parasitoids to locate their insect hosts, because the pollutants: i) react with and chemically  
53 change the VOCs released by plants and insects in the air, and ii) cause physiological changes to the  
54 plants, altering the VOCs that the plants release [6, 7]. While these laboratory studies have been  
55 critical for identifying the mechanisms by which air pollution affects herbivore-parasitoid  
56 interactions, little is known about the ecological impacts of air pollution on these odour-mediated  
57 interactions, and whether the efficiency of pest-regulation (i.e., parasitoid recruitment) under field  
58 conditions would be affected by their disruption. Recent reviews [8, 9] have called for studies to  
59 bridge the gap in our knowledge on how VOCs influence the process of host location by parasitoids  
60 at larger spatial scales. In addition, there have also been calls for more work on the combined effects  
61 of different air pollutants [10]; emissions of NO<sub>x</sub> occur alongside elevations in O<sub>3</sub> [11 and references  
62 therein], yet no studies to date have considered their combined effects on multi-trophic  
63 interactions.

64

65 The effects of air pollution on VOC-mediated interactions is of increasing concern because global  
66 tropospheric background O<sub>3</sub> concentrations are rising [12]. Concentrations of O<sub>3</sub> are typically higher  
67 in rural areas than urban areas because in urban areas there is more NO<sub>x</sub> pollution from vehicles and  
68 industry, and O<sub>3</sub> and NO<sub>x</sub> readily react with one another, reducing O<sub>3</sub> concentrations [13]. NO<sub>x</sub>

69 emissions remain a serious problem, with areas throughout the UK continually exceeding limits  
70 imposed by the EU Ambient Air Quality Directive [14]. Transportation accounts for the majority of  
71 NO<sub>x</sub> emissions in the UK (e.g., 47% in 2017; [15]) and many of the latest 'Euro 6' diesel cars that have  
72 been approved for sale continue to exceed air pollution limits (RDE test data; [16]) so diesel exhaust  
73 pollution is likely to remain a problem for decades to come [17]. Furthermore, as urbanisation and  
74 traffic congestion increases, those critical ecosystem services delivered by insects that rely upon  
75 odour-mediated interactions, such as pest-regulation and pollination, may be at increased risk [13,  
76 14, 18]. Long-term, this risk may diminish as diesel exhaust emission sources reduce, at which point  
77 urban environments and polluted rural areas (e.g. those next to major roads) will have to contend  
78 with relatively higher levels of O<sub>3</sub> (because less O<sub>3</sub> is quenched by NO<sub>x</sub> [19, 20]) and its effect on the  
79 natural ecosystem services that we rely on [10].

80

81 Using a unique set of Free-Air Diesel and Ozone Exposure (FADOE) rings over two years, this study  
82 aimed to determine how changes in diesel exhaust and O<sub>3</sub> pollution, individually and in combination,  
83 shape populations of insect herbivore pests (cabbage aphids, *Brevicoryne brassicae* Linnaeus.) and  
84 their parasitoids (specifically, *Diaeretiella rapae* MacIntosh). We used oilseed rape (or OSR; *Brassica*  
85 *napus* L.) as our model plant, which is commonly attacked by *B. brassicae* and is the most  
86 economically important brassica species in Europe [21]. The native European *B. brassicae*, now  
87 distributed throughout the world, are specialist feeders on Brassicaceous crops (e.g., OSR, cabbage,  
88 broccoli, cauliflower etc.). The parasitoid *D. rapae* commonly targets *B. brassicae* and has been  
89 shown to be attracted to aphid- and plant-released glucosinolate hydrolysis products that act as  
90 indicators of host presence [22-24], making these insects ideal model species for investigating the  
91 mechanisms underpinning the effects of diesel exhaust and O<sub>3</sub> on tri-trophic interactions. We  
92 identified the total number of parasitoids attracted to aphid-infested and non-infested plants under  
93 each pollution scenario (diesel exhaust, O<sub>3</sub>, diesel exhaust plus O<sub>3</sub>, and control) and recorded the  
94 abundance and oviposition success of the parasitoid, *D. rapae*. As such, we hypothesised that air  
95 pollution would result in reduced abundances of all species of parasitoid, including *D. rapae*, by  
96 reacting with and depleting the VOCs that parasitoids use to locate their aphid hosts, and as a result  
97 decreased parasitism rates of their aphid hosts. Phloem-feeding aphids tend to respond positively, in  
98 terms of growth/reproductive rate, to pollution-mediated changes in plant quality due to stress-  
99 related increases in nitrogen-containing compounds and/or decreases in plant defensive compounds  
100 [25]; consequently, reductions in parasitism may act to further increase their pest status [18, 26].  
101 We therefore hypothesised that pollution-mediated increases in aphid abundance (resulting in  
102 increases of aphid-emitted VOCs) would counteract the negative effects of air pollution on

103 parasitoid abundance. We also examined whether the concentrations of specific glucosinolates in  
104 leaf tissue changed as a result of exposure to pollution and, if so, whether they were correlated with  
105 any changes in parasitoid abundance. Understanding how air pollution could modify pest–parasitoid  
106 interactions in the field at temporal and spatial scales that are relevant to the insects could provide  
107 greater insight into how current or future levels of air pollution may mediate and influence insect  
108 pest outbreaks.

109

110

## 111 **Materials and methods**

### 112 *Insect cultures and plant material*

113 Four *Brevicoryne brassicae* aphid cultures were established from a single parthenogenetic adult  
114 female collected from an OSR field at Sonning Farm (latitude 51.480330, longitude -0.899504).  
115 Cultures were maintained at 20 °C on propagated OSR (cv. Tamarin, sourced from Senova,  
116 Cambridge, UK) for at least six generations (c. 8 weeks) prior to the experiment. For the experiment,  
117 OSR plants (cv. Tamarin) were grown from seed in 100 mL round cell trays in glasshouse rooms  
118 receiving natural light. After four weeks, plants were transplanted to 18 cm diameter pots containing  
119 c. 2.7 kg of vegetable topsoil (Quality Garden Supplies Ltd., Staffordshire, UK) and white mesh  
120 (organza) nets (55 x 75 cm) were placed over the plants and attached tightly around the rim of all  
121 pots to prevent any insect damage under field conditions. Bamboo sticks were placed inside the nets  
122 to prevent contact between the leaves and the mesh.

123

### 124 *Field conditions and experimental procedures*

125 In 2018, eight FADOE octagonal rings (8 m in diameter) were constructed at the University of  
126 Reading's Sonning farm within a field of winter wheat (*Triticum aestivum* cv. Skyfall), which  
127 maximised weed control (i.e. prevented weeds from growing, which themselves could have emitted  
128 different VOCs that may have altered the odour landscape inconsistently across the field). The  
129 centre of each ring was positioned 46 m from the centre of the field (latitude 51.482853, longitude -  
130 0.897749) in an octagonal formation, such that each ring was separated by a distance of at least 30  
131 m. Full details of the FADOE configuration and layout are reported in [11]. Two rings were assigned  
132 to each of four treatments: i) diesel exhaust (D), ii) O<sub>3</sub>, iii) diesel exhaust and O<sub>3</sub> combined (D+O<sub>3</sub>),  
133 and iv) ambient air control. Concentrations of nitric oxide (NO), nitrogen dioxide (NO<sub>2</sub>), nitrogen  
134 oxides (NO<sub>x</sub> = NO + NO<sub>2</sub>) and O<sub>3</sub> were monitored continuously and automatically maintained at field-  
135 realistic levels. The target concentrations were 120 ppb NO<sub>x</sub> (based on average concentrations  
136 adjacent to major UK roadways and urban areas; [27]) and 90 ppb O<sub>3</sub> (based on peak concentrations

137 recorded in rural European sites in 1990-2012; [28]) but average concentrations achieved within the  
138 rings were significantly lower than these, as described in the results. Diesel and O<sub>3</sub> generators were  
139 turned on for up to 17 hours of the day (between 4.30 am and 9.30 pm), during which oviposition  
140 rates of *D. rapae* parasitoids are highest (females oviposit over 96% of their total eggs during the  
141 photophase; [29]). In 2019, the FADOE rings were moved to an adjacent field of wheat (latitude  
142 51.482374, longitude -0.895855) and rotated within the field to account for the effects of pseudo-  
143 replication. A total of three experimental runs (described below) were undertaken, one in  
144 September-October 2018 and two in September-October 2019. Natural environmental conditions,  
145 including air temperature, wind speed and wind direction were monitored continuously throughout  
146 the experiment.

147

148 Netted OSR plants (16 plants in the first and third experimental runs and 28 plants in the second  
149 experimental run) were placed in each of the eight FADOE rings in four random groups (four plants  
150 per group in the first and third experimental runs and seven plants per group in the second  
151 experimental run; Fig. 1a). When plants were five weeks old (i.e. one week after plants were  
152 transplanted into pots within the rings), each plant in two groups (aphid treatments A10 and OPEN)  
153 was inoculated with 10 teneral adult *B. brassicae* aphids. A further group was inoculated with 50  
154 aphids (A50) and the final group remained insect-free (CON). The aphids were left to establish for  
155 one week on the netted plants before placing a sticky trap (22 cm x 10 cm) on a stake in the centre  
156 of each group (outside of the nets), in order to capture naturally-occurring parasitoids. After a  
157 further week, the first set of sticky traps were stored at -20°C and replaced in all but the OPEN  
158 group. The nets from the OPEN group were removed so that the aphids were exposed (Fig. 1a). After  
159 a further week, the plants in the OPEN group were re-netted and a sticky trap was positioned inside  
160 the net of each plant to catch emerging parasitoids. The second set of sticky traps were collected  
161 from the three other treatments (A10, A50 and CON) on the same day and stored at -20°C until  
162 required. Aphids were removed from each plant within these three groups using a pooter and stored  
163 in 60 mL pots at -20°C before being freeze-dried for 72 h and weighed. Plants were oven-dried at  
164 70°C and weighed. The sticky traps from inside each of the nets of the OPEN plants were collected  
165 10 days later and stored at -20°C. *Diaretiella rapae*, as well as all other parasitoids, were identified  
166 and counted from all sticky traps, from which *D. rapae* was easily identified by its distribution and  
167 wing structure [30, 31]. Therefore, parasitism rate (i.e. parasitoid emergence of *D. rapae*) was  
168 recorded from the OPEN treatment only, and parasitoid abundance (i.e. total parasitoid abundance  
169 and the abundance of *D. rapae*) was recorded from the three other treatments (A10, A50 and CON).  
170 The timeline of experimental events is visualised in Fig. 1b.



171

## 172 *Chemical analysis*

173 For the second experimental run, three additional plants were added to each treatment within each  
174 ring for purposes of chemical analyses. Therefore, all seven plants in the A10, A50 and OPEN  
175 treatments were inoculated with aphids (described above). Upon harvest, three plants were  
176 selected from each of the A50 and CON treatments (Fig. 1a), from which four intact leaves were  
177 removed, freeze-dried and ground for glucosinolate analysis using liquid chromatography-mass  
178 spectrometry (LC-MS), following the protocol set out by [32]. Seven glucosinolates were detected  
179 using this method. Based on their side-chain structure and amino acid precursors [33], these  
180 glucosinolates were classified as aliphatic (glucoalyssin, progoitrin, glucobrassicinapin, gluconapin)  
181 and indolic (glucobrassicin, neoglucobrassicin, 4-methoxyglucobrassicin).

182

183 Solid phase micro-extraction gas chromatography mass spectrometry (SPME-GC-MS) was used to  
184 determine VOC relative abundances in fresh OSR leaf samples collected from three plants in each of  
185 the A50 and CON (i.e. no aphids) treatments within each ring. Sample preparation, headspace  
186 extraction from macerated leaves, chromatography and mass spectrometry conditions were as  
187 presented by [34]. VOCs were identified or tentatively identified by comparison of each mass  
188 spectrum with authentic compounds, or the NIST mass spectral database (NIST/EPA/NIH Mass  
189 Spectral database, 2014). A spectral quality value >80 was used alongside linear retention index (LRI)  
190 to support the identification of compounds where no authentic standards were available. All peak  
191 areas were normalised. LRI was calculated for each VOC using the retention times of a homologous  
192 series of C<sub>6</sub>-C<sub>25</sub> *n*-alkanes and by comparing the LRI with those of authentic compounds analysed  
193 under similar conditions.

194

## 195 *Statistical analysis*

196 Air pollution and aphid treatment effects on plant mass, final aphid population mass, parasitoid  
197 abundance and emergence, and glucosinolate concentrations were analysed using mixed models in  
198 the *lme4* statistical package [35] within the R statistical interface v4.1.1. The fixed effects included  
199 air pollution treatment (control, O<sub>3</sub>, D and D+O<sub>3</sub>) and aphid treatment (No aphids, A10 and A50) as  
200 well as the two-way interaction between these terms. The random terms included year, run and ring  
201 location to account for seasonal and spatial differences that could confound any treatment effects  
202 and their inclusion was confirmed by model reductions using AIC and QQ plots. Negative binomial  
203 models were used for dependent variables with count data (numbers of *D. rapae* and other  
204 parasitoids and numbers of *D. rapae* that emerged from parasitised aphids) based on model

205 deviance and critical chi-squared values. Where appropriate, response variables were transformed  
206 before analysis (Table S1) to standardise residuals, which were confirmed with AIC and QQ plots. The  
207 model df, total number of observations not accounting for replication ( $N_{\text{obs}}$ ) and group N (i.e.  $N_{\text{group}}$ ;  
208 Year/Run/Ring = 24) are included in all reported model statistics. Parasitoids captured in sticky traps  
209 within the OPEN group prior to net removal were not included in these analyses. Pairwise  
210 comparisons of means for treatment effects were made with Tukey's post hoc tests utilising the *glht*  
211 function in R's 'multcomp' package [36] Pearson's correlation tests using the R base function  
212 *cor.test* were used to determine whether indolic, aliphatic and total glucosinolate concentrations  
213 were correlated with average *D. rapae* abundances for each ring. Normalized VOC abundances were  
214 analysed using XLSTAT (Addinsoft, Paris, France) protected Analysis of Variance (ANOVA) with post  
215 hoc Tukey's Honestly Significant Difference (HSD) pairwise comparison ( $P < 0.05$ ). As such, if the  
216 ANOVA model is not significant, post hoc pairwise comparisons are 'protected' from  
217 overinterpretation (e.g. through the generation of Type I statistical errors) by not being calculated,  
218 and simply stated as being non-significant. This approach has been used previously in relation to  
219 glucosinolate and VOC data in Brassicaceae plants [37].

220

221

## 222 **Results**

### 223 *Air pollutant concentrations*

224 Concentrations of  $\text{NO}_x$  and  $\text{O}_3$  recorded during the experimental period of September 2018 and 2019  
225 are reported and analysed in [38]. In short, individual pollution rings averaged low to moderate  
226 concentrations (as defined by DEFRA Air Quality Index; [39]) of  $48.63 (\pm 1.39 \text{ SE})$  ppb  $\text{NO}_x$  and  $38.85$   
227  $(\pm 1.30 \text{ SE})$  ppb  $\text{O}_3$  in the individual D and  $\text{O}_3$  treatment rings, respectively, compared with  $7.35 \pm$   
228  $0.18$  ppb  $\text{NO}_x$  and  $21.74 \pm 0.28$  ppb  $\text{O}_3$  in the ambient (control) rings. In the combined (D+ $\text{O}_3$ )  
229 pollution treatment,  $\text{NO}_x$  concentrations ( $34.14 \pm 1.14$  ppb) were significantly lower than those in D,  
230 associated with the interaction between atmospheric  $\text{NO}_x$  and  $\text{O}_3$  and the conversion of nitrogen  
231 dioxide to nitric oxide (see [11, 38] for details). Moreover, the concentrations of  $\text{O}_3$  in the D+ $\text{O}_3$   
232 treatment ( $20.38 \pm 0.27$  ppb) were 48% lower than the  $\text{O}_3$  treatment, decreasing to levels equivalent  
233 to those in the control treatment.

234

### 235 *Aphid population and plant mass under air pollution and aphid treatments*

236 Aphid population mass was not significantly affected by air pollution but was significantly higher in  
237 the A50 treatment compared with the A10 treatment (Fig. 2). Air pollution and aphid treatments,  
238 individually and in interaction, had no significant effects on plant mass (Table S1).

239

240 *Parasitoid responses to air pollution and aphid treatments*

241 *Diaeretiella rapae* parasitoid abundance was significantly affected by air pollution ( $\chi^2_{3,10} = 20.00$ ,  
242  $P < 0.001$ ,  $N_{\text{obs}} = 72$ ,  $N_{\text{group}} = 24$ ), whereby *D. rapae* decreased under  $O_3$  but increased under  $D+O_3$   
243 compared with ambient (control) conditions (Fig. 3). The abundance of other parasitoids, in contrast,  
244 significantly decreased under all three pollution treatments relative to the control treatment ( $\chi^2_{3,10} =$   
245  $8.35$ ,  $P = 0.039$ ,  $N_{\text{obs}} = 72$ ,  $N_{\text{group}} = 24$ ). Air pollution had a significant effect on the percentage of all  
246 parasitoids that were *D. rapae* ( $\chi^2_{3,10} = 25.68$ ,  $P < 0.001$ ,  $N_{\text{obs}} = 72$ ,  $N_{\text{group}} = 24$ ), which increased  
247 significantly under both diesel treatments (D and  $D+O_3$ ) but not under  $O_3$  (Fig. 3).

248

249 The abundance of *D. rapae* significantly increased when aphids were present (i.e., plants that were  
250 initially infested with 10 and 50 aphids) compared with plants that were not inoculated with aphids  
251 ( $\chi^2_{2,10} = 16.60$ ,  $P < 0.001$ ,  $N_{\text{obs}} = 72$ ,  $N_{\text{group}} = 24$ ). The abundance of other parasitoids increased when  
252 plants were infested with 50 aphids but not when plants were infested with 10 aphids. There were  
253 no interactive effects of air pollution and aphid treatment on parasitoids. Full statistical results are  
254 shown in Table S1.

255

256 The number of *D. rapae* that successfully emerged from parasitised aphids (OPEN plants) was  
257 significantly affected by air pollution ( $\chi^2_{3,8} = 25.39$ ,  $P < 0.001$ ,  $N_{\text{obs}} = 120$ ,  $N_{\text{group}} = 24$ ), whereby *D.*  
258 *rapae* emergence decreased under  $O_3$  and increased under  $D+O_3$  (Fig. 4).

259

260 *Plant glucosinolate concentrations*

261 Total glucosinolate concentrations in leaves of OSR increased in the combined  $D+O_3$  treatment ( $\chi^2_{3,8}$   
262  $= 11.89$ ,  $P = 0.008$ ,  $N_{\text{obs}} = 133$ ,  $N_{\text{group}} = 24$ ), which was driven by increases in aliphatic glucosinolates  
263 ( $\chi^2_{3,8} = 14.57$ ,  $P = 0.002$ ,  $N_{\text{obs}} = 133$ ,  $N_{\text{group}} = 24$ ), especially gluconapin (3-butenyl-glucosinolate: Fig.  
264 5). Concentrations of indolic glucosinolates did not vary significantly between pollutants or aphid  
265 treatments. Full statistical results are shown in Table S1. Concentrations of aliphatic glucosinolates  
266 were positively correlated with the abundance of *D. rapae* that were attracted to aphid-infested  
267 plants, which was driven by the positive association between gluconapin and *D. rapae* abundance  
268 (Fig. 6).

269

270 *Plant volatile organic compound relative abundances*

271 Pollution treatment and aphid infestation had a significant effect on 21 of the 44 VOC compounds  
272 identified (Table S2) and there was a two-way interactive effect of air pollution and aphid treatment

273 on six VOC compounds (Table S3). In general, VOCs from plants that were infested with aphids and  
274 subjected to both O<sub>3</sub> and diesel exhaust combined were significantly higher than other aphid and/or  
275 pollution treatment combinations. For example, normalized peak areas of four methyl esters (methyl  
276 hexanoate, 2-hexenoic acid, methyl octanoate and (Z)-3-hexenyl isobutyrate), one alcohol (2-hexen-  
277 1-ol), two aldehydes ((E)-tiglaldehyde and (E,E)-2,4-heptadienal), dimethyl disulfide and hexanoic  
278 acid were significantly higher in aphid-infested D+O<sub>3</sub>-fumigated plants compared with un-infested  
279 D+O<sub>3</sub>-fumigated plants. Furthermore, normalized peak areas of three methyl esters (methyl  
280 hexanoate, 2-hexenoic acid and methyl octanoate), two ketones (3-pentanone and β-ionone), two  
281 aldehydes ((E)-tiglaldehyde and (E,E)-2,4-heptadienal) and dimethyl disulfide were significantly  
282 higher in aphid-infested D+O<sub>3</sub>-fumigated plants compared with aphid-infested plants under ambient  
283 (control) conditions (Table S2).

284

## 285 **Discussion**

286 Exposure to diesel exhaust and O<sub>3</sub> pollutants had no clear effect on the population mass of aphids  
287 that were not exposed to natural enemies, yet these pollutants, in isolation, had opposing effects on  
288 the parasitism rate of *D. rapae* and their attraction to aphid-infested plants. In particular, we  
289 demonstrated negative effects of O<sub>3</sub> on parasitoid recruitment, mirroring effects which have been  
290 reported by others [18, 40, 41] and that are generally considered to be a result of the degradation of  
291 behaviourally important plant-released VOCs [42]. However, it is also possible that air pollution  
292 could physiologically alter VOC perception and directly impair insect health or motility [43-46]. In the  
293 current study, plants exposed to diesel exhaust, alone and in combination with O<sub>3</sub>, were generally  
294 more attractive to *D. rapae*, and those aphids on plants exposed to both pollutants experienced  
295 higher rates of parasitism. This change in attraction, which was especially pronounced under the  
296 combined pollution treatment, occurred regardless of whether the plants were aphid-infested or  
297 not, indicating that changes in plant-released VOCs, as opposed to insect-emitted VOCs (i.e., those  
298 released from aphids directly), are more likely be responsible for the increased attraction of *D.*  
299 *rapae*. It is also possible that compounds within the pollution mix attracted *D. rapae* directly and, as  
300 such, further studies examining the direct impacts of concurrent air pollutants on the behaviour of  
301 parasitoids will be essential for mechanistically determining how parasitoids will respond to changes  
302 in the atmosphere as we shift away from fossil fuel dependence.

303

304 The combined pollution treatment significantly increased plant glucosinolate concentrations,  
305 especially of the aliphatic glucosinolate gluconapin (3-butenyl-glucosinolate). These changes in leaf  
306 tissue concentrations suggest that the plants modified their glucosinolate production as a stress-

307 induced response to their exposure to both pollutants simultaneously [47, 48]. Increases in  
308 concentrations of gluconapin were positively correlated with increases in abundance of *D. rapae*.  
309 The hydrolysis product of gluconapin, 3-butenyl isothiocyanate, has been shown to act as an  
310 attractant for *D. rapae* in previous studies [23, 24]. We did not identify any glucosinolate hydrolysis  
311 products in the headspace of macerated OSR leaves, although it is possible that parasitoids may be  
312 attracted to hydrolysis products (i.e., isothiocyanates) that were below the detection threshold of  
313 instrumentation. OSR has typically been bred to contain low concentrations of glucosinolates and  
314 isothiocyanates [49], making them challenging to detect; however, further studies using non-  
315 destructive headspace sampling from whole living tissue (e.g., [5, 50]) could more effectively mimic  
316 the VOC emissions that the parasitoids would be exposed to and provide the identities of additional  
317 VOCs that may contribute to parasitoid recruitment [49]. The increase in some VOCs within the leaf  
318 tissue of OSR when aphids were present and when plants were fumigated with both pollutants  
319 provides further evidence to suggest a stress-induced systemic impact on the secondary metabolism  
320 of OSR.

321

322 Air pollution-mediated changes in the proportion of *D. rapae* in the parasitoid assemblage has the  
323 potential to impact the structure of insect communities associated with OSR and other brassica  
324 species via changes in pest regulation [51]. Parasitoids other than *D. rapae* differed in their response  
325 to air pollution and tended to decrease in all pollution treatments. These contrasting responses of *D.*  
326 *rapae*, a specialist parasitoid of *Brassica*-feeding aphids [23, 52], when compared with responses of  
327 other parasitoids to air pollution suggests that differences could be species-specific or a function of  
328 their diet (i.e., whether they are specialist or generalist feeders that target few or multiple host  
329 Orders, respectively). As specialist parasitoids (i.e. those with preferred prey limited to one or a few  
330 related host species [53, 54]), *D. rapae* may be more likely to respond to air pollution-mediated  
331 changes in VOCs because they tend to respond to a restricted set of stimuli, specific to their aphid  
332 hosts and the plants they feed upon [41, 52, 55]. Generalist parasitoids that target multiple hosts  
333 that may feed on many different plants, in contrast, may be less likely to rely on specific stimuli [38,  
334 56]) but instead utilise a range of different VOCs that may be more prone to being degraded by  
335 oxidising air pollutants. It is also possible that specific VOCs are induced by host herbivory, therefore  
336 enhancing the signal that conveys host presence for *D. rapae* specifically.

337

338 Regardless of the specific mechanism used, parasitoids may be able to adapt to forage in polluted  
339 atmospheres by learning to associate altered VOC emission profiles with their target host [57]. As  
340 such, studies comparing parasitoids originating from polluted and unpolluted environments could

341 quantify their ability to adapt, which is also likely to differ according to their diet specialisation.  
342 Comparing generalist and specialist parasitoids originating from urban environments with those  
343 originating from more rural environments would be a useful next step. In general, mechanistically  
344 identifying how groups of generalist and specialist parasitoid species will respond to air pollution,  
345 using a combination of controlled laboratory studies and longer-term population studies, could  
346 contribute to the targeted formation and/or release of specific compounds that effectively attract  
347 natural enemies and reduce herbivore populations. This would be especially valuable for protected  
348 (i.e. crops grown under glass or plastic) and high-value crops that more often rely on biological  
349 control for herbivore pest management [58, 59]. From a wider ecological perspective, declines in the  
350 abundance of parasitoids other than *D. rapae* within polluted environments is likely to enhance the  
351 pest status of aphids and other plant pests in general, with negative consequences for food security.

352

### 353 **Conclusions**

354 Both diesel exhaust and O<sub>3</sub> pollution, individually and in combination, had deleterious effects on the  
355 abundance of parasitoid species other than *D. rapae*. In contrast, we demonstrated significant  
356 increases in *D. rapae* parasitoid recruitment under the combined effects of diesel exhaust and O<sub>3</sub>  
357 pollution, which contrasted with our hypothesis that both pollutants would deplete the VOCs that  
358 these parasitoids use to find their aphid hosts. This attraction response by *D. rapae* to diesel  
359 exhaust- and O<sub>3</sub>-polluted environments is likely associated with an increase in the aliphatic  
360 glucosinolate, gluconapin, in OSR leaves, which is the precursor of an isothiocyanate which is  
361 attractive to *D. rapae*. These results stress the importance of studies incorporating the effects of  
362 multiple pollutants occurring in tandem in the natural environment. Concentrations of NO<sub>x</sub> and O<sub>3</sub>  
363 emitted from the FADOE rings were lower than those considered safe under current air quality  
364 standards, emphasising how only moderate levels of air pollution can have significant impacts on  
365 plant-parasitoid dynamics. Shifting to sustainable energy generation and electrifying the fleet of  
366 diesel vehicles within the next two decades will again significantly alter the levels of atmospheric  
367 pollutants at times of peak daily activity of important ecosystem service providers, including those  
368 providing important pest-regulation services. A mechanistic understanding of how these service  
369 providers will respond to air pollution is, therefore, a vital, but hitherto neglected, component  
370 required to aid understanding and prediction of pest outbreaks.

371

372

### 373 **Data accessibility**

374 Data deposited in the EIDC Digital Repository (doi to follow).

375

### 376 **Authors' contributions**

377 JMWR conceived the experimental design, carried out data collection, carried out data analyses and  
378 drafted the manuscript. LMB participated in data collection. NJM contributed to the maintenance of  
379 the FADOE facility. LB and JJ performed chemical analysis and LB critically revised the manuscript.  
380 JDB participated in the design of the study and critically revised the manuscript. RDG participated in  
381 the design of the study and participated in drafting the manuscript. All authors gave final approval  
382 for publication and agree to be held accountable for the work performed therein.

383

### 384 **Competing interests**

385 We declare we have no competing interests.

386

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393

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398

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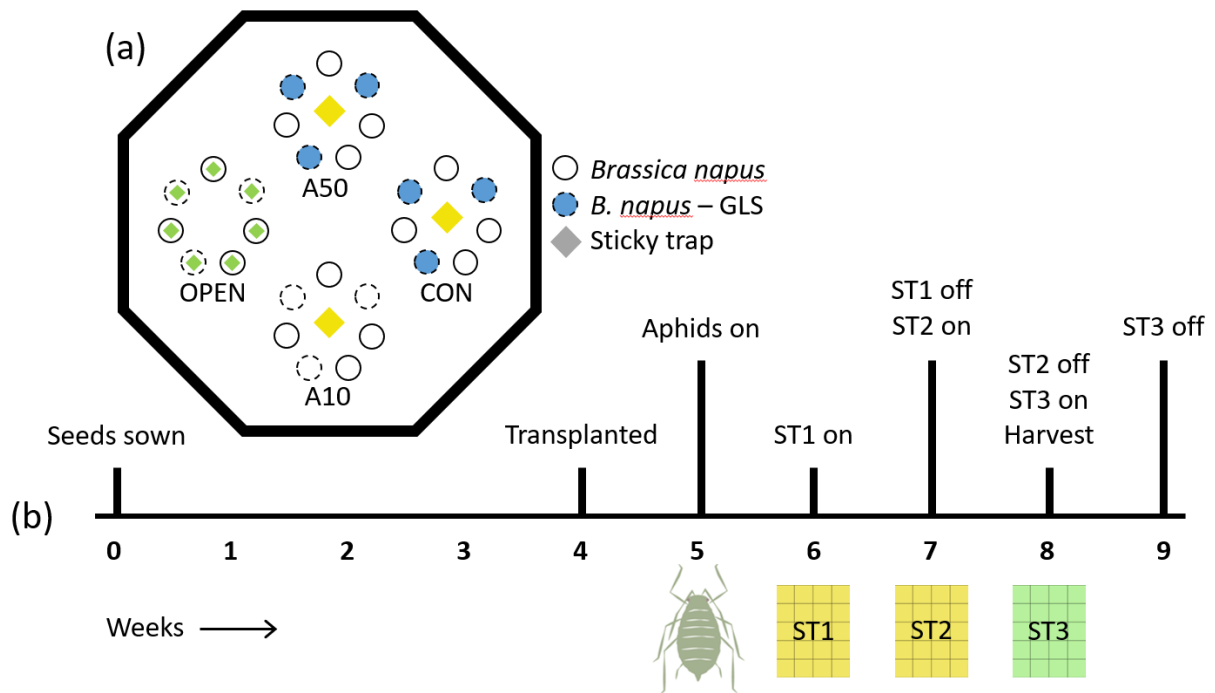
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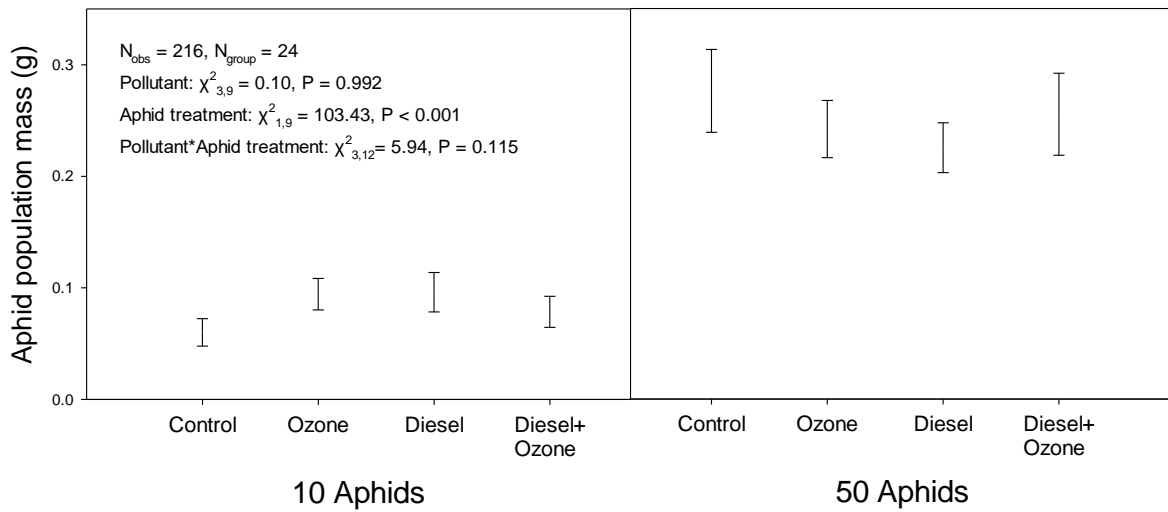
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564

565 **Fig. 1.** Experimental layout and timeline of events. Plants and aphid treatments within the eight  
566 individual rings are shown for the second experimental run (a), with “*B. napus* – GLS” referring to  
567 OSR plants that were selected for glucosinolate analysis. The first and third experimental runs  
568 included four plants for each aphid treatment. Therefore, circles with dotted lines indicate plants  
569 that were included in the second experimental run only. The timeline of events (b) was the same for  
570 all three experimental runs. “ST” refers to sticky trap. Parasitoid numbers on ST1 and ST2 (yellow  
571 diamonds) were pooled, and ST3 (green diamonds) was used to trap emerging parasitoids from  
572 OPEN plants only.

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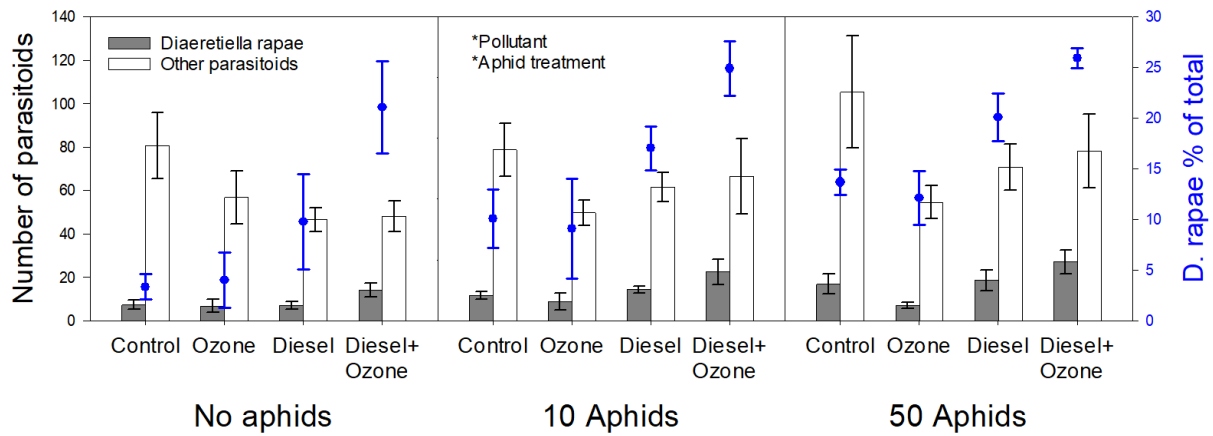
10 Aphids

50 Aphids

575 **Fig. 2.** Aphid (*Brevicoryne brassicae*) final population mass under air pollution (control, ozone, diesel  
 576 exhaust or diesel exhaust and ozone) and aphid treatments (either 10 aphids or 50 aphids added as a  
 577 parent population at the start of the experiment to *Brassica napus* plants) three weeks after aphid  
 578 inoculation. Values are means  $\pm$  SE. Statistical effects of treatments, and their interaction, on aphid  
 579 population mass shown.  $N_{obs}$  = total number of observations.  $N_{group}$  = group number associated with  
 580 the random effects of Year/Run/Ring.

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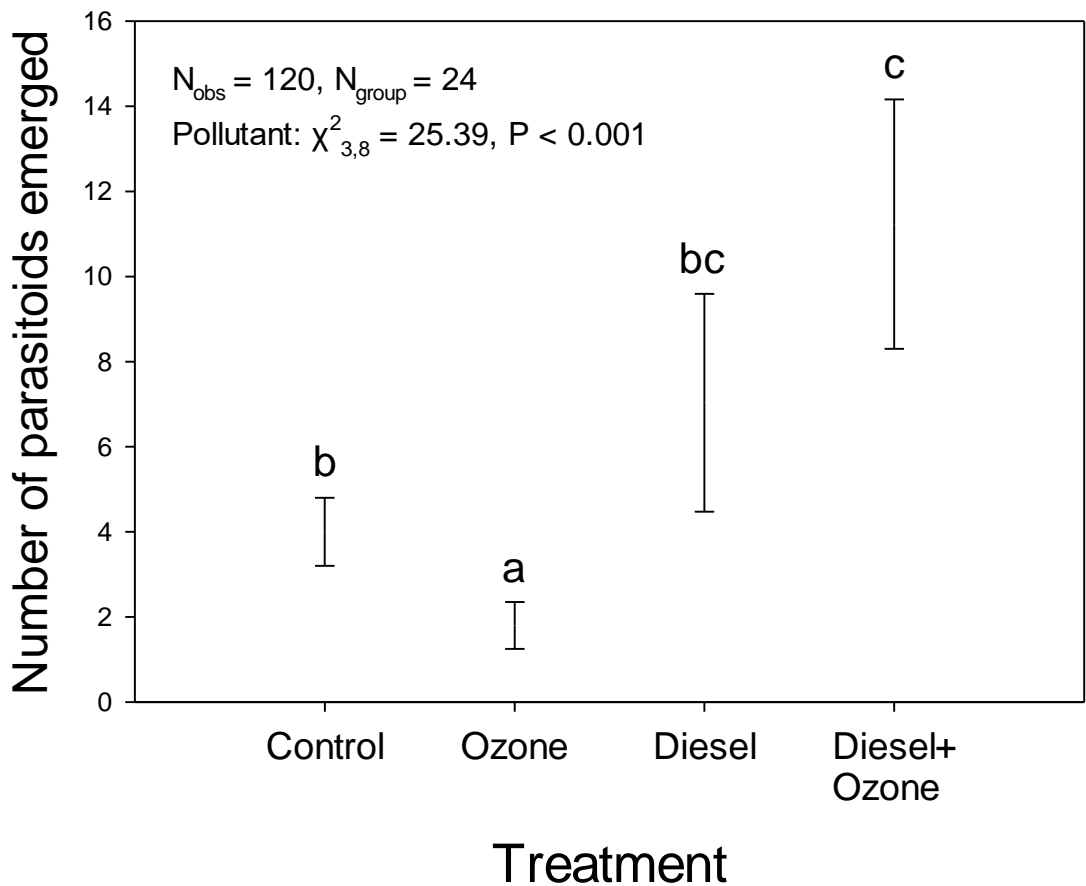
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583

584 **Fig. 3.** Parasitoid abundance (left axis) and the percentage of total parasitoids that were *Diaeretiella*  
585 *rapae* (right axis, blue) under air pollution (control, ozone, diesel exhaust or diesel exhaust and  
586 ozone) and aphid treatments (i.e., plots with no *Brevicoryne brassicae* aphids, and either 10 aphids  
587 or 50 aphids added as a parent population to *Brassica napus* plants) after two weeks. Values are  
588 means  $\pm$  SE. \* indicates significant effects ( $P > 0.05$ ) for all three dependent variables. Pairwise  
589 comparisons for the individual effects of air pollution and aphid treatments are shown in Figure S1.

590

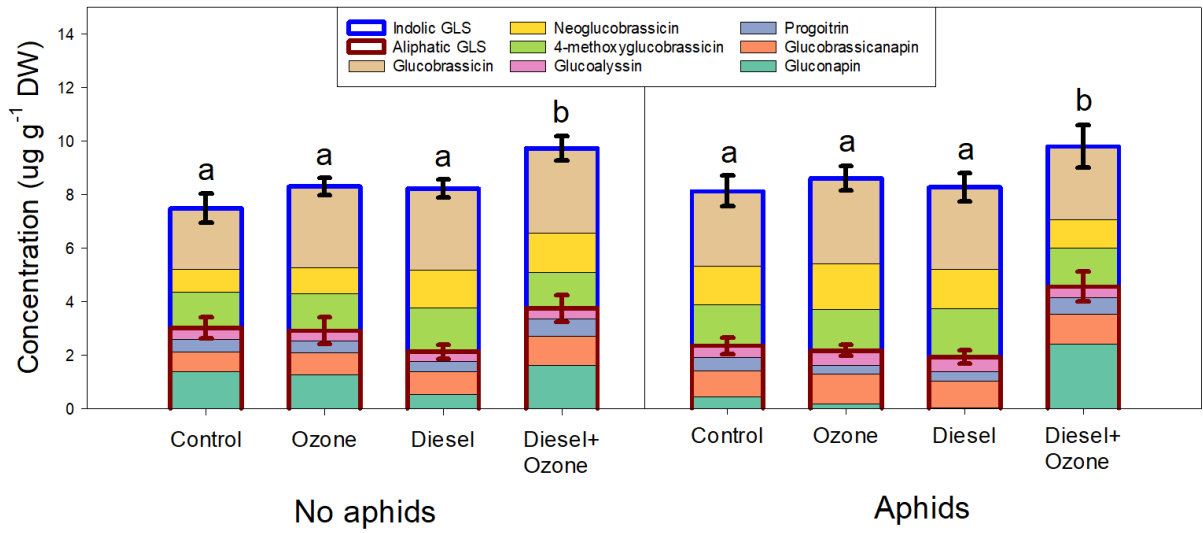


591

592 **Fig. 4.** The effects of air pollution treatment (control, ozone, diesel exhaust or diesel exhaust and  
 593 ozone) on the number of *Diaeretiella rapae* parasitoids that emerged from parasitised aphids on  
 594 exposed (OPEN) *Brassica napus* plants. Values are means  $\pm$  SE. Statistical effects of air pollution on  
 595 parasitoid emergence shown. Bars with the same letters were not significantly different ( $P < 0.05$ ).

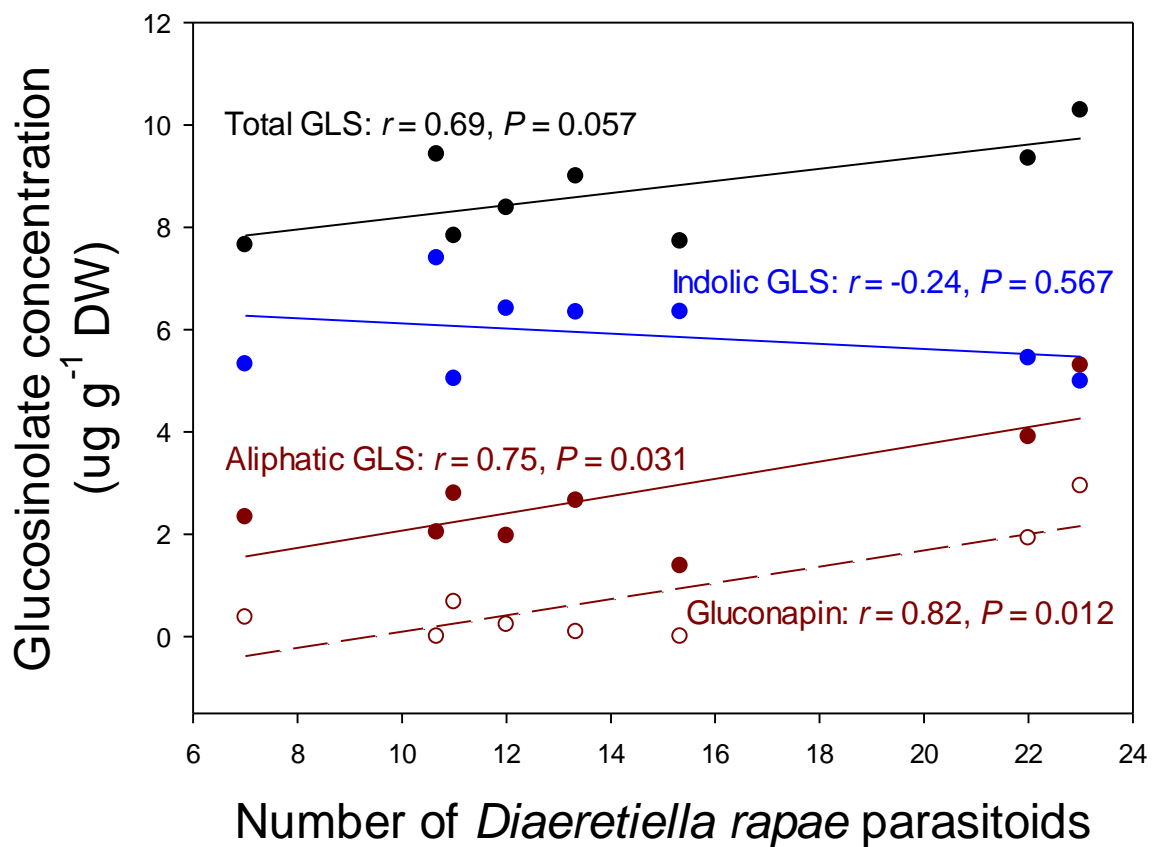
596  $N_{\text{obs}}$  = total number of observations.  $N_{\text{group}}$  = group number associated with the random effects of  
 597 Year/Run/Ring.

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**Fig. 5.** Indolic (highlighted blue) and aliphatic (highlighted red) glucosinolate concentrations under air pollution treatments (control, ozone, diesel exhaust or diesel exhaust and ozone) and aphid treatments (control *Brassica napus* plants with no aphids and A50 plants with aphids). Black letters indicate significant differences between air pollution treatments for total glucosinolate concentrations; bars with the same letters were not significantly different ( $P < 0.05$ ).

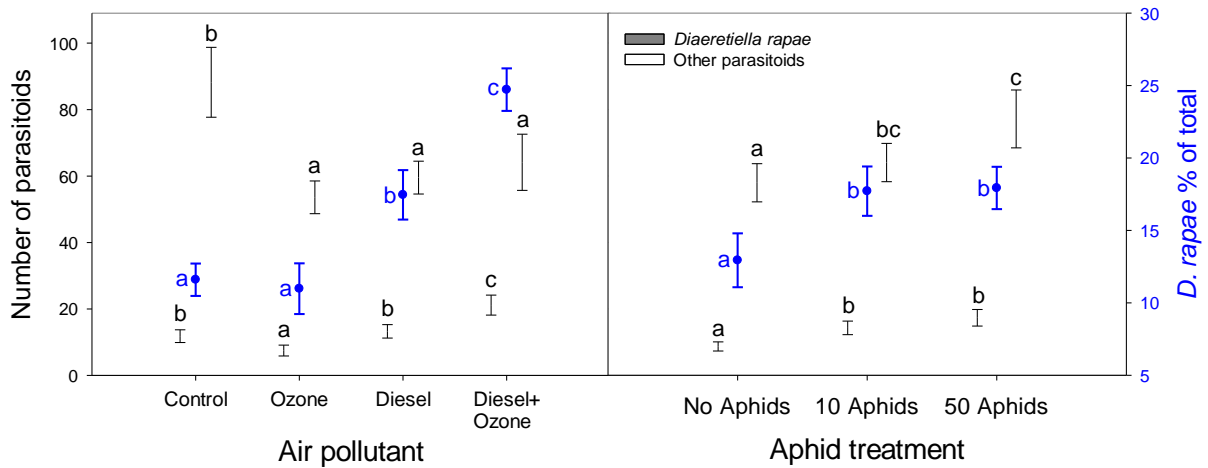


606

607 **Fig. 6.** Correlations between the mean number of *D. rapae* parasitoids counted on sticky traps within  
 608 each ring and mean glucosinolate concentrations (GLS) per ring. N = 8. Correlation test statistics  
 609 shown. Significant correlations between individual aliphatic GLS and parasitoid abundance (i.e.,  
 610 Gluconapin) are displayed. Other individual GLS were not significantly correlated with parasitoid  
 611 abundance.

612





613

614 **Figure S1.** Individual effects of air pollution (left panel) and aphid treatment (right panel) on  
 615 parasitoid abundance (left axis) and the percentage of total parasitoids that were *Diaeretiella rapae*  
 616 (right axis, blue). Values are means  $\pm$  SE. Bars with the same letters for each dependent variable (i.e.  
 617 *Diaeretiella rapae* abundance, the abundance of other parasitoids and *D. rapae* % of total) within  
 618 each panel were not significantly different ( $P < 0.05$ ).

619

## Supplementary materials

**Table S1.** Effects of air pollution and aphid treatments on plant and insect measurements. <sup>a</sup>indicates variables that were square-root transformed. ‘Diesel’ refers to diesel exhaust pollution. Statistically significant effects ( $P < 0.05$ ) are highlighted in bold. Means  $\pm$  SE are shown for individual treatments. Models for plant mass and insect characteristics include random effects of Year, Run and Ring location ( $N = 24$ ). Models for glucosinolates include random effects of Ring location and plant replicate ( $N = 8$ ).  $N_{\text{obs}}$  refers to total observed  $N$  in statistical models. Treatment df followed by the number of model parameters is provided in subscript.

Response variable	Air pollution (Pollutant)				Aphid treatment (Aphid)			Statistical analyses
	Control	Ozone	Diesel	Diesel+Ozone	None	A10	A50	
<b>PLANT CHARACTERISTICS</b>								
Plant mass (g) $N_{\text{obs}} = 312$	4.89 $\pm 0.19$	4.60 $\pm 0.19$	4.32 $\pm 0.23$	4.62 $\pm 0.20$	4.72 $\pm 0.17$	4.76 $\pm 0.16$	4.30 $\pm 0.20$	Pollutant: $\chi^2_{3,10} = 1.48, P = 0.686$ Aphid: $\chi^2_{2,10} = 4.79, P = 0.091$ Pollutant $\times$ Aphid: $\chi^2_{6,16} = 7.93, P = 0.244$
Indolic glucosinolates ( $\mu\text{g g}^{-1}$ DW) $N_{\text{obs}} = 133$	5.10 $\pm 0.31$	5.87 $\pm 0.34$	6.20 $\pm 0.28$	5.60 $\pm 0.43$	5.47 $\pm 0.25$	-	5.91 $\pm 0.24$	Pollutant: $\chi^2_{3,8} = 5.40, P = 0.145$ Aphid: $\chi^2_{1,8} = 2.42, P = 0.120$ Pollutant $\times$ Aphid: $\chi^2_{3,11} = 5.79, P = 0.122$
Aliphatic glucosinolates ( $\mu\text{g g}^{-1}$ DW) $N_{\text{obs}} = 133$	2.70 $\pm 0.25$	2.57 $\pm 0.29$	2.04 $\pm 0.18$	4.16 $\pm 0.38$	2.95 $\pm 0.22$	-	2.81 $\pm 0.23$	Pollutant: $\chi^2_{3,8} = 14.57, P = \mathbf{0.002}$ Aphid: $\chi^2_{1,8} = 0.37, P = 0.542$ Pollutant $\times$ Aphid: $\chi^2_{3,11} = 2.66, P = 0.447$
Total glucosinolates ( $\mu\text{g g}^{-1}$ DW) $N_{\text{obs}} = 133$	7.80 $\pm 0.40$	8.44 $\pm 0.27$	8.24 $\pm 0.29$	9.76 $\pm 0.45$	8.42 $\pm 0.23$	-	8.72 $\pm 0.31$	Pollutant: $\chi^2_{3,8} = 11.89, P = \mathbf{0.008}$ Aphid: $\chi^2_{1,8} = 0.54, P = 0.463$ Pollutant $\times$ Aphid: $\chi^2_{3,11} = 0.45, P = 0.931$
<b>INSECT CHARACTERISTICS</b>								
Aphid population mass (g) <sup>a</sup> $N_{\text{obs}} = 216$	0.16 $\pm 0.02$	0.16 $\pm 0.02$	0.15 $\pm 0.02$	0.16 $\pm 0.02$	-	0.08 $\pm 0.01$	0.25 $\pm 0.02$	Pollutant: $\chi^2_{3,9} = 0.10, P = 0.992$ Aphid: $\chi^2_{1,9} = 103.43, P < \mathbf{0.001}$ Pollutant $\times$ Aphid: $\chi^2_{3,12} = 5.94, P = 0.115$
<i>Diaeretiella rapae</i> abundance $N_{\text{obs}} = 72$	11.83 $\pm 1.91$	7.50 $\pm 1.63$	13.28 $\pm 2.02$	21.17 $\pm 3.01$	8.71 $\pm 1.38$	14.29 $\pm 2.04$	17.33 $\pm 2.49$	Pollutant: $\chi^2_{3,10} = 20.00, P < \mathbf{0.001}$ Aphid: $\chi^2_{2,10} = 16.60, P < \mathbf{0.001}$ Pollutant $\times$ Aphid: $\chi^2_{6,16} = 5.16, P = 0.524$
Abundance of other parasitoids $N_{\text{obs}} = 72$	88.22 $\pm 10.51$	53.61 $\pm 4.92$	59.56 $\pm 4.94$	64.17 $\pm 8.45$	57.96 $\pm 5.74$	64.04 $\pm 5.77$	77.17 $\pm 8.71$	Pollutant: $\chi^2_{3,10} = 8.35, P = \mathbf{0.039}$ Aphid: $\chi^2_{2,10} = 9.31, P = \mathbf{0.010}$ Pollutant $\times$ Aphid: $\chi^2_{6,16} = 5.81, P = 0.445$
<i>D. rapae</i> % of total parasitoids <sup>a</sup> $N_{\text{obs}} = 72$	11.61 $\pm 1.12$	10.99 $\pm 1.75$	17.46 $\pm 1.71$	24.73 $\pm 1.47$	12.94 $\pm 1.86$	17.72 $\pm 1.71$	17.93 $\pm 1.46$	Pollutant: $\chi^2_{3,10} = 25.68, P < \mathbf{0.001}$ Aphid: $\chi^2_{2,10} = 16.18, P < \mathbf{0.001}$ Pollutant $\times$ Aphid: $\chi^2_{6,16} = 1.82, P = 0.935$

**Table S2.** Relative abundances of volatile organic compounds present in Solid Phase Microextraction headspace samples of *Brassica napus* leaves, from plants exposed to ambient air (Control), diesel exhaust (Diesel) and ozone pollution (Ozone) with and without aphids present (Aphids and No aphids, respectively). N = 3 per group. Identified and tentatively identified compounds are presented with PubChem Compound ID (CID) numbers and Linear Retention Indices (LRI).

Compound identification	CID number	LRI <sup>§</sup>	ID*	Normalized peak areas																P-value
				Control + No aphids		Control + Aphids		Ozone + No aphids		Ozone + Aphids		Diesel + No aphids		Diesel + Aphids		Diesel + Ozone + No aphids		Diesel + Ozone + Aphids		
<i>Methyl esters</i>																				
Methyl acetate	5971	<650	A	2680465	ns	3618619	ns	2488018	ns	3250038	ns	2574370	ns	2464986	ns	1465211	ns	3280177	ns	0.469
Methyl propionate	11124	<650	B	6807725	ns	2880745	ns	3792547	ns	9855903	ns	6928412	ns	2356247	ns	10664845	ns	6161987	ns	0.270
Methyl hexanoate	7824	925	A	4651735	a	45931758	ab	1031120	a	168531302	ab	3201879	a	120384488	ab	2778975	a	291137635	b	0.012
Methyl (Z)-3-hexenoate	5362819	932	B	5505523	ns	3881537	ns	3499786	ns	8120145	ns	5161060	ns	4451698	ns	5359849	ns	9875749	ns	0.124
2-hexenoic acid methyl ester	61310	967	B	2992757	ab	1345694	a	816817	a	1166615	a	2337748	a	980430	a	1818024	a	6672197	b	0.001
Methyl octanoate	8091	1124	B	879752	a	617730	a	258554	a	1419375	a	660633	a	1067536	a	1437922	a	5579506	b	0.000
(E)-3-hexenyl isobutyrate	5352539	1144	A	0	ns	0	ns	0	ns	0	ns	0	ns	0	ns	207631	ns	293393	ns	0.094
Methyl nonanoate	15606	1224	B	1095996	ns	214333	ns	714351	ns	1067931	ns	652062	ns	961201	ns	481155	ns	0	ns	0.503
Methyl decanoate	8050	1324	B	806253	a	223430	a	177845	a	687557	a	240224	a	391658	a	615085	a	1038469	a	0.027
(Z)-3-hexenyl hexanoate	5352543	1382	A	0	a	385096	ab	0	a	0	a	0	a	359490	ab	0	a	1350403	b	0.011
Methyl dodecanoate	8139	1524	B	806278	a	2473008	a	281385	a	11415225	a	557081	a	10813071	a	722498	a	8868901	a	0.008
Methyl tetradecanoate	31284	>1500	B	472840	a	7428844	ab	280134	a	50215128	b	609502	a	21461074	ab	858763	a	19861324	ab	0.007
<i>Alcohols</i>																				
Propanol	1031	<650	B	1455039	ns	1040798	ns	926693	ns	992870	ns	1468841	ns	922762	ns	661157	ns	1753979	ns	0.639
1-pentanol	6276	768	A	722875	ns	0	ns	518438	ns	0	ns	971131	ns	169945	ns	258840	ns	1366039	ns	0.094
(E)-2-penten-1-ol	5364919	769	A	3553966	ns	659865	ns	1046175	ns	645411	ns	1563366	ns	766700	ns	1451780	ns	1646934	ns	0.429
(Z)-2-penten-1-ol	5364919	771	A	4222093	ns	2580591	ns	4057377	ns	1108192	ns	3170362	ns	2700248	ns	5147437	ns	9424105	ns	0.209
(E)-2-hexen-1-ol	5318042	853	B	0	ns	0	ns	0	ns	1519454	ns	0	ns	1464711	ns	0	ns	0	ns	0.069
(Z)-3-hexen-1-ol	5281167	859	B	282424102	ns	198931122	ns	208071497	ns	197718831	ns	274509395	ns	164771891	ns	228653504	ns	399423283	ns	0.239
2-hexen-1-ol	5318042	867	A	18130245	a	10626701	a	17917639	a	3951654	a	17781634	a	4746952	a	24396497	ab	67228684	b	0.002
1-hexanol	8103	869	A	43600425	ns	13075272	ns	12908507	ns	14435390	ns	21868001	ns	10899992	ns	17090560	ns	38317179	ns	0.201
1-octen-3-ol	18827	980	B	4782584	ns	6547814	ns	4843795	ns	11103838	ns	5444648	ns	4463238	ns	2068140	ns	2748408	ns	0.072

<i>Ketones</i>																				
3-methyl-2-butanone	11251	674	B	973495	ns	1873301	ns	703707	ns	2720059	ns	1324203	ns	1979601	ns	1375508	ns	976932	ns	0.388
3-pentanone	7288	695	B	7225975	ab	2996176	a	3325887	a	3035797	a	5658284	ab	3463528	ab	6011825	ab	10381135	b	0.009
2-heptanone	8051	891	B	0	ns	710655	ns	0	ns	1392410	ns	0	ns	1302596	ns	0	ns	510025	ns	0.087
3-octanone	246728	987	B	3048703	ns	1740145	ns	1761972	ns	3425317	ns	2662196	ns	1941437	ns	2502971	ns	3158367	ns	0.610
2-nonanone	13187	1093	B	139461	a	1256735	a	0	a	944475	a	0	a	0	a	230382	a	0	a	0.009
<i>Aldehydes</i>																				
(E)-tiglaldehyde	5321950	755	B	2100028	ab	0	a	601920	a	0	a	1245495	ab	205703	a	1624857	ab	3951388	b	0.004
(Z)-3-hexenal	643941	799	A	0	ns	566877	ns	602488	ns	0	ns	468309	ns	0	ns	0	ns	0	ns	0.079
(E)-4-oxohex-2-enal	6365145	959	B	0	a	0	a	0	a	0	a	0	a	0	a	366724	a	1759040	a	0.040
(E,E)-2,4-heptadienal	5283321	1014	B	289838	a	0	a	340743	a	0	a	516128	a	0	a	472887	a	2372520	b	0.003
<i>Alkenes</i>																				
3-methyl-1,2-butadiene	11714	759	B	2093392	ab	1183270	ab	1133180	ab	953428	a	1635177	ab	990837	a	1839283	ab	3050479	b	0.022
3-ethyl-1,5-octadiene	5353002	948	B	2216815	ns	647329	ns	831741	ns	224053	ns	750351	ns	356473	ns	3890492	ns	5541592	ns	0.108
(E)-2-tetradecene	5352912	1292	B	833643	ns	809982	ns	428129	ns	929175	ns	971692	ns	780927	ns	685227	ns	1049354	ns	0.591
(E)- $\beta$ -farnesene	5281517	1462	A	0	a	0	a	0	a	2316851	a	0	a	0	a	0	a	1048928	a	0.028
<i>Alkanes</i>																				
1,1-dimethylcyclopropane	74202	847	B	676049	ns	0	ns	0	ns	0	ns	0	ns	0	ns	403397	ns	1743730	ns	0.097
2-ethyl-3-vinylloxirane	534767	854	B	40921089	ns	6310846	ns	17041630	ns	1672222	ns	15187877	ns	2006088	ns	22372794	ns	93476322	ns	0.064
<i>Cyclo-alcohols</i>																				
1-cyclohexene-1-methanol	317542	1021	B	1294308	ab	791125	ab	202423	a	3236687	ab	274425	a	1527304	ab	533178	ab	3745310	b	0.005
2-methylenecyclopentanol	550922	1061	B	1073340	a	350773	a	0	a	0	a	309680	a	649465	a	85189	a	1563017	a	0.044
<i>Other</i>																				
Methyl thiocyanate	11168	714	B	0	ns	0	ns	1180570	ns	0	ns	1586797	ns	0	ns	0	ns	0	ns	0.078
Dimethyl disulfide	1068	746	B	0	a	229887	a	0	a	521310	ab	0	a	0	a	0	a	1197123	b	0.001
Hexanoic acid	8892	978	B	1561109	a	6883846	ab	3322245	ab	9288844	ab	1358160	a	2510199	a	825824	a	17423583	b	0.009
(Z)-3-hexenyl acetate	5363388	1006	B	5657761	ns	7006784	ns	8665609	ns	1819434	ns	13229012	ns	2391923	ns	5214277	ns	14803118	ns	0.228
1-chlorooctane	8142	1065	B	421837	ns	750760	ns	387697	ns	0	ns	258777	ns	0	ns	219243	ns	901789	ns	0.265
$\beta$ -ionone	638014	1502	B	2683596	ab	498871	a	703767	ab	0	a	883199	ab	365862	a	1791658	ab	3354479	b	0.003

<i>Unknown</i>																				
<unknown 1>	-	664	-	2873258	ns	3129185	ns	3685174	ns	965650	ns	4301699	ns	1097426	ns	4044021	ns	5975188	ns	0.666
<unknown 4>	-	1234	-	2343247	ns	907894	ns	836757	ns	223509	ns	1299205	ns	375311	ns	1614294	ns	1902493	ns	0.090
<unknown 6>	-	1327	-	254217	ns	0	ns	0	ns	0	ns	309367	ns	0	ns	255865	ns	304227	ns	0.324
<unknown 7>	-	1513	-	0	a	299943	ab	0	a	4900756	c	0	a	2991504	bc	0	a	320200	ab	<0.0001

§ = LRI on a HP-5MS. \* = A, compound mass spectrum and LRI agrees with authentic compound; B, mass spectrum agrees with reference spectrum in the NIST/EPA/NIH database and LRI agree with literature sources, tentatively identified. ns = not significant according to protected ANOVA with Tukey's Honestly Significant Difference pairwise comparison test; different letters within rows signify significant differences at the  $P < 0.05$  threshold.

**Table S3.** ANOVA with *post hoc* Tukey HSD test levels of significance for the two-way interaction between aphid treatment (Aphid) and air pollution (Pollutant). Statistically significant effects ( $P < 0.05$ ) are highlighted in bold.

Compound	P-value		
	Aphid	Pollutant	Aphid x Pollutant
<i>Methyl esters</i>			
Methyl acetate	<b>0.016</b>	0.912	0.451
Methyl propionate	0.517	0.401	0.191
Methyl hexanoate	<b>0.041</b>	0.217	0.245
Methyl (Z)-3-hexenoate	0.151	0.270	0.126
2-hexanoic acid methyl ester	0.273	<b>0.023</b>	<b>0.004</b>
Methyl octanoate	0.145	0.138	0.143
(E)-3-hexenyl isobutyrate	0.445	0.962	0.727
Methyl nonanoate	0.064	0.185	0.058
Methyl decanoate	0.987	0.243	0.309
(Z)-3-hexenyl-hexanoate	0.100	0.100	0.100
Methyl dodecanoate	0.116	0.822	0.640
Methyl tetradecanoate	0.778	0.998	0.456
<i>Alcohols</i>			
Propanol	0.682	0.838	0.443
1-pentanol	0.814	0.753	0.302
(E)-2-penten-1-ol	0.286	0.587	0.617
(Z)-2-penten-1-ol	0.768	<b>0.021</b>	<b>0.026</b>
(E)-2-hexen-1-ol	0.698	0.707	0.855
(Z)-3-hexen-1-ol	0.855	0.311	0.126
2-hexen-1-ol	0.494	<b>0.008</b>	<b>0.025</b>
1-hexanol	0.283	0.687	0.096
1-octen-3-ol	0.272	0.494	0.328
<i>Ketones</i>			
3-methyl-2-butanone	<b>0.000</b>	0.096	0.270
3-pentanone	0.577	<b>0.011</b>	<b>0.035</b>
2-heptanone	0.778	0.594	0.708
3-octanone	0.389	0.900	0.199
2-nonanone	0.124	<b>0.033</b>	0.120
<i>Aldehydes</i>			
(E)-tiglaldehyde	0.690	0.250	0.180
(Z)-3-hexenal	<b>0.005</b>	<b>0.001</b>	<b>0.004</b>
(E)-4-oxohex-2-enal	0.349	0.954	0.823
(E,E)-2,4-heptadienal	0.235	0.408	0.155
<i>Alkenes</i>			
3-methyl-1,2-butadiene	0.882	<b>0.041</b>	0.115
3-ethyl-1,5-octadiene	0.693	0.082	0.972
(E)-2-tetradecene	0.862	0.699	0.261
(E)- $\beta$ -farnesene	0.696	0.696	0.696

<i>Alkanes</i>			
1,1-dimethylcyclopropane	0.157	0.980	0.580
2-ethyl-3-vinyloxirane	0.695	0.209	0.137
<i>Cyclo-alcohols</i>			
1-cyclohexene-1-methanol	<b>0.043</b>	0.668	0.300
2-methylenecyclopentanol	0.936	0.788	0.265
<i>Other compounds</i>			
Methyl thiocyanate	0.464	0.171	0.149
Dimethyl disulfide	0.134	0.392	<b>0.038</b>
Hexanoic acid	0.481	0.260	0.377
(Z)-3-hexenyl acetate	0.363	0.461	0.113
1-chlorooctane	0.219	0.313	0.489
$\beta$ -ionone	0.845	0.112	0.270
<i>Unknown compounds</i>			
<unknown 1>	0.646	<b>0.006</b>	<b>0.001</b>
<unknown 4>	0.120	0.057	0.230
<unknown 6>	0.476	0.844	0.727
<unknown 7>	0.128	0.509	0.128