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1 **Ozone alters the chemical signal required for plant – insect pollination: the**
2 **case of the Mediterranean fig tree and its specific pollinator.**

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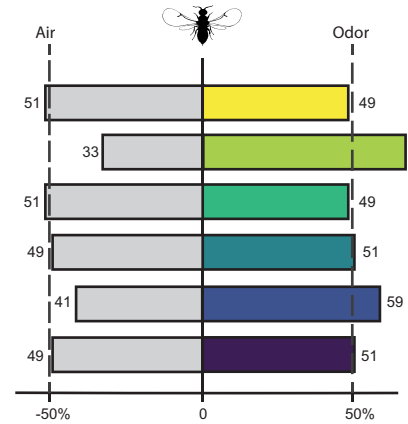
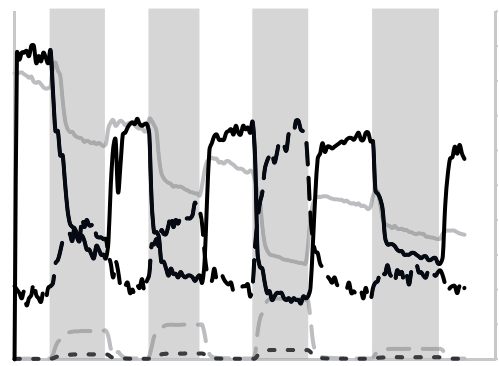
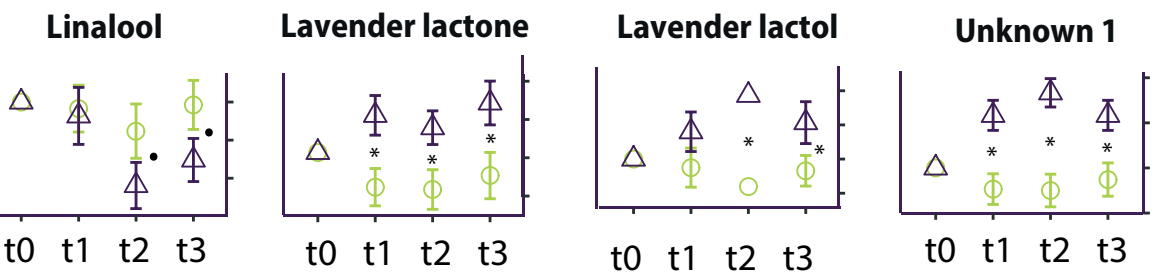
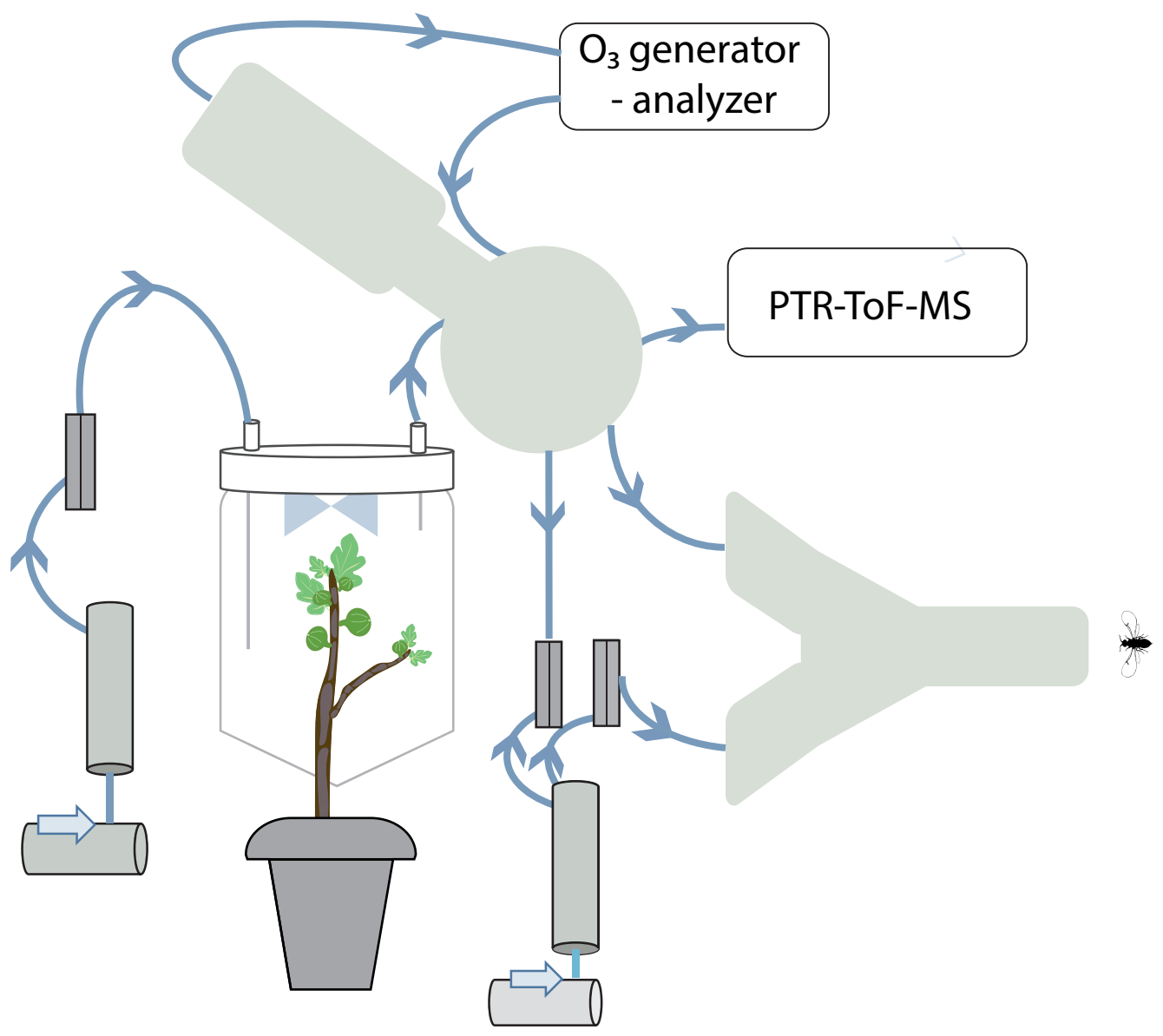
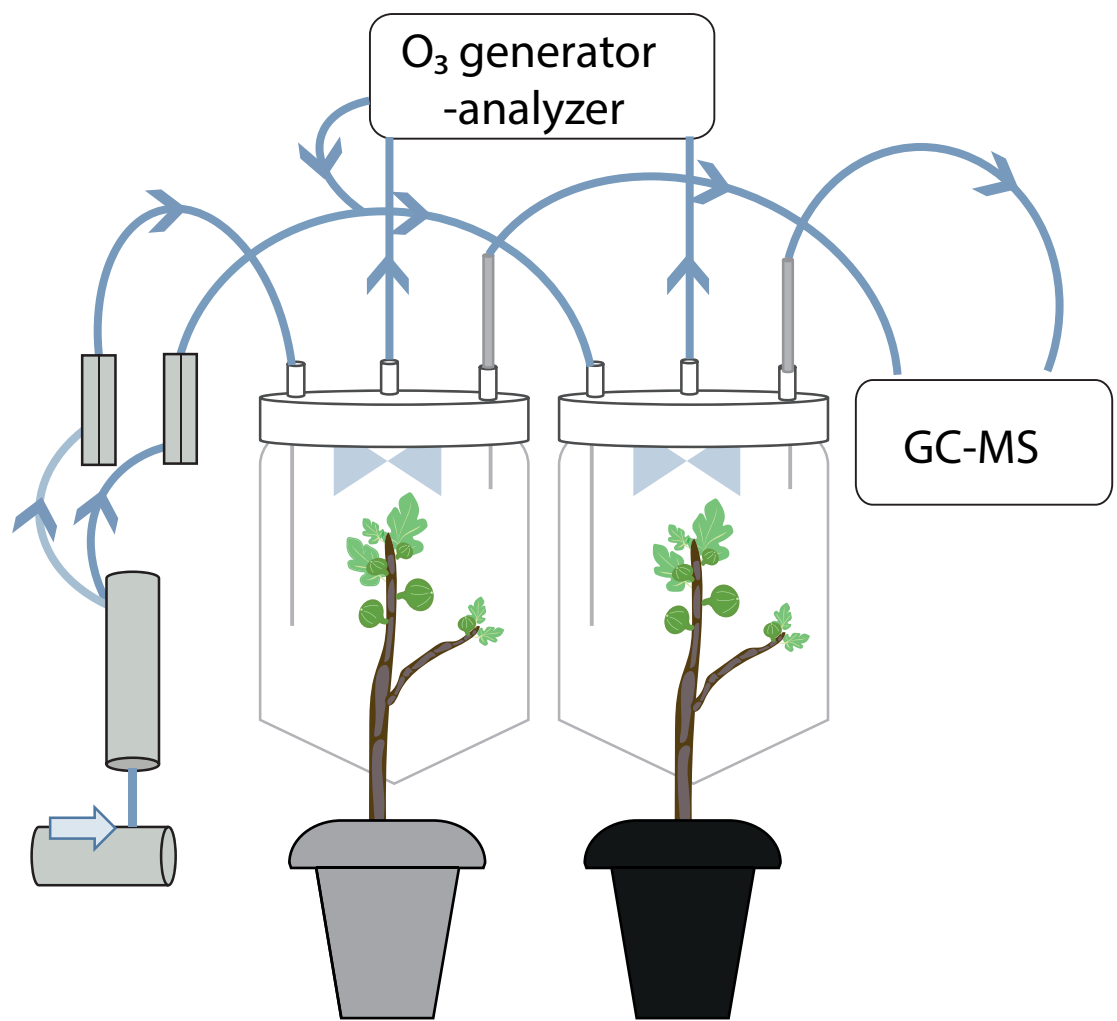
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11



12 Abstract

13 Tropospheric ozone (O₃) is likely to affect the chemical signal emitted by flowers to attract their
14 pollinators through its effects on the emission of volatile organic compounds (VOCs) and its
15 high reactivity with these compounds in the atmosphere. We investigated these possible effects
16 using a plant-pollinator interaction where the VOCs responsible for pollinator attraction are
17 known and which is commonly exposed to high O₃ concentration episodes: the Mediterranean
18 fig tree (*Ficus carica*) and its unique pollinator, the fig wasp (*Blastophaga psenes*). In
19 controlled conditions, we exposed fig trees bearing receptive figs to a high-O₃ episode (5h) of
20 200 ppb and analyzed VOC emission. In addition, we investigated the chemical reactions
21 occurring in the atmosphere between O₃ and pollinator-attractive VOCs using real-time
22 monitoring. Finally, we tested the response of fig wasps to the chemical signal when exposed
23 to increasing O₃ mixing ratios (0, 40, 80, 120 and 200 ppb). The exposure of the fig tree to high
24 O₃ levels induced a significant decrease in leaf stomatal conductance, a limited change in the
25 emission by receptive figs of VOCs not involved in pollinator attraction, but a major change in
26 the relative abundances of the compounds among pollinator-attractive VOCs in ozone-enriched
27 atmosphere. Fig VOCs reacted with O₃ in the atmosphere even at the lowest level tested (40
28 ppb) and the resulting changes in VOC composition significantly disrupted the attraction of the
29 specific pollinator. These results strongly suggest that current O₃ episodes are probably already
30 affecting the interaction between the fig tree and its specific pollinator.

31 *Keywords:* *Ficus carica, flowers, Mediterranean ecosystems, plant-pollinator interaction, air*
32 *pollution, VOCs*

33 1 Introduction

34 Plants produce and emit into the atmosphere a great diversity of volatile organic compounds
35 (VOCs) that belong to different chemical classes (Dudareva et al., 2013; Knudsen et al., 2006).
36 These VOCs are involved in numerous ecological functions, such as mediation of plant-plant
37 interactions (Brosset & Blande, 2022; Ueda et al., 2012), protection of plants against pathogens,
38 herbivores and abiotic stresses (Dicke & Baldwin 2010; Heil, 2008; Ormeño et al., 2020), and
39 also attraction of pollinators (Hossaert-McKey et al., 2010; Pichersky & Gershenzon, 2002;
40 Raguso, 2008). These multiple functions of plant VOCs create a complex chemical
41 environment around the plant in which interacting species, and particularly insects, must extract
42 the relevant cues that provide them with crucial information on resources for their survival and
43 reproduction (Conchou et al., 2019). If these chemical cues have evolved in plants with the
44 function of exchanging specific information with other living organisms, then they are
45 considered signals (Van Der Kooi et al., 2023). For instance, at receptive stage, flowers emit
46 chemical signals to advertise the presence of a reward (e.g. nectar, pollen or oviposition site) in
47 order to attract pollinators (Van Der Kooi et al., 2023). This chemical signal responsible for
48 plant-pollinator communication is generally composed of a blend of VOCs in particular
49 proportions (Proffit et al., 2020; Raguso, 2008; Wright et al., 2005). Many abiotic factors can
50 affect the emission of VOCs by plants, such as inducing increased or decreased emission of
51 certain compounds (Blande et al., 2014; Peñuelas & Staudt, 2010; Pinto et al., 2010). For
52 instance, an increase in temperature can have significant but contrasting effects on the emission
53 of floral VOCs between different species, as well as on the emission of different chemical
54 compounds within a given species, therefore affecting the proportions of VOCs emitted (Farré-
55 Armengol et al., 2014). If these modifications affect the emission of some of the VOCs
56 constituting the chemical signal responsible for pollinator attraction, this can have dramatic
57 consequences for the maintenance of plant-pollinator interactions.

58 Recent studies have shown that pollutants present in the troposphere can have
59 significant effects on the emission of floral VOCs (Masui et al., 2023; Saunier & Blande, 2019;
60 Yuan et al., 2021). Among these pollutants, tropospheric ozone (O₃) is one of the major
61 phytotoxic pollutants observed in suburban and rural areas near metropolitan areas (Cooper *et*
62 *al.*, 2014). This highly oxidizing molecule can cause physiological stress in plants (Black et al.,
63 2007; Leitao et al., 2007; Long & Naidu, 2002). Ozone enters plants mainly through stomata,
64 leading to the formation of reactive oxygen species (ROS) that cause lipid peroxidation, protein
65 oxidation, and eventually cell death (reviewed in Iriti & Faoro, 2009 and Tausz et al., 2007).
66 Plants have developed a wide range of responses to limit this oxidative stress. For instance,
67 stomatal closure is a direct and very effective way of limiting the damage caused by O₃ (Morales
68 et al., 2021; Reiling & Davison 1995). On the other hand, stomatal closure leads to a drop in
69 photosynthesis, affecting the plant's entire metabolism, including the emission of VOCs (Masui
70 et al., 2023). In addition, exposure to oxidative stress can induce plants to produce enzymatic
71 and non-enzymatic antioxidants, including VOCs (Oikawa & Lerdau, 2013). As with other
72 abiotic factors, while many studies have determined the effects of O₃ on the VOC emissions
73 from vegetative plant parts (Blande et al., 2014; Peñuelas & Staudt, 2010; Pinto et al., 2010),
74 to our knowledge only three studies have investigated the effects on floral VOC emissions
75 (Dubuisson et al., 2022; Saunier & Blande, 2019; Yuan et al., 2021). They have shown (i) no
76 significant direct effects on the emission of VOCs by flowering *Lavandula angustifolia* plants
77 exposed to 200 ppb (parts per billion) for 5h (Dubuisson et al., 2022); (ii) significant but plant
78 species-specific effects on the emission of VOCs by Brassicaceae species exposed to 80 and
79 120 ppb of O₃ during daylight hours for 5 consecutive days (Saunier & Blande, 2019); and (iii)
80 varietal-specific effects on the emission of VOCs by *Rosa* spp. exposed to 40 ppb for 35 days
81 (Yuan et al., 2021). Although the last two studies suggest that changes in the emission of floral
82 VOCs induced by increased tropospheric O₃ levels may disrupt pollinator attraction, neither

83 study specifically investigated the effects of these O₃ exposures on the VOCs emitted by flowers
84 and responsible for the attraction of their associated pollinators.

85 Once emitted into the atmosphere, VOCs can react with gaseous oxidants such as O₃
86 and form secondary pollutants more oxidized than the parent compounds. Atmospheric
87 chemistry studies have investigated the reactions of many VOCs, including some of the major
88 floral VOCs, with O₃ (Atkinson & Arey, 2003; Calogirou et al., 1999). As the reaction time of
89 the different VOCs with O₃ is extremely variable, progressive and significant changes in the
90 proportions of VOCs in floral plumes are expected in the atmosphere especially during periods
91 with elevated O₃ level. Several modeling and experimental studies have confirmed this
92 prediction and have also shown that the attraction of bumblebees, honeybees and tobacco
93 hawkmoth to the flowers of plants they pollinate, or to mixtures of synthetic versions of VOCs
94 present in these flowers, was affected if these compounds were exposed to high O₃
95 concentrations (Cook et al., 2020; Farré-Armengol et al., 2016; Fuentes et al., 2016;
96 McFrederick et al., 2008; Saunier et al., 2023; Langford et al., 2023). These studies have also
97 shown that pollinators are able to identify new odor bouquets formed from ozone-altered floral
98 VOCs, which could mitigate the effect of this oxidative pollution on pollination (Cook et al.,
99 2020; Saunier et al., 2023). In those studies, significant effects of O₃ on pollinator attraction
100 behavior were observed at 100-120 ppb, although no lower mixing-ratios were tested. These
101 various studies on pollinator orientation following the alteration of floral odors by O₃ are
102 important, but have all been carried out on model insects, which use learning to orient
103 themselves, and which are not necessarily representative of the diversity of pollinators
104 encountered in nature. A recent experimental field study conducted in an O₃-enriched
105 environment revealed a reduction in the number of pollinating individuals attracted to flowering
106 plants of the black mustard, the extent of the reduction differing depending on the insect family
107 (Ryalls et al., 2022). These results confirm that some plant-pollinator interactions are more

108 likely to be negatively affected by air pollution than others, particularly those that are
109 specialized on particular plants and where the pollinator has a limited learning capacity. As O₃
110 concentrations have significantly increased since the pre-industrial era (Sicard et al., 2021;
111 Vingarzan, 2004) and high-O₃ episodes have become more frequent (Vautard et al., 2005), it is
112 crucial to understand how rising tropospheric O₃ levels can affect chemical communication
113 between plants and pollinators.

114 Due to high levels of human activity and sunlight, the Mediterranean region is one of
115 the areas experiencing the highest levels of O₃ pollution in the world, with frequent high-O₃
116 episodes in which O₃ mixing-ratio can reach 200 ppb (Millán, 2000). In the present study, we
117 used an emblematic interaction from this region, that between the Mediterranean fig tree (*Ficus*
118 *carica* L., Moraceae) and its sole pollinator, the fig wasp (*Blastophaga psenes* L., Hymenoptera,
119 Agaonidae) as a model system to evaluate the effects of exposure to high but realistic O₃ mixing
120 ratios on the chemical communication between plant and pollinator. The fig wasps locate
121 receptive figs (i.e., those ready to be pollinated) of their host using a blend of four VOCs
122 (linalool, benzyl alcohol, (*Z*)-linalool oxide furanoid and (*E*)-linalool oxide furanoid) in
123 particular proportions (Proffit *et al.*, 2020). Because of their short lifespan and the fact that they
124 can only visit figs of a single tree during their lifetime, fig wasps have no opportunity to base
125 their choices on learning. Previous studies have shown that exposure of fig wasps to high levels
126 of O₃ has a significant effect on the detection of VOCs by their antennae, on their attraction to
127 the odors of receptive figs, on their mobility in general, and even on their survival
128 (Vanderplanck et al., 2021 a,b). To complete these studies, we aimed to assess the effect of
129 acute exposure to high O₃ levels on the VOCs emitted by receptive *F. carica* figs, in particular
130 those VOCs responsible for attracting pollinators, and the integrity of this chemical signal in
131 the atmosphere. To do so, we simulated, under controlled conditions, an episode of high, but
132 realistic, O₃ level to investigate whether: (i) exposure of fig trees to 200 ppb of O₃ for 5 h

133 induces plant stress and affects the emission of VOCs by receptive figs; (ii) this exposure alters
134 the integrity of floral VOCs emitted in the atmosphere around the plants and in particular that
135 of the four VOCs responsible for fig wasp attraction; (iii) O₃ reacts with these four VOCs, and
136 if so, determine the O₃ mixing ratio (0 ppb, 40 ppb, 80 ppb, 120 ppb and 200 ppb) at which
137 significant variations in the proportions of these compounds are observed; and finally (iv) these
138 changes impact pollinator attraction.

139 2 Materials and methods

140 2.1 Model system and site

141 *Ficus carica* is a gynodioecious but functionally dioecious fig species. Male trees bear figs in
142 which fig wasps oviposit and their larvae develop. Female trees bear figs that produce only
143 seeds (for more details on the reproductive biology of figs and fig wasps, see Kjellberg & Lesne,
144 2020; Proffit et al., 2020). As pollinators reproduce exclusively within figs, reproduction of
145 each partner depends on that of the other.

146 The study was carried out in 2018, 2019 and 2021, in i) the Terrain d'Experience (TE)
147 technical platform of the Laboratoire d'Excellence Centre Méditerranéen Environnement et
148 Biodiversité (LabEx CeMEB) in the Centre d'Ecologie Fonctionnelle et Evolutive (CEFE,
149 Montpellier, France) and ii) the Laboratoire Chimie Environnement (LCE, Marseille, France).
150 We performed three experiments in spring (April-May) when male trees bear receptive figs for
151 the first time in the year. For all the behavioral tests, we used only female fig wasps, because
152 they are the only ones to disperse from one fig to another, the males being apterous.

153 2.2 Effect of plant exposure to high O₃ level on VOC emission (Experiment 1)

154 This first experiment was conducted in the greenhouses of the TE under controlled temperatures
155 (day/night: 25°/15°C), natural light conditions and ambient O₃ conditions (~20ppb). We used a
156 dynamic enclosure system to individually expose potted trees with figs at the receptive stage to

157 O₃ (see Fig. S1 and Dubuisson *et al.* (2022) for more details about the experimental setup).
158 These 3–4-year-old trees were obtained from cuttings taken on male wild fig trees provided by
159 nurserymen (Pépinières Baud™, <http://www.fig-baud.com>). During a set of experiments,
160 potted trees were exposed in different chambers in parallel: one control plant, exposed to
161 approximately 0 ppb of O₃, and one exposed to 200 ppb O₃ for 5 h between 10:00 am and 15:00
162 h, corresponding to the period of fig wasp activity in the field at this season (M. Hossaert-
163 McKey & M. Proffit, unpublished data) as well as the maximum daily O₃ mixing ratio recorded
164 in the region (Atmo Occitanie, Observatoire Régional de l'Air en Occitanie, www.atmo-occitanie.org). VOCs were collected from both chambers four times: (t0) before O₃ exposure,
165 (t1) 3 h after the beginning of O₃ exposure, (t2) 5 h after the beginning of O₃ exposure and (t3)
166 around 1 h following O₃ exposure. VOCs were collected as described in Dubuisson *et al.* (2022).
167 In addition, prior to each experimental exposure, VOC samples were collected from the empty
168 chamber for background subtraction. The experiment was repeated 16 times (32 plants in total).
169 As a marker of stress, stomatal conductance (Damour *et al.*, 2010) was measured on three leaves
170 of each plant with an AP4-Porometer (Delta-T Devices, Cambridge, England) before and after
171 introduction of plants into the chambers.
172

173 2.3 Effect of VOC-ozone mixtures on pollinator attraction (Experiment 2)

174 We aimed to isolate the VOCs emitted from about 30 receptive figs borne on cut branches of
175 wild male fig trees placed in a 42 L PTFE chamber. The resulting gaseous mixture flowed to a
176 reactor of 2 L (Hei-Vap Core model, Heidolph Instruments GmbH & Co. KG, Regensburg,
177 Germany) in the dark, where the VOCs could be exposed to different O₃ mixing ratios (0, 40,
178 80, 120 and 200 ppb generated by the same O₃ generator/analyzer as for the first experiment).
179 Respectively, 0, 20, 50, 80 and 110 ppb were measured at the outlet of the reactor. The difference
180 in O₃ mixing ratio between the inlet and the outlet of the reactor confirmed that O₃ was reacting
181 with VOCs in the reactor (loss of ~50% of the O₃ mixing ratio, but a loss of only ~25% when only

182 O₃ was present in the reactor). After the reaction, the gaseous mixture was pushed out of the
183 reactor to an olfactometer to test the attraction of fig wasps to this odor (details of the system
184 are presented in Fig. 1a). A light source (400-700 nm SpectraBulb artificial light, E27 LED,
185 FloraLed, France) was used to illuminate the branches hidden from the sight of insects.
186 Temperature and relative humidity were respectively 22 ± 0.04 °C and 38 ± 0.38 % during the
187 experiment.

188 We tested whether females of fig wasps were attracted to the gaseous mixture emitted by
189 receptive figs after reaction of VOCs with O₃, using a Y-tube olfactometer experimental
190 protocol previously described by Proffit et al. (2020). Odor sources were connected to both
191 lateral arms of the Y-tube with the reactor on one side (odor) and purified and humidified air
192 with activated charcoal and distilled water on the other side (control) (Fig. 1a). Each gaseous
193 stream (odor and control) flowed into the olfactometer at 0.2 L min^{-1} . Emerging adult female
194 wasps were collected from mature figs of male trees in natural populations less than 40 km
195 distant from Montpellier and were naïve to the odor sources. In total, 303 females were tested
196 in six different two-choice tests: (i) ozonated fig VOCs exposed to 0, 40, 80, 120 or 200 ppb O₃
197 against control or (ii) O₃ only at 200 ppb against control. Between two choice modalities, VOCs
198 in the reactor were sampled using the same adsorbent traps as in Experiment 1 at a flow rate of
199 0.2 L min^{-1} for 5 min to assess qualitatively and quantitatively the odor source sent into the
200 olfactometer (between eight and thirteen VOC samples per modality). Treatments and
201 modalities assayed were alternated over the course of the day and experiments were repeated
202 for 20 days.

203 2.4 Determination of reaction rate coefficients of pollinator-attractive VOCs
204 with O₃ (Experiment 3)

205 The experiment consisted of enclosing branches from wild male trees bearing about 30
206 receptive figs in a 42 L PTFE chamber. The branches were collected less than 1 hour before the
207 beginning of the experiments. Throughout experiments, emitted VOCs were transferred in the
208 dark into a glass reactor for O₃ exposure (Fig. **1b**). Ozone was injected at the inlet of the reactor
209 and measured at the outlet of the reactor using the same O₃ generator-analyzer as in
210 Experiments 1 and 2. Ozone exposure started 2 hours after the branches were enclosed. To
211 calculate mean reaction kinetics for attractive compounds, VOCs were exposed to five different
212 O₃ mixing ratios from 120 to 900 ppb in two different reaction volumes (1.975 L and 2.975 L).
213 Three replicates per exposure and volume were performed. Due to the flow rates of VOCs
214 through the reactors (1.65 L.min⁻¹) and their respective volumes (ranging from 1.975 to 2.975
215 L), exposure times varied between 71.8 s and 108 s depending on the experiments.

216 To specifically study the ozonolysis reactions of VOCs and prevent the other type of
217 reactions, several precautions were taken. Firstly, the transfer of VOCs and their exposure to
218 O₃ were conducted in the dark to avoid photolysis reactions. Furthermore, to prevent the
219 accumulation of oxidants (such as the hydroxyl radical), the reactor under use was a flow-
220 reactor, which allowed for the renewal of air every 71.8 s or 108 s, depending on the reactor
221 volume (see above). Finally, to eliminate any radicals that might still form, cyclohexane, inert
222 to O₃ but highly reactive with the hydroxyl radical, was continuously injected into the reactor.
223 The efficiency of this experimental setup was validated by comparing the ozonolysis kinetic
224 rate constants of linalool obtained with this device to those reported in the literature (see Table
225 1). Real-time monitoring of VOCs in the reactor was carried out using a proton-transfer-reaction
226 time-of-flight mass spectrometer (PTR-ToF-MS 8000, Ionicon Analytik GmbH, Innsbruck,
227 Austria) (Hansel et al., 1995; Lindinger et al., 1998). In the PTR-ToF-MS, the reaction chamber
228 pressure was set at 2.2 mbar, drift tube voltage at 460 V and drift tube temperature at 110°C
229 corresponding to an electric field strength applied to the drift tube (E) to buffer gas density (N)

230 ratio of ≈ 120 Td. Mass spectra were recorded up to m/z 500 at 1 minute integration time. Data
231 were post-processed using the commercial software Tofware V2.4.5 (TOFWERK AG, Thun,
232 Switzerland) that allows extracting time series of the high-resolution fitted peaks. Ions
233 corresponding to the protonated ions were chosen to extract the time series (see table 1). The
234 VOC mixing ratios were determined using the transmission-corrected ion counts of protonated
235 VOC and H_3O^+ and the reaction rate constants, as described by Cappelin et al. (2012). A mixture
236 of 14 aromatic organic compounds (TO-14A Aromatic Mix, Restek Corporation, Bellefonte,
237 USA, 100 ± 10 ppb in nitrogen), was used to assess the relative ion transmission efficiency.

238 We determined the kinetic rate constants with O_3 (k_{O_3}) of each ion detected by plotting
239 the Napierian logarithm of the ratio of its corresponding fitted concentration (see Methods S1
240 for calculation details) to experimental concentration according to the O_3 concentration $[O_3]$
241 generated in the different reaction volumes. Then the second-order constant was obtained with:

$$242 \quad \text{Ln} \left(\frac{[mass\ i]_{fit}}{[mass\ i]_{experimental}} \right) = \frac{k_i}{t} [O_3] \quad \text{Eqn 1}$$

243 where k_i is the slope of the regression line for the ion of mass i , t the reaction time in the
244 reactor (in seconds), $[O_3]$ is the O_3 concentration in the reactor (in molecules cm^{-3}), with k_i/t
245 corresponding to the second-order reaction constant of the ion of mass i (in cm^3 molecule $^{-1}$ s $^{-1}$).
246

247 2.5 VOC analysis by GC-MS

248 All VOC analyses by GC-MS (Gas Chromatography - Mass Spectrometry for identification and
249 quantification) were conducted following the method detailed by Proffit et al. (2020) and
250 Dubuisson et al. (2022). From these GC-MS data, we calculated for each sample the relative
251 proportion of each VOC in the total blend. Preliminary analyses revealed that there was no good
252 correlation between the quantity of VOCs emitted by receptive figs and their dry weight

253 (unpublished data). Therefore, in order to estimate variations in the absolute quantity of VOCs
254 emitted, for each compound we used an index of variation in the amount of VOCs at a given
255 time (t1, t2 and t3) relative to the amount of VOCs present in the samples at the beginning of
256 the experiment (t0), using the following equation:

$$257 \quad \text{Variation index of } VOC_{ti} = \frac{(\text{area of } VOC_{ti+1}) - (\text{area of } VOC_{t0+1})}{(\text{area of } VOC_{ti+1}) + (\text{area of } VOC_{t0+1})} \quad \text{Eqn 2}$$

258 where *area of VOC_{ti}* stands for the peak area of a given compound at a given time i (i:
259 from t0 to t3) and *area of VOC_{t0}* stands for the peak area of a given compound in the sample of
260 the corresponding t0. The variation index varied between -1 and 1, with 0 corresponding to the
261 initial VOC concentration at t0.

262 2.6 Statistical analyses

263 All the data visualization and analyses were performed in R (v. 3.6.2; R Development Core
264 Team; URL <http://www.R-project.org>) with the following packages: ade4 (Thioulouse et al.,
265 2018), car (Fox & Weisberg, 2019), vegan (Oksanen et al., 2020), mixOmics (Rohart et al.,
266 2017), Hotelling (Curran, 2021), RVAideMemoire (Hervé, 2021), contrast (O’Callaghan et al.,
267 2020) and nlme (Pinheiro et al., 2021).

268 2.6.1 Experiment 1

269 The effects of the two factors, treatment (“control”, “ozone”), and time (“before”, “after”
270 exposure) and their interaction on stomatal conductance were tested using linear mixed models
271 with individual plant as a random factor. Conductance values were log-transformed prior to the
272 analysis to achieve normality of the residuals. An analysis of deviance was performed on the
273 model. Pairwise comparisons using contrasts were performed between all modalities of the
274 factors.

275 Regarding the VOCs, linear mixed models for each compound were fitted with
276 treatment (“control” or “ozone”), time (“t0”, “t1”, “t2” and “t3”) and their interaction as fixed
277 factors and the pair of plants as random factor to evaluate the effect of treatments on their
278 variation indices. Then, analyses of deviance were used to test the significance of the effects of
279 these factors on the variation indices. Finally, pairwise comparisons using contrasts were
280 performed between modalities of the factors.

281 Furthermore, the relative proportion of each VOC in the total blend (excluding isoprene)
282 were analyzed by partial redundancy analysis (pRDA) by fitting one multivariate linear
283 regression of the relative proportions of VOCs according to plant treatment, time, and the
284 interaction of these two factors considering the pair of plants as a random factor in the model
285 (Hervé et al., 2018). Then, we performed a constrained principal component analysis (PCA) on
286 the fitted values of the regression model followed by permutation tests to assess (i) the
287 significance of the effects of the fixed factors and (ii) the real differences between modalities
288 of the factors. P-values were adjusted with the “false discovery rate” (fdr) method (Benjamini
289 & Hochberg, 1995).

290 2.6.2 Experiment 2

291 We compared the composition of VOCs presented to the fig wasps during the bioassays by
292 performing a pRDA on the proportions of all VOCs in the reactor using O₃ mixing ratio (0, 40,
293 80, 120 and 200 ppb) as a fixed factor and the fig branch replicate as a random factor.
294 Permutation F-tests were executed to assess the significance of the effect of the fixed factor and
295 between modalities of the factor with adjusted p-values (fdr method).

296 We also performed analyses of deviance on linear regressions to test the effect of O₃
297 level (fixed factor) on the relative proportions of each of the compounds in the attractive
298 mixture with the fig tree branch as a random factor. Pairwise comparisons using contrasts were
299 then conducted between the modalities of the factors.

300 Finally, to determine the significance of preferences of fig wasps for one odor source
301 compared to the control in the two-choice tests, we performed two-tailed exact binomial tests
302 (with expected $P = 0.5$).

303 3 Results

304 Linalool was the major compound emitted when plants (Experiment 1) or VOCs (Experiment
305 2) were not exposed to high O₃ level. On average, linalool accounted for 48.88 ± 5.41 % and
306 65.28 ± 10.58 % of the total emission of the gaseous mixtures constituted of 31 and 16 VOCs
307 in Experiments 1 and 2 respectively (see Supporting Information Tables S1 and S2). The four
308 VOCs responsible for fig wasp attraction (linalool, two linalool oxides and benzyl alcohol) were
309 the dominant compounds in the odor bouquets emitted by the figs in absence of O₃ (controls)
310 (mean proportion of these four VOCs among all VOCs emitted throughout both experiments:
311 64.66 ± 6.01 %).

312 3.1 Effect of plant exposure to high O₃ level on VOC emission (Experiment 1)

313 Time (before or after exposure) had a significant effect on stomatal conductance ($x^2 = 9.91$, P
314 $= 0.002$). But this effect depended on the O₃ treatment (for the interaction $x^2 = 16.22$, $P < 0.001$),
315 even though treatment alone was not significant ($x^2 = 0.57$, $P = 0.45$) (Fig. S3). In pairwise
316 comparisons, stomatal conductance values were similar for the two groups of fig trees (control
317 and ozonated) before O₃ exposure ($t = -1.94$, $P = 0.25$) but significantly differed after exposure
318 ($t = 3.24$, $P = 0.02$). Indeed, stomatal conductance decreased significantly after plants were
319 exposed to O₃ ($t = 4.99$, $P = 0.005$).

320 Ozone exposure significantly affected the estimated quantity of seven of the 31 VOCs
321 detected in the chamber (Fig. 2a). Pairwise comparisons showed a significant decrease in the
322 estimated quantity of two terpenes ((E)- β -ocimene and germacrene D) at 3 h and 5 h of plant
323 exposure to 200 ppb O₃ (for both, $P < 0.007$), while 2-ethyl-hexan-1-ol estimated quantity

324 decreased significantly only after 5 h (t2) of O₃ exposure ($P = 0.03$). Linalool tended to decrease
325 significantly after 5 h of exposure to O₃ ($P = 0.08$). In contrast, the estimated quantity of three
326 compounds, the unknown 1, lavender lactone (5-methyl-5-vinyl-dihydrofuran-2(3H)-one), and
327 its alcohol form lavender lactol (5-methyl-5-vinyl-dihydrofuran-2(3H)-ol), increased
328 significantly when fig trees were exposed to 200 ppb O₃ for 3 and 5 h (t1 and t2) compared to
329 the control (for both $P < 0.03$). One hour after O₃ exposure was stopped, estimated quantity of
330 lavender lactone ($P = 0.01$), lavender lactol ($P = 0.03$), 2-ethyl-hexan-1-ol ($P = 0.02$), oct-1-
331 ene ($P < 0.001$) and germacrene D ($P = 0.008$) were still significantly different from those in
332 the control plants.

333 Estimated quantity of ten other compounds varied over time (from t0 to t3) regardless
334 of the O₃ exposure ($P < 0.01$) (Fig. **2b**), suggesting variation in fig tree emissions over the
335 course of the day. In contrast, estimated quantity of the attractive compounds for fig wasps did
336 not vary significantly over the day (linalool: $P > 0.31$, (*Z*)-linalool oxide: $P > 0.37$, (*E*)-linalool
337 oxide: $P > 0.47$, and benzyl alcohol: $P > 0.54$).

338 As a result, the relative proportions of VOCs varied significantly according to the O₃
339 exposure (permutation test: $F_{1,115} = 3.89$, $P = 0.001$) and time (permutation test: $F_{3,115} = 2.83$,
340 $P = 0.001$) but the interaction between the two factors was not significant (permutation test:
341 $F_{3,115} = 1.74$, $P = 0.52$). All fixed factors combined explained 11% of the variability. The
342 random factor “pair of plants” explained 7.8 % of this variation. Pairwise comparison of VOC
343 emissions highlighted significant differences in the relative proportions of VOCs emitted by fig
344 trees in the morning (“control: t0” and “ozone: t0”) and in the afternoon (“control: t1”, “control:
345 t2” and “control: t3”) ($P < 0.01$). In addition, in pairwise comparisons samples collected from
346 plants exposed to O₃ for 3 and 5 h (“ozone: t1” and “ozone: t2”) were significantly different
347 from those collected in control chambers (“control: t0” to “control: t3”) and from the exposed
348 plants that were no longer exposed to O₃ (“ozone:t3”) (for all $P < 0.01$).

349 These significant effects of the O₃ exposure level and time on the relative proportions
350 of different VOCs were obvious in the PCA of the pRDA (Fig. 3a), where the samples from
351 these different groups were clearly separated. Indeed, on the first component of the PCA (52%
352 of the variation explained), samples collected from plants exposed to O₃ for 3 and 5 h were
353 clearly separated from those collected in control chambers (“control: t0” to “control: t3”) and
354 from the exposed plants that were no longer exposed to O₃ (“ozone:t3”). On the second
355 component of the PCA (25 % of the variation explained), the samples collected at mid-day were
356 separated from those collected in the afternoon. The correlation circle of the constrained PCA
357 (Fig. 3b) highlights these effects on the proportions of different compounds. Germacrene D,
358 (*E*)-β-ocimene and α-pinene were among the main VOCs emitted by plants one hour after ozone
359 exposure (“ozone: t3”). In contrast, (*E*)-linalool oxide fur., lavender lactone, lavender lactol and
360 the unknown 1 were positively correlated with the first component, suggesting that their
361 proportions increased after exposure to 200 ppb of O₃ for 3 and 5 h (“ozone: t1” and “ozone:
362 t2”). Moreover, benzyl alcohol and two linalool oxides (furanoid and pyranoid) were positively
363 correlated with the second component, indicating that their relative abundances were higher in
364 odor bouquets of plants sampled at mid-day, whereas aliphatic compounds were present in
365 higher proportions in odor bouquets of the same plants sampled in the afternoon.

366

367 3.2 Effect of VOC-ozone mixtures on pollinator attraction (Experiment 2)

368 In this second experiment, where only VOCs were ozonated, we were able to characterize the
369 odor sent to the pollinators in the Y-tube olfactometers. As in the control samples (65.28 ±
370 10.58 %), linalool was still the major compound in the ozonated samples with 40 and 80 ppb
371 of O₃ but in a lower proportion (43 ± 11 % and 36.3 ± 11.1 % respectively). As in Experiment
372 1, new compounds dominated the mixture for higher O₃ exposure levels. At 120 ppb O₃, linalool
373 was no longer the major compound in the mixture and was replaced by the (*Z*)-linalool oxide

374 (32.3 ± 14 %). At 200 ppb of O₃, lavender lactol and lavender lactone were the major
375 compounds, with relative proportions of 22.5 ± 6.7 % and 22.2 ± 9 %, respectively, while the
376 relative proportion of linalool dropped to 8.0 ± 3.8 % (Table S2).

377 The O₃ mixing ratio had a significant effect on the relative proportions of VOCs in the
378 reactor (permutation test: $F_{4,29} = 2.74$, $P = 0.001$). Seventeen percent of the variation in the
379 relative abundance of VOCs was explained by the O₃ mixing ratio in the reactor (0, 40, 80, 120
380 and 200 ppb) (Fig. **4a**). Forty-six percent of this variation was explained by the random factor
381 (i.e. fig branch). Pairwise comparisons showed that the VOC proportions were significantly
382 different between the ozonated and control (0 ppb), whatever the O₃ level applied ($P < 0.004$).
383 Among the ozonated samples, the relative proportions of the VOCs was significantly different
384 between samples exposed to 40 ppb and all other O₃ mixing ratios ($P < 0.003$) and there were
385 no significant differences for O₃ mixing ratios ranging between 80 and 200 ppb ($P > 0.12$) (Fig.
386 **4a**).

387 A total of 86 % of the constrained variance was explained by the first and the second
388 components of the PCA (64% and 22 % respectively). The effect of O₃ mixing ratio can be
389 observed on the first component, where ozonated samples > 40 ppb were positively correlated
390 with the first component. Lavender lactol, lavender lactone and the unknown compound 1 were
391 all positively correlated with the first component (Fig. **4b**). Their relative proportions were
392 higher in the odor bouquets exposed to high O₃ levels than in control ones. On the contrary,
393 linalool, (*E*)- and (*Z*)- linalool oxides furanoid were negatively correlated with the first
394 component, suggesting that their proportions were higher in the 0 ppb-exposed odor bouquet
395 than in ozonated ones. Benzyl alcohol was positively correlated with the second component,
396 suggesting that its proportion was higher in 40 ppb ozonated odors compared to the control but
397 also the higher O₃ levels.

398 When only the four VOCs attractive to fig wasps were considered, the O₃ mixing ratio
399 had a significant effect on their relative proportions, mainly those of linalool and (*E*)-linalool
400 oxide fur. at 120 ppb and 200 ppb O₃ compared with the proportions of these compounds at
401 lower ozone levels (Fig. 5).

402 In olfactometer tests, interestingly, O₃ alone had no significant effect on pollinator choice
403 ($n = 49, P = 1$). In contrast, as expected, fig wasps were significantly attracted by the 0 ppb-
404 ozonated mixture emitted by receptive figs (binomial test, $n = 55, P = 0.03$). On the other hand,
405 they showed no significant attraction to fig odors that had been altered by exposure to high O₃
406 mixing ratios (binomial test, 40 ppb: $n = 48, P = 1$; 80 ppb: $n = 52, P = 1$; 120 ppb: $n = 50, P =$
407 0.3; 200 ppb: $n = 49, P = 1$) (Fig. 6).

408 3.3 Determination of reaction rate coefficients of pollinator-attractive VOCs 409 with O₃ (Experiment 3)

410 This set of experiments confirmed the effects of O₃ on VOC amount and relative abundance in
411 the gaseous mixture, including (i) decreases in amount of linalool and sesquiterpenes and (ii)
412 increases in quantity of linalool oxide and benzyl alcohol. It also demonstrated the formation
413 of reaction products, especially lavender lactone (Fig. S4). Reaction rate coefficients were
414 calculated and are listed in Table 1. For linalool, the reaction rate coefficient was of the same
415 order of magnitude as found in the literature, validating our experimental setup (Table 1). For
416 sesquiterpenes, the comparison with the literature was approximate because we did not identify
417 these compounds individually but rather their global concentration. However, since
418 sesquiterpenes all have reaction kinetics similar in order of magnitude, the comparison with the
419 literature remains relevant (Table 1). Finally, for benzyl alcohol, no comparison with the
420 literature was possible since we observed a production of benzyl alcohol and not a degradation,

421 as in other studies, which means that under our experimental conditions, the production rate of
422 benzyl alcohol was higher than its consumption rate.

423 4 Discussion

424 In this study, under controlled conditions of O₃ exposure in a specialized plant-pollinator
425 interaction where the chemical signal responsible for pollinator attraction is known, we were
426 able to demonstrate the major effects of an O₃ pollution episode on the emission of floral VOCs,
427 and the chemical degradation of floral VOCs after their emission into the atmosphere. We were
428 able to characterize precisely the variations in the proportions of pollinator-attractive VOCs
429 under different O₃ mixing ratios, and the threshold at which pollinator attraction is modified.

430 4.1 Effect of acute exposure to high levels of O₃ on the emission of VOCs by 431 receptive figs of *Ficus carica*

432 In the three experiments carried out in this study, the four compounds responsible for pollinator
433 attraction (linalool, benzyl alcohol, (*Z*)- and (*E*)-linalool oxides furanoids), dominated the odor
434 bouquet of receptive figs, a result in line with previous studies (Grison-Pigé et al., 2002; Soler
435 et al., 2012; Proffit et al., 2020). Moreover, during VOC sampling, corresponding to the period
436 of the specific pollinator's activity, and in the absence of pollution by O₃, the estimated quantity
437 and relative abundances of the four attractive VOCs did not change, while those of other VOCs
438 varied significantly. More precisely, the number of compounds detected in Experiment 1, on
439 whole plants bearing receptive figs maintained in greenhouses, and Experiment 2, on branches
440 bearing figs maintained under constant temperature and humidity conditions, was slightly
441 different. It is quite possible that these differences owe to the fact that, in the case of Experiment
442 1, the plants were exposed to greater variations in temperature and especially light than in
443 Experiment 2, where these two parameters were controlled. Furthermore, in the case of

444 Experiment 1, significant variation in the estimated quantity of certain compounds was
445 detected. For instance, the estimated quantities of undecanal, oct-1-ene and decanal increased,
446 whereas those of myrcene and terpinolene decreased over time during fig receptivity. In
447 specialized plant-pollinator interactions, the attractive signal (especially the relative proportions
448 of attractive compounds) is expected to be homogeneous during the period of pollinator activity,
449 during the day, but also throughout all the duration of fig receptivity (Delle-Vedove et al.,
450 2017). However, phenotypic plasticity in the emission of other VOCs, often related to
451 environmental conditions (e.g. light and temperature), is expected (Farré-Armengol et al.,
452 2014). It would be interesting to follow precisely the dynamics of emission of VOCs from
453 receptive figs over the day, but also during the whole period of receptivity. Technological
454 advances in analytical chemistry allow such monitoring, especially with the fast analysis of
455 VOCs provided by PTR-ToF-MS (Hansel et al., 1995; Lindinger et al., 1998).

456 Our study highlighted significant variation in the estimated quantity of some VOCs
457 during high O₃ level episodes, revealing the possible effects of this pollutant on physiological
458 processes related to the emission of plant VOCs and/or chemical reactions between the emitted
459 VOCs and O₃ in the atmosphere. After the whole plant was exposed for a short period (5 h) to
460 high O₃ mixing-ratio (200 ppb), a significant decrease in leaf stomatal conductance was
461 detected. Similar physiological responses have been reported for other plant species (Reiling &
462 Davison 1995; Yu & Blande, 2022). Hence, in our experiment, stomatal closure may be a
463 mechanism of oxidative stress-avoidance adopted by fig trees under high O₃ level episodes.
464 Immediately after the 5h of exposure, when no more O₃ was in the chamber, a significant
465 decrease in estimated quantity of some VOCs (e. g. germacrene D) was observed
466 simultaneously with an increase in estimated quantity of other compound (e. g. lavender
467 lactone, lavender lactol and unknown 1). This result suggests that the O₃ exposure affected, at
468 the plant level, the emission of some VOCs. An alternative explanation, which does not exclude

469 the previous one, could be that O₃ reacted with VOCs in the inter-cellular cavities after VOCs
470 were produced, as has been reported in other plant species (Wedow et al., 2021). In other
471 studies, lavender lactone and lavender lactol have been reported as the main degradation
472 products formed by the reaction of linalool with O₃ (Lee et al., 2006; Shu et al., 1997). One
473 way to test more accurately the effect of O₃ on the emission of VOCs would be to examine the
474 expression of the genes involved in their biosynthesis. In any case, our study shows that a 5-
475 hour exposure to 200 ppb O₃ mixing ratio does not affect the emission of VOCs attractive to
476 pollinators.

477 4.2 Effect of O₃ on VOCs of receptive figs

478 The different experiments we carried out enabled us to distinguish very clearly between the
479 possible effects of O₃ on the emission of VOCs from receptive figs, and the direct effects of
480 ozone on these VOCs in the atmosphere. Thus, when only the VOCs emitted by fig trees were
481 ozonated, and not the whole plant, results were similar to those obtained during exposure of the
482 whole plant: a decrease in the relative abundance of terpenes, including linalool and some
483 sesquiterpenes (α -copaene and one unknown sesquiterpene), an increase in the relative
484 abundance of linalool oxides, and the occurrence of three new compounds (lavender lactone,
485 lavender lactol and unknown 1). These results suggest that during a short O₃ episode this
486 pollutant has a greater effect on the integrity of the odor of receptive figs in the atmosphere,
487 due to its high reactivity with these VOCs, than on the emission of these compounds by the
488 plant.

489 Regarding the effect of O₃ on the VOCs constituting the attractive signal for the
490 pollinator after their emission in the atmosphere, the reaction constants of linalool and benzyl
491 alcohol have already been determined (Calogirou *et al.*, 1999; Atkinson & Arey, 2003).
492 However, the reaction constant of linalool oxides with O₃ was not known prior to our study.
493 Our calculations of the reaction rate coefficients of VOCs attractive to pollinators confirmed a

494 high but contrasting reactivity with O₃ (Table 1). When the mixture of attractive VOCs was
495 exposed to O₃, the amount of linalool decreased significantly while the amount of linalool
496 oxides increased, highlighting the effect of O₃ on changes in the relative abundances of VOCs
497 in the attractive mixture. One or two of the isomers of linalool oxides furanoid ((*Z*)-linalool
498 oxide and (*E*)-linalool oxide) are produced by the chemical reaction of linalool with O₃ but
499 unfortunately, the PTR-ToF-MS technology does not allow quantifying them individually.
500 However, thanks to the identification of degradation products of linalool (linalool oxides but
501 lavender lactone) it could be possible to determine the branching ratio of these compounds in
502 the reaction of linalool with O₃ under our experimental conditions (Seakins, 2007). The reaction
503 rate coefficient of lavender lactone was also estimated for the first time. These results give an
504 estimation of how quickly the attractive blend changes when exposed to O₃.

505 4.3 Effects of ozone-related changes in floral VOCs on the attraction of 506 pollinators

507 At concentrations as low as 40 ppb of O₃, which is a mixing-ratio commonly observed in spring
508 and summer in the Mediterranean region, a significant effect was observed on the relative
509 abundances of the pollinator-attractive VOCs. In our behavioral tests, these changes in the
510 proportions of VOCs inhibited the attraction of fig wasps to odors of receptive figs. This O₃
511 level is well below those of other experimental studies that have sought to assess experimentally
512 the impact of ozone-induced degradation of floral VOCs and consequently on pollinator
513 attraction (Cook et al., 2020; Farré-Armengol et al., 2016; Saunier et al., 2023). To date, the
514 only experimental study to have tested the effect of O₃ degradation of floral VOCs on insect
515 attraction with a mixing-ratio range as wide as our study was carried out on an herbivore, the
516 striped cucumber beetle (*Acalymma vittatum*, Chrysomelidae) (Fuentes et al., 2016). Using a
517 setup very similar to that used in the present study, these authors showed an alteration in the

518 attraction of this herbivore to floral odors at an O₃ mixing ratio of 80 ppb; below this level, the
519 insect was significantly attracted to floral odors. As in this study, our olfactometer tests with O₃
520 suggest that this pollutant alone has no direct effect on the attraction behavior of fig wasps.
521 Another possible effect of O₃ that is intrinsic to our experimental set-up is that it can directly
522 affect insect physiology and behavior, as already demonstrated (Demares et al., 2022; Demares
523 et al., 2024; Vanderplanck et al., 2021 a,b). In the case of the direct effect of O₃ on the
524 physiology and behavior of fig wasps, a significant effect was only demonstrated after a one-
525 hour exposure to 120 ppb; below this value, attraction behavior was not modified
526 (Vanderplanck et al., 2021 a). This finding confirms that the effect observed in the present study
527 from 40 ppb of O₃ is indeed due to the degradation of the VOCs of the receptive figs and most
528 probably to the change in the proportions of the four pollinator-attractive VOCs. These results
529 are in accordance with previous results of Proffit et al. (2020), showing that the change in the
530 proportions of VOCs in the attractive odor completely inhibited the attraction of the pollinating
531 fig wasps.

532 An important new question that arises from our results is whether pollinators can detect
533 the degradation products of the reaction of fig VOCs with O₃, and whether these novel
534 compounds can affect their behavior. A recent study showed that the model species *Drosophila*
535 *melanogaster* is able to detect via its antennae some of the degradation products of VOCs
536 generated by O₃ oxidation, and that these products induced various behavioral responses in this
537 species (Venkateswaran et al., 2023). One might expect the pollinator to develop some new
538 form of learning of the attractive odor degraded by the O₃, as has been demonstrated for tobacco
539 hawkmoth and bumblebees (Cook et al., 2020; Saunier et al., 2023). Unfortunately, unlike
540 generalist insects, specialist insects have limited phenotypic plasticity, including for their
541 learning abilities (Jones & Agrawal, 2017). Moreover, in the case of fig wasps, whose
542 individuals are short-lived (about one day, Proffit *et al.*, 2020), once they are attracted by the

543 signal emitted by receptive figs and enter within the fig to lay eggs, they generally lose their
544 wings as well as part of their antennae (Van Noort & Compton, 1996), preventing them from
545 visiting a new fig and learning new odors. Thus, the impact of O₃, or other phytotoxic pollutants,
546 on plant-pollinator interactions is highly variable among species of both plants and pollinators
547 (Agathokleous et al., 2022; Masui et al., 2023; Ryalls et al., 2022; Saunier & Blande, 2019),
548 and certainly depends on their ecology. The study of these effects cannot therefore be limited
549 to certain model species, and there is now an urgent need for comparative studies between
550 different plant-pollinator interactions.

551 5 Conclusion

552 The present study provides a complete picture of the possible effects of acute O₃ episodes on
553 the integrity of the chemical signal emitted by a plant to attract its specific pollinator during the
554 exposure phase and immediately thereafter. The exposure of the Mediterranean fig tree to high
555 O₃ levels induced a significant decrease in leaf stomatal conductance and a limited change in
556 the emission of VOCs not involved in pollinator attraction, but more importantly, a major
557 change in the relative abundances of the pollinator-attractive VOCs in the atmosphere. The
558 results of the present study, coupled with those of others carried out on the direct effects of O₃
559 on fig wasps (Vanderplanck et al., 2021 a,b), indicate that in the case of an acute O₃ episode,
560 of the three stages of chemical plant-pollinator communication—emission by plants, diffusion
561 into the air, detection by pollinators—it is the reaction of VOCs with O₃ in the air, affecting
562 detection by pollinators, that most affects the interaction between the fig tree and its pollinator.
563 This reactivity of fig VOCs with O₃ in the atmosphere was observed at the lowest O₃ level
564 tested (40 ppb O₃) and these changes in VOC composition significantly altered the attraction of
565 the specific pollinator. These alarming results suggest that the O₃ episodes currently occurring
566 in the Mediterranean region are probably already affecting chemical communication between
567 the fig tree and its specific pollinating wasp. Experiments now need to be carried out to assess

568 whether such changes in pollinator attraction behavior can affect the reproduction of both
569 partners.

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856 Figure titles

857 **Fig. 1 Schematic diagram of the fig tree volatile organic compounds (VOCs) exposed to**
858 **increasing ozone mixing ratio (a) coupled with behavioral tests on the fig wasp in**
859 **Experiment 2 and (b) analyzed by proton-transfer-reaction time-of-flight-mass**
860 **spectrometer (PTR-ToF-MS) in Experiment 3.** In addition, VOCs were sampled in the
861 reactor in each experiment through adsorbent traps for 5 min at a flow rate of 0.2 L min⁻¹. Air
862 purified by charcoal filter, or an air generator, was pushed into the chamber at 2 L min⁻¹
863 (reactor). Y-tube olfactometers in panel a were made of glass (4 cm in diameter) with each
864 lateral arm being 20 cm long and the central arm being 15 cm long. White triangles represent
865 the air flow directions.

866 **Fig. 2 Variation indices of volatile organic compounds (VOCs) from fig trees that showed**
867 **significant variation with (a) ozone treatment or (b) time.** Mean ± SE is presented from each
868 time. VOC samples from control fig trees are represented in strong rounded grey lines and
869 VOCs samples from ozonated fig trees are represented in black dashed lines with black
870 triangles. Black asterisks indicate significant differences between control and ozonated samples
871 based on pairwise comparisons ($P < 0.05$) and black dots correspond to statistical tendencies
872 ($P \leq 0.08$).

873 **Fig. 3 (a) Score plot of the constrained Principal Component Analysis from Partial**
874 **Redundancy Analysis (pRDA) of the relative proportions of fig tree volatile organic**
875 **compounds (VOCs) in chambers as a function of ozone treatment and time in Experiment**
876 **1 and (b) the associated correlation circle.** Black symbols on the score plot correspond to the
877 odor bouquet of ozonated fig trees, grey symbols correspond to that of control fig trees, and the
878 shape of symbols indicate the time of VOC collection in the chambers (circle = t0, triangle =

879 t1, square = t2, cross = t3). Groups with different letters next to the centroids showed
880 statistically significant different chemical profiles based on pairwise comparisons ($P < 0.05$).

881 **Fig. 4 (a) Score plot of the constrained Principal Component Analysis from Partial**
882 **Redundancy Analysis (pRDA) of the relative proportions of fig tree volatile organic**
883 **compounds (VOCs) in the reactor as a function of ozone mixing ratio in Experiment 2 and**
884 **(b) the associated correlation circle.** Empty circles correspond to 0 ppb of O₃ and full black
885 circles, cross, triangles and squares correspond respectively to 200, 120, 80 and 40 ppb of O₃.
886 Letters next to the centroids show the significant differences between each group of odors based
887 on pairwise comparisons ($P < 0.003$).

888 **Fig. 5 Bar plots of the proportions of attractive VOCs.** Different letters mean significant
889 differences between proportions within one compound (linear model followed by contrasts, P
890 < 0.04).

891 **Fig. 6 Attraction responses of fig wasps to different odors emitted by receptive figs**
892 **exposed to different ozone mixing ratio.** Choice tests were conducted in Y-tube olfactometers
893 where fig wasps were allowed to choose between fig odor in one branch, and control odor (air)
894 in the other. In a second set of tests, fig wasps could choose between O₃ alone at 200 ppb and
895 air. P = results of the two-tailed exact binomial tests (NS means non-significant and * means
896 $P < 0.05$). n = number of tested individuals. Number of wasps that did not make a choice is
897 indicated in parentheses.

898

899

900 **Table 1. Reaction rate coefficients (cm³ molecules⁻¹ s⁻¹) of pollinator-attractive volatile**
 901 **organic compounds (VOCs), sesquiterpenes and major identified reaction products.** Mass
 902 over change (m/z) values used to monitor each VOC using a proton-transfer-reaction time-of-
 903 flight mass spectrometer (PTR-ToF-MS) are indicated. Reaction rate coefficients found in the
 904 literature are indicated with the corresponding references.

<i>VOC</i>	<i>m/z</i>	Reaction rate coef. with O ₃	Type	Literature mean value	References
<i>linalool</i>	137	(3.16 ± 0.63) x 10 ⁻¹⁶	D	(4.10 ± 1.0) x 10 ⁻¹⁶	Bernard et al. (2012)
<i>linalool oxides</i>	171	(2.65 ± 1.07) x 10 ⁻¹⁵	P	NA	NA
<i>benzyl alcohol</i>	109	(6.50 ± 1.94) x 10 ⁻¹⁶	P	< 2.00 x 10 ⁻¹⁹	Bernard et al. (2013)
<i>sesquiterpenes</i>	205	(8.33 ± 2.67) x 10 ⁻¹⁶	D	[10 ⁻¹⁴ ; 10 ⁻¹⁶]	Richters et al. (2015) β-caryophyllene, α-humulene, α-cedrene and isolongifolene
<i>lavender</i>	127	(1.75 ± 0.76) x 10 ⁻¹⁵	P	NA	NA
<i>lactone</i>					

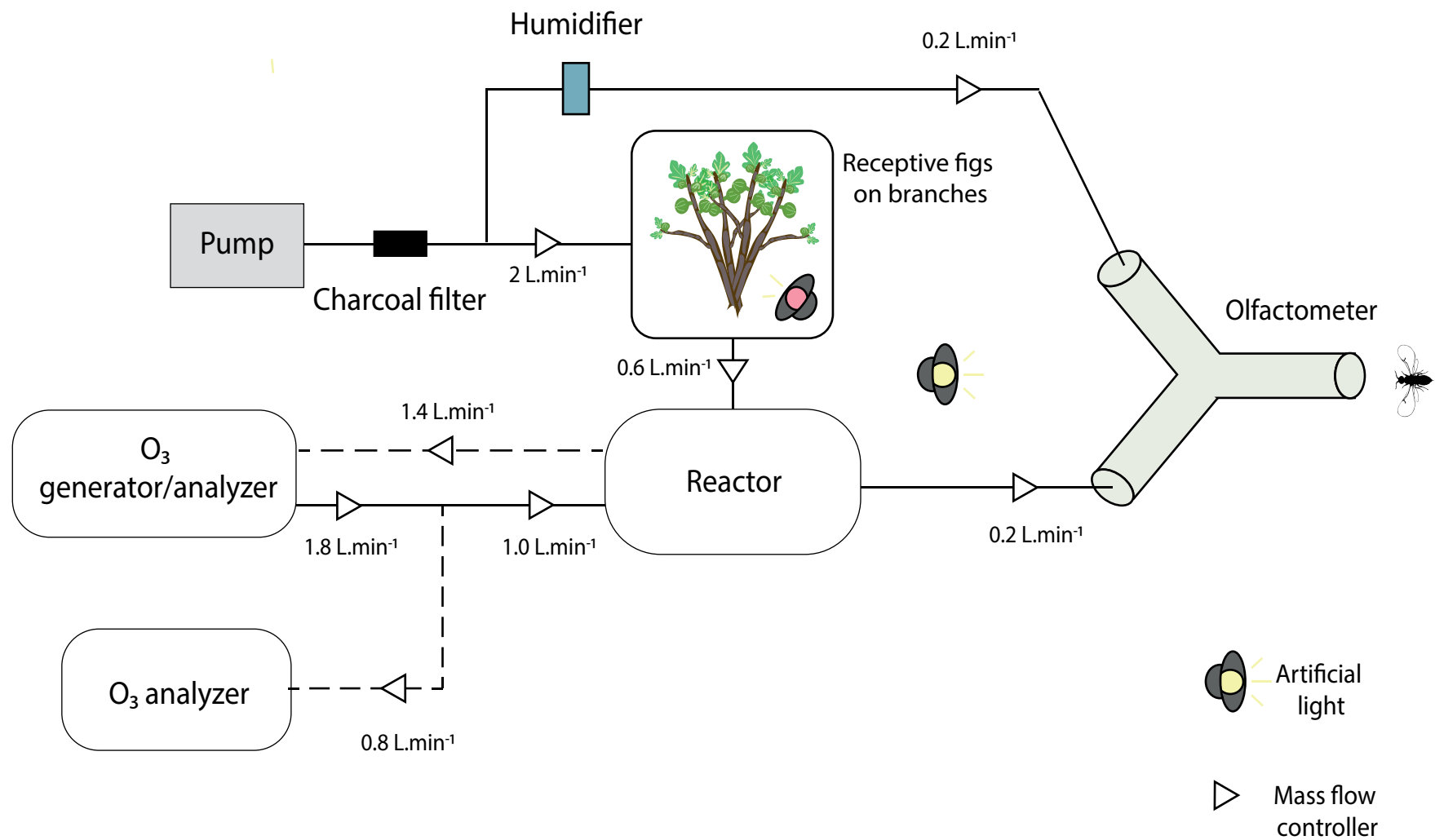
905 m/z corresponds to the protonated ion MH⁺

906 NA no reference found in the literature.

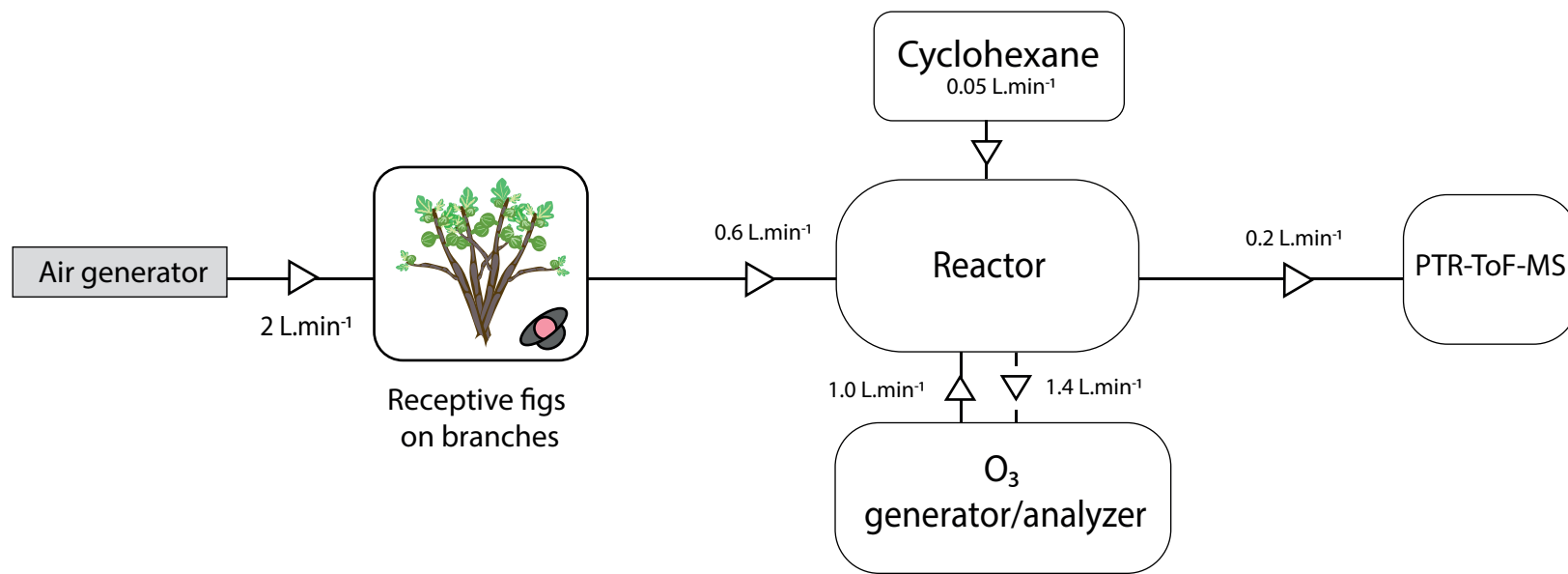
907 D degradation

908 P production

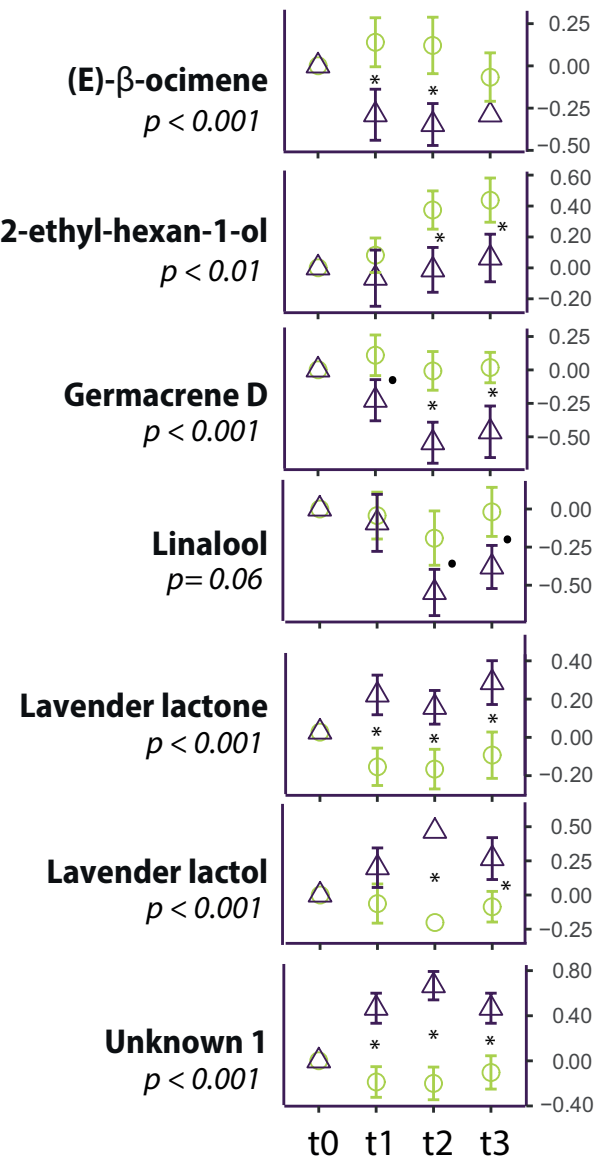
(a)



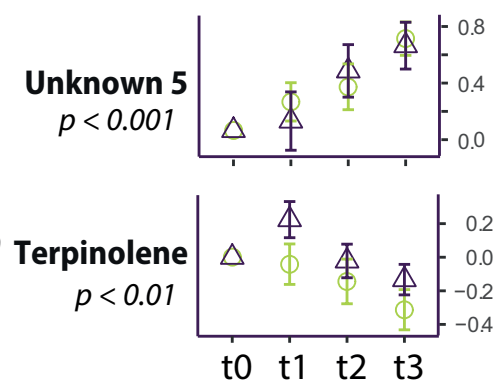
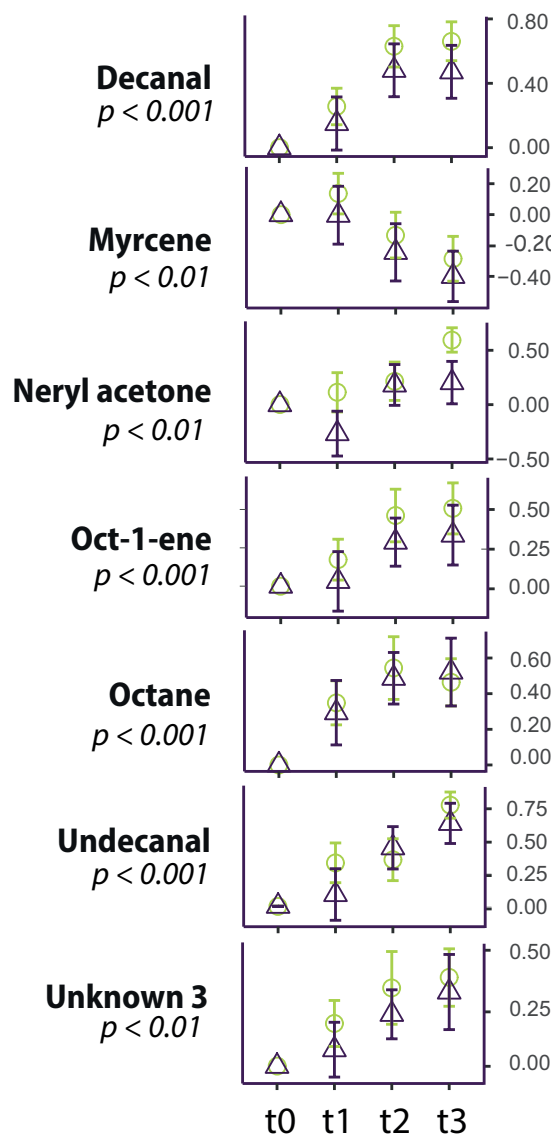
(b)



Treatment effect



Time effect



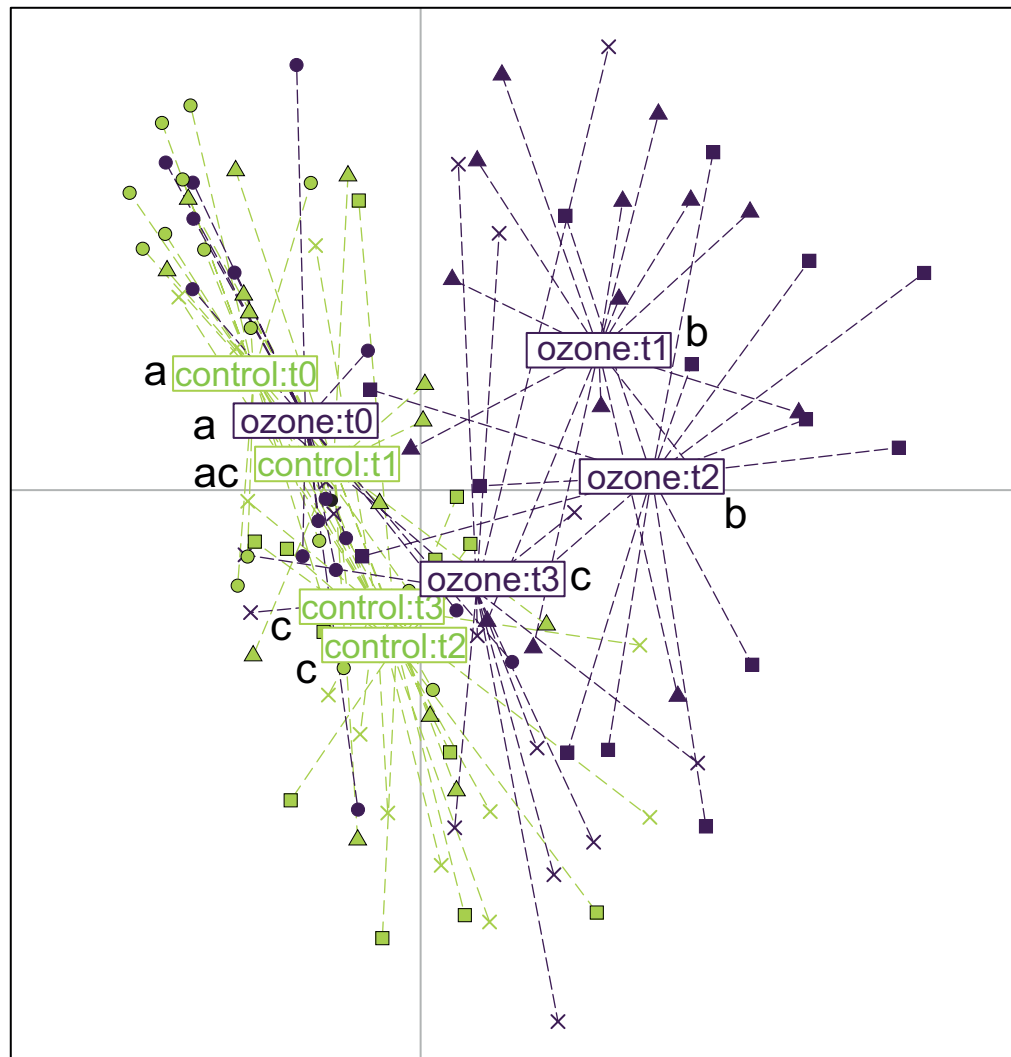
○ Control
△ Ozone

• $p = 0.06$
** $p < 0.01$
*** $p < 0.001$

Variation index

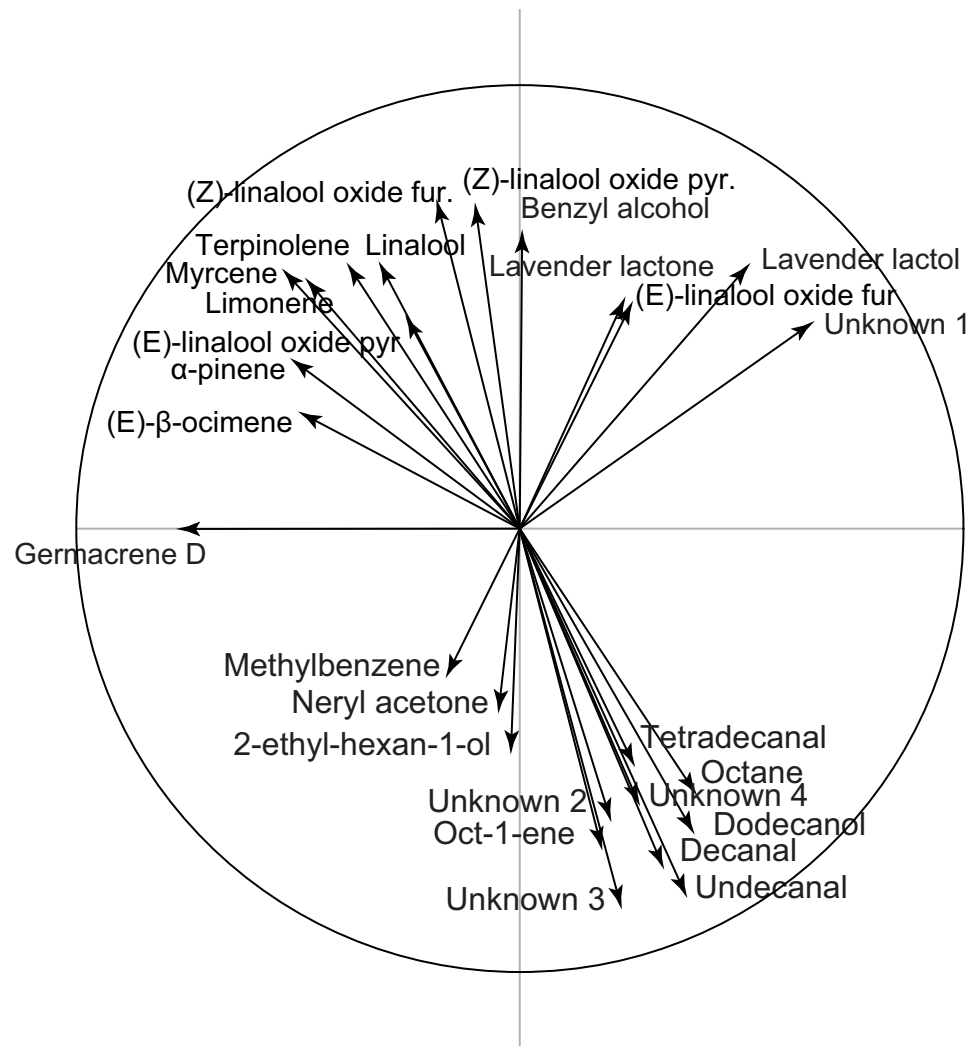
(a)

Component 2: 25.20%

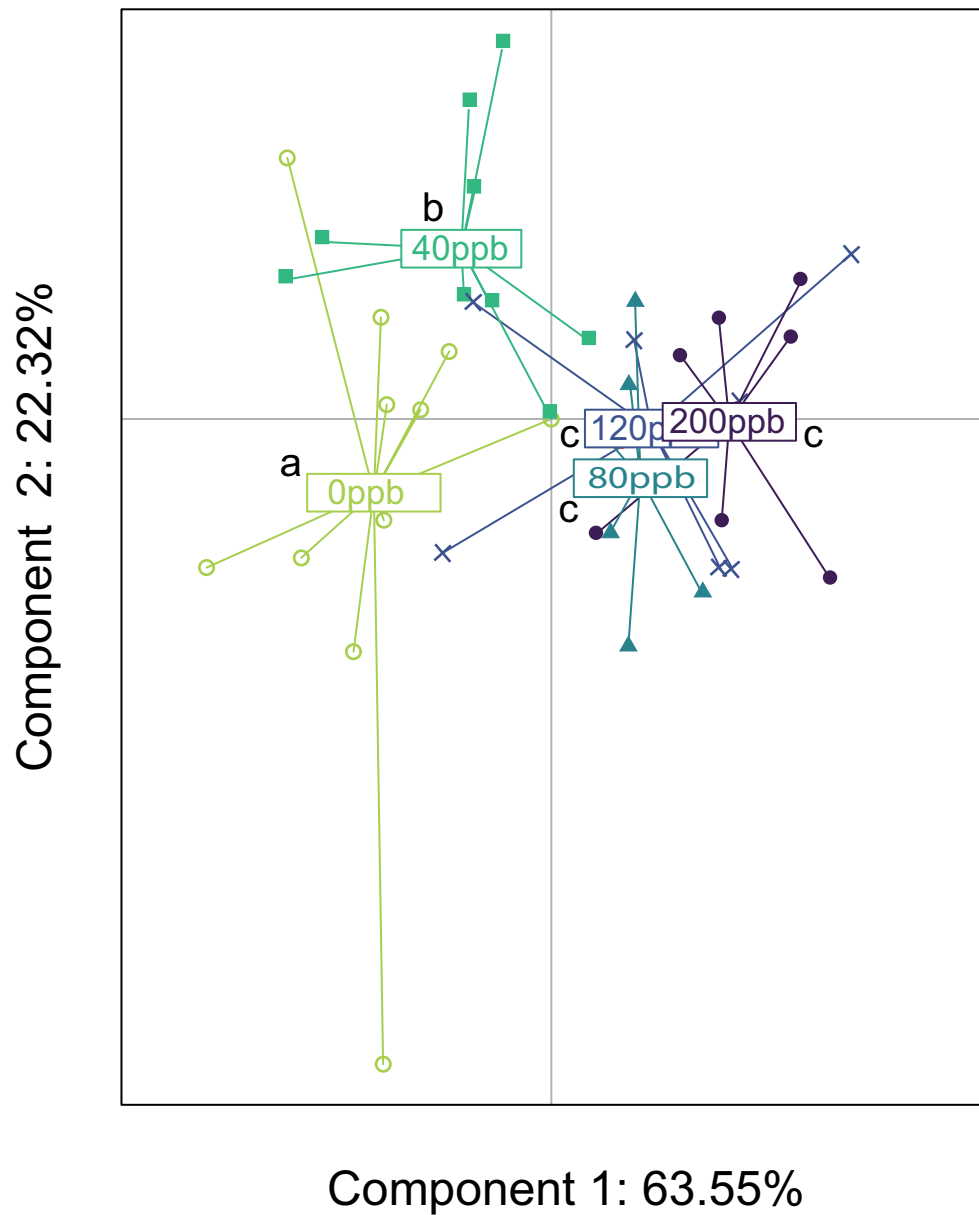


Component 1: 52.02%

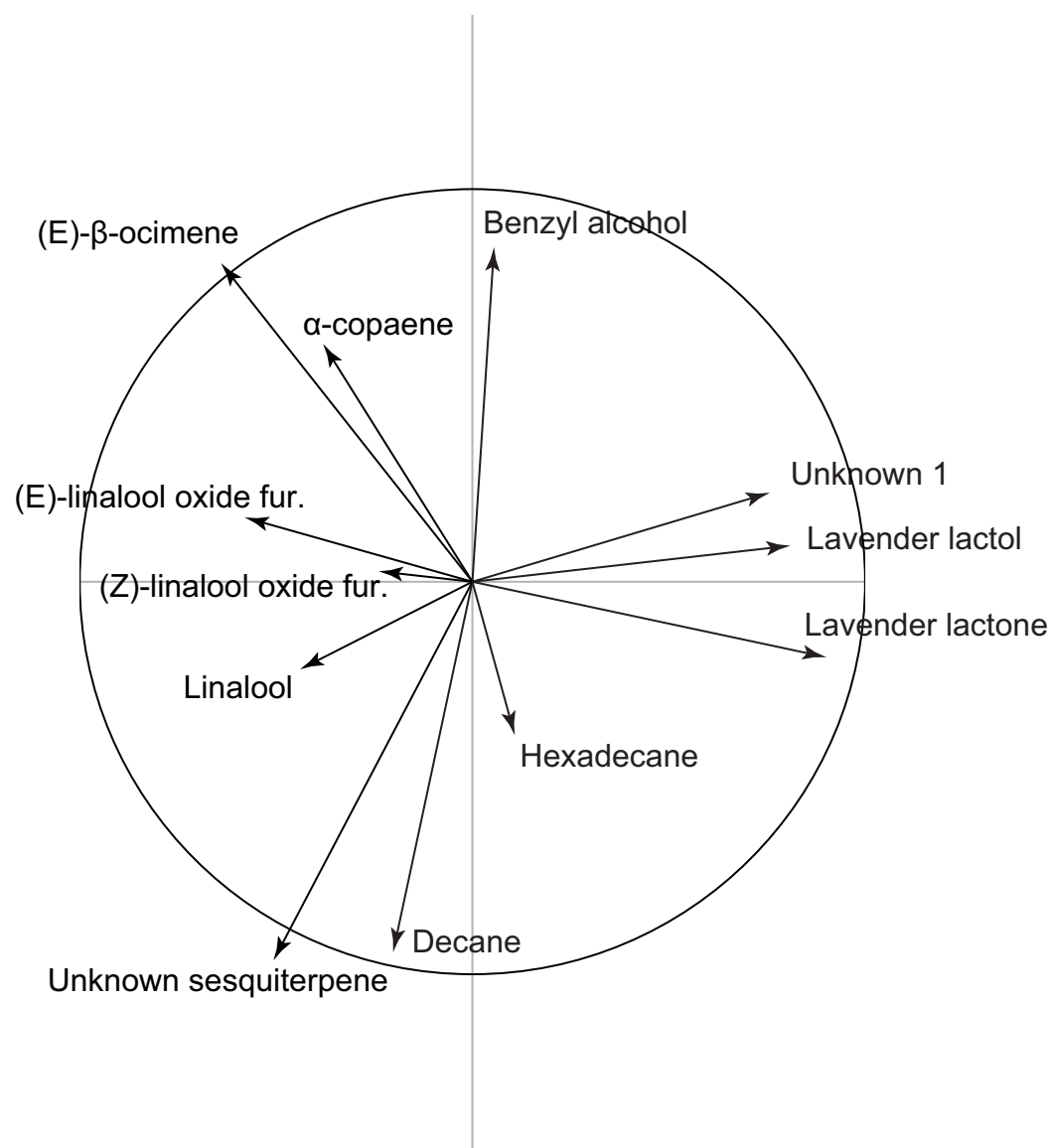
(b)



(a)



(b)



Percentage of attractive VOCs

