



1 **Measurement report: Age-dependent BVOC emissions in *Eucalyptus urophylla*: a**
2 **comparison of leaf cuvette and branch chamber measurements**

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11



12 **Abstract**

13 Biogenic volatile organic compound (BVOC) emission factors (E_s) underpin air quality and climate models,
14 yet current databases intermingle data from both seedlings and mature trees and from two enclosure
15 techniques, leaf cuvettes and dynamic branch chambers, whose comparability has rarely been rigorously
16 tested. Here we quantified BVOC emissions from *Eucalyptus urophylla* by pairing the two methods on a
17 statistically representative number of 2-month-old seedlings in the laboratory and 2-year-old trees measured
18 at a managed plantations in subtropical China. Leaf-cuvette and branch-chamber determination of isoprene
19 E_s matched within 5% for both age classes, demonstrating method equivalence. In contrast, tree age exerted
20 a significant impact on both the magnitude and speciation of emissions. Seedlings emitted ~50% more
21 isoprene and were enriched in cyclic monoterpenes like α -pinene and 1,8-cineole, whereas field-grown trees
22 shifted toward highly reactive acyclic monoterpenes, with β -ocimenes accounted for over 85% of the terpene
23 flux and a double rise in sesquiterpenes. These ontogenetic shifts imply that one-third of the entries in global
24 E_s compilations, which are derived from seedling studies, likely overestimate local isoprene fluxes while
25 under-representing the atmospheric reactivity of mature canopies. Our results validate the use of either
26 chamber type for measuring isoprene E_s , highlight the need for improved analytical sensitivity before
27 extending this equivalence to terpenes, and call for systematic, large-sample, branch-level measurements of
28 adult trees to produce representative E_s values. Incorporating age-resolved emission factors into models will
29 refine estimates of ozone and secondary organic aerosol formation in fast-growing subtropical plantations
30 and other managed forests worldwide.

31



32 **1 Introduction**

33 Terrestrial vegetation release on the order of $\sim 1,000$ Tg yr⁻¹ for biogenic volatile organic compounds
34 (BVOCs), representing $\sim 90\%$ of global non-methane VOCs injected to the troposphere (Guenther et al., 1995,
35 2012). Owing to their high reactivity with atmospheric lifetimes of only minutes to hours (Atkinson and Arey,
36 2003), BVOCs can strongly modulate the oxidative capacity of the troposphere and drive the production of
37 ozone and secondary organic aerosols (Di Carlo et al., 2004; Peñuelas and Staudt, 2010; Scott et al., 2017;
38 Pfannerstill et al., 2024; Weber et al., 2024). Reliable quantification of these fluxes is therefore essential for
39 assessing their impacts on air quality and climate.

40 Current bottom-up BVOC emission inventories rely on species-specific emission factors (E_s) that are applied
41 within models such as the Model of Emissions of Gases and Aerosols from Nature (MEGAN; Guenther et
42 al., 2012, 2020). Inaccurate E_s are now recognized as a leading source of uncertainty in regional and global
43 estimates of BVOC budgets (Guenther et al., 2012; Zhang et al., 2020; Wang et al., 2023). Decades of
44 enclosure studies produced several widely used databases, like the Sheffield (Hewitt and Street, 1992),
45 UCAR (Wiedinmyer et al., 2004), and most recently, the tropical plant compilation of Mu et al. (2022).
46 However, two methodological and biological issues remain unresolved.

47 Both leaf cuvettes and dynamic branch chambers have been used for measuring E_s . Guenther et al. (1994)
48 suggested, from a literature survey, that isoprene E_s with leaf cuvette are approximately 75% higher than
49 branch-based values, but a rigorous, side-by-side validation on the same trees is still lacking. Whether the
50 two techniques can be used interchangeably is therefore uncertain.

51 As for the tree age, approximately one-third of database entries originate from greenhouse or growth-chamber
52 seedlings (Guenther, 2013), yet seedling's physiology differs markedly from that of mature trees. Limited
53 case studies on *Eucalyptus spp.* point to pronounced ontogenetic shifts in both the magnitude and speciation
54 of BVOC emissions (Street et al., 1997; He et al., 2000; Winters et al., 2009). Meanwhile, considerable
55 uncertainty may result from the potential intraspecific variability and chemodiversity of BVOC emissions
56 (Loreto et al., 2009; Staudt and Visnadi, 2023; Zeng et al., 2024). Previous investigations, however, employed
57 small sample sizes and heterogenous protocols, leaving the statistical representativeness of age effects largely
58 unconstrained.

59 Addressing these gaps requires large-sample, method-controlled measurements that span contrasting
60 developmental stages. Here we report parallel cuvette and branch chamber determination of BVOC emissions
61 from *Eucalyptus urophylla* seedlings grown under laboratory conditions (2-month-old) and saplings (2-year-
62 old) measured in situ at a managed plantation in subtropical China. The objectives are to 1) quantitatively
63 test the equivalence of the two chamber types for isoprene and, where detection limits permit, for terpenes;
64 2) disentangle how tree age influences both emission factors and chemical composition; and 3) assess the



65 implications for constructing representative E_s databases and for simulating ozone and secondary organic
66 aerosol formation in rapidly expanding plantation forests. By combining method inter-comparison with
67 statistically robust sampling across ontogeny, our work provides critical benchmark for future BVOC
68 inventories and modeling frameworks.

69 **2 Materials and methods**

70 **2.1 Study sites and plant materials**

71 Laboratory measurements were performed at the Guangzhou Institute of Geochemistry (GIG, 23.145° N,
72 113.364° E). Field measurements were carried out in a managed *E. urophylla* plantation at Heshan (22.649°
73 N, 112.904° E), Guangdong province, China. The study region experiences a humid subtropical monsoon
74 climate, with a 30-year mean temperature of 22 °C and annual precipitation of ~1,700 mm (Mu et al., 2023;
75 Zeng et al., 2024). As shown in Table 1, two age classes, 2-month-old and 2-year-old, were investigated, with
76 seedlings measured in laboratory and 2-year-old trees measured *in situ* at the plantation. Seedlings were
77 purchased from a local nursery and measured by both leaf cuvette and dynamic branch chamber. Field trees
78 were randomly chosen from >8 ha of homogeneous plantation to ensure spatial representativeness.

79 **2.2 Enclosure measurements**

80 **2.2.1 Leaf cuvette**

81 Leaf cuvette fluxes were obtained with a LI-6800 portable photosynthesis system (LI-COR, Lincoln, NE,
82 USA) fitted with a 6800-01A fluorometer head (aperture 6 cm², air flow 500 μmol s⁻¹). Environmental set
83 points were 30 °C leaf temperature, 1000 μmol m⁻² s⁻¹ PAR, 400 μmol mol⁻¹ CO₂ and 55% relative humidity
84 to produce emission factors (E_s). After 5 min stabilization of photosynthesis, 200 mL min⁻¹ of outlet air was
85 diverted for 2 min through Tenax TA/Carbograph 5TD adsorbent cartridges (Markes International Ltd,
86 Bridgend, UK) using a dual-channel pump (ZC-QL, Zhejiang Hengda Instrumentation Ltd., Zhejiang, China).
87 Detailed leaf handling, leaf area and dry mass determination are provided in Text S1 and are also described
88 in Zeng et al. (2024, 2025ab).

89 **2.2.2 Dynamic branch chamber**

90 A cylindrical PMMA chamber (Ø 25 cm × 28 cm, 13.7 L) internally coated with FEP film was used for
91 branch-level measurements. The charcoal- and KI-scrubbed ambient air (9 L min⁻¹) was supplied by a mass-
92 flow controller (Alicat Scientific, Inc., Tucson, AZ, USA) coupled with an oil-free pump (MPU2134-N920-
93 2.08; KNF, Freiburg, Germany), then it was well mixed with PTFE-bladed fans in the chamber (Zeng et al.,
94 2022a). Sunlit branches (3-5 m above ground) were enclosed; fluxes were allowed to stabilize for 1-2 h before
95 sampling. Outlet air (and inlet blanks) was drawn at 200 mL min⁻¹ for 10 min by an automatic sampler
96 (JEC921; Jectec Science and Technology, Co., Ltd, Beijing, China) onto the same adsorbent tubes as above.



97 Concurrent meteorological and radiometric variables were logged continuously (Rotronic HC2A-S RH/T
98 probes; LI-1500 PAR sensor; OMEGA/ RKC thermocouples). More details about the branch sampling are
99 provided in Text S2 and Zeng et al. (2022a).

100 **2.3 Thermal desorption-GC/MS analysis**

101 Tubes were analyzed within 7 days with a TD-100 system (Markes) coupled to an Agilent 7890 GC-
102 5975 MSD. Primary cartridge desorption was 280 °C, cold-trapping at -10 °C, then desorption at 320 °C.
103 Separation employed an HP-5 MS (30 m × 0.25 mm × 0.25 μm) column. The GC oven temperature program
104 was started at 35 °C (3 min), 5 °C min⁻¹ to 100 °C (1 min), 10 °C min⁻¹ to 120 °C (12 min), and 20 °C min⁻¹
105 to 260 °C (2 min). Identification used authentic standards, while quantification based on calibration curves.
106 More information about the analysis and quantification are given in Text S3 and Zeng et al. (2022a, 2022b).

107 **2.4 Calculation of emission rates and emission factors**

108 For branch data, emission rates (E , μg g⁻¹ h⁻¹) were calculated as

$$109 \quad E = \frac{F(C_{out} - C_{in})}{g_{dw}}$$

110 where F is chamber flow (L h⁻¹) and g_{dw} the dry leaf mass. Real-world emission rates were standardized to
111 30 °C and 1000 μmol m⁻² s⁻¹ PAR using the MEGAN algorithms for light-dependent compounds and a
112 temperature-only function for light-independent monoterpenes ($\beta = 0.10$ K⁻¹) and sesquiterpenes ($\beta = 0.17$ K⁻¹).
113 For cuvette data, the LI-6800 set points already represent standard conditions, so E is equal to E_s after
114 correcting for leaf dry weight. More details about the calculation of emission factors are provided in Text S4
115 and in Zeng et al. (2023).

116 **2.5 Statistical analysis**

117 All statistics were performed in Excel 2019. Isoprene and terpene E_s were log-normal, and they were log-
118 transformed before testing. Equality of means between age classes or methodologies was evaluated with
119 Welch's t-test; distributions that failed Shapiro-Wilk normality ($p < 0.05$) were compared with Mann-
120 Whitney U. Significance was accepted at $p < 0.05$. Results are reported as mean ± 1 SD unless otherwise
121 specified.

122 **3. Results and discussion**

123 **3.1 Method inter-comparison for isoprene E_s**

124 Side-by-side measurements of *E. wrophylla* seedlings (2 months old) and 2-years-old trees showed that the
125 two dominant enclosure techniques yielded statistically indistinguishable isoprene E_s . As shown in Fig. 1, for
126 seedlings measured at GIG, cuvette-derived isoprene E_s averaged at 102.4 ± 34.1 μg g⁻¹ h⁻¹ (N=50) versus



127 107.7±34.9 $\mu\text{g g}^{-1} \text{h}^{-1}$ from branch chambers (N=15), a non-significant 5% difference ($p=0.61$). In the field,
128 2-year-old trees exhibited similarly close agreement: 66.9±31.4 $\mu\text{g g}^{-1} \text{h}^{-1}$ (N=114) from leaf cuvettes and
129 69.8±21.2 $\mu\text{g g}^{-1} \text{h}^{-1}$ (N=26) from branch chambers ($p=0.57$). A direct test in which the same branch was first
130 sub-sampled leaf-by-leaf and then enclosed intact confirmed parity within analytical uncertainty (Fig. S1).

131 Leaf cuvette and dynamic branch chamber are the two most widely employed techniques for measuring plant
132 BVOC emissions (Niinemets et al., 2011). The present study provides the first large-sample validation that
133 leaf cuvette and dynamic branch chamber protocols are interchangeable for isoprene, contradicting the ~75 %
134 bias inferred from the earlier literature meta-analysis (Guenther et al., 1994). The convergence arises despite
135 distinct air flow regimes (0.75 L min^{-1} vs. 9 L min^{-1}) and path lengths, implying that adsorptive/ozonolysis
136 losses are negligible for this highly volatile compound. Because branch chambers integrate six to ten leaves
137 per branch, their representativeness is at least as good as that of cuvettes as previously demonstrated for
138 Eucalyptus by Zeng et al. (2024), and they remain advantageous when mature crowns are inaccessible.

139 Establishing methodological equivalence for isoprene enables age effects to be probed with confidence using
140 the more logistically efficient branch chamber dataset (Sect. 3.2). Whether the same holds for lower-volatility
141 monoterpenes and sesquiterpenes remains an open question, as current detection limits preclude rigorous
142 comparison; systematic tests with larger sample volumes are warranted.

143 **3.2 Ontogenetic controls on the magnitude and speciation of BVOC emissions**

144 **3.2.1 Speciation diversity**

145 Dynamic branch chamber measurements revealed 12 BVOC species in 2-month-old seedlings (isoprene, 8
146 monoterpenes, 3 sesquiterpenes; Fig. S2) versus 17 species in 2-year-old trees (isoprene, 11 monoterpenes,
147 5 sesquiterpenes; Table 2). Thus, chemical richness increased by ~40% with age, consistent with the view
148 that metabolic complexity develops as trees mature (Satake et al., 2024).

149 **3.2.2 Emission magnitude**

150 Isoprene dominated the flux from both age classes but its standardized E_s declined significantly ($p<0.001$)
151 from 107.7±34.9 $\mu\text{g g}^{-1} \text{h}^{-1}$ in seedlings to 69.8±21.2 $\mu\text{g g}^{-1} \text{h}^{-1}$ in two-year-old trees. When normalized by
152 leaf area, however, the two groups were indistinguishable (Fig. 2a), indicating that the mass-based contrast
153 mainly reflects an increase in leaf mass per area (LMA) with tree age (Fig. S3). Seedlings therefore allocated
154 a larger fraction of assimilated carbon to isoprene (Fig. 2c) despite exhibiting lower net photosynthesis (P_n ,
155 Fig. 2b), suggesting a typical growth-defense trade-off of early ontogeny. Consistent with our findings,
156 isoprene E_s for the 1-year-old *E. globulus* was 5-fold higher than that for the 7-year-old individual (Street et
157 al., 1997). Winters et al. (2009) also documented lower isoprene E_s in four 10-year-old eucalyptus species
158 compared to their seedlings measured by He et al. (2000).



159 3.2.3 Terpene speciation shift

160 Total monoterpene emissions rose nearly 6-fold with tree age (1.09 vs. 6.14 $\mu\text{g g}^{-1} \text{h}^{-1}$), but the increase was
161 almost entirely due to acyclic β -ocimenes, whose E_s leapt from 0.13 ± 0.06 to 5.33 ± 4.61 $\mu\text{g g}^{-1} \text{h}^{-1}$ (Table 2).
162 By contrast, E_s for cyclic α -pinene, limonene, and 1,8-cineole declined by 30-65% (Fig. 3). Consequently,
163 terpene composition shifted from 75% cyclic (seedlings) to 85% acyclic (2-year-old trees) (Fig. 3).
164 Sesquiterpenes doubled in absolute terms but remained a minor (<1%) proportion of total BVOCs.

165 Such a cyclic-to-acyclic transition has been observed in other *Eucalyptus* species and might be attributed to
166 age-dependent expression of terpene synthesis and to selective pressures from biotic/abiotic stress in the
167 field (Monson et al., 2021; Pollastri et al., 2021). For example, most previous studies reported that cyclic α -
168 pinene and 1,8-cineole were the dominant MTs in stressless lab-grown seedlings (Evans et al., 1982;
169 Guenther et al., 1991; He et al., 2000; Tsui et al., 2009; Malik et al., 2019), whereas acyclic MTs like β -
170 ocimenes were generally low or even undetectable. In contrast, most stress-rich field-grown trees could emit
171 large amounts of acyclic β -ocimenes (Street et al., 1997; Nunes and Pio, 2001; Sørensen et al., 2020; Purser
172 et al., 2020, 2021; Nagalingam et al., 2023).

173 Both isoprene and β -ocimenes are known to play key roles in plant antioxidant defense. Isoprene helps
174 scavenge reactive oxygen species (ROS) (Jardine et al., 2014), maintain membrane stability (Sharkey and
175 Singaas, 1995; Pollastri et al., 2019, 2021), and regulate antioxidative processes (Zuo et al., 2019; Monson
176 et al., 2021), whereas the highly reactive β -ocimene may act as an even more efficient, direct antioxidant
177 against ROS. The highly reactive β -ocimenes can quench ROS more efficiently than isoprene
178 (Pollastri et al., 2021), providing a plausible advantage for mature trees exposed to stronger light, heat,
179 drought, and herbivory.

180 4. Conclusions

181 Parallel measurements on *E. urophylla* seedlings (2 months old) and saplings (2 years old) demonstrate that
182 leaf-level cuvettes and dynamic branch chambers yield statistically indistinguishable isoprene E_s . This large
183 sample validation may resolve a long-standing methodological debate and confirms that both enclosure types
184 can be used interchangeably for the most abundant BVOC when appropriate QA/QC protocols are followed
185 (Zeng et al., 2022a, 2025c). For monoterpenes and sesquiterpenes, assessment of method equivalence was
186 precluded by their lower fluxes and the limited air volumes sampled. Because terpene emissions are more
187 sensitive to wall losses and analytical detection limits than isoprene, systematic inter-comparison studies with
188 larger sample volumes and a broader range of plant species are still required.

189 Emission profiles changed markedly among age classes. Seedlings emitted ~50 % more mass-based isoprene
190 and were enriched in cyclic monoterpenes (α -pinene and 1,8-cineole), whereas saplings produced 6-fold
191 higher total monoterpenes dominated by highly reactive acyclic β -ocimenes and exhibited a double rise in



192 sesquiterpenes. This shift likely implies a developmental re-allocation of carbon from generalized antioxidant
193 protection (isoprene) towards compounds better suited to coping with the more intense and severe biotic and
194 abiotic stresses experienced by field-grown trees.

195 Roughly one-third of the global BVOC E_s entries now feeding chemistry-climate models originate from
196 greenhouse seedlings. Our results show that these data tend to overestimate canopy-scale isoprene fluxes and
197 under-represent atmospheric reactivity by excluding large β -ocimene emissions from mature canopies.
198 Seedling measurements are therefore inappropriate as generic surrogates for natural forests. Incorporating
199 age-resolved E_s and prioritizing statistically robust and in-situ sampling of branches from adult trees,
200 particularly in rapidly expanding subtropical plantations, will reduce inventory uncertainty and refine
201 predictions of ozone and secondary organic aerosol formation.

202 Future studies should extend age-stratified measurements to other high isoprene genera (e.g. *Quercus*,
203 *Populus*) and to tropical species that dominate global BVOC budgets, evaluate method comparability for
204 low-volatility terpenes using larger-volume or adsorption-minimized chambers, and couple physiological
205 measurements with transcriptomics to unravel the molecular basis of the observed metabolic shift from cyclic
206 to acyclic terpenes.

207

208 **Data availability.** The measurement data used in this study can be available at
209 <https://doi.org/10.17632/jw8g8gkm5t.1> (Zeng, 2025).

210 **Supplement.** The related supplement is published alongside this article.

211 **Author contributions.** JZ and XT designed and carried out the experiments with the support of WP, YL, HR,
212 ZM, HG, and WS. JZ and XT analyzed the samples in the lab. JZ and XT analyzed the data and prepared the
213 original manuscript. XW and YZ revised the manuscript.

214 **Conflict of interest.** The authors declare no conflicts of interest relevant to this study.

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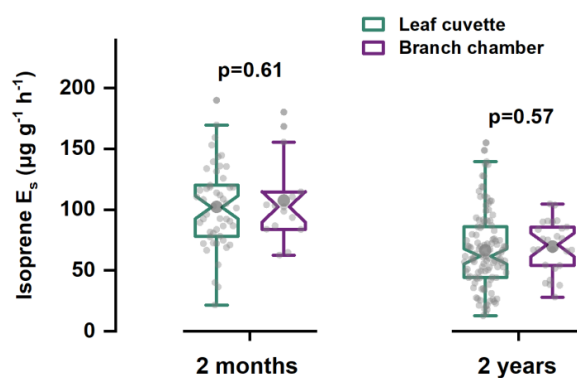
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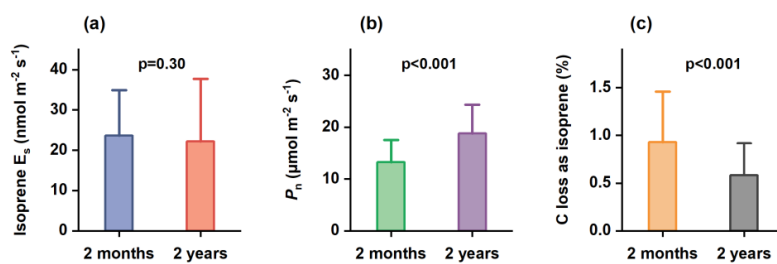
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370

371 **Figure 1.** Comparison of isoprene E_s from leaf cuvettes with those from dynamic branch chambers for both
372 2-month-old and 2-year-old trees.

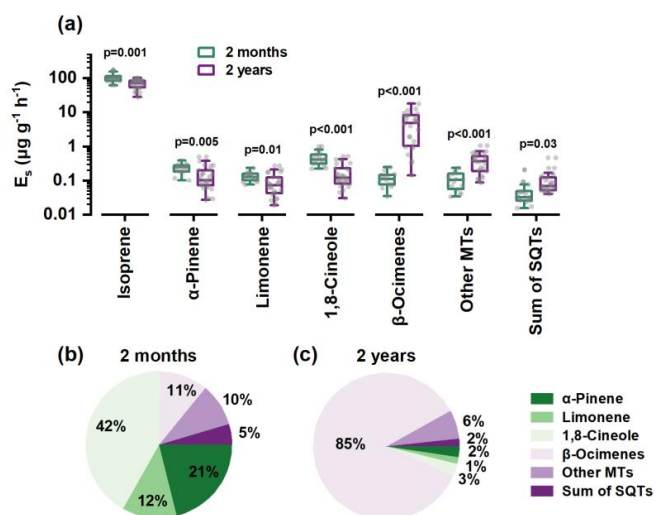
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375 **Figure 2.** Comparison of area-based isoprene E_s (a), net photosynthetic rate (P_n , b), and carbon loss fraction
376 as isoprene emission (c) for 2-month-old trees with those for 2-year-old ones. These data were from the leaf
377 cuvette measurements.

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380 **Figure 3.** Comparison of emission factors between 2-month-old and 2-year-old trees (a). Terpene

381 composition for the 2-month-old trees (b) and 2-year-old trees (c).

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383

Table 1. Two age classes of *Eucalyptus urophylla* investigated in this study

Site	Laboratory	Field
Age class	2 months	2 years
Height (m)	0.3-0.4	10-12
Cuvette (no. of replicate)	50	114
Branch chamber (no. of replicate)	15	26
Cuvette LMA (g m^{-2})	57 ± 7	82 ± 13
Branch-chamber LMA (g m^{-2})	55 ± 8	78 ± 11

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385



386

Table 2. Comparison of BVOC emission factors among two age groups

Compounds	2 months old	2 years old
Isoprene	107.72±34.93	69.75±21.15
α -Pinene	0.24±0.07	0.15±0.12
β -Pinene	0.01±0.01	n.d.
β -Myrcene	0.01±0.01	0.17±0.13
α -Phellandrene	n.d.	0.04±0.03
Limonene	0.14±0.03	0.09±0.07
1,8-Cineole	0.48±0.22	0.17±0.13
cis- β -Ocimene	0.07±0.04	0.37±0.30
trans- β -Ocimene	0.06±0.02	4.96±4.31
3,6-Dimethyl-1,3,7-octatriene	n.d.	0.06±0.04
Linalool	0.08±0.06	0.07±0.05
3,4-Dimethyl-2,4,6-octatriene	n.d.	0.05±0.04
Alloocimene	n.d.	0.02±0.02
Sum of MTs	1.09±0.35	6.14±5.23
α -Longipinene	0.02±0.01	0.01±0.01
α -Copaene	0.03±0.03	0.03±0.02
β -Caryophyllene	0.01±0.01	0.04±0.05
α -Humulene	n.d.	0.01±0.01
Alloaromadendrene	n.d.	0.01±0.01
Sum of SQTs	0.05±0.05	0.10±0.10

387

n.d.: not detected