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Lavender sensitivity to water stress

1 **Lavender sensitivity to water stress: comparison between eleven varieties**
2 **across two phenological stages**

3

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16

17 **Highlight**

- 18 • *L. angustifolia* and *L. x intermedia* featured a drought-tolerant strategy toward water
19 stress
20 • *L. angustifolia* is more tolerant to water stress than *L. x intermedia*
21 • Some particular compounds such as bornyl acetate could be considered as defense
22 compounds
23 • Water stress could be a treat to the quantity and the quality of essential oil
24 • The varieties Sumian and Rapido are the best candidates for cultivation under water
25 stress

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34 **Abstract**

35 Essential oils from *Lavandula angustifolia* Mill. and *Lavandula x intermedia* Emeric x Loisel
36 composed of concentrated terpene mixtures, and are highly produced due to their economic
37 value. In the Mediterranean area, the production is threatened by the increasing frequency and
38 intensity of drought events, leading to the loss of crops. Thus, we aimed to evaluate the
39 response to water stress and the tolerance of the main French cultivated varieties of *L.*
40 *angustifolia* and *L. x intermedia* during flowering period, based on the response of their
41 primary metabolic traits (e.g. growth, photosynthesis, stomatal conductance) and specialized
42 metabolic traits (terpene storage and emissions related to the plant defense system). Two
43 treatments were applied: a control where plants received 300 mL every day (≈ 75 % of
44 substrate field capacity) and a stress where plants received 100 mL every two days (≈ 25 % of
45 substrate field capacity). Our results showed that both species featured a drought-tolerant
46 strategy to cope with water stress with a more competitive strategy in *L. angustifolia*. Water
47 deficit modified the amounts of stored terpenes in both species which could be economically
48 harmful for the sector related to essential oil production. Moreover, some compounds such as
49 bornyl acetate were highlighted as potential defense compounds. The study also highlighted
50 that the varieties ‘Rapido’ (*L. angustifolia*), arecent variety developed in France, and
51 ‘Sumian’ (*L. x intermedia*), could be the best candidates to cultivate under intense drought.

52

53 Keywords: *L. angustifolia*, *L. x intermedia*, abiotic stress, sensitivity, terpenes, water stress

54 **1. Introduction**

55 For several centuries, fine lavender has been part of the rural economy of the dry mountains
56 of Provence, where lavenders naturally occur (Guitton 2010). Their economic value is mainly
57 explained by the vast amounts of terpenes they accumulate in the aerial parts which allows
58 production of lavender essential oil (EO) after distillation. The lavender EO production was
59 developed with the rise of the perfume industry in Grasse (South of France) during the 18th
60 and 19th centuries. Nowadays, the increasing use of lavender EO (e.g. perfumery,
61 phytotherapy, laundry industries) entails their massive cultivation in France and other
62 countries (Bulgaria, China). Two principal lavender species, originating from the South of
63 France, are cultivated worldwide for their EO: *Lavandula angustifolia* Mill. (called true
64 lavender or fine lavender) and a natural and sterile hybrid, *Lavandula x intermedia* Emeric ex
65 Loisel (called lavandin), which is a cross between two *Lavandula* species: *L.*
66 *angustifolia* subsp. *angustifolia* Mill. and *L. latifolia* Medik. At the global scale, lavender and
67 lavandin EO from France represented respectively 26 % and 95 % of the world market in
68 2016 (Giray 2018).

69 These EO are complex mixture of hundreds of monoterpenes and sesquiterpenes stored in
70 glandular trichomes, like in other Lamiaceae species (Park *et al.* 2016). These specialized
71 structures of the epidermis are abundant on leaves and on calices of inflorescences. The main
72 compounds of lavender EO are linalool and its ester linalyl acetate, which represent more than
73 half of the total EO content. Quality and yield of the EO depend on the genotype and the
74 environmental conditions (Aprotosoiaie *et al.* 2017).

75 Since the severe drought in 2003 (Ciais *et al.* 2005), French plantations experience an excess
76 of mortality due to the combination of both intense and long-lasting drought periods and the
77 worsening symptoms of yellow disease. This disease is due to the *Stolbur* phytoplasma, a
78 bacteria without cell wall living in plant phloem (Christensen *et al.* 2005; Sémétey *et al.*

79 2018). and whose main vector is the phloem-sucking leafhopper *Hyalesthes obsoletus* Sign.
80 (Weintraub & Beanland 2006). Typical symptoms of the yellow disease include progressive
81 leaf yellowing (or chlorosis), drying and eventually death.

82 Those symptoms can be intensified during summer water deficit, typical in the Mediterranean
83 region. Climatic models predict an intensification of water deficit in this area consisting of an
84 average increase of the annual mean temperature by 3.4 °C and, an elongation of the water
85 deficit period by 2100 resulting in a 30 % decrease of summer natural rainfall (Giorgi &
86 Lionello 2008; Christensen *et al.* 2013; Polade *et al.* 2014). This intensification disturbs
87 primary metabolic processes including limitation of the stomata aperture and thus carbon
88 assimilation in the short term (days) (Chaves *et al.* 2009) and growth in the middle term
89 (weeks, months). Limited carbon assimilation can also affect (at short and middle time scales)
90 specialized metabolic processes such as terpene metabolism which provides defenses for the
91 plant against numerous environmental constraints (read hereafter). Water deficit modifies
92 both, terpene storage or concentration within plant organs (Llusià & Peñuelas 1998; Delfine *et*
93 *al.* 2005) and terpene emissions from those organs (Yani *et al.* 1993; Ormeno *et al.* 2007;
94 Llusià *et al.* 2009; Saunier *et al.* 2017) which impacts plant health and interaction with its
95 environment.

96 On the one hand, increasing terpene concentration within plant organs contributes to plant
97 protection against biotic pressures (pathogen attack, herbivorous feeding), due to the
98 decreased palatability, or increased toxicity of plants when ingested by herbivores (Bassolé &
99 Juliani 2012). On the other hand, emitted and stored terpenes are involved in plant defense
100 against the oxidative pressure generated by abiotic and biotic pressures (Vickers *et al.* 2009).
101 Terpenes limit cell oxidation both, directly - as scavengers of reactive oxygen species
102 (Vickers *et al.* 2009; Velikova *et al.* 2011) - and more likely indirectly since their synthesis
103 allows getting rid of the energy excess (Ormeño *et al.* 2020). This energy excess occurs when

Lavender sensitivity to water stress

104 photosynthesis is strongly limited and terpene affinity for lipid tissues contributes to
105 restructuring damaged thylakoid membranes (Sharkey & Monson 2017; Ormeño *et al.* 2020).
106 Furthermore, it has been shown on several Lamiaceae species that water stress induced
107 changes in terpene content suggesting a role in plant protection to cope water scarcity. For
108 example, Mahdavi *et al.* (2020) found differences between sensitive and tolerant cultivars of
109 *Thymus vulgaris* L. The sensitive plants presented a trade-off between growth and defense
110 whereas tolerant ones exhibited increases of α -phellandrene, o-cymene, γ -terpinene and β -
111 caryophyllene concentrations. In *Ocimum basilicum* L., some water stress-tolerant varieties
112 showed accumulation of terpenes in response to water stress (Kalamartzis *et al.* 2020). These
113 examples suggest that terpenes may contribute to cope with water stress in Lamiaceae.

114 To cope with future climatic evolution in the Mediterranean area, assessing which lavender
115 varieties are more naturally resistant to drought is of crucial economic and ecological interest.
116 Cultivation of the most drought-resistant cultivars could also be a way to minimize the
117 combined effect of water stress and yellow disease. Varieties with little resistance to drought
118 would probably be less competitive to face the concomitant action of both pressures.

119 In the present study, we investigated the capacity of the principal varieties of *L. angustifolia*
120 and *L. x intermedia* cultivated in Southern France to cope with water deficit through changes
121 in their *i*) primary metabolic traits (e.g. stomatal conductance, net photosynthesis,
122 transpiration) and growth (estimated through floral and foliar biomasses and leaf mass area
123 ratio), *ii*) specialized metabolic traits through terpene storage (floral and foliar terpene
124 content) and emissions of entire individuals, *iii*) plasticity of primary and specialized
125 metabolic traits. Based on those responses, we highlighted the most resistant varieties to water
126 deficit which would be the most appropriate varieties to cultivate in the Mediterranean area
127 for a sustainable production of EO. We hypothesized that *L. angustifolia* and *L. x intermedia*
128 would exhibit different strategies in front of water deficit according to primary and

129 specialized metabolic traits and that some terpenes would be highly accumulated under water
130 stress and could act as defense compounds.

131

132 **2. Material and methods**

133 **2.1. Plant material**

134 The two lavender species that represent the highest tonnages in terms of EO production
135 worldwide were used in this study: the true lavender *L. angustifolia* and its sterile hybrid, the
136 lavandin *L. x intermedia*. Within each species, we chose the most cultivated varieties (also
137 called cultivars) in France together with more recent ones (Diva, Rapido, Sara) selected for
138 their tolerance to both, water stress and yellow disease as observed by the CRIEPPAM
139 (Centre Régionalisé Interprofessionnel d'Expérimentation en Plantes à Parfum Aromatiques
140 et Médicinales). Six varieties of *L. angustifolia* and five varieties of *L. x intermedia* were used
141 with respectively: CDR05, Matheronne, 77-13, Diva, Rapido and Sara; Abrial, Grosso,
142 Grosso ada, Grosso certitude and Sumian (Table 1). These cultivars corresponded to clonal
143 plants obtained by cutting except Rapido and Sara which corresponded to synthetic
144 populations obtained by breeding of 7 parental plants.

145 All plants were 1-year-old, provided by the CRIEPPAM and certified healthy plants (i.e. not
146 infected by yellow disease). They were grown in 1.2 L pots with a soil composed of 1/3 sand
147 and 2/3 brown soil. Plants were placed in a greenhouse with an average temperature ranging
148 between 16-18 °C/30-35 °C (night/day respectively) and an average air relative humidity
149 ranging between 75-85 %/35-50 % (night/day respectively). Climatic conditions inside the
150 greenhouse were recorded with a datalogger (HOBO U12-012, Onset, USA). The watering
151 treatment began at T0. T0 corresponds to the morphological stage of elongation of floral
152 stems. At this stage, we found no significant difference for diameters, height, the number of

Lavender sensitivity to water stress

153 floral stems and the volume of aerial parts (see figure S1 in supplementary files) indicating
154 that all plants were at a similar phenological stage when the experiment started.

155 The control treatment (C) consisted of plant watering every day with 300 mL water
156 corresponding to 75 % of substrate field capacity (FC), that is, the maximal water retention
157 capacity of the substrate, previously measured according to Veihmeyer and Hendrickson
158 (1949). The drought stress treatment (S) consisted of supplying 100 mL water every 2 days,
159 corresponding to 25 % of FC. Due to phenological stage heterogeneity at T1 and T2 (see
160 below), water treatment started on May 17th 2013 for *L. angustifolia* varieties and May 24th
161 2013 for *L. x intermedia* varieties, and ended, for both species, on July 22nd 2013 (Fig. 1).

162 Physiological and metabolic traits were measured under these two watering treatments over
163 the two phenological stages, T1 and T2: T1 is the stage where 50 % of flowers were opened
164 when the inflorescences are traditionally harvested to produce EO and T2 corresponds to the
165 fructification stage (and the end of the experimentation). To catch up those stages,
166 measurements during T1 on *L. angustifolia* took place from June 10th to July 4th 2013 while
167 measurements on *L. x intermedia* were performed from June 19th to July 4th 2013 since
168 flowering occurred 1 week later in this second species. For both species, T2 was comprised
169 between 9th –22nd July 2013. Because measures were destructive, we used a total of 20 plants
170 per variety to reach n=5 samples per measurement session and treatment. Different
171 experiments on clonal and populations of *L. angustifolia* showed that sampling 3-5 plants per
172 variety gave a good level of inter individual variability allowing to compare several varieties
173 (Guitton 2010; Despinasse *et al.* 2020). Thus, a total of 220 plants were analyzed during the
174 experiment.

175

176 **2.2. Dynamic enclosures at the entire plant-scale**

177 Dynamic enclosures were used for monitoring gas exchanges of CO₂, H₂O and terpene
178 emissions as fully described in Genard-Zielinski *et al.* (2015) with slight modifications as
179 described in Saunier *et al.* (2017). Each entire plant together with the pot was introduced in
180 the enclosure 1 h prior to measurements. Each enclosure was \approx 30 L volume and consisted of
181 a PTFE (polytetrafluoroethylene) frame closed by a 50 μ m thick PTFE film (for a picture; see
182 Ormeño *et al.* 2020). At least 2 h before enclosure, the substrate and the pot of each plant
183 were surrounded with a PTFE sheet to avoid substrate/pot emissions and minimize stem
184 handling/mechanical stress during enclosure (Ormeño *et al.* 2010). Air inside the chamber
185 was renewed every 3 min thanks to an airflow of 9 L min⁻¹ using a PTFE pump
186 (N840.1.2FT.18, KNF®, Germany). Biogenic volatile organic compounds (BVOCs) were
187 removed from the inlet air using activated charcoal and ozone was also removed from inlet air
188 by placing PTFE filters impregnated with sodium thiosulfate (Na₂S₂O₃, Sigma®) according to
189 Pollmann *et al.* (2005) as applied in numerous BVOC-related studies (Ormeño *et al.* 2010;
190 Fares *et al.* 2012; Saunier *et al.* 2017). A PTFE fan, placed inside the chamber, ensured a
191 rapid mixing of the air. All measurements were performed in the laboratory under fixed
192 Photosynthetically Active Radiation (PAR) ($1000 \pm 200 \mu\text{mol m}^{-2} \text{s}^{-1}$) - provided by a 400 W
193 light bulb (HQI-BT 400W/D Daylight E40, Osram, Germany) placed over each enclosure
194 system - and constant room temperature ($30 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$).

195

196 **2.3. CO₂ and H₂O exchanges**

197 Exchanges of CO₂ and H₂O from the enclosed plants were monitored every minute using two
198 InfraRed Gas Analysers (IRGA 840A, LI-COR, USA), placed at the air inlet and air outlet,
199 respectively. All gas exchanges were averaged between 12:00 and 15:00 (local time) when
200 plants were the most physiologically active. Net photosynthesis (P_n, $\mu\text{molCO}_2 \text{ m}^{-2} \text{ s}^{-1}$),

201 stomatal conductance to water (G_w , $\text{mmolH}_2\text{O m}^{-2} \text{s}^{-1}$) and transpiration (E , $\text{mmolH}_2\text{O m}^{-2} \text{s}^{-1}$) were calculated using equations described by Von Caemmerer and Farquhar (1981) as
202 detailed in Genard-Zielinski *et al.* (2015).
203

204

205 **2.4. Terpene emissions**

206 After one hour of air homogenization, terpene emissions were collected in pre-conditioned
207 cartridges placed at the outlet of the dynamic enclosure chamber. Cartridges contained 2
208 absorbents: Tenax TA (50 mg, Agilent technologies) and Carbotrap B (120 mg, Sigma-
209 Aldrich). Collection took place for 5 min with an air flow of 0.3 L min^{-1} . One cartridge was
210 sampled for each plant. After collection, cartridges were stored at $-20 \text{ }^\circ\text{C}$ before analysis.
211 Blanks at the inlet air allowed to periodically check for VOC scrubber efficiency.

212

213 **2.5. Growth and water potential**

214 Once gas exchanges (CO_2 , H_2O) and terpene emission collection had been studied,
215 measurements of growth were carried out. Growth was characterized through three traits
216 including total dry foliar biomass, total dry floral biomass, and leaf mass per area ratio (LMA,
217 g m^{-2}). LMA, a proxy of leaf density, was measured using 10 leaves per individual. The
218 surface of each fresh leaf was measured with a leaf area meter (AM350, ADC Bioscientific
219 Ltd., UK). Dry mass of leaves and inflorescences were measured by weighting after oven-
220 drying at $70 \text{ }^\circ\text{C}$ for 2 days. The average ratio between leaf dry mass and surface was thereby
221 calculated for each plant to obtain the LMA.

222 Stem water potential (Ψ) was measured at midday with a Scholander pressure chamber (PMS
223 instrument Co., USA, range : 0- 7 MPa). Measurements were carried out using 2 stems by
224 individual. A third measurement was performed only when the difference between the two
225 values was higher than -0.3 MPa.

226

227 **2.6. Terpene content in leaves and inflorescences**

228 Leaves and inflorescences were collected after gas exchange measurements and immediately
229 frozen in liquid nitrogen. They were then stored at -80 °C until terpene extractions were
230 carried out in the laboratory. Terpenes extraction from leaves were carried out according to
231 (Ormeno *et al.* 2007) with slight modifications (read hereafter) and according to Guitton
232 (2010) for inflorescences.

233 For both, inflorescence and leaf terpenes, methyl undecanoate ($12\text{ng}\cdot\mu\text{L}^{-1}$) was used as
234 internal standard and added to the extraction solvent. After the extraction, leaves and
235 inflorescences were collected and oven-dried for 2 days at 70 °C after solvent evaporation in
236 order to express results in a terpene mass per dry mass basis. The total terpene content
237 corresponded to the sum of the quantity of each terpene.

238

239 **2.7. Terpene analysis by GC-MS**

240 Analyses of terpene emissions from entire plants were performed by Gas Chromatography
241 (GC, model 6890N, Agilent technology) coupled with a Mass Selective Detector (MSD,
242 model 5973, Agilent Technology) after thermodesorption (TDS3, Gerstel). Analyses of leaf
243 terpene content were performed by GC (model 7890B, Agilent technology) coupled with an
244 MSD (model 5977A, Agilent Technology) using an injection volume of 1 μL in splitless
245 mode. For both analyses, the column used was an HP5-MS (30 m, Agilent Technology) and
246 the carrier gas was helium at a flow rate of 1 mL min^{-1} . The chromatographic program was

Lavender sensitivity to water stress

247 fixed, for both analyses, as follows : 40 °C at the beginning of the run and maintained during
248 5 min. The temperature rate was set to 3 °C min⁻¹ to reach 245 °C, then 7 °C min⁻¹ to reach
249 300 °C. This maximal temperature was maintained during 7 min to clean to column.

250 For the inflorescence terpene content, compounds were separated and analysed by GC
251 (Agilent 6850 equipment) using a 30 m long capillary DB-5MS coupled with a MSD (model
252 5973, Agilent Technology). Chromatographic conditions were as follows: helium was used as
253 the carrier gas at a flow rate of 1.2 mL min⁻¹; oven was set at 40 °C after injection followed
254 by a 3 °C min⁻¹ temperature ramp to 245 °C. Temperature was then hold at 245 °C for 4 min;
255 the injection volume was 2 µL in split mode with a 2:1 split ratio.

256 Compound identification was checked through the injection of analytical standards (Sigma-
257 Aldrich), the calculation of Kovats index (using the alkane series C₈-C₄₀) and the comparison
258 of experimental mass spectra to the NIST and Wiley databases and with MSeasy (R package,
259 Nicolè *et al.* 2012) as previously described for *Lavandula* species (Guitton *et al.* 2018;
260 Despinasse *et al.* 2020) or other Mediterranean plants (Ormeno *et al.* 2009).

261

262 **2.8. Statistical analyses**

263 Statistical data analyses were performed with R (version 3.3.2). First, we compared the
264 species (with merged varieties within each species) to determine their global strategy to cope
265 with water stress. Second, we analysed the differences among varieties to highlight the most
266 resistant to water stress.

267 The effect of phenology (T1 and T2), treatment (control and stress), species (varieties
268 merged) and their interaction were tested on all primary and specialized plant traits using
269 Redundancy Analysis (RDA) and associated permutation test with 999 permutations (vegan
270 package) on all measured plant traits. To go further and to determine the major differences
271 between species to cope with water stress, we tested the effect of species and treatment on

272 each parameter separately and within each phenological stage using a two-ways ANOVA
273 (after validating normality and homoscedasticity) to highlight the main differences between *L.*
274 *angustifolia* and *L. x intermedia* to cope with water stress. These tests allowed to determine
275 the effect of a prolonged period of water stress. When pairwise comparisons were needed, a
276 Tuckey *post hoc* test was performed. If interactions were occurred, Student tests were
277 performed to the for the effect of each factor separately. Moreover, the chemical data were
278 processed separately for each species to highlight the potential role of terpenoids as defense
279 compounds. The effect of phenology, treatment, and their interaction were tested on each
280 dataset of terpene composition (leaf, inflorescence, and emission) using RDA. To determine
281 the effect of water stress on total terpene content (floral and foliar) and total emission, two
282 ways ANOVA (watering treatment and phenological stages as factors) were
283 performed followed by a Tuckey *post hoc* for pairwise comparison. Then, to highlight
284 potential defense compounds (i.e. compounds showing an accumulation under water stress
285 through), Partial Least Square – Discriminant Analyses were performed on terpene content
286 (floral and foliar) and Variable Important for Projection (VIP) scores.

287

288 Then, to classify the varieties of *L. angustifolia* and *L. x intermedia* according to their
289 tolerance to water stress, we used the plasticity index (PI). The PI to abiotic factors is an
290 important parameter to predict plant responses, especially in the context of climate change
291 (Richter *et al.* 2012). The PI is a relative difference of trait means across environmental
292 conditions. To compare the response of varieties to water stress, we calculated the PI on Pn,
293 dry floral biomass and floral terpene content. The PI was calculated with the following
294 formula (Valladares *et al.* 2006; Fernandez *et al.* 2016):

$$295 \quad PI = \frac{\text{mean } S - \text{mean } C}{\text{max mean } (S \text{ or } C)}$$

296 where *S* stands for water stress treatment and *C* for the control treatment. The index scales
297 from -1 to 1 where -1 indicates a maximal effect of the treatment with $mean\ C > mean\ S$; 1
298 indicates a maximal effect of the treatment with $mean\ S > mean\ C$. An index close to 0
299 indicates that the treatment has no effect. A negative value of PI indicates a decrease of the
300 phenotypic response under water stress and consequently, a positive value indicates an
301 increase of the phenotypic response under water stress. To support the PI, Student tests were
302 performed on Pn, dry floral biomass and floral terpene content to identify significant
303 difference between control and treatment. The tests were performed for each variety and
304 phenological stage.

305

306 **3. Results**

307 **3.1. Differences in primary metabolic traits between *L. angustifolia* and *L. x*** 308 ***intermedia* under water stress**

309 The RDA approach allowed to test and visualize the effect of species, phenological stages and
310 treatment on all measured traits (Fig. 2). The two first axis of the RDA explained more than
311 80 % of the total variance of the data. The interaction between phenological stage, species and
312 treatment was significant ($F=2.4, P=0.03$), with all single factors being also significant (Table
313 2). Plants were separated between treatments on the axis 1, control plants being on the left
314 axis side while plants experiencing water stress were on the right axis side. Control plants
315 were characterized by high values of primary metabolic processes (CO_2 assimilation, stomatal
316 aperture, and transpiration) and low values of stem Ψ whereas plants experiencing water
317 stress showed the opposite pattern. The species effect was on a diagonal axis from the bottom
318 left to the top right, with *L. x intermedia* on the bottom left and *L. angustifolia* on the top
319 right. *L. x intermedia* was associated with high floral biomass and high terpene production
320 (storage and emission) compared to *L. angustifolia*. The phenological effect was materialized

321 on the other diagonal from top left to bottom right, except for *L. angustifolia* control. The
322 plants at flowering stage were characterized by higher values of Gw, E and Pn. To go further
323 and highlight the main differences between species to cope with water stress, two-ways
324 ANOVAs with species and treatment as explaining factors were performed for each
325 phenological stage on all parameters (Table S1 in supplementary files). Ψ , LMA and the foliar
326 biomass were the three variables with significant interactions between species and treatment.
327 Ψ was strongly reduced with water stress but the decrease was stronger for *L. x intermedia*
328 between control and stress conditions (from -1.0 to -3.0 MPa for *L. angustifolia* and from -
329 0.7 to -2.7 MPa for *L. x intermedia*, in C and S plants respectively, Fig. 3A and 3B). At T2, *L.*
330 *x intermedia* had higher LMA than *L. angustifolia* (Fig. 3C and 3D), but it decreased with
331 water stress while it increased for *L. angustifolia*. The response of foliar biomass showed
332 clear differences between the two species. Water stress reduced more foliar biomass of *L. x*
333 *intermedia* (-37 %, Fig. 3F) than *L. angustifolia* (-17 %; Fig. 3E). For these three parameters,
334 *L. x intermedia* was more impacted by water stress.

335

336 **3.2. Different impact of water stress on the specialized metabolic traits of *L.*** 337 ***angustifolia* and *L. x intermedia***

338 We detected a total of 42, 54 and 42 compounds in the different chemical profiles (emissions,
339 floral and foliar terpene content, respectively). Their concentrations for each condition are
340 detailed in supplementary files (Tables S2 – S7). Total leaf terpene content did not vary with
341 water stress in either species (Fig. 4A and 4B). The content increased at T2 for *L. x*
342 *intermedia* while it decreased for *L. angustifolia*. Total floral terpene content decreased
343 significantly with water stress (at T2 for *L. x intermedia* and at T1 for *L. angustifolia*, Fig. 4C
344 and 4D). Terpene emissions were drastically reduced (~ -80 %) at T2 under water stress for

345 both species (Fig. 4E and 4F) while emissions reached the highest values under optimal
346 watering.

347 The qualitative variation of the chemical profiles (tested through RDA) revealed that the floral
348 terpene composition and the chemical profile of emissions were significantly impacted by
349 water stress while leaf terpene composition was only modified by phenology. Excepting the
350 terpene emission of *L. angustifolia*, all terpene compositions differed significantly between
351 full flowering and end of flowering (Table S8 in supplementary file). To pinpoint the
352 compounds responsible for the changes in these chemical profiles with water stress, we
353 performed PLSDA when RDA revealed a significant treatment effect. We selected floral
354 compounds with the highest VIP scores and focused on those showing an increase of their
355 concentrations with water stress because they could act more likely as defense compounds
356 (Table 3, Fig. S2 in supplementary files). For the emissions at T2, none of the terpene
357 compounds increased with water stress. For *L. angustifolia*, concentration of some floral
358 compounds increased with water stress at T2 (o-cymene, p-cymene, bornyl acetate and
359 piperitone). The strong effect of water stress at T1 on floral terpene content consisted of
360 decreases in compound abundance. For *L. x intermedia*, p-cymene, linalool oxide, muurol-5-
361 en-4-one were highly discriminant and showed higher concentrations at T1 and T2 under
362 water stress. Moreover, α -cadinol increased in the inflorescences under water stress only at T1
363 whereas, p-cymen-8-ol was found only at T2. It should be noted that trans-carveol and p-
364 cymen-8-ol were induced by water stress since they were not detected under control
365 conditions in *L. angustifolia* and *L. x intermedia*, respectively.

366

367 **3.3. Comparison of water stress-tolerance among varieties**

Lavender sensitivity to water stress

368 To allow the comparison of the different varieties in terms of water stress-tolerance, we
369 calculated the phenotypic plasticity Index (PI) for three traits directly related to the yield of
370 EO: Pn, floral biomass and floral terpene content (Fig. 5, Table S9 in supplementary files).

371 All values of PI for Pn were negative independently of the variety and the phenological stage,
372 indicating a reduction of Pn with water stress (Fig. 5A). At T1, varieties showed values
373 around -0.4 except CDR05 and Diva (-0.2) and Grosso and G. ada reached -0.7. All varieties
374 showed lowest values of PI at T2 except Grosso and G. ada that maintained -0.7. At T2, PI
375 values were around -0.7 except CDR05, Diva and Rapido which were at -0.5.

376 From an agroecology perspective, floral biomass is a crucial parameter as it is directly related
377 to the yield of essential oil. Almost all varieties showed a negative PI of floral biomass at full
378 flowering (T1, Fig 5B, Table S10 in supplementary files), indicating decreases of their floral
379 biomass with water stress ($-0.6 < \text{PI} < -0.2$). Floral biomass of Rapido was not affected and
380 showed a PI near to 0. CDR05, Sara, Grosso, Abrial and Sumian showed intermediate
381 response with PI values around -0.3. At the end of flowering, the floral biomass decrease was
382 more drastic, except for Rapido and Matheronne ($\text{PI} \approx -0.3$ and $\text{PI} \approx -0.45$, respectively). The
383 floral biomass of Matheronne was similarly impacted by water stress at T1 and T2. Diva, G.
384 ada and Abrial presented the lowest floral biomass PI, around -0.8, whereas the remaining
385 varieties showed values around -0.6.

386 As floral biomass, floral terpene content is also an important parameter related to EO yield.
387 Only Abrial showed a neutral PI value at T1 (Fig. 5C, Table S11 in supplementary files). The
388 lowest values were observed for Sara and CDR05 ($\text{PI} < -0.4$) whereas all the other varieties
389 showed intermediate values ($-0.3 < \text{PI} < -0.1$). Many varieties showed the same PI values at
390 T1 and T2. At T2, PI was close to zero in CDR05 and Sumian and only 77-13 presented a
391 lower PI value. Surprisingly, at T2, Rapido showed a positive PI value (≈ 0.4), indicating an
392 increase of inflorescence terpene content with water stress.

393

394 **4. Discussion**

395 **4.1. Strategies of *L. angustifolia* and *L. x intermedia* to cope with drought**

396 Our results showed that both species, *L. angustifolia* and *L. x intermedia*, are affected by
397 water stress. In terms of gas exchanges, most varieties of both species showed a similar
398 strategy to cope with water stress consisting of a simultaneous decrease in stomatal
399 conductance and leaf water potential. This strategy, typical of many drought-tolerant
400 Mediterranean plants (Levitt 1980; Martínez-Ferri *et al.* 2000; Barzilai *et al.* 2021), leads to a
401 reduction in CO₂ assimilation which implies a limited functioning of the plant. When water
402 stress was applied over a period of seven weeks, it implied very low Ψ accompanied by a
403 dramatic Pn decline in all varieties comparable to those reached in the field in the
404 Mediterranean area. Our observations are in accordance with previous studies that have
405 described lavender species, *L. angustifolia* and *L. stoechas* L., as moderately tolerant to water
406 stress compared to other Mediterranean species (Nogués & Baker 2000; Zollinger *et al.*
407 2006).

408 Moreover, *L. x intermedia* is a hybrid well known to produce more biomass than *L.*
409 *angustifolia* (Lis-Balchin 2002) but such an investment could be an unfavorable strategy
410 under water stress since less carbon is then available to produce defense compounds (Herms
411 & Mattson 1992). Leaf biomass production of *L. x intermedia* was reduced after 1 month of
412 water stress in contrast to *L. angustifolia* which is only reduced after 2 months of drought
413 treatment. Thus, *L. x intermedia* varieties seemed to be more affected by drought than *L.*
414 *angustifolia*, probably due to their high biomass production of *L. angustifolia* (Lamacque *et*
415 *al.* 2020).

416

417 **4.2. Floral terpene production under drought and impact on essential oil quality**

418 From an ecophysiological perspective, water stress implies an oxidative stress (Reddy *et al.*
419 2004; Gill & Tuteja 2010) which plants counteract through numerous mechanisms including
420 terpene production (emission, storage) (Vickers *et al.* 2009). Terpenoids have been suggested
421 to act as antioxidants both directly and indirectly, by reducing ROS formation (Ormeño *et al.*
422 2020). A direct antioxidant effect is suspected, whereby terpenoids act as ROS scavengers
423 (Sharkey & Monson 2017). However, it is more plausible that terpenoid-related ROS
424 reduction is due to two indirect modes of action. The most supported hypothesis is that
425 terpenoid production (emission and storage) allows the dissipation of excess energy when
426 environmental stressors prevent this dissipation through optimal photosynthesis activity. It
427 has also been indicated that terpenoids stabilize damaged membranes (such as the thylakoid
428 membrane) because they are localized in damaged lipidic cell structures (Velikova *et al.*
429 2011). Our hypothesis was that terpenoid production could increase under water stress as
430 shown for several Mediterranean species such as *Pistacia lentiscus* L. (Anacardiaceae, Llusà
431 & Peñuelas 1998), *Cistus monspeliensis* L. (Cistaceae, Llusà & Peñuelas 1998) or
432 *Rosmarinus officinalis* L. (now *S. rosmarinus*, Lamiaceae, Delfine *et al.* 2005). Nevertheless,
433 total foliar terpene content did not change with water stress in our study. These results could
434 be explained by the decrease of net photosynthesis, reducing carbon availability for their
435 production. These terpenes are stored in glandular trichomes physically protected by
436 numerous non-glandular trichomes responsible of leaf grey color in lavender. Lavenders
437 belong to the Lamiaceae family and our results suggest that foliar terpenes were produced in
438 young leaves before the stress treatment like in other species from this family such as
439 peppermint (Battaile & Loomis 1961).

440 Unlike foliar terpenes, floral terpene production was reduced under water stress for both
441 species. This observation is consistent with results obtained on other Lamiaceae species
442 (García-Caparrós *et al.* 2019) [or on other varieties](#) of *Lavandula latifolia* Medik. (Gorgini

443 Shabankareh *et al.* 2021). At the subspecies level, the populational variety Rapido was able to
444 increase inflorescence terpene content with water stress. This is an important result for
445 producers. Concerning the quality of EO, a standardized qualitative composition is needed for
446 each variety. These norms imply less than 20 terpenoids with linalool and linalyl acetate
447 representing more than 50 % of the EO. The minor compounds are numerous, more than 80
448 had been detected (Shellie *et al.* 2002) and they also participate to the olfactory quality of the
449 oil. *L. x intermedia* varieties produced two other terpenoids in high proportion, camphor and
450 1,8-cineol (Lis-Balchin 2002; Guitton 2010). These molecules are undesirable for perfume
451 industry and decrease the economic value of *L. x intermedia* EO. Our results indicate that the
452 major compounds are not modified by the water stress. However, our study highlighted that
453 some compounds seem to be accumulated in inflorescences while other compounds remained
454 unchanged or decreased with water stress. For instance, p-cymene and bornyl acetate showed
455 higher concentrations under water deficit. It has been shown that p-cymene can be produced
456 by the γ -terpinene synthase (Crocchi *et al.* 2010) and accumulated under mild water stress
457 (Morshedloo *et al.* 2017) in *Origanum vulgare* L. (Lamiaceae). Mohammadi *et al.* (2018) also
458 found the increase of p-cymene pathway in *Thymus vulgaris* L. (Lamiaceae) in response to
459 water stress. Bornyl acetate, ester of the borneol, is produced in *Lavandula spp.* by an
460 enzymatic pathway beginning with a terpene synthase, the bornyl diphosphate synthase
461 (Landmann *et al.* 2007; Despinasse *et al.* 2017), also responsible for the production of
462 camphor and borneol. Our results suggest that γ -terpinene synthase and the bornyl
463 diphosphate synthase were enhanced under water stress and that those associated compounds
464 could be considered as water stress defense compounds. In *Salvia officinalis* L. (Lamiaceae),
465 expression of the bornyl diphosphate synthase gene was upregulated at the beginning of water
466 stress and an increase of camphor leaf content was observed during the water stress (Radwan
467 *et al.* 2017). Further research is needed to confirm the overexpression of those terpene

468 synthases by molecular methods. Compared to our results, the changes in terpenes highlighted
469 in those studies were clearer. This can be explained by the difference of water stress
470 application or by the action of other defense compounds such as osmoprotectants and
471 phenolic compounds (Al-Huqail *et al.* 2020) or enzymatic antioxidants (Szekely-Varga *et al.*
472 2020) that could also help to cope with water stress.

473 It seems that water stress, especially when occurring over a long period of time, could reduce
474 the EO yield (in addition to the decrease in floral biomass production, see below) but also
475 alter its quality (Chrysargyris *et al.* 2016), especially if the borneol/camphor pathway is up-
476 regulated under water stress. Indeed, those compounds depreciate the lavender EO quality
477 (Aprotosoae *et al.* 2017).

478 Terpene emissions from whole plants also decreased with water stress. As observed for other
479 Mediterranean species, effect of drought on terpenoid emission are species-specific
480 (Haberstroh *et al.* 2018). In this study, these decreases observed in both species could also be
481 explained by the reduction in net photosynthesis driving to a decrease of carbon availability
482 for terpene biosynthesis. The stomatal closure could also stop their emission (Niinemets *et al.*
483 2004). Besides, the response of terpene emissions to water stress also depends on the type of
484 compounds. For instance, Ormeno *et al.* (2007) showed that sesquiterpene emissions
485 decreased under severe water stress (Ψ close to -4 MPa) in a Lamiaceae species (*R.*
486 *officinalis*, now *S. rosmarinus*) while monoterpenes remained unchanged. The observed
487 differences with our study can be explained by the shorter application of water stress (11
488 days) in Ormeno *et al.* (2007) compared to 1 or 2 months in our study.

489

490 **4.3. Different sensitivities to drought according to varieties**

491 Our results also revealed different drought tolerances between *L. angustifolia* or *L. x*
492 *intermedia* varieties. For instance, varieties CDR05 and Rapido showed higher values of Pn

493 compared to the other varieties. Then, Rapido maintained its floral biomass production at T1
494 and only a slight decrease was observed at T2. Besides, this variety maintained a high
495 production of floral terpenes after one month of drought and even increased after two months
496 of drought. This populational variety has been selected before for its tolerance to water stress
497 and yellow disease and seems to be a good candidate to cope with climate change (Table 4).
498 Unfortunately, in our study, this variety produced a small number of inflorescences compared
499 to the other varieties. The selection of Rapido is recent but this variety presents more
500 heterozygosis than a clonal variety and probably more genetic potential for stress tolerance
501 (Pardo 2010). Our results are really encouraging and need to be confirmed under field
502 conditions. To a lesser extent, Matheronne, which is currently one of the most cultivated
503 varieties in Southern France (source: CRIEPPAM), could also be a good candidate for EO
504 production with the context of rapid climate change in the Mediterranean area. This variety
505 exhibited a higher floral production after 2 months of water deficit application compared to
506 the other varieties. However, Matheronne is highly sensitive to yellow disease (source:
507 CRIEPPAM), which could counterbalance its relative high tolerance to water stress.

508 Even though our results indicated a lesser tolerance to water stress of *L. x intermedia*
509 compared to *L. angustifolia* (because of the higher biomass production of *L. x intermedia*, see
510 above), it seems that Abrial and Sumian could be good *L. x intermedia* candidates in terms of
511 water stress resistance, especially for a short period of drought (< 1 month). However, like
512 Matheronne, Abrial is also highly sensitive to yellow disease and has disappeared from
513 French fields. On the contrary, Sumian appears to be tolerant to yellow disease and it could be
514 interesting to maintain its cultivation for a sustainable production of EO in the Mediterranean
515 area (source: CRIEPPAM). In the recent study from Gorgini Shabankareh *et al.* (2021),
516 *Lavandula stricta*, native to Iran, presented the best tolerance to water stress compared to two
517 *Lavandula angustifolia* cultivars. The linalool richness of this species could be interesting for

518 future breeding program in semi-arid areas. Breeding different *Lavandula* sp., around
519 different climatic belts, could be possible due to the large polymorphism of terpene
520 production in the genus *Lavandula* (Guitton *et al.* 2018). Today, the variability of lavender
521 EO is limited due to industrial purposes and interests. Nevertheless, the natural variability of
522 the EO depends on genotypes and the environmental factors as recently documented (Bayati
523 *et al.* 2020; Faria & Rodrigues 2021). In the context of climate change, the natural variability
524 of EO from new genotypes or new environmental conditions will have to be evaluated by
525 scientists and appreciated by consumers.

526

527 **5. Conclusion**

528 In this study, most varieties responded to water stress with a drought-tolerant strategy by
529 closing their stomata when leaf water potential dropped, resulting in limited gas exchanges
530 and floral and foliar biomass productions. Water stress led to an important decrease in terpene
531 emissions at the whole plant scale, while the floral terpene production decreased less
532 drastically. Moreover, our results indicated that some terpenes such as borneol and p-cymene
533 could be considered as defense compounds against water stress, although future mechanistic
534 tests are necessary to validate this role.

535 To conclude, based on their drought tolerance, it seems that Rapido and Sumian varieties of *L.*
536 *angustifolia* and *L. x intermedia* respectively are the best candidates for sustainable
537 agriculture in the context of climate change. Further investigations are required to validate our
538 results on Rapido and Sumian under field conditions as well as in conjunction with yellow
539 disease. Some traits measured in our study could be easily followed at the field scale such as
540 dry floral biomass and total floral terpene content.

541

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548

549 **Author contribution**

550 EO, SM, CF, FN and ABM designed the experiments. AS, EO, SM, ER, SD and YD
551 conducted the research. AS, EO, SM and FN performed the data treatment and statistical
552 analyses. EO, CF, SB, FN and ABM supervised the realization of the study. CF, FN and
553 ABM acquired the funding and AS, EO, SM, CF, FN and ABM wrote, reviewed and edited
554 the article.

555

556 **Data availability statement**

557 All data supporting the findings of this study are available within the paper and within its
558 supplementary materials published online.

559

560 **Conflict of interest**

561 The authors declare that they have no conflict of interest.

562

563 **References**

- 564 Al-Huqail A., El-Dakak R.M., Sanad M.N., Badr R.H., Ibrahim M.M., Soliman D. & Khan F.
565 (2020). Effects of Climate Temperature and Water Stress on Plant Growth and
566 Accumulation of Antioxidant Compounds in Sweet Basil (*Ocimum basilicum*
567 L.) Leafy Vegetable. *Scientifica*, 2020, 3808909.
- 568 Aprotosoiaie A.C., Gille E., Trifan A., Luca V.S. & Miron A. (2017). Essential oils of
569 *Lavandula* genus: a systematic review of their chemistry. *Phytochemistry Reviews*, 16,
570 761-799.

- 571 Barzilai O., Avraham M., Sorek Y., Zemach H., Dag A. & Hochberg U. (2021). Productivity
572 versus drought adaptation in olive leaves: Comparison of water relations in a modern
573 versus a traditional cultivar. *Physiologia Plantarum*.
- 574 Bassolé I.H.N. & Juliani H.R. (2012). Essential oils in combination and their antimicrobial
575 properties. *Molecules*, 17, 3989-4006.
- 576 Battaile J. & Loomis W. (1961). Biosynthesis of terpenes: II. The site and sequence of terpene
577 formation in peppermint. *Biochimica et biophysica acta*, 51, 545-552.
- 578 Bayati P., Karimmojeni H. & Razmjoo J. (2020). Changes in essential oil yield and fatty acid
579 contents in black cumin (*Nigella sativa* L.) genotypes in response to drought stress.
580 *Industrial Crops and Products*, 155, 112764.
- 581 Chaves M.M., Flexas J. & Pinheiro C. (2009). Photosynthesis under drought and salt stress:
582 regulation mechanisms from whole plant to cell. *Annals of botany*, 103, 551-560.
- 583 Christensen J.H., Kanikicharla K.K., Aldrian E., An S.I., Cavalcanti I.F.A., de Castro M.,
584 Dong W., Goswami P., Hall A. & Kanyanga J.K. (2013). Climate phenomena and
585 their relevance for future regional climate change. In: *Climate Change 2013 the*
586 *Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report*
587 *of the Intergovernmental Panel on Climate Change*. Cambridge University Press, pp.
588 1217-1308.
- 589 Christensen N.M., Axelsen K.B., Nicolaisen M. & Schulz A. (2005). Phytoplasmas and their
590 interactions with hosts. *Trends in plant science*, 10, 526-535.
- 591 Chrysargyris A., Laoutari S., Litskas V.D., Stavrinides M.C. & Tzortzakis N. (2016). Effects
592 of water stress on lavender and sage biomass production, essential oil composition and
593 biocidal properties against *Tetranychus urticae* (Koch). *Scientia horticultrae*, 213,
594 96-103.
- 595 Ciais P., Reichstein M., Viovy N., Granier A., Ogée J., Allard V., Aubinet M., Buchmann N.,
596 Bernhofer C. & Carrara A. (2005). Europe-wide reduction in primary productivity
597 caused by the heat and drought in 2003. *Nature*, 437, 529-533.
- 598 Crocoll C., Asbach J., Novak J., Gershenzon J. & Degenhardt J. (2010). Terpene synthases of
599 oregano (*Origanum vulgare* L.) and their roles in the pathway and regulation of
600 terpene biosynthesis. *Plant molecular biology*, 73, 587-603.
- 601 Delfine S., Loreto F., Pinelli P., Tognetti R. & Alvino A. (2005). Isoprenoids content and
602 photosynthetic limitations in rosemary and spearmint plants under water stress.
603 *Agriculture, ecosystems & environment*, 106, 243-252.
- 604 Despinasse Y., Fiorucci S., Antonczak S., Moja S., Bony A., Nicolè F., Baudino S., Magnard
605 J.-L. & Jullien F. (2017). Bornyl-diphosphate synthase from *Lavandula angustifolia*: a
606 major monoterpene synthase involved in essential oil quality. *Phytochemistry*, 137,
607 24-33.
- 608 Despinasse Y., Moja S., Soler C., Jullien F., Pasquier B., Bessièrè J.-M., Noûs C., Baudino S.
609 & Nicolè F. (2020). Structure of the Chemical and Genetic Diversity of the True
610 Lavender over Its Natural Range. *Plants*, 9, 1640.
- 611 Fares S., Park J., Gentner R., Weber R., Ormeño E., Karlik J. & Goldstein A. (2012).
612 Seasonal cycles of biogenic volatile organic compound fluxes and concentrations in a
613 California citrus orchard. 12, 9865-9880.
- 614 Faria J. & Rodrigues A.M. (2021). Metabolomic Variability in the Volatile Composition of
615 Essential Oils from *Pinus pinea* and *P. pinaster*. In: *Biology and Life Sciences Forum*.
616 Multidisciplinary Digital Publishing Institute, p. 14.
- 617 Fernandez C., Monnier Y., Santonja M., Gallet C., Weston L.A., Prévosto B., Saunier A.,
618 Baldy V. & Bousquet-Mélou A. (2016). The impact of competition and allelopathy on
619 the trade-off between plant defense and growth in two contrasting tree species.
620 *Frontiers in plant science*, 7, 594.

- 621 García-Caparrós P., Romero M.J., Llanderal A., Cermeño P., Lao M.T. & Segura M.L.
 622 (2019). Effects of drought stress on biomass, essential oil content, nutritional
 623 parameters, and costs of production in six Lamiaceae species. *Water*, 11, 573.
- 624 Genard-Zielinski A.-C., Boissard C., Fernandez C., Kalogridis C., Lathière J., Gros V.,
 625 Bonnaire N. & Ormeño E. (2015). Variability of BVOC emissions from a
 626 Mediterranean mixed forest in southern France with a focus on *Quercus pubescens*.
 627 *Atmospheric Chemistry & Physics*, 15, 431-446.
- 628 Gill S.S. & Tuteja N. (2010). Reactive oxygen species and antioxidant machinery in abiotic
 629 stress tolerance in crop plants. *Plant physiology and biochemistry*, 48, 909-930.
- 630 Giorgi F. & Lionello P. (2008). Climate change projections for the Mediterranean region.
 631 *Global and planetary change*, 63, 90-104.
- 632 Giray F.H. (2018). An analysis of world lavender oil markets and lessons for Turkey. *Journal*
 633 *of Essential Oil Bearing Plants*, 21, 1612-1623.
- 634 Gorgini Shabankareh H., Khorasaninejad S., Soltanloo H. & Shariati V. (2021). Physiological
 635 response and secondary metabolites of three lavender genotypes under water deficit.
 636 *Scientific reports*, 11, 1-22.
- 637 Guitton Y. (2010). Diversité des composés terpéniques volatils au sein du genre *Lavandula*:
 638 aspects évolutifs et physiologiques. In. Université Jean Monnet Saint-Etienne.
- 639 Guitton Y., Nicolè F., Jullien F., Caissard J.-C., Saint-Marcoux D., Legendre L., Pasquier B.
 640 & Moja S. (2018). A comparative study of terpene composition in different clades of
 641 the genus *Lavandula*. *Botany Letters*, 165, 494-505.
- 642 Haberstroh S., Kreuzwieser J., Lobo-do-Vale R., Caldeira M.C., Dubbert M. & Werner C.
 643 (2018). Terpenoid emissions of two Mediterranean woody species in response to
 644 drought stress. *Frontiers in plant science*, 9, 1071.
- 645 Herms D.A. & Mattson W.J. (1992). The dilemma of plants: to grow or defend. *The quarterly*
 646 *review of biology*, 67, 283-335.
- 647 Kalamartzis I., Dordas C., Georgiou P. & Menexes G. (2020). The Use of Appropriate
 648 Cultivar of Basil (*Ocimum basilicum*) Can Increase Water Use Efficiency under Water
 649 Stress. *Agronomy*, 10, 70.
- 650 Lamacque L., Charrier G., Farnese F.d.S., Lemaire B., Améglia T. & Herbette S. (2020).
 651 Drought-induced mortality: Branch diameter variation reveals a point of no recovery
 652 in lavender species. *Plant physiology*, 183, 1638-1649.
- 653 Landmann C., Fink B., Festner M., Dregus M., Engel K.-H. & Schwab W. (2007). Cloning
 654 and functional characterization of three terpene synthases from lavender (*Lavandula*
 655 *angustifolia*). *Archives of Biochemistry and Biophysics*, 465, 417-429.
- 656 Levitt J. (1980). *Responses of Plants to Environmental Stress, Volume 1: Chilling, Freezing,*
 657 *and High Temperature Stresses*. Academic Press.
- 658 Lis-Balchin M. (2002). *Lavender: the genus Lavandula*. CRC press.
- 659 Llusà J. & Peñuelas J. (1998). Changes in terpene content and emission in potted
 660 Mediterranean woody plants under severe drought. *Canadian Journal of Botany*, 76,
 661 1366-1373.
- 662 Llusà J., Penuelas J., Prieto P. & Estiarte M. (2009). Net ecosystem exchange and whole
 663 plant isoprenoid emissions by a mediterranean shrubland exposed to experimental
 664 climate change. *Russian Journal of Plant Physiology*, 56, 29-37.
- 665 Mahdavi A., Moradi P. & Mastinu A. (2020). Variation in Terpene Profiles of *Thymus*
 666 *vulgaris* in Water Deficit Stress Response. *Molecules*, 25, 1091.
- 667 Martínez-Ferri E., Balaguer L., Valladares F., Chico J. & Manrique E. (2000). Energy
 668 dissipation in drought-avoiding and drought-tolerant tree species at midday during the
 669 Mediterranean summer. *Tree Physiology*, 20, 131-138.

- 670 Mohammadi H., Ghorbanpour M. & Brestic M. (2018). Exogenous putrescine changes redox
671 regulations and essential oil constituents in field-grown *Thymus vulgaris* L. under
672 well-watered and drought stress conditions. *Industrial Crops and Products*, 122, 119-
673 132.
- 674 Morshedloo M.R., Craker L.E., Salami A., Nazeri V., Sang H. & Maggi F. (2017). Effect of
675 prolonged water stress on essential oil content, compositions and gene expression
676 patterns of mono-and sesquiterpene synthesis in two oregano (*Origanum vulgare* L.)
677 subspecies. *Plant physiology and biochemistry*, 111, 119-128.
- 678 Nicolè F., Guitton Y., Courtois E.A., Moja S., Legendre L. & Hossaert-McKey M. (2012).
679 MSeasy: unsupervised and untargeted GC-MS data processing. *Bioinformatics*, 28,
680 2278-2280.
- 681 Niinemets Ü., Loreto F. & Reichstein M. (2004). Physiological and physicochemical controls
682 on foliar volatile organic compound emissions. *Trends in plant science*, 9, 180-186.
- 683 Nogués S. & Baker N.R. (2000). Effects of drought on photosynthesis in Mediterranean plants
684 grown under enhanced UV-B radiation. *Journal of experimental botany*, 51, 1309-
685 1317.
- 686 Ormeno E., Cespedes B., Sanchez I.A., Velasco-García A., Moreno J.M., Fernandez C. &
687 Baldy V. (2009). The relationship between terpenes and flammability of leaf litter.
688 *Forest Ecology and Management*, 257, 471-482.
- 689 Ormeño E., Gentner D.R., Fares S., Karlik J., Park J.H. & Goldstein A.H. (2010).
690 Sesquiterpenoid emissions from agricultural crops: correlations to monoterpenoid
691 emissions and leaf terpene content. *Environmental science & technology*, 44, 3758-
692 3764.
- 693 Ormeno E., Mevy J., Vila B., Bousquet-Mélou A., Greff S., Bonin G. & Fernandez C. (2007).
694 Water deficit stress induces different monoterpene and sesquiterpene emission
695 changes in Mediterranean species. Relationship between terpene emissions and plant
696 water potential. *Chemosphere*, 67, 276-284.
- 697 Ormeño E., Viros J., Mévy J.-P., Tonetto A., Saunier A., Bousquet-Mélou A. & Fernandez C.
698 (2020). Exogenous Isoprene Confers Physiological Benefits in a Negligible Isoprene
699 Emitter (*Acer monspessulanum* L.) Under Water Deficit. *Plants*, 9, 159.
- 700 Pardo J.M. (2010). Biotechnology of water and salinity stress tolerance. *Current Opinion in*
701 *Biotechnology*, 21, 185-196.
- 702 Park C.G., Jang M., Yoon K.A. & Kim J. (2016). Insecticidal and acetylcholinesterase
703 inhibitory activities of Lamiaceae plant essential oils and their major components
704 against *Drosophila suzukii* (Diptera: Drosophilidae). *Industrial crops and products*,
705 89, 507-513.
- 706 Polade S.D., Pierce D.W., Cayan D.R., Gershunov A. & Dettinger M.D. (2014). The key role
707 of dry days in changing regional climate and precipitation regimes. *Scientific reports*,
708 4, 1-8.
- 709 Pollmann J., Ortega J. & Helmig D. (2005). Analysis of atmospheric sesquiterpenes:
710 Sampling losses and mitigation of ozone interferences. *Environmental science &*
711 *technology*, 39, 9620-9629.
- 712 Radwan A., Kleinwächter M. & Selmar D. (2017). Impact of drought stress on specialised
713 metabolism: biosynthesis and the expression of monoterpene synthases in sage (*Salvia*
714 *officinalis*). *Phytochemistry*, 141, 20-26.
- 715 Reddy A.R., Chaitanya K.V. & Vivekanandan M. (2004). Drought-induced responses of
716 photosynthesis and antioxidant metabolism in higher plants. *Journal of plant*
717 *physiology*, 161, 1189-1202.

- 718 Richter S., Kipfer T., Wohlgemuth T., Guerrero C.C., Ghazoul J. & Moser B. (2012).
 719 Phenotypic plasticity facilitates resistance to climate change in a highly variable
 720 environment. *Oecologia*, 169, 269-279.
- 721 Saunier A., Ormeño E., Wortham H., Temime-Roussel B., Lecareux C., Boissard C. &
 722 Fernandez C. (2017). Chronic drought decreases anabolic and catabolic BVOC
 723 emissions of *Quercus pubescens* in a Mediterranean forest. *Frontiers in plant science*,
 724 8, 71.
- 725 Sémétey O., Gaudin J., Danet J.-L., Salar P., Theil S., Fontaine M., Krausz M., Chaisse E.,
 726 Eveillard S. & Verdin E. (2018). Lavender decline in France is associated with chronic
 727 infection by lavender-specific strains of “Candidatus *Phytoplasma solani*”. *Applied*
 728 *and environmental microbiology*, 84.
- 729 Sharkey T.D. & Monson R.K. (2017). Isoprene research—60 years later, the biology is still
 730 enigmatic. *Plant, cell & environment*, 40, 1671-1678.
- 731 Shellie R., Mondello L., Marriott P. & Dugo G. (2002). Characterisation of lavender essential
 732 oils by using gas chromatography–mass spectrometry with correlation of linear
 733 retention indices and comparison with comprehensive two-dimensional gas
 734 chromatography. *Journal of Chromatography A*, 970, 225-234.
- 735 Szekely-Varga Z., Gonzalez-Orenga S., Cantor M., Boscaiu M. & Vicente O. (2020).
 736 Antioxidant responses to drought and salinity in *Lavandula angustifolia* Mill. *Notulae*
 737 *Botanicae Horti Agrobotanici Cluj-Napoca*, 48, 1980-1992.
- 738 Valladares F., Sanchez-Gomez D. & Zavala M.A. (2006). Quantitative estimation of
 739 phenotypic plasticity: bridging the gap between the evolutionary concept and its
 740 ecological applications. *Journal of ecology*, 94, 1103-1116.
- 741 Veihmeyer F. & Hendrickson A. (1949). Methods of measuring field capacity and permanent
 742 wilting percentage of soils. *Soil science*, 68, 75-94.
- 743 Velikova V., Várkonyi Z., Szabó M., Maslenskova L., Nogues I., Kovács L., Peeva V.,
 744 Busheva M., Garab G. & Sharkey T.D. (2011). Increased thermostability of thylakoid
 745 membranes in isoprene-emitting leaves probed with three biophysical techniques.
 746 *Plant Physiology*, 157, 905-916.
- 747 Vickers C.E., Gershenzon J., Lerdau M.T. & Loreto F. (2009). A unified mechanism of action
 748 for volatile isoprenoids in plant abiotic stress. *Nature chemical biology*, 5, 283-291.
- 749 Von Caemmerer S.v. & Farquhar G.D. (1981). Some relationships between the biochemistry
 750 of photosynthesis and the gas exchange of leaves. *Planta*, 153, 376-387.
- 751 Weintraub P.G. & Beanland L. (2006). Insect vectors of phytoplasmas. *Annu. Rev. Entomol.*,
 752 51, 91-111.
- 753 Yani A., Pauly G., Faye M., Salin F. & Gleizes M. (1993). The effect of a long-term water
 754 stress on the metabolism and emission of terpenes of the foliage of *Cupressus*
 755 *sempervirens*. *Plant, Cell & Environment*, 16, 975-981.
- 756 Zollinger N., Kjelgren R., Cerny-Koenig T., Kopp K. & Koenig R. (2006). Drought responses
 757 of six ornamental herbaceous perennials. *Scientia Horticulturae*, 109, 267-274.
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Lavender sensitivity to water stress

759 **Table 1:** Characteristics of the studied varieties according to their known sensitivity or resistance to both yellow disease and drought (Eric
760 Chaisse, CRIEPPAM, pers. com).

Species	Variety name	Nature (regeneration)	Characteristics	Use
<i>L. angustifolia</i>	CDR05	Clone (cuttings)	highly sensitive to yellow disease	Blue lavender selected for bouquet
	Matheronne	Clone (cuttings)	sensitive to yellow disease	essential oil
	77-13	Clone (cuttings)	highly sensitive to yellow disease	essential oil
	Diva	Clone (cuttings)	recently selected for water stress & yellow disease tolerance	essential oil
	Rapido	Population (seed germination)	recently selected for water stress & yellow disease tolerance	essential oil
	Sara	Population (seed germination)	recently selected for water stress & yellow disease tolerance	essential oil
<i>L. x intermedia</i>	Abrial	Clone (cuttings)	highly sensitive to yellow disease	essential oil
	Grosso	Clone (cuttings)	yellow disease tolerant	essential oil
	Grosso ada	Clone (cuttings)	yellow disease tolerant	essential oil
	Grosso certitude	Clone (cuttings)	yellow disease tolerant	essential oil
	Sumian	Clone (cuttings)	yellow disease tolerant	essential oil

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Lavender sensitivity to water stress

769 **Table 2:** F and p-value from RDA with treatments, phenological stages and species (all varieties
 770 merges) on the whole data set with n=25 and n=30 for *L. x intermedia* and *L. angustifolia*,
 771 respectively.

Factors	F	P
Phenological stage	30.1	0.001
Species	59.3	0.001
Treatment	53.1	0.001
Phenological stage x Species	6.1	0.001
Phenological stage x Treatment	10.8	0.001
Species x Treatment	6.0	0.001
Phenological stage x Species x Treatment	2.4	0.03

772 **Table 3:** Compounds showing an accumulation of concentration (mg g^{-1}) under water stress and highlighted with VIP scores extracted from
 773 PLSDA where C1 is the component 1 and C2 is the component 2. They were highlighted in inflorescences at T1 and T2 for *L. x intermedia* and
 774 only at T2 for *L. angustifolia*. Wilcoxon tests were performed to test the differences between treatments with varieties merged ($n = 30$ for *L.*
 775 *angustifolia*, $n = 25$ for *L. x intermedia*), (*): $0.05 < P < 0.1$, *: $0.01 < P < 0.05$, **: $0.001 < P < 0.01$ and ***: $P < 0.001$. n.d.: not detected. Values
 776 represent the mean \pm SD.

<i>L. angustifolia</i>					
	Compounds	VIP scores	C	S	P-value
Floral terpene content (T2)	o-cymene	C1: 2.6; C2: 2.5	0.02 ± 0.06	0.2 ± 0.1	***
	p-cymene	C1: 2.0; C2: 1.9	0.3 ± 0.1	0.4 ± 0.2	***
	trans-carveol	C1: 2.0; C2: 1.9	n.d.	0.04 ± 0.07	**
	bornyl acetate	C1: 2.0; C2: 1.7	0.01 ± 0.03	0.1 ± 0.1	**
	piperitone	C1: 1.5; C2: 1.4	0.005 ± 0.025	0.03 ± 0.06	*
<i>L. x intermedia</i>					
	Compounds	VIP scores	C	S	P-value
Floral terpene content (T1)	linalool oxide	C1: 2.4; C2: 1.9	0.1 ± 0.2	0.4 ± 0.2	**
	α -cadinol	C1: 2.4; C2: 1.7	1.4 ± 0.3	1.8 ± 0.5	**
	muurol-5-en-4-one	C1: 2.0; C2: 1.5	0.3 ± 0.3	0.5 ± 0.3	**
	p-cymene	C1: 1.4; C2: 1.1	0.01 ± 0.06	0.1 ± 0.2	(*)
Floral terpene content (T2)	muurol-5-en-4-one	C1: 2.6; C2: 2.4	0.1 ± 0.1	0.5 ± 0.3	***
	p-cymene	C1: 2.2; C2: 2.0	0.01 ± 0.06	0.2 ± 0.2	***
	linalool oxide	C1: 2.2; C2: 1.9	0.1 ± 0.2	0.5 ± 0.3	***
	p-cymen-8-ol	C1: 1.0	n.d.	0.1 ± 0.2	*

Lavender sensitivity to water stress

Table 4: Summary of the response to the main traits used to classify the different varieties according to their resistance to water deficit. Classification is performed within *L. angustifolia* and *L. x intermedia* based on plasticity indices (PI). ↗ : PI > 0 ; ≈ : PI ≈ 0 ; ↘ : -0.3 < PI < 0 ; ↙↘ : -0.6 < PI < -0.3; ↙↙↘ : PI < -0.6.

Species	Varieties	Pn		Dry floral biomass		Total floral terpene content	
		T1	T2	T1	T2	T1	T2
<i>L. angustifolia</i>	Rapido	↙↘	↙↘	≈	↘	↘	↗
	Matheronne	↙↘	↙↙↘	↙↘	↙↘	↘	↘
	Diva	↘	↙↙↘	↙↘	↙↙↘	↘	↘
	CDR05	↘	↙↙↘	↘	↙↙↘	↙↘	≈
	Sara	↙↘	↙↙↘	↘	↙↙↘	↙↘	↘
	77-13	↙↘	↙↙↘	↙↘	↙↙↘	↘	↙↘
<i>L. x intermedia</i>	Sumian	↙↘	↙↙↘	↘	↙↙↘	↘	≈
	Abrial	↙↘	↙↙↘	↙↘	↙↙↘	≈	↘
	G. ada	↙↘	↙↙↘	↙↘	↙↙↘	↘	↘
	Grosso	↙↙↘	↙↙↘	↙↘	↙↙↘	↘	↘
	G. certitude	↙↙↘	↙↙↘	↙↙↘	↙↙↘	↘	↘

Figures legends:

Figure 1: Scheme of the experimental design. For each variety of *L. angustifolia* and *L. x intermedia*, T0 corresponds to the phenological stage where the floral stem starts to grow, T1 corresponds to the phenological stage where around 50% of flowers are opened and T2 is the fructification. Measurements on *L. angustifolia* at T1 were performed between 10 June and 4 July whereas for *L. x intermedia*, they were performed between 19 June and 4 July. Measurements at T2 were performed between 9 and 22 July, for both species.

Figure 2: Redundancy Analysis (RDA) was performed on primary metabolic traits (dry foliar and floral biomasses, LMA, Gw, Pn, E and absolute Ψ) and specialized metabolic traits (total foliar terpene content, total floral terpene content and total terpene emission) according to phenological stage, treatment and species (all varieties merged). Solid squares represent *L. angustifolia* whereas empty squares represent *L. x intermedia*. Blue represents individuals under control at T1, green is control at T2, orange is stress at T1 and red is stress at T2. Gas exchanges are represented in the up left part whereas chemical and growth parameters are linked to the down left. Absolute values of Ψ are represented at the down right.

Figure 3: Boxplots for water potential (Ψ , **A** and **B**), leaf mass per area (LMA) (**C** and **D**) and dry foliar biomass (**E** and **F**) according to treatment and phenological stage for each species (with all varieties merged). Two-ways ANOVA were performed followed by a Tukey post hoc test. Asterisks denote the effects of factors with ns: non-significant, *: $0.01 < P < 0.05$; **: $0.001 < P < 0.01$ and ***: $P < 0.001$ and $a > b$. When interaction occurred between factors, Student test were performed according to treatment for each phenological stage. Bars represent means \pm SE with $n = 25$ and $n = 30$ for *L. x intermedia* and *L. angustifolia*, respectively.

Figure 4: Boxplots for total foliar terpene content (**A** and **B**), total floral terpene content (**C** and **D**) and total terpene emissions (**E** and **F**) according to treatment and phenological stage for each species (with all varieties merged). Two-ways ANOVA followed by a Tuckey *post hoc*. Asterisks denote the effects of factors with ns: non-significant, *: $0.01 < P < 0.05$; ***: $P < 0.001$ and $a > b > c$. When interaction occurred between factors, Student test were performed according to treatment for each phenological stage. Bars represent means \pm SE with $n = 25$ and $n = 30$ for *L. x intermedia* and *L. angustifolia*, respectively

Figure 5: Plasticity index (PI) performed on net photosynthesis (**A**), dry floral biomass (**B**) and total floral terpene content (**C**) of the varieties at T1 (squares) and T2 (rounds). A negative value of PI indicates a decrease of the phenotypic response under water stress and consequently, a positive value indicates an increase.

Lavender sensitivity to water stress

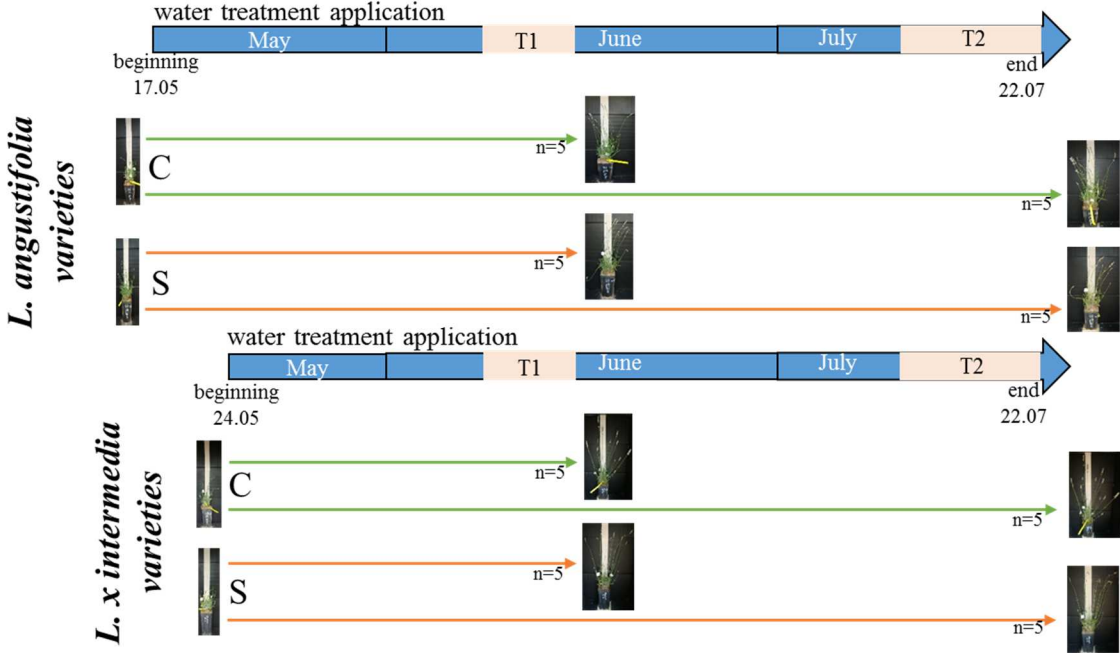


Figure 1:

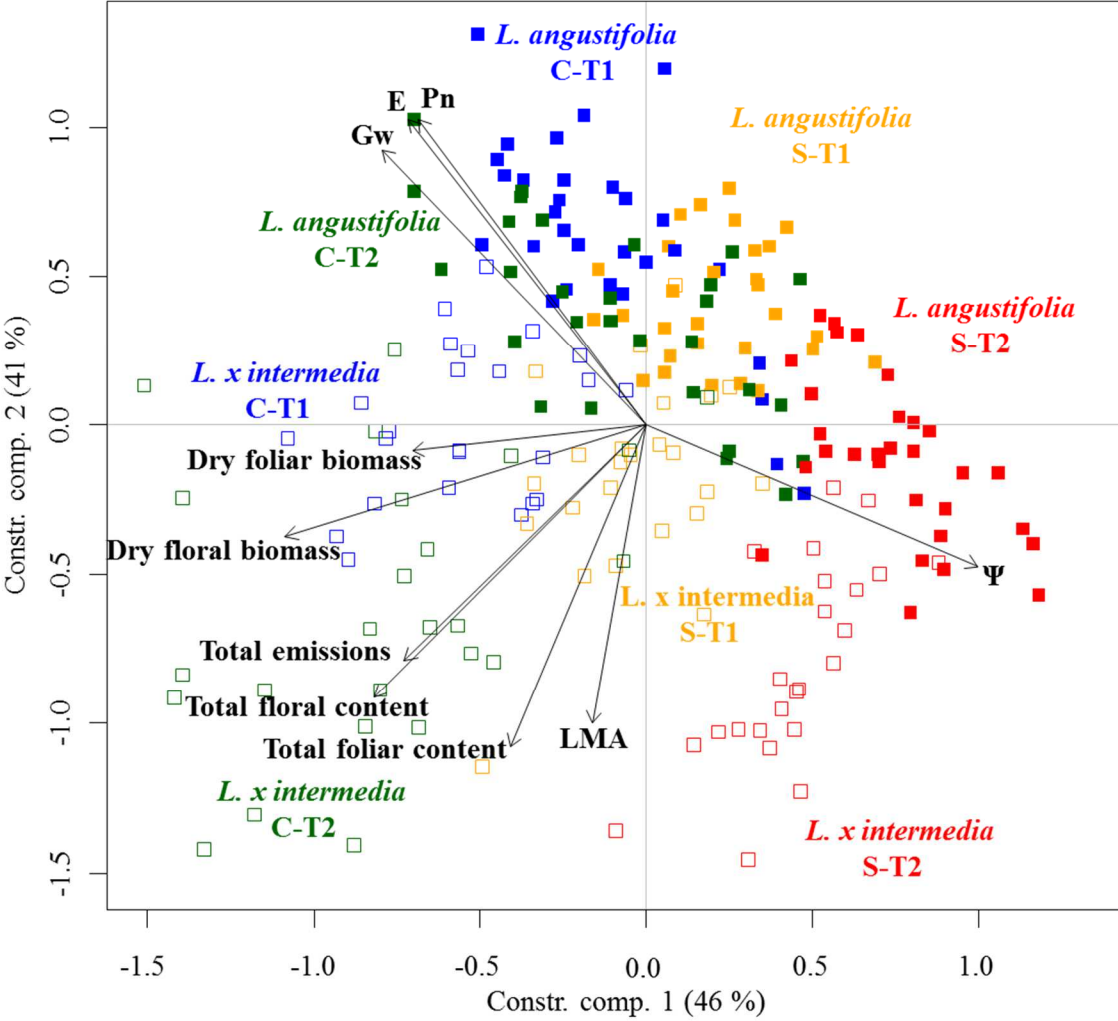


Figure 2:

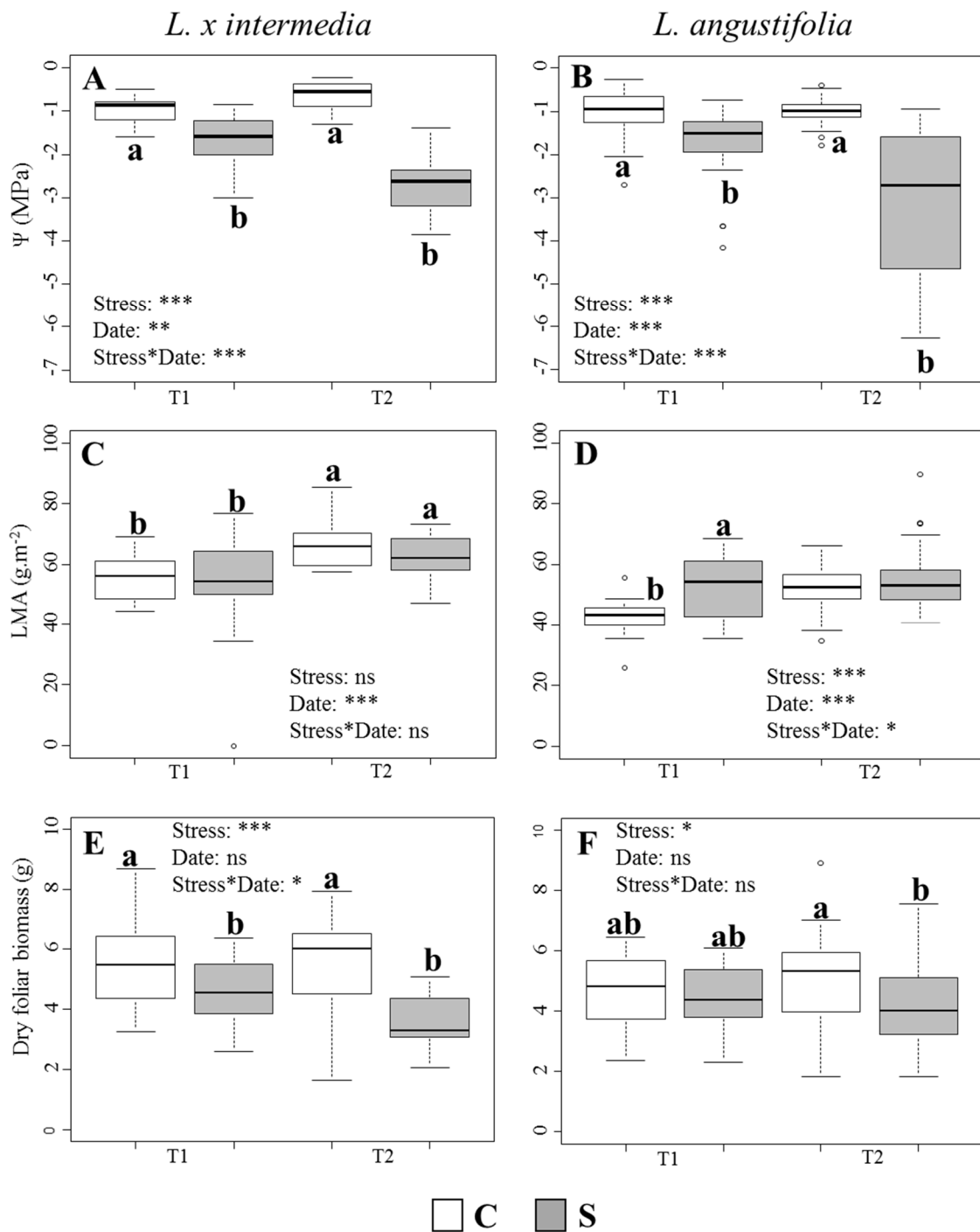


Figure 3:

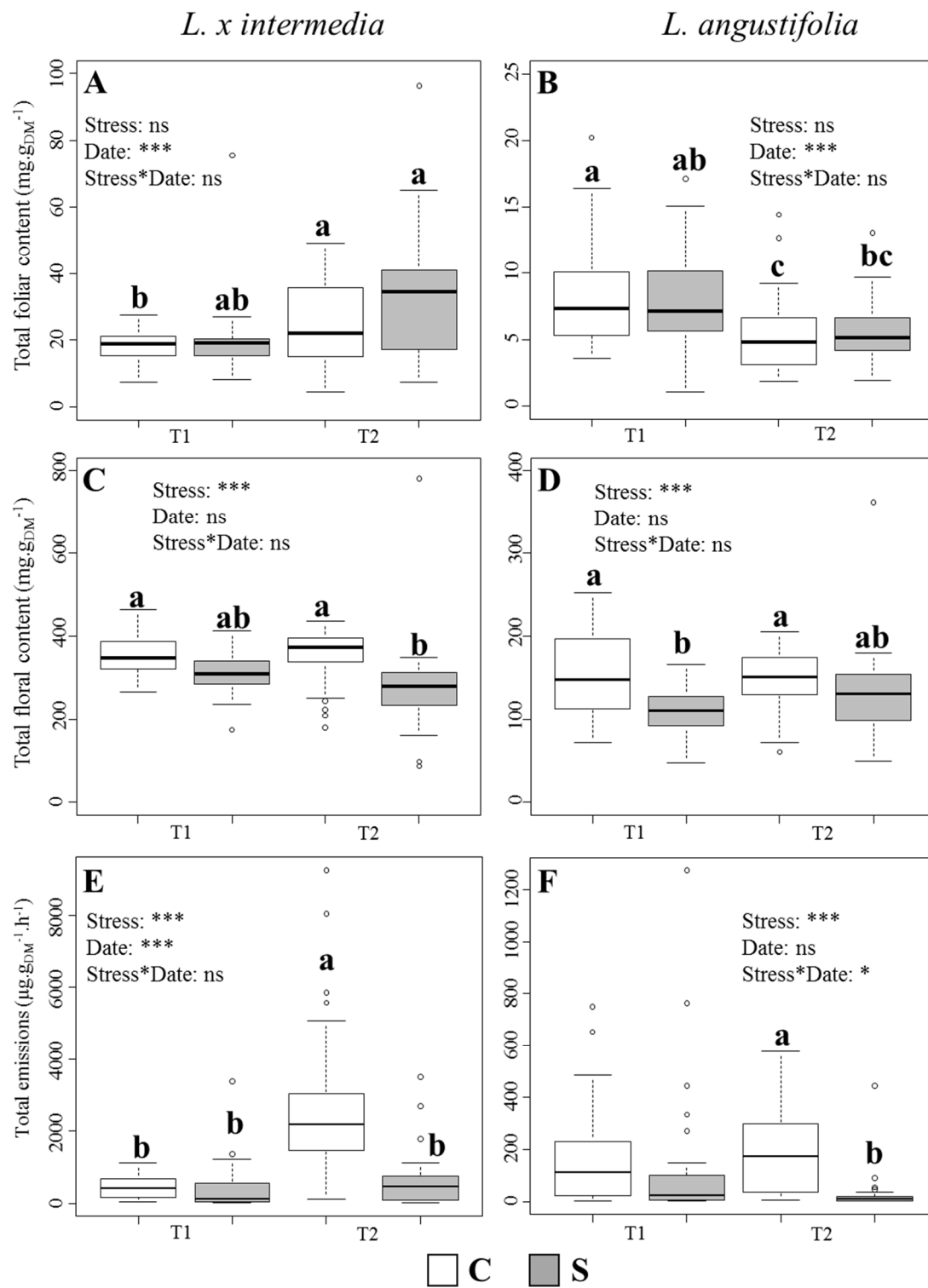


Figure 4:

Lavender sensitivity to water stress

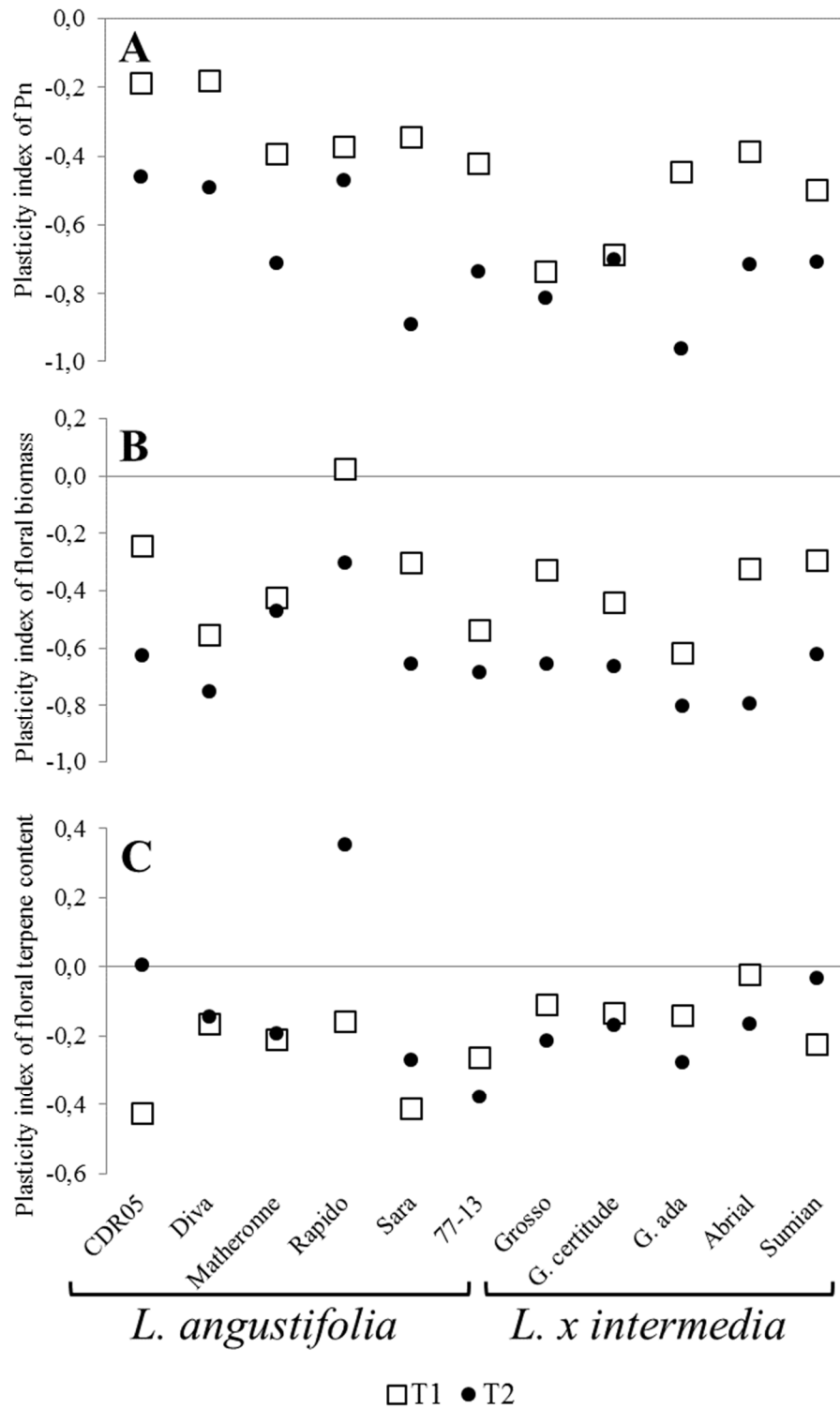


Figure 5: