

Article

Selecting Non-VOC Emitting Cork Oaks—A Chance to Reduce Regional Air Pollution

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Abstract

Cork oak is a strong emitter of volatiles, namely monoterpenes, which are important precursors of secondary air pollutants. Past studies have revealed distinct chemotypes in emitting as well as non-emitting individuals. Promoting non-emitters in afforestation and urban greening could improve air quality, but their rarity suggests that they are less resilient. To gain insight into this, we screened natural descendants from two non-emitting cork oaks for emissions and ecophysiological traits ($\text{CO}_2/\text{H}_2\text{O}$ -gas exchange variables, budburst date, growth) and tested whether emitting and non-emitting descendants differ in their resistance to temperature and light fluctuations (sun-flecks). Both half-sib populations were composed of the same chemotypes in similar frequencies, comprising 32% of non-emitters and 50 and 18% of two emitting chemotypes with overall moderate emission rates. Based on this distribution, we identified an inheritance mode and compared it with the chemotype frequency of the mother population. In terms of ecophysiological traits, all chemotypes performed similarly, and non-emitters were as resistant to sun-flecks as emitters. We conclude that the chemotypes in emitters reflect a common polymorphism in monoterpene-emitting plants that is not related to adaptive selection. We also conclude that non-emission is heritable and that its phenotype should be evaluated in reforestation studies.

Keywords: *Quercus suber*; volatile organic compounds; isoprene; monoterpene; air quality; intraspecific variability; chemotype; heritability; half-sibs

1. Introduction

The emissions of biogenic volatile organic compounds (BVOCs) from vegetation contribute to the significant mass of reactive carbon that is being introduced into the troposphere [1]. Since their carbon skeletons contain often unconjugated double bonds (e.g., isoprene), BVOCs become rapidly oxidized, and in the presence of nitrogen oxides and solar radiation, this can lead to the net formation of ozone, peroxyacyl nitrates, and other secondary air pollutants [2]. All of these pollutants contribute to millions of premature deaths worldwide [3–5]. In addition, the consumption of atmospheric oxidants, such as the OH radical, reduces the breakdown of less reactive compounds, including methane. Since methane and ozone are important greenhouse gases, BVOC emissions indirectly favor global warming [6]. However, BVOC oxidation products, especially those derived from large molecules, also contribute to the formation of secondary organic aerosols and cloud condensation nuclei [7]. These can have a cooling effect by increasing the albedo of the Earth's troposphere [8]. Whether the cooling effect associated with BVOCs will outweigh their warming effect in the future depends on which BVOC class increases in emission



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in response to climate and land use changes [9]. Given these impacts on air quality and climate, there is an interest in considering the BVOC emission potentials of vegetation in reforestation and city greening plans [10,11].

Trees are a major contributor to the VOC load because of their high biomass and because many species express a high potential for constitutive VOC production [12]. Oaks (*Quercus* spp.) and other genera of the Fagaceae family are among the strongest known BVOC emitters. The leaves of the majority of oaks which have been screened for VOC emissions thus far produce high amounts of the C5-hemiterpene isoprene. However, a few species that all belong to the *Quercus* subspecies *Cerris* do not produce isoprene, instead producing either large amounts of monoterpenes (C10) or nothing [13,14]. The reasons why the constitutive production of volatile isoprenoids diverged during oak evolution are not currently understood. A very recent study on the molecular evolution of terpene synthases suggested that isoprene-forming enzymes evolved independently twice within the oak subgenus *Quercus*, possibly from an ancestral monoterpene synthase producing predominantly ocimenes [15].

Both isoprene and monoterpenes are synthesized at high rates under light conditions inside the chloroplasts of mature oak leaves via the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, which uses primary carbon substrates and energetic co-factors originating from ongoing photosynthetic processes [16,17]. Under most environmental conditions, the synthesis of these volatiles consumes less than 1–2% of net-assimilated carbon, but the percentage can be higher under stress conditions such as drought, in which photosynthesis is more constrained than isoprenoid biosynthesis. There is ample literature showing that the chloroplast production of volatile isoprenoids helps the leaves to cope with stress, in particular its thermotolerance during canopy sun-flecks when temperature and radiation can suddenly increase [18,19]. The mechanisms underlying its protective properties are still a matter of debate, but the majority of recent studies have highlighted signaling and regulatory functions that affect various metabolic pathways [20].

The evergreen Mediterranean cork oak (*Quercus suber* L.) is one of the oaks that emits monoterpenes at high rates [21–23]. As cork oak is widely used for cork production, as well as for ornamental and shade trees [24], it essentially contributes to regional VOC emissions. Similarly to other monoterpene-emitting oaks, cork oak leaves predominantly release five monoterpenes: α -pinene, β -pinene, sabinene, limonene, and myrcene. Studies investigating the intraspecific variability of its BVOC emissions have revealed persistent compositional differences among individuals [23,25], which cluster into three distinct chemotypes: a pinene type, which emits predominantly α - and β -pinene and sabinene; a limonene type, which emits predominantly limonene; and a mixed type, which shows an intermediate emission profile. Additionally, earlier emission studies conducted on a small number of individuals in Italy reported cork oak as a non-emitting species [26,27]. A recent screening study encompassing ten cork oak provenances corroborated the existence of non-emitting specimens, yet emphasized that they are present in very low numbers in natural populations, at a frequency ranging from 0 to 10% [14].

Reforestation using low- or non-emitting lineages within emitting populations offers an interesting way to reduce the atmospheric VOC load from forests. This is particularly interesting for cork oak forests, as they are maintained and exploited for cork production and cannot therefore be replaced by other species. However, their rarity suggests that they are subject to negative selection. To better understand the underlying reasons, we have grown natural half-sibling populations from acorns originating from two non-emitting cork oaks. Cork oak is wind pollinated and each individual tree has both male and female flowers (monoecious). Self-pollination leading to successful seed set is rare in natural populations due to incompatible pollen–pistil interactions, and because male

flowers develop earlier and release pollen before female flowers become receptive [28]. The cork oak's nuclear genome comprises a diploid set of 24 chromosomes, on which genes encoding terpene synthases are located [29–31]. Therefore, monoterpene synthase genes are biparentally inherited. Consequently, the chemodiversity of VOC emissions in the progeny of a mother tree should essentially reflect the diversity brought by the pollen of the surrounding trees, i.e., the different paternal contributions in addition to the maternal contribution and potential environmental effects. Thus, analyzing the natural offspring of non-emitting mother trees can provide valuable information about the inheritance mode and differences in the progeny's fitness. To this end, we measured BVOC emissions and the photosynthetic and growth performances of 157 cork oak saplings from two maternal half-sib populations. We also tested a subset of contrasting chemotypes for their resistance to extreme sun-flecks.

2. Materials and Methods

2.1. Plant Culture

In a previous study [23], a few apparent non-emitting individuals were detected within a cork oak population of the Albères mountains in the Eastern coastal Pyrenees of the French department Pyrénées Occidentales (42°29'19" N 2°51'32" E). The forest type is a Mediterranean broadleaf evergreen forest, consisting of 80% cork oak trees that are managed for cork production. The non-emitting individuals were morphologically and genetically indiscernible from other emitting individuals. 90% of the neighbor cork oak trees assayed for VOC emissions were strong monoterpene emitters. In autumn, we collected mature acorns from two non-emitting individuals growing approximately 100 m away on the western slope of the population. The collected acorns were potted in 5 L pots with a mix of commercial potting soil and silica sand and grown outside in the institute garden for 4 years, where they were irrigated during summer and occasionally fertilized with Osmocote Plus 12–14 M (15% N, 3.5% P, 9% K, 1.2% Mg plus trace elements).

2.2. Screening for VOC Emissions

In spring prior to the experiments, the saplings were transferred to a climate-controlled greenhouse to promote budding and maintain stable temperature conditions, since oak trees' ability to produce VOCs can vary with the seasons, depending on prevailing climate conditions [32]. VOC emission and gas exchanges were measured during summer on the terminal leaves of an intact branch with a homemade environmentally controlled dynamic chamber system (Figure S1). The chamber volume was ca 0.11 L, and it was continuously flushed with charcoal filtered ambient air at a net flow rate of 0.7 L min^{−1}. CO₂/H₂O gas concentrations from the air entering and leaving the chamber were continuously measured with two infrared gas analyzers—a Li7000 running in differential mode combined with a Li840 in absolute mode (LI-COR, Lincoln, NE, USA). The chamber air temperature was set to 30 °C. The average leaf temperature measured with a thermocouple positioned under the lamina of an enclosed leaf was 30.2 ± 0.6 °C SD. The chamber was illuminated with a LED lamp (LX60 Heliospectra AB, Göteborg, Sweden) to achieve an incident photosynthetic photon flux density (PPFD) of around 1000 μmol m^{−2} s^{−1} at chamber height, as measured by a quantum sensor (PAR-SB 190, LI-COR, Lincoln, NE, USA) positioned outside close to the chamber (mean PPFD: 1050 ± 42 μmol m^{−2} s^{−1}). Temperature, PPFD, and CO₂/H₂O data were recorded on a 21X Micrologger (Campbell Scientific Ltd., Shepshed, Loughborough, UK).

The leaves were acclimatized to chamber conditions for at least 30 min. When leaves were photosynthesizing at steady state, VOC sampling was initiated. If photosynthesis remained low, acclimation was stopped and the plant was replaced by another.

For VOC measurement, one liter of chamber air was sampled by means of a pump and a mass flow controller at a flow rate of 0.1 L min^{-1} for 10 min on a glass cartridge filled with 200 mg Tenax TA (20–35 mesh, Agilent, Geneva, Switzerland). Trapped VOCs were analyzed with a Chrompack CP9003 GC-FID after injection via a two-stage Chrompack TCT4002 thermo-desorber (all Varian Inc., Palo Alto, CA, USA) on a Chrompack Sil 8CB low-bleed capillary column ($30 \text{ m} \times 0.25 \text{ mm}$) using He as carrier gas (1 mL min^{-1}) and the following oven temperature program: 3 min at 40°C , 3°C min^{-1} to 100°C , $2.7^\circ\text{C min}^{-1}$ to 140°C , $2.4^\circ\text{C min}^{-1}$ to 180°C , 6°C min^{-1} to 250°C . To ensure peak identification, additional samples were occasionally taken for GC analyses coupled with mass spectrometry (GC-MS) using a Shimadzu QP2010-SE instrument equipped with a TD-20 thermal desorption system (Shimadzu, Kyoto, Japan) (see [33] for more details).

After measurements were obtained, the chlorophyll content of the measurement leaves was estimated with a Minolta SPAD 502 m before being harvested to determine dry weights on a micro balance after drying for at least 48 h in a ventilated oven at 60°C . Projected leaf area was measured and calculated using a scanner and Image J5 software (National Institutes of Health, Bethesda, MD, USA). Finally, we assessed the aboveground growth of the saplings by counting the total number of leaves per cohort and the number of ramifications.

On the whole, we acquired emission data of 89 and 68 descendants from two non-emitting mother trees. Replicate measurements were performed for five trees to assess the extent to which the quantity and quality of emissions of a given sapling was conserved. We further tested a subset of 48 saplings for isoprene emissions by directing a fraction of chamber air to an AirmoVOC C2-C6 gas chromatograph equipped with a flame ionization detector (Chromatotec, Saint-André de Cubzac, France). This online instrument sampled air at a flow rate of 12 mL min^{-1} from the chamber outlet on an internal cooled adsorbent trap. The trapped VOCs were thermally injected into a fused silica PLOT $\text{Al}_2\text{O}_3/\text{KCl}$ column, which was heated according to the following temperature program: 1 min at 40°C , 15°C increase per min up to 180°C , then 20 min at 180°C .

2.3. Stress Resistance Experiment

To see whether non-emitting and emitting descendants differ in their ability to withstand abiotic stress, we exposed the leaves of a terminal twig of five strong emitting individuals and five apparent non-emitting individuals of the same descendent population to fast temperature and light fluctuations (sun-flecks). The following protocol was applied: firstly, acclimation to 30°C and $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PPFD for 30 min; then, measurement of chlorophyll fluorescence and VOC sampling; next, exposures to 43°C and $1800 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PPFD five times, with a 5 min recovery phase at initial conditions following each exposure; finally, acclimation to 30°C and $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PPFD for 30 min, with measurement of chlorophyll fluorescence and VOC sampling, after the last stress exposure. Changes in the incident PPFD were achieved by altering the distance and intensity of the light source to the plant chamber. Fast and reproducible changes in chamber temperature were achieved by stopping/activating the chamber-integrated water cooling and an external fan heater, supported by the congruent changing heating effect of the light source. Thus, target temperatures were reached and stabilized in less than 1 min.

$\text{CO}_2/\text{H}_2\text{O}$ gas exchanges were measured as described beforehand and recorded during each phase. Chlorophyll fluorescence measurements were realized by a pulse-modulated fluorometer PAM-2000 (Walz, Effeltrich, Germany) to deduce the following variables [34]: (i) at the beginning and at the end of the experiment on leaves adapted 30 min to darkness: the maximum quantum efficiency of photosystem 2 $F_v/F_m = (F_m - F_o) F_m^{-1}$, where F_o is the initial fluorescence measured under weak red modulated irradiance, and F_m is the max-

imum fluorescence under a saturated pulse of white light (approx. $10,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD); (ii) on light-adapted leaves during each phase: the actual quantum efficiency of photosystem 2 $\Phi\text{PSII} = (F_m' - F_s) F_m'^{-1}$, where F_s is the steady-state fluorescence at light (here, 400 PPFD) and F_m' the maximum fluorescence. F_m and F_m' data were further used to calculate the non-photochemical quenching (NPQ) as $(F_m' - F_m) F_m'^{-1}$. NPQ reflects the fraction of absorbed light energy dissipated as heat from PSII as a protective mechanism against the absorption of excessive light energy, which otherwise leads to photodamage (decrease in F_v/F_m). Furthermore, chlorophyll concentrations of the leaves were measured on the leaves before and after the stress experiment.

2.4. Calculations and Statistics

The VOC emission rate was calculated as the difference between the VOC air concentration in the chamber enclosing the leaves and the mean concentration measured in the empty chambers multiplied by chamber airflow rate and divided by the projected leaf area or by the leaf dry mass. The mean background concentration of the major emitted VOCs ranged between 0.05 ± 0.02 and $2.74 \pm 0.62 \text{ ng L}^{-1}$. Based on these, we estimated the realistic detection limit of VOC emissions in our measurement system to be around $20 \text{ ng m}^{-2} \text{s}^{-1}$.

$\text{CO}_2/\text{H}_2\text{O}$ gas exchange variables were calculated according to [35]. These included photosynthesis (net CO_2 -assimilation), transpiration, leaf water vapor conductance (stomatal conductance), and water use efficiency (WUE is the ratio of photosynthesis to transpiration). Given that foliar monoterpene emissions from cork oak are directly linked to their biosynthesis in chloroplasts from primary carbon substrates and energetic co-factors stemming from ongoing photosynthetic processes, we also calculated the percentage loss of net assimilated mol carbon by VOC emission (C-loss). Although the partitioning of photosynthates into monoterpene synthesis is more complex than a proportional relationship between emission and photosynthesis (see, e.g., [33]), considering the C-loss is useful for discerning between quantitative chemotypes. Further, we calculated the leaf mass per area (LMA, g m^{-2}) as the ratio of leaf dry mass to projected leaf area (also referred to as the specific leaf weight).

We analyzed emission data of the half-sib populations with factorial discriminant analysis to differentiate chemotypes. Further, we performed two-way analysis of variance to test whether measured variables significantly differed between the observed chemotypes and descendent populations alone or in interactions. Regarding stress resistance, we applied t-tests to detect differences between emitting and non-emitting saplings and repeated measures t-tests to detect differences before and after stress within the two groups. Data were transformed to ranks if tests for normal distribution and equal variance failed (Shapiro–Wilk and Levene tests were used for this). All statistical analyses were performed with the Addinsoft XLSTAT statistical and data analysis solution. The significance levels of differences between groups of measured variables are annotated as *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$, (*) $0.05 < p < 0.1$, and NS: Not Significant $p > 0.1$.

3. Results

3.1. VOC Emissions of the Half-Sib Populations

The leaves of 107 saplings out of 157 (68%) screened for VOCs emissions released monoterpenes with a mean rate of $788 \pm 44 \text{ SE ng m}^{-2} \text{s}^{-1}$ ($5.8 \pm 0.3 \text{ nmol m}^{-2} \text{s}^{-1}$ or $24.8 \pm 1.0 \mu\text{g g}^{-1} \text{h}^{-1}$), composed of $99 \pm 1\%$ of α -pinene, sabinene, β -pinene, myrcene, and limonene (Figure 1). In addition, low amounts of eucalyptol and β -ocimenes were occasionally detected. The remaining 50 saplings (32%) emitted no or only low amounts of monoterpenes ($31 \pm 4 \text{ ng m}^{-2} \text{s}^{-1}$) close to our estimated detection limit

($20 \pm 4 \text{ ng m}^{-2} \text{ s}^{-1}$). Isoprene was not consistently detected in the chamber air with cork oak leaves and was found only at very low concentrations, which were somewhat higher than those in the empty chamber (0.31 ± 0.15 vs. $0.11 \pm 0.02 \text{ ng L}^{-1}$) and were indicative of very low isoprene emissions for this oak species, if any ($0.35 \pm 0.27 \text{ ng m}^{-2} \text{ s}^{-1}$ or $5 \pm 4 \text{ pmol m}^{-2} \text{ s}^{-1}$). Since the presence of isoprene could not be confirmed by GC-MS, we consider these emissions to be tentative.

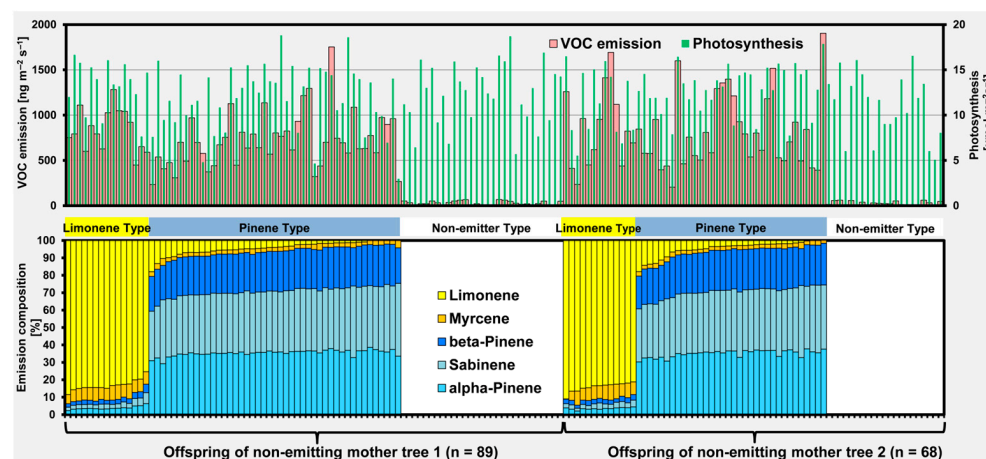


Figure 1. The chemodiversity of the VOCs emitted by the natural offspring of two non-emitting mother trees. The upper chart shows total VOC emission and net photosynthesis rates and the lower chart shows the proportions of the five major emitted monoterpenes (sum of the 5 = 100%). In both descendant populations, half-sibs cluster in three chemotypes with similar abundances: limonene type (17 and 19%) predominately emits limonene; pinene type (51 and 50%) predominately emits α -, β -pinene and sabinene; and non-emitters (33 and 31%) release VOCs close to or under the realistic detection limit of emissions in our measurement system.

There was no significant difference in photosynthesis between monoterpene-emitting and non-emitting saplings (12.4 ± 0.3 vs. $11.9 \pm 0.5 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$, $p = 0.001$), resulting in highly significant different C-losses (0.573 ± 0.023 vs. 0.024 ± 0.003 , $p < 0.001$). Within the group of emitters, emission rates were weakly correlated with photosynthesis rates, which explained about 20% of the emission variability (Figure S2). The proportions of the five monoterpenes in the emissions varied discontinuously among individuals (Figure 1) with one group (frequency 50%) emitting predominantly α -pinene, sabinene, and β -pinene in relative stable proportions of, respectively, $35.3 \pm 0.2\%$, $35.2 \pm 0.2\%$, and $22.6 \pm 0.1\%$. In this group, myrcene and limonene were present only in small fractions ($2.4 \pm 0.1\%$ and $4.5 \pm 0.5\%$). By contrast, the second group (frequency 18%) emitted predominantly limonene ($83.7 \pm 0.5\%$) with minor contributions of myrcene ($7.1 \pm 0.3\%$) and very little pinenes and sabinene (2.6–3.7%). These groups correspond to the three chemotypes observed in previous studies, which are referred to hereafter as the pinene, limonene and non-emitter types. Discriminant analysis based on the absolute emission rates of the five main monoterpenes and associated C-losses confirmed this classification (see Figure S3). Furthermore, repeated measurements conducted on the same saplings corroborated their inherent characteristics (Figure S4). The frequencies of each chemotype were very similar in both descendent populations, with the pinene-type appearing the most frequently (51 and 50%), followed by the non-emitter (33 and 31%) and limonene type (17 and 19%). Regarding leaf ecophysiology and plant growth (Table 1), ANOVAs did not reveal significant differences between chemotypes except higher LMA for the limonene-type leaves in one descendant population. Furthermore, regardless of chemotype, the two descendant populations differed in their foliage mass and number of ramifications accompanied by a trend of divergence in the timing of budburst.

Table 1. Foliar VOC emissions, CO₂/H₂O gas exchange, leaf structure and growth features of two natural half-sib cork oak populations (D1, D2) descending from two non-VOC emitting mother trees growing at a site in French Catalonia. Each descendant population consists of three chemotypes based on their VOC emissions: limonene-emitter (L), pinene-emitter (P) and non-emitter (N) (see Figures 1 and S2). VOC and CO₂/H₂O gas exchanges were measured on current-year leaves at the same temperature and light conditions of 30 °C and 1000 µmol m^{−2} s^{−1} PPFD. G_{H2O} is the leaf water vapor conductance and C-loss is the % loss of assimilated carbon by VOC emission. The water use efficiency (WUE) is the ratio between photosynthesis and transpiration. LMA is the leaf dry mass per area and chlorophyll represents the leaf chlorophyll concentration estimated by an optical method (SPAD arbitrary values). Statistics indicate significant differences between descendant populations, chemotypes, and interactions. Significance levels: *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$, (*) $0.05 < p < 0.1$, NS $p > 0.1$.

Descendants/ Chemotype (No of Trees)	VOC Emission ng m ^{−2} s ^{−1}	Photosynthesis µmol m ^{−2} s ^{−1}	C-Loss %	Transpiration mmol m ^{−2} s ^{−1}	G _{H2O} mmol m ^{−2} s ^{−1}	WUE mmol mol ^{−1}	LMA g m ^{−2}	Chlorophyll Content SPAD	Foliage Mass No of Leaves	Ramification No of Twigs	Budburst Day of Year
D1/Limonene (15)	846 ± 60	13.3 ± 0.7	0.57 ± 0.04	4.6 ± 0.2	259 ± 25	3.0 ± 0.2	123 ± 3.4	38.3 ± 0.6	115 ± 12	7.3 ± 1.1	82.7 ± 1.6
D2/Limonene (13)	857 ± 121	11.3 ± 1.1	0.66 ± 0.06	3.8 ± 0.4	246 ± 35	3.1 ± 0.2	111 ± 2.3	37.9 ± 0.7	154 ± 14	9.3 ± 1.3	79.5 ± 2.8
D1/Pinene (45)	725 ± 45	11.9 ± 0.5	0.56 ± 0.04	4.0 ± 0.2	218 ± 11	3.0 ± 0.1	113 ± 1.9	37.3 ± 0.5	120 ± 11	7.4 ± 0.8	82.7 ± 0.8
D2/Pinene (34)	820 ± 70	12.9 ± 0.4	0.56 ± 0.04	4.2 ± 0.1	235 ± 12	3.1 ± 0.1	112 ± 2.5	38.2 ± 0.6	152 ± 10	9.4 ± 0.8	81.6 ± 1.0
D1/Non (29)	33 ± 5	12.4 ± 0.6	0.02 ± 0.004	4.5 ± 0.2	227 ± 13	2.8 ± 0.1	117 ± 2.2	38.3 ± 0.6	125 ± 12	6.1 ± 0.9	84.6 ± 1.3
D2/Non (21)	28 ± 5	11.1 ± 0.8	0.02 ± 0.004	3.8 ± 0.3	202 ± 15	2.9 ± 0.1	119 ± 3.1	37.6 ± 0.9	176 ± 25	10.1 ± 1.4	81.6 ± 1.1
D1 total (89)	520 ± 44	12.3 ± 0.4	0.39 ± 0.03	4.3 ± 0.1	228 ± 8	2.9 ± 0.1	116 ± 1.3	37.8 ± 0.3	121 ± 7	7.0 ± 0.5	83.1 ± 0.6
D2 total (68)	582 ± 61	12.1 ± 0.4	0.41 ± 0.04	4.0 ± 0.1	227 ± 10	3.1 ± 0.1	114 ± 1.6	38.0 ± 0.4	160 ± 9	9.6 ± 0.7	81.8 ± 0.8
Limonene total (28)	851 ± 64	12.4 ± 0.6	0.61 ± 0.04	4.2 ± 0.2	253 ± 20	3.1 ± 0.1	117 ± 2.4	38.1 ± 0.4	133 ± 10	8.3 ± 0.9	81.3 ± 1.5
Pinene total (79)	766 ± 40	12.4 ± 0.4	0.56 ± 0.03	4.1 ± 0.1	225 ± 8	3.0 ± 0.1	113 ± 1.5	37.7 ± 0.4	134 ± 8	8.2 ± 0.6	82.2 ± 0.6
Non total (50)	31 ± 4	11.9 ± 0.5	0.02 ± 0.003	4.2 ± 0.2	217 ± 10	2.9 ± 0.1	118 ± 1.8	38.0 ± 0.5	146 ± 13	7.8 ± 0.8	83.3 ± 0.9
Statistics	***	NS	***	NS	NS	NS	*	NS	*	(*)	NS
Descendant	NS	NS	NS	NS	NS	NS	NS	NS	D1 < D2 **	D1 < D2 **	D1 > D2 *
Chemotype	N < L, P ***	NS	N < L, P ***	NS	NS	NS	N > P (*)	NS	NS	NS	NS
D × C	NS	NS	NS	NS	NS	NS	D1L > D2L (*)	NS	NS	NS	NS

3.2. Resistance to Simulated Sun-Flecks

There was no significant difference between emitters and non-emitters for any measured variable except for their mean chlorophyll content (SPAD), which was lower in the group of non-emitters (44.2 ± 1.1 vs. 47.4 ± 0.6 , $p = 0.038$) and was not affected by stress (i.e., no before–after stress difference). Also, there was no significant difference in the MT emission rates measured before and after sun-fleck exposures in both groups (emitters: 170 ± 21 vs. 161 ± 28 $\text{ng m}^{-2} \text{s}^{-1}$, $p = 0.82$; non-emitters: 3.7 ± 0.7 vs. 3.4 ± 0.8 $\text{ng m}^{-2} \text{s}^{-1}$, $p = 0.66$). However, there were several significant stress effects on other variables with few marginal differences between emitters and non-emitters (Figure 2).

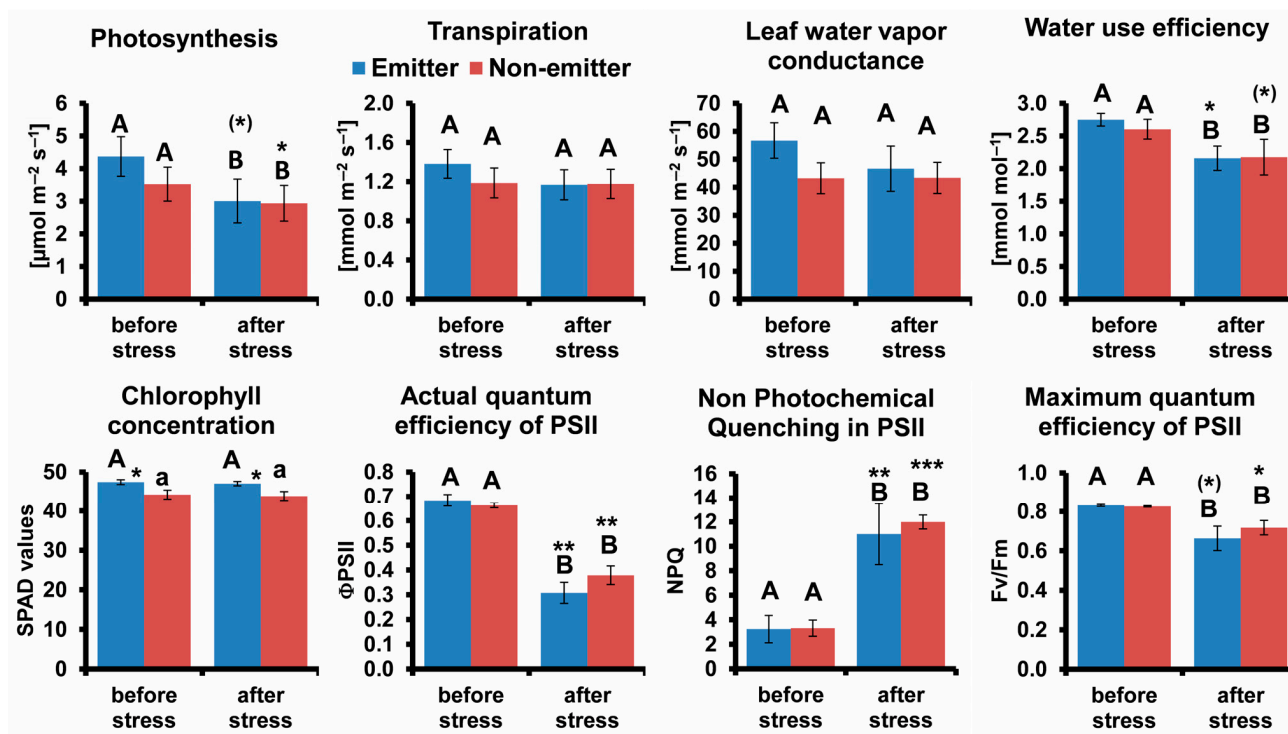


Figure 2. The resistance of emitting (blue) and non-emitting (brown) D1 descendants during simulated sun-flecks: After acclimatizing to 30 °C and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, an intact twig was exposed to 43 °C and 1800 PPFD five times for five minutes, with a five-minute recovery phase at the initial conditions between each exposure (Figure S4). $\text{CO}_2/\text{H}_2\text{O}$ gas exchange was recorded during each phase. VOC emissions, chlorophyll fluorescence variables, and chlorophyll concentration (SPAD) were measured once at the beginning and at the end of the experiment. The figures show the mean values + SE ($n = 5$) of emitting and non-emitting descendants measured before and after stress (recovery 5) in the same conditions (30 °C, 400 PPFD). Superscript letters indicate significant differences between the results before and after stress (A, B) and between emitter and non-emitter (upper, lower case). Significance levels: *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$, (*) $0.05 < p < 0.1$.

Regarding $\text{CO}_2/\text{H}_2\text{O}$ gas exchanges, the temperature and irradiation changes from 30 °C and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD to 43 °C and 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD strongly decreased photosynthesis (Figure S5). Photosynthesis recovered during each recovery phase to a similar level in both chemotypes. After the final recovery, the mean drop in photosynthesis was less significant, though it was higher in emitters than in non-emitters due to their somewhat higher and more variable photosynthesis rate before stress (emitters: 4.37 ± 0.61 vs. 3.01 ± 0.67 $\mu\text{mol m}^{-2} \text{s}^{-1}$, $p = 0.082$; non-emitters: 3.52 ± 0.52 vs. 2.94 ± 0.54 $\mu\text{mol m}^{-2} \text{s}^{-1}$, $p = 0.044$). Transpiration increased dramatically during each stress phase due to the sudden increase in the vapor pressure deficit (decrease in relative air humidity) and because stomata reacted with delay to temperature and PPFD changes.

In fact, stomatal conductance generally lowered after stress during the recovery phases, with the apparent closure diminishing with repeated exposure to stress. At the end, there was no significant difference in transpiration and conductance compared to pre-stress values. Water use efficiency was very low during stress phases and gradually decreased in the recovery phases. After the final recovery, the water use efficiency remained more reduced in emitters (2.75 ± 0.10 vs. 2.16 ± 0.19 mmol mol⁻¹, $p = 0.026$) than in non-emitters (2.60 ± 0.15 vs. 2.18 ± 0.25 , $p = 0.060$). Chlorophyll fluorescence measurements showed a significant drop of Φ_{PSII} in both groups ($p < 0.01$), along with a strong increase in NPQ ($p < 0.01$ and < 0.001). F_v/F_m was reduced in both groups, with a smaller but slightly more significant reduction in non-emitters (0.83 ± 0.01 vs. 0.72 ± 0.04 , $p = 0.044$) than in emitters (0.83 ± 0.01 vs. 0.66 ± 0.06 , $p = 0.051$). The individual losses of F_v/F_m were clearly correlated with their losses in photosynthesis, indicating that persistent photodamage had affected CO₂ assimilation (Figure S6). However, no visible signs of damage, such as discoloration, developed in the days afterwards. Overall, cork oak leaves showed good resistance to extreme sun-flecks, with no clear difference between the emitters and non-emitters.

4. Discussion

4.1. Inheritance Mode

Given that the two pinene isomers and sabinene strongly covaried, the major bulk of constitutive monoterpenes could essentially be produced by two type of monoterpene synthases, one producing mainly limonene and one producing pinenes plus sabinene, whose activities differed among chemotypes. Environmental factors such as temperature might influence the product pattern of terpene synthases [36], a potential bias we avoided as much as possible in our study by screening all plants during the same season and under common assay conditions. The same chemotypes were already observed in previous screening studies involving cork oak, including on saplings of similar provenance (Figure S7a) and on adult trees from the population of the two non-emitting mother trees (Figure S7b). Compared to these, the emissions of the two half-sibs differed in three main ways: (i) the average emission rates of the half-sib emitters were two to three times lower; (ii) the frequency of non-emitters was much higher; and (iii) the mixed chemotype is missing. This intermediate chemotype emits high proportions of both pinenes and limonene and was also the most prevalent chemotype in the mother population (Figure S7b). Furthermore, we observed that both half-sib populations were composed of the same chemotypes with very similar frequencies, confirming that both mother trees shared the same genotype for constitutive VOC emissions and that the emission configuration in their offspring was determined by a similar mixture of pollen they received from the surrounding trees in the population. The strong discontinuous, binary distribution of the emission compositions among half-sib emitters suggests a simple inheritance mode. We hypothesize that the functional monoterpene-synthase genes for limonene and pinenes plus sabinene are dominant alleles that are located at the same chromosomal locus on different chromosome homologues, hereafter referred to as L and P alleles. In non-emitting trees, both alleles would be non-functional (N alleles), either because they are not expressed under assay conditions or because they express enzymes with strongly reduced activity; for example, due to altered substrate affinity. Accordingly, the non-emitter type would be always homozygous (NN), the mixed emitter type would always be heterozygous (LP), and the limonene and pinene emitter types could be either homozygous (LL and PP) or heterozygous (LN and PN). Since pollen could bring only one of the three putative alleles (P, L, or N) to the N-allele of the ovule of the non-emitting trees, all emitting descendants must be heterozygotes PN or LN, which might explain the overall lower emissions compared to natural populations, in which PP-, LL- and PL-genotypes are frequent. Of 21 trees of the mother population screened for

VOC emissions, [23] reported that 9 were mixed types, 8 were pinene types, and four were non-types (Figure S7b,c). Assuming that this chemotype frequency is representative and all trees produced similar amounts of pollen, the pollen received by the two non-emitting mother trees would comprise ca 21% of the L-type, between 40 and 60% of the P-Type, and between 19 and 38% of the N-type. This estimation, based on a simple inheritance mode, fits the observed frequencies of chemotypes in the two offspring populations relatively well (pinene type: 51% and 50%, limonene type: 17% and 19%; non-emitter: 33% and 31%), especially considering that a small proportion of non-emitters among the half-sibs may have resulted from self-pollination.

In contrast to the emission composition, the quantities of monoterpenes released by the emitters varied widely (see Figures 1 and S2). Replicate measurements made on the same saplings showed rate changes of up to twofold, indicating a high level of plasticity in the foliar synthesis rates (Figure S4). This plasticity can be attributed to limitations imposed by substrate availability, which are linked to rearrangements in the partitioning of photosynthates to metabolic sinks and/or feedback mechanisms within the MEP pathway [33,37,38]. The expression of monoterpene synthase genes and the resulting enzyme levels may also vary over time depending on prevailing weather conditions and leaf age [32,39]. Furthermore, these genes are likely to occur in multiple copies as gene clusters on the same chromosome [15]. Such gene arrays may result from multiple tandem duplications over evolutionary time, which could differ between populations and individuals. However, the hypothetical inheritance mode we deduced from the binary distribution of the emission composition implies that little or no crossing-overs or mutations leading to functional diversification occurred, such that the putative P and L allele clusters have essentially conserved their product specificity and genomic locations, as stated by [15].

4.2. Do Chemotypes Differ in Their Fitness?

The results of the offspring screening confirm that there are cork oak phenotypes with inhibited constitutive monoterpene production and that this trait is inheritable. It represented almost one-third of the half-sibs, which is much higher than the frequency usually observed in cork oak populations ranging between 0 and 10% [14]. This rarity let us believe that non-emitters are subject to strong negative selection. However, neither the results of the stress experiments (Figure 2) nor the ecophysiological data of the half-sib screening (Table 1) provided clear evidence of fitness differences. However, the present study had several limitations with regard to detecting fitness differences between chemotypes. Firstly, we only compared chemotypes that all descended from non-emitting mother trees. Chemotypes that descend from emitting mother trees may generally have stronger emissions and perhaps stronger vigor. Secondly, the sun-fleck stress experiment was conducted in autumn, at a time when the VOC synthesis of the saplings may already have been down-regulated, providing little support for thermotolerance. Thirdly, we did not monitor the germination rates of the collected acorns, which may have been reduced in non-emitters due to feedback in the synthesis of essential isoprenoids or other metabolites [40] during early seedling development. Furthermore, in addition to, or instead of, playing a beneficial role in resistance to abiotic stress, the constitutive production of VOCs in cork oak may directly or indirectly contribute to resistance to biotic stress, particularly from defoliating insects [41].

However, similar chemotypes to those found in cork oak have been observed in the emissions of other Mediterranean oak species [23,25,27,42,43]. Chemotypes contrasting in their limonene and pinene proportions were also reported for distantly related species such as *Abies grandis* [44], *Cannabis sativa* [45], and Cotton [46]. In fact, the major monoterpenes emitted by cork oak are emitted by various other plant species (e.g., [47–51]) and are the

most common monoterpenes in the atmosphere (e.g., [52–54]). Given the ubiquity of the monoterpenes emitted by cork oak and the common diversification pattern, one must question their ability to fulfill specific ecological functions in trophic interactions, particularly in highly specialized systems [55,56]. We argue that the occurrence and frequency of chemotypes in cork oak may be largely unrelated to fitness. If paralogous monoterpene synthase genes cluster in tandem arrays on oak chromosomes, multiple independent mutations would be necessary to completely suppress monoterpene production. These mutations would have a low probability of co-occurring and the large majority of trees produce monoterpenes, albeit at variable rates.

5. Conclusions

This study addressed a gap in our understanding of how the quality and quantity of constitutive VOC emissions vary intrinsically within cork oak populations. Offspring populations from non-emitting individuals exhibited similar chemical polymorphisms, characterized by a high proportion of non-emitters and a discontinuous binary distribution of emitters in two chemotypes, with relatively low mean emission rates. These observations suggest that two monoterpene synthase genes, which differ in their product pattern, are located on different chromosome homologues and that functional and non-functional alleles exist within these genes. This putative inheritance mode is consistent with the frequency of chemotypes observed in the mother population.

In line with our previous studies, chemotypes did not differ in terms of photosynthetic or growth performance and exhibited similar resistance to sun-flecks. While the large proportion of non-emitters observed in half-sib populations provides additional evidence that they are suppressed in natural cork oak populations, where they are very rare, our results do not suggest that this is due to a lack of competitiveness. Thus, our study supports the idea of using non-emitters in reforestation and urban greening programs to reduce the input of precursors involved in atmospheric ozone and particle formation. Cork oak forests cover an area of 2.3 million hectares, two-thirds of which are in Europe while one-third are in Africa [24]. Although their leaf area index of around 2 [57] is moderate compared to temperate and tropical forests, cork oak forests likely contribute significantly to the regional BVOC load because cork oak is always the predominant species in forests managed for cork production. Furthermore, the high emission potential of their evergreen foliage, along with high leaf temperatures and incident radiation, favors strong, year-round emissions. Further research is needed to understand the underlying mechanisms and adaptive role of VOC diversification in cork oak. For instance, it would be interesting to conduct common garden experiments with defined chemotypes along an edaphic–climatic gradient in order to identify the combinations of abiotic and biotic constraints under which chemotypes may perform better. Furthermore, field studies are needed in cork oak forests to assess their real role as a source or sink of ozone, particles, and associated precursors.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/environments13020070/s1>, Figure S1: Photos of the leaf chamber; Figure S2: Relation between VOC emission and photosynthesis; Figure S3: Results of discriminant analysis; Figure S4: Replicate measurements on five saplings; Figure S5: CO₂/H₂O gas exchanges, temperature and light during sun-fleck experiment; Figure S6: relation between photosynthesis and Fv/Fm reductions; Figure S7: Monoterpene emissions from related cork oak populations and estimation of monoterpene synthase allele frequency in the pollen of the mother population.

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References

1. Guenther, A.B.; Jiang, X.; Heald, C.L.; Sakulyanontvittaya, T.; Duhl, T.; Emmons, L.K.; Wang, X. The Model of Emissions of Gases and Aerosols from Nature version 2.1 (MEGAN2.1): An extended and updated framework for modeling biogenic emissions. *Geosci. Model Dev.* **2012**, *5*, 1471–1492. [\[CrossRef\]](#)
2. Borbon, A.; Fornaro, A.; Oliveira, A.P.; Souza, S.R.; de Brito, J.F.; Jaffrezo, J.-L.; Staudt, M.; Ynoue, R.Y.; Codato, G.; Sánchez, M.P.; et al. The BIOMASP+ project on biosphere-atmosphere exchanges and their role in air pollution in the subtropical megacity of São Paulo: Motivations, methods and preliminary observations. *Bull. Am. Meteorol. Soc.* **2025**, *in press*. [\[CrossRef\]](#)
3. WHO. *WHO Global Air Quality Guidelines. Particulate Matter (PM_{2.5} and PM₁₀), Ozone, Nitrogen Dioxide, Sulfur Dioxide and Carbon Monoxide*; World Health Organization: Geneva, Switzerland, 2021; ISBN 978-92-4-003422-8.
4. Burnett, R.; Chen, H.; Szyszkowicz, M.; Fann, N.; Hubbell, B.; Pope, C.A., 3rd; Apte, J.S.; Brauer, M.; Cohen, A.; Weichenthal, S.; et al. Global estimates of mortality associated with long-term exposure to outdoor fine particulate matter. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 9592–9597. [\[CrossRef\]](#)
5. Zhang, J.; Wei, Y.; Fang, Z. Ozone Pollution: A Major Health Hazard Worldwide. *Front. Immunol.* **2019**, *10*, 2518. [\[CrossRef\]](#)
6. Boy, M.; Zhou, P.; Kurtén, T.; Chen, D.; Xavier, C.; Clusius, P.; Roldin, P.; Baykara, M.; Pichelstorfer, L.; Foreback, B.; et al. Positive feedback mechanism between biogenic volatile organic compounds and the methane lifetime in future climates. *npj Clim. Atmos. Sci.* **2022**, *5*, 72. [\[CrossRef\]](#)
7. Dada, L.; Stolzenburg, D.; Simon, M.; Fischer, L.; Heinritzi, M.; Wang, M.; Xiao, M.; Vogel, A.L.; Ahonen, L.; Amorim, A.; et al. Role of sesquiterpenes in biogenic new particle formation. *Sci. Adv.* **2023**, *9*, eadi5297. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Thornhill, G.; Collins, W.; Olivie, D.; Skeie, R.B.; Archibald, A.; Bauer, S.; Checa-Garcia, R.; Fiedler, S.; Folberth, G.; Gjermundsen, A.; et al. Climate-driven chemistry and aerosol feedbacks in CMIP6 Earth system models. *Atmos. Chem. Phys.* **2021**, *21*, 1105–1126. [\[CrossRef\]](#)
9. Masson-Delmotte, V.P.; Zhai, A.; Pirani, S.L.; Connors, C.; Péan, S.; Berger, N.; Caud, Y.; Chen, L.; Goldfarb, M.I.; Gomis, M.; et al. (Eds.) *IPCC, 2021: Climate Change 2021: The Physical Science Basis; Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2021; 2391p. [\[CrossRef\]](#)
10. Behnke, K.; Grote, R.; Brüggemann, N.; Zimmer, I.; Zhou, G.; Elobeid, M.; Janz, D.; Polle, A.; Schnitzler, J.-P. Isoprene emission-free poplars—A chance to reduce the impact from poplar plantations on the atmosphere. *New Phytol.* **2012**, *194*, 70–82. [\[CrossRef\]](#)
11. Chang, T.-W.; Tokiwa, S.; Masui, N.; Tani, A. Quantifying variabilities in monoterpene emission among natural individuals of an urban-greening species (*Quercus phylliraeoides*) using a rapid measuring method. *Urban For. Urban Green.* **2024**, *94*, 128245. [\[CrossRef\]](#)
12. Kesselmeier, J.; Staudt, M. Biogenic volatile organic compounds (VOC): An overview on emission, physiology and ecology. *J. Atmos. Chem.* **1999**, *33*, 23–88. [\[CrossRef\]](#)
13. Monson, R.K.; Jones, R.T.; Rosenstiel, T.N.; Schnitzler, J.-P. Why only some plants emit isoprene. *Plant Cell Environ.* **2013**, *36*, 503–516. [\[CrossRef\]](#)
14. Staudt, M.; Rivet, C.; Erdogan, M. Diversity of Volatile Emissions From Cork Oak: Quantity and Quality Vary Independently Across Its Range. *Ecol. Evol.* **2025**, *15*, e72093. [\[CrossRef\]](#)
15. Ikezaki, Y.; Kudo, S.N.; Nakata, T.; Koita, S.; Munakata, R.; Yazaki, K.; Torimaru, T.; Tomaru, N.; Isobe, S.; Hirakawa, H.; et al. Molecular evolution of terpene synthase underlying the diversification of isoprene emission in Fagaceae. *bioRxiv* **2025**. [\[CrossRef\]](#)

16. Delwiche, C.F.; Sharkey, T.D. Rapid appearance of ^{13}C in biogenic isoprene when $^{13}\text{CO}_2$ is fed to intact leaves. *Plant Cell Environ.* **1993**, *16*, 587–591. [[CrossRef](#)]
17. Loreto, F.; Ciccioli, P.; Cecinato, A.; Brancaleoni, E.; Frattoni, M.; Fabozzi, C.; Tricoli, D. Evidence of the photosynthetic origin of monoterpenes emitted by *Quercus ilex* L. leaves by ^{13}C labeling. *Plant Physiol.* **1996**, *110*, 1317–1322. [[CrossRef](#)] [[PubMed](#)]
18. Singsaas, E.L.; Sharkey, T.D. The regulation of isoprene emission responses to rapid leaf temperature fluctuations. *Plant Cell Environ.* **1998**, *21*, 1181–1188. [[CrossRef](#)]
19. Behnke, K.; Loivamäki, M.; Zimmer, I.; Rennenberg, H.; Schnitzler, J.-P.; Louis, S. Isoprene emission protects photosynthesis in sunfleck exposed Grey poplar. *Photosynth. Res.* **2010**, *104*, 5–17. [[CrossRef](#)] [[PubMed](#)]
20. Zuo, Z.; Weraduwa, S.M.; Huang, T.; Sharkey, T.D. How volatile isoprenoids improve plant thermotolerance. *Trends Plant Sci.* **2025**, *30*, 1237–1250. [[CrossRef](#)]
21. Pio, C.; Nunes, T.; Brito, S. Volatile hydrocarbon emissions from common and native species of vegetation in Portugal. In *Air Pollution Report 47*; Slanina, J., Angeletti, G., Beilke, S., Eds.; Guyot SA: Brussels, Belgium, 1993; pp. 291–298.
22. Pio, C.A.; Silva, P.A.; Cerqueira, M.A.; Nunes, T.V. Diurnal and seasonal emissions of volatile organic compounds from cork oak (*Quercus suber*) trees. *Atmos. Environ.* **2005**, *39*, 1817–1827. [[CrossRef](#)]
23. Staudt, M.; Mir, C.; Joffre, R.; Rambal, S.; Bonin, A.; Landais, D.; Lumaret, R. Isoprenoid emissions of *Quercus* spp. (*Q. suber* and *Q. ilex*) in mixed stands contrasting in interspecific genetic introgression. *New Phytol.* **2004**, *163*, 573–584. [[CrossRef](#)] [[PubMed](#)]
24. Pausas, J.G.; Pereira, J.S.; Aronson, J. The Tree. In *Cork Oak Woodlands on the Edge*; Aronson, J., Pereira, J.S., Pausas, J.G., Eds.; Island Press: Washington, DC, USA, 2009; pp. 11–21.
25. Welter, S.; Bracho-Nunez, A.; Mir, C.; Zimmer, I.; Kesselmeier, J.; Lumaret, R.; Schnitzler, J.-P.; Staudt, M. The diversification of terpene emissions in Mediterranean oaks: Lessons from a study of *Quercus suber*, *Quercus canariensis* and its hybrid *Quercus afares*. *Tree Physiol.* **2012**, *32*, 1082–1091. [[CrossRef](#)]
26. Steinbrecher, R.; Hauff, K.; Rabong, R.; Steinbrecher, J. Isoprenoid emission of oak species typical for the Mediterranean area: Source strength and controlling variables. *Atmos. Environ.* **1997**, *31*, 79–88. [[CrossRef](#)]
27. Csiky, O.; Seufert, G. Terpenoid emissions of mediterranean oaks and their relation to taxonomy. *Ecol. Appl.* **1999**, *9*, 1138–1146. [[CrossRef](#)]
28. Boavida, L.C.; Silva, J.P.; Feijo, J.A. Sexual reproduction in the cork oak (*Quercus suber* L.). II. Crossing intra- and interspecific barriers. *Sex. Plant Reprod.* **2001**, *14*, 143–152. [[CrossRef](#)]
29. Bohlmann, J.; Meyer-Gauen, G.; Croteau, R. Plant terpenoid synthases: Molecular biology and phylogenetic analysis. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 4126–4133. [[CrossRef](#)] [[PubMed](#)]
30. Fischbach, R.J.; Zimmer, W.; Schnitzler, J.-P. Isolation and functional analysis of a cDNA encoding a myrcene synthase from holm oak (*Quercus ilex* L.). *Eur. J. Biochem.* **2001**, *268*, 5633–5638. [[CrossRef](#)] [[PubMed](#)]
31. Ramos, A.M.; Usié, A.; Barbosa, P.; Barros, P.M.; Capote, T.; Chaves, I.; Simões, F.; Abreu, I.; Carrasquinho, I.; Faro, C.; et al. The draft genome sequence of cork oak. *Sci. Data* **2018**, *5*, 180069. [[CrossRef](#)] [[PubMed](#)]
32. Staudt, M.; Joffre, R.; Rambal, S. How growth conditions affect the capacity of *Quercus ilex* leaves to emit monoterpenes. *New Phytol.* **2003**, *158*, 61–73. [[CrossRef](#)]
33. Staudt, M.; Daussy, J.; Ingabire, J.; Dehimeche, N. Growth and actual leaf temperature modulate CO_2 responsiveness of monoterpene emissions from holm oak in opposite ways. *Biogeosciences* **2022**, *19*, 4945–4963. [[CrossRef](#)]
34. Maxwell, K.; Johnson, G.N. Chlorophyll fluorescence—A practical guide. *J. Exp. Bot.* **2000**, *51*, 659–668. [[CrossRef](#)]
35. von Caemmerer, S.; Farquhar, G.D. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **1981**, *153*, 376–387. [[CrossRef](#)]
36. Koo, H.J.; Vickery, C.R.; Xu, Y.; Louie, G.V.; O'Maille, P.E.; Bowman, M.; Nartey, C.M.; Burkart, M.D.; Noel, J.P. Biosynthetic potential of sesquiterpene synthases: Product profiles of Egyptian Henbane premnaspirodiene synthase and related mutants. *J. Antibiot.* **2016**, *69*, 524–533. [[CrossRef](#)] [[PubMed](#)]
37. Niinemets, Ü.; Rasulov, B.; Talts, E. CO_2 -responsiveness of leaf isoprene emission: Why do species differ? *Plant Cell Environ.* **2021**, *44*, 3049–3063. [[CrossRef](#)]
38. Sahu, A.; Mostofa, M.G.; Weraduwa, S.M.; Sharkey, T.D. Hydroxymethylbutenyl diphosphate accumulation reveals MEP pathway regulation for high CO_2 -induced suppression of isoprene emission. *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e2309536120. [[CrossRef](#)] [[PubMed](#)]
39. Fischbach, R.J.; Staudt, M.; Zimmer, I.; Rambal, S.; Schnitzler, J.P. Seasonal pattern of monoterpene synthase activities in leaves of the evergreen tree *Quercus ilex*. *Physiol. Plant.* **2002**, *114*, 354–360. [[CrossRef](#)] [[PubMed](#)]
40. Rasulov, B.; Bichele, I.; Laisk, A.G.U.; Niinemets, Ü. Competition between isoprene emission and pigment synthesis during leaf development in aspen. *Plant Cell Environ.* **2014**, *37*, 724–741. [[CrossRef](#)] [[PubMed](#)]
41. Sánchez-Osorio, I.; Robles, D.; Tapias, R. Presence of *Quercus suber* Soft-Leaf Defoliators on Trees with Distinct Foliar Monoterpene Emission Profiles. *Appl. Sci.* **2024**, *14*, 1112. [[CrossRef](#)]

42. Staudt, M.; Mandl, N.; Joffre, R.; Rambal, S. Intraspecific variability of monoterpene composition emitted by *Quercus ilex* leaves. *Can. J. For. Res.* **2001**, *31*, 174–180. [\[CrossRef\]](#)
43. Staudt, M.; Visnadi, I. High chemodiversity in the structural and enantiomeric composition of volatiles emitted by Kermes oak populations in Southern France. *Elem. Sci. Anth.* **2023**, *11*, 00043. [\[CrossRef\]](#)
44. Katoh, S.; Croteau, R. Individual variation in constitutive and induced monoterpene biosynthesis in grand fir. *Phytochemistry* **1998**, *47*, 577–582. [\[CrossRef\]](#)
45. Fishedick, J.T. Identification of Terpenoid Chemotypes Among High (-)-*trans*- Δ^9 -Tetrahydrocannabinol-Producing *Cannabis sativa* L. Cultivars. *Cannabis Cannabinoid Res.* **2017**, *2*, 34–47. [\[CrossRef\]](#)
46. Clancy, M.V.; Mamin, M.; Flückiger, G.; Quijano-Medina, T.; Pérez-Nino, B.; Abdala-Roberts, L.; Turlings, T.C.J.; Bustos-Segura, C. Terpene chemotypes in *Gossypium hirsutum* (wild cotton) from the Yucatan Peninsula, Mexico. *Phytochemistry* **2023**, *205*, 113454. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Geron, C.; Rasmussen, R.; Arnts, R.R.; Guenther, A. A review and synthesis of monoterpene speciation from forests in the United States. *Atmos. Environ.* **2000**, *34*, 1761–1781. [\[CrossRef\]](#)
48. Owen, S.; Boissard, C.; Hewitt, C.N. Volatile organic compounds (VOCs) emitted from 40 Mediterranean plant species: VOC speciation and extrapolation to habitat scale. *Atmos. Environ.* **2001**, *35*, 5393–5409. [\[CrossRef\]](#)
49. Malik, T.G.; Gajbhiye, T.; Pandey, S.K. Some insights into composition and monoterpene emission rates from selected dominant tropical tree species of Central India: Plant-specific seasonal variations. *Ecol. Res.* **2019**, *34*, 821–834. [\[CrossRef\]](#)
50. Zeng, J.; Song, W.; Zhang, Y.; Mu, Z.; Pang, W.; Zhang, H.; Wang, X. Emissions of isoprenoids from dominant tree species in subtropical China. *Front. For. Glob. Change* **2022**, *5*, 1089676. [\[CrossRef\]](#)
51. Chen, X.; Gong, D.; Lin, Y.; Xu, Q.; Wang, Y.; Liu, S.; Li, Q.; Ma, F.; Li, J.; Deng, S.; et al. Emission characteristics of biogenic volatile organic compounds in a subtropical pristine forest of southern China. *J. Environ. Sci.* **2025**, *148*, 665–682. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Yáñez-Serrano, A.M.; Nölscher, A.C.; Bourtsoukidis, E.; Gomes Alves, E.; Ganzeveld, L.; Bonn, B.; Wolff, S.; Sa, M.; Yamasoe, M.; Williams, J.; et al. Monoterpene chemical speciation in a tropical rainforest: variation with season, height, and time of day at the Amazon Tall Tower Observatory (ATTO). *Atmos. Chem. Phys.* **2018**, *18*, 3403–3418. [\[CrossRef\]](#)
53. Liu, Y.; Schallhart, S.; Taipale, D.; Tykkä, T.; Räsänen, M.; Merbold, L.; Hellén, H.; Pellikka, P. Seasonal and diurnal variations in biogenic volatile organic compounds in highland and lowland ecosystems in southern Kenya. *Atmos. Chem. Phys.* **2021**, *21*, 14761–14787. [\[CrossRef\]](#)
54. Borsdorf, H.; Bentele, M.; Müller, M.; Rebmann, C.; Mayer, T. Comparison of Seasonal and Diurnal Concentration Profiles of BVOCs in Coniferous and Deciduous Forests. *Atmosphere* **2023**, *14*, 1347. [\[CrossRef\]](#)
55. Rowen, E.; Kaplan, I. Eco-evolutionary factors drive induced plant volatiles: A meta-analysis. *New Phytol.* **2016**, *210*, 284–294. [\[CrossRef\]](#)
56. Douma, J.C.; Ganzeveld, L.N.; Unsicker, S.B.; Boeckler, G.A.; Dicke, M. What makes a volatile organic compound a reliable indicator of insect herbivory? *Plant Cell Environ.* **2019**, *42*, 3308–3325. [\[CrossRef\]](#)
57. Zribi, L.; Chaar, H.; Khaldi, A.; Henchi, B.; Mouillot, F.; Gharbi, F. Estimate of biomass and carbon pools in disturbed and undisturbed oak forests in Tunisia. *For. Syst.* **2016**, *25*, e060. [\[CrossRef\]](#)

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