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Laboratory and field measurements of enantiomeric monoterpene emissions as a function of chemotype, light and temperature

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Abstract

Plants emit significant amounts of monoterpenes into the Earth's atmosphere where they react rapidly to form a multitude of gas phase species and particles. Many monoterpenes exist in mirror images forms or enantiomers. In this study the enantiomeric monoterpene profile for several representative plants (*Quercus ilex* L., *Rosmarinus officinalis* L., and *Pinus halepensis Mill.*) was investigated as a function of chemotype, light and temperature both in the laboratory and in the field. Analysis of enantiomeric monoterpenes from 19 *Quercus ilex* individuals from Southern France and Spain revealed four regiospecific chemotypes (genetically fixed emission patterns). In agreement with previous work, only *Quercus ilex* emissions increased strongly with light. However, for all three plant species no consistent enantiomeric variation was observed as a function of light, and the enantiomeric ratio of α-pinene was found vary by less than 20 % from 100 and 1000 μmol m^{-2} s^{-1} PAR. The rate of monoterpene emission increased with temperature from all three plant species, but little variation in the enantiomeric distribution of α-pinene was observed with temperature. There was more enantiomeric variability between individuals of the same species than could be induced by either light or temperature. Field measurements of α-pinene enantiomer mixing ratios in the air taken at a *Quercus ilex* forest in Southern France, and several other previously reported field enantiomeric ratio diel cycle profiles are compared. All show smoothly varying diel cycles (some positive and some negative) even over changing wind directions. This is surprising in comparison with variations of enantiomeric emission patterns shown by individuals of the same species.

1 Introduction

Terrestrial vegetation is an important global source of reactive volatile organic compounds (VOCs) contributing circa 1 Pg (1×10^{15} g) of carbon annually (Guenther, 2002), approximately ten times more than the estimated sum of anthropogenic VOC emissions including fossil fuel and biomass burning (Piccot et al., 1992). Some of these VOCs may serve to attract pollinators, fruit dispersers and parasitoids of herbivores, as well as to repel herbivores, warn neighboring plants of imminent herbivore and pathogen attack (Baldwin et al., 2006; Degenhardt and Gershenzon, 2000; Gershenzon and Dudareva, 2007; Hopke et al., 1994; van Dam et al., 2010). Moreover, some BVOCs have been shown to provide protection from environmental stresses such as ozone and high temperatures (Sharkey and Singsaas, 1995; Loreto and Velikova, 2001). BVOCs are produced in various plant tissues and compartments above and below ground as the result of many biochemical pathways. Different plant families emit different subsets of these VOCs and these emissions may vary in intensity in response to endogenous and exogenous factors such as time of day, temperature, light, age, etc. (Kesselmeier and Staudt, 1999).

Among BVOCs, isoprene (C5), monoterpenes (C10) and sesquiterpenes (C15), which are often more than 90 % of the total plant VOCs emission, deserve special attention because of their high volatility, reactivity in the atmosphere and their large fractional contribution (Aaltonen et al., 2011; Staudt and Lhoutellier, 2011). Generally, all isoprenoids are derived from isopentenyl diphosphate (IPP, C5) and its isomer dimethylallyl diphosphate (DMAPP, C5). These precursors are either synthesized by the plastidic 1-deoxy-D-xylulose-5-phosphate (DXP) pathway (also known as methylerythritol phosphate (MEP) pathway) in the case of isoprene and monoterpenes or in the cytosolic mevalonate pathway in the case of sesquiterpenes (Mahmoud and Croteau, 2002). Isoprene and monoterpenes represent a large part of the non-methane BVOC flux with global flux estimates between 454 and 601 $\mathrm{Tg\,C\,y^{-1}}$ and between 32 and 127 $\mathrm{Tg\,C\,y^{-1}}$, respectively (Arneth et al., 2008). Although there are large uncertainties in the magnitude of emission rates of individual and total BVOCs, in general, isoprene and monoterpenes are thought to be the strongest biogenic emissions and are the most commonly measured compounds (Staudt and Lhoutellier, 2011). The main driving variables for the emission of BVOCs are photosynthetically active radiation (PAR) and temperature which form the basis of all emission models (Arneth et al., 2008; Guenther et al., 2006;

Monson et al., 2012 and references therein). However, other environmental factors such as the effect of seasonality, the CO_2 level, the ozone level, mechanical stress and drought also have been studied (Blanch et al., 2007; Curci et al., 2009; McKinney et al., 2011; Penuelas and Staudt, 2010; Staudt et al., 2000, 2002) and incorporated into emission models recently (Arneth et al., 2007; Arneth and Niinemets, 2010; Grote et al., 2010; Keenan et al., 2009). In the past decade, great efforts have been made to improve regional and global models which estimate the source strength of BVOCs and in particular of isoprenoids (Grote and Niinemets, 2008; Guenther et al., 2006; Niinemets et al., 2002; Schurgers et al., 2009; Zimmer et al., 2000). While there is a generally accepted empirical emission algorithm for isoprene, which has been established to be proportional to both temperature and light (Guenther et al., 1995, 2006), for monoterpenes the situation is less clear – with some monoterpene emitters responding to temperature, and others to both light and temperature (Fuentes et al., 2000). In some species of plants, monoterpenes are synthesized and stored in secretory organs, such as the resin ducts within the leaves of conifers or the glandular trichomes surrounding the leaves and the stems of many Lamiaceae (Grote and Niinemets, 2008). The emissions rely on vaporization and diffusion from these extensive storage pools. In this case the emission rate of the monoterpene to the atmosphere is observed to increase exponentially with increasing leaf temperature. In other species such as the Mediterranean oak (*Quercus ilex*), no significant storage pool exists and the monoterpene emission occurs (in similar fashion to isoprene) when light is present (Guenther et al., 1993; Loreto et al., 1996a; Staudt and Seufert, 1995). Typically, the monoterpene emission from these plants respond to light following a rectangular hyperbola similar to that of photosynthesis, while its response to temperature resembles a double Arrhenius function perhaps reflecting the activity of specific enzymes limiting the monoterpene biosynthesis (Fischbach et al., 2002; Staudt and Bertin, 1998). There is increasing evidence that both pools, de-novo synthesis and storage pools, can coexist in plants each one significantly contributing to the total emission of monoterpenes (e.g. Ghirardo et al., 2010). In that case emission should show an intermediate behaviour with respect to light and temperature responses. For example emissions increase with increasing light but do not cease in darkness.

Numerous monoterpenes and sesquiterpenes occur in two enantiomeric forms, for example α-pinene exists as (+)-α-pinene and (−)-α-pinene, see Fig. 1. Typically such enantiomeric pairs have been measured and modeled together as one substance in ambient atmospheric chemistry studies (e.g. Guenther et al., 1995, 2006; Grote and Niinemets, 2008) as both enantiomers react at the same rates with ozone and OH. However, for the biosphere (insect and plants) the two enantiomers can be very different distinct molecules that may elicit opposite responses in pollinators and predators alike (Rostelien et al., 2005; Tooker and Hanks, 2004). Furthermore, variation in the individual enantiomers in ambient air may has the potential to provide clues to variable monoterpene sources within the plant cover through their individual responses to stimuli and thereby represents a means to better link current empirical models to internal plant processes. Over the past 10 yr, as chiral chromatography columns (such as cyclodextrin) have become commercially available more chirally resolved studies have begun to emerge, providing captivating new information. Pioneering works on tree and plant emissions have shown very distinct enantiomeric fingerprints in different tree species (Yassaa et al., 2000) and even different chemotypes within the same species (Yassaa and Williams, 2007). It has been shown that clear changes in enantiomeric ratios occur when trees are subjected to mechanical stress, allowing processes upwind to be deduced (Eerdekens et al., 2009; Yassaa and Williams, 2007). Regiospecific enantiomeric ratios have been reported from tropical and Boreal forests (Williams et al., 2007), and enantiomeric signatures have been used to constrain aerosol formation from isoprene (Noziere et al., 2011). Possible heterogeneous enantiomeric enrichment mechanisms on aerosols (Ebben et al., 2011; Stokes et al., 2009) have been investigated and new enantiomerically resolved marine sources of monoterpenes (Yassaa et al., 2008) have provided exciting new information and questions for this emerging field.

In some plant species the two enantiomers may be produced via different enzymes (Phillips et al., 1999). The individual enantiomer can have different biological activities. It might be advantageous to the plant to be able to alter the enantiomeric ratios it emits in order to communicate, via the atmosphere, to predators, pollinators and neighbouring plants. Indeed several biochemical studies have shown that monoterpene producing enzymes (i.e. monoterpene synthases) are enantiomer specific (Martin et al., 2004). Multiple enzymes producing a similar blend of terpenes but with distinct chirality can co-exist in plants and may be subject to different environmental controls (Faldt et al., 2006). This implies the presence of more than one underlying mechanism in their production and this unresolved mechanistic complexity may help to explain the current difficulties encountered in establishing a reliable empirical monoterpene emission algorithm.

In this study, we screened the enantiomeric monoterpene footprints emitted by different holm oak individuals under standard conditions (30 °C and 1000 PAR) in order to assess the extent of chemotype diversity in the species. Furthermore, we examined enantiomeric monoterpene emissions as a function of temperature and light under controlled laboratory conditions using three plant species that represent different types of monoterpene producers: *Quercus ilex*, an evergreen sclerophyllous oak, abundant in the Mediterranean basin, was chosen as a representative emitter of non-stored monoterpenes whose emission is essentially controlled by the rate of biosynthesis. Further, the conifer *Pinus halepensis* and the *Lamiaceae Rosmarinus officinalis*, two widespread monoterpene storing species, whose emissions are thought to mainly result from temperature driven diffusion processes of the stored monoterpenes out of storage organs independent of physiological processes. The enantiomeric response will be examined to find clues of different metabolic origins of monoterpenes and whether the two possible pools of monoterpenes synthesized in the plant can be differentiated (Loreto and Schnitzler, 2010) namely: (1) synthesis without storage in the chloroplasts of the green tissues; (2) synthesis in plastids of glandular organs (trichomes, resin ducts) with storage; (3) mixed type of (1) and (2). Thus, if no de-novo synthesis pools exist in the foliage of these plants, the effects of temperature on the gas vapour pressure in plant tissue and on the resistance along the emission pathway should be the dominant parameter of the emission rates. It is possible that in *Pinus halepensis* and *Rosmarinus officinalis* two pools exist as has been demonstrated for Norway spruce and Scots pine (Ghirardo et al., 2010). If two pools exist that produce exactly the same enantiomers, emissions should show no light dependence in enantiomeric ratio (i.e. there are emissions in the dark that increase with light but with the same enantiomeric pattern). If, however, two pools exist that produce different enantiomers, the enantiomeric ratio in the emission should change in response to both light and temperature. Furthermore, we report for comparison the diurnal profile of the enantiomeric monoterpenes over different ecosystems. In particular, focus is set on α-pinene whose enantiomeric ratios have been most commonly reported in field studies (Williams et al., 2007; Yassaa and Williams, 2005, 2007). The underlying question is whether enantiomeric signatures may be used to better understand the multitude of metabolic origins of monoterpenes that may exist in plants and to improve emission algorithms. For more accurate ecosystem response modelling, especially with regard to future climate changes, it will be necessary to link atmospheric chemistry models to ecological models including biological, mechanical and environmental stresses.

2 Experiment

2.1 Plant material and the dynamic leaf enclosure cuvette set up

A total of 27 potted plants were studied at the CEFE-CNRS in Montpellier (France) during the months of April to July in 2009 and May to June in 2010, including the three non-deciduous trees, and shrubs *Quercus ilex* L., *Rosmarinus officinalis* L., and *Pinus halepensis Mill*.

Plants or seeds were collected from the field (Southern France and Spain) and were potted and gown outside at the institute. The individuals were 2–4 yr old and more than three individuals of each species were studied. In March, the plants were put in a greenhouse in Montpellier at an approximate day/night temperature of 25/15 °C to initiate bud break.

A dynamic temperature and light controlled chamber system (see Fig. 2) was used to determine VOC exchanges at the leaf level. The enclosure chamber (approximately 105 mL in volume) was constantly flushed with air at 500 mL min^{-1} resulting in an air exchange of the enclosure system on average every 5 s. The in-flow air was first passed through a clean air generator (AIRMOPURE, Chromatotec, France) that purified and dried the ambient air. It was then re-humidified by passing a variable portion of the air stream through a water filled bottle. Homogenous mixing of the air in the enclosure was maintained by a Teflon fan at the bottom of the cuvette. Leaf and cuvette temperatures were monitored with two thermocouples (Chrom-Constantan, OMEGA). These two temperatures were rather close with an average difference of 0.7 °C during experiments. Cuvette and plants were illuminated with a white light source (OSRAM 1000 W) filtered by a 5 cm water bath. PAR was measured with a quantum sensor (Licor, PAR-SB 190, Lincoln, NE, USA) located next to the chamber. All sampling lines that were made of Teflon were maintained at a constant temperature of 45 °C.

Before experiments, terminal shoots consisting of 4 to 7 leaves were prepared for fixation in the cuvette by removing some leaves at the insertion point. This was done at least one week before the measurement to minimize disturbance effects. In order to ensure homogenous light repartition on the adaxial surface of the leaves, several terminal leaves of an individual plant were placed horizontal to the light.

In order to ensure adaptation of the plants to the chamber environment, all measurement shoots of *Quercus ilex* were placed in the chamber at least 45 min prior to the measurements. Since the leaves of the conifer (*Pinus halepensis*) and the aromatic shrub (*Rosmarinus officinalis*) possess glands and ducts storing VOCs, mechanical stress can cause large bursts of VOCs from these plants (Niinemets et al., 2011). To avoid masking temperature and light driven effects by the stressed emission, these two species were enclosed at least 12 h before measurements began. To ensure that the enclosed leaves show normal physiological activity, photosynthesis and transpiration were measured (data not shown) by directing a constant portion of the inlet and outlet air through a CO_2/H_2O infrared gas analyzer (LI-COR Inc. 7000, Lincoln, Nebraska, USA).

First of all, as an assessment of chemotype variance within a given species, a total of nineteen *Quercus ilex* individuals were measured for enantiomeric monoterpenes in steady state under standard conditions (30 °C and 1000 $\mu mol\,m^{-2}\,s^{-1}$ Photosynthetic Active Radiation (PAR)). To study light and temperature responses of emissions, the cuvette condition were varied in terms of light (0, 100, 200, 400, 800 and 1600 $\mu mol\,m^{-2}\,s^{-1}$ PAR) and temperature (20, 25, 30, 35, 40, 45 °C). The responses to light were determined by stepwise increases of 0 to approx. 1600 $\mu mol\,m^{-2}\,s^{-1}$ PAR at a constant temperature of 30±0.2 °C. The responses to temperature were measured by increasing the enclosure temperature in 5 °C increments from 20 to 45 °C under 1000 PAR. At the end of each experiment the studied leaves were harvested and leaf area and dry weight were determined. Projected leaf area was determined with an optical area meter (Delta-T Devices Ltd., Cambridge, UK) and leaf dry weights after drying at 60 °C for at least 48 h.

2.2 Sampling and analysis

Two types of adsorbent filled cartridges (Carbograph I/II or Tenax and carbograph, MARKES) were used for this study. The cartridges were conditioned by purging with Helium 6.0 for 2 h at 350 °C and 30 min at 380 °C prior to use. Cuvette air was drawn at around 100 $mL\,min^{-1}$ through the cartridges and the sample flow passed over the sorbent for 10 min. Directly before analysis, cartridges were pre-purged for 5 min with Helium to reduce the water content and were then thermally desorbed by flushing the heated tubes at 200 °C for 10 min. The desorbed VOCs were trapped at 10 °C on a low dead volume cold trap. The cold trap was 2 mm in diameter and filled by a 60 mm long bed of sorbent (Tenax TA and Carbograph I) supported by quartz wool. The cold trap was then heated to 200 °C rapidly and then held there for 5 min while sample was transferred to the GC column. In this work, a β-cyclodextrin column (30 m, 0.256 mm I.D., 0.25 µm film; J&W Scientific, CA, USA) has been used for the separation of enantiomeric and non-enantiomeric monoterpenes, xylene isomers, and other VOCs. The column temperature was initially held for 5 min at 40 °C and raised to 120 °C at 1.5 °C min^{-1} then to 200 °C at a rate of 30 °C min^{-1}.

The measurement systems were calibrated with either a pressurized gas standard mixture (National Physical Laboratory, UK) containing enantiomeric monoterpenes or liquid standards which were prepared from pure authentic standards (Fluka, Aldrich, Bedoukan) dissolved in methanol. In the later case, a microliter level liquid standard was injected into the cartridge and flushed with helium for 5 min. Calibrations were performed every 8–10 samples. Multipoint calibrations for all reported species for both gas and liquid standards revealed a good linear dependency of peak area to the respective compound concentration. Both datasets have been combined here. Empty cuvette air was measured each time before installing a new plant and this showed no significant levels of the compounds discussed. Table 1 shows the retention time, precision and overall uncertainty of the selected BVOCs which are the main focus of this work. The overall uncertainty was calculated based on the calibration standard (stated accuracy 5 %, National Physical Laboratory) and the precision of the chemical.

3 Results and discussion

3.1 Monoterpene emission composition for *Quercus ilex* under standard conditions – an assessment of chemotype diversity

Although BVOC emissions are strongly modulated by the biotic and abiotic environment, their compositional fingerprints have been used as chemotaxonomic markers in order to better understand the geographical distribution of certain species (Loreto et al., 2009). *Quercus ilex* emits large amounts of monoterpenes but small to non-detectable amounts of isoprene (Plaza et al., 2005; Staudt and Bertin, 1998). Previous studies have shown the compositional profile of the emissions is mainly genetically controlled (Staudt et al., 2001, 2003, 2004), but none of these have taken enantiomers into account. Figure 3 shows the percent composition of individual monoterpenes emitted from 19 *Quercus ilex* individuals that originated from two different areas (Southern Spain and Southwest France) at standard temperature (30 °C) and light (1000 PAR) conditions. These contrasting emissions strongly suggest the existence of distinct chemotypes (genetically fixed) within this tree species rather than environmental induced emission differences. The results provide a limited assessment of the geographic differences in the BVOC emission profile and an overview of potential chemotypes prevalent in the region. A total of 11 chiral and non-chiral monoterpene hydrocarbons including enantiomeric pairs of α-pinene, β-pinene, camphene and limonene have been identified in all samples. The main monoterpene compounds emitted were α-pinene, β-pinene, limonene, myrcene and sabinene. As can be seen in Fig. 3, there are generally two types of emitter within this selection of *Quercus ilex* individuals: a limonene dominated emitter (e.g. QS1) and a pinene dominated emitter (e.g. QF1). Within the six individuals originating from Spain both limonene and pinene dominated individuals were identified. Thus, if we ignore for a moment the enantiomeric speciation, no clear regiospecific monoterpene emission pattern exists within the group studied here. For those individuals from Spain identified as a limonene dominated emitter, (−)-limonene and (+)-α-pinene were the overwhelmingly dominant enantiomers. In contrast for the pinene chemotype, (−)-α-pinene and (+)-limonene (with some (−)-β-pinene) are the predominant enantiomers. For those originally from France, almost all of the pinene dominated chemotype showed a clear (+)-enantiomer preference in the case of α-pinene and (+)-β-pinene preference. Only two individuals from France were found to be limonene emitters, and these showed an enantiomeric excess of the (−)-enantiomer for all of the three chiral monoterpenes namely α-pinene, β-pinene and limonene. Table 2 lists the chemotypes of studied individuals. As can be seen clearly, four distinct chemotypes can be ascertained if the enantiomeric speciation is taken into account: (I) high limonene with high (−)-α-pinene (all were from France); (II) high limonene with high (+)-α-pinene (all were from Spain); (III) high pinene with high (−)-α-pinene (most were from Spain); (IV) high pinene with high (+)-α-pinene (all were from France).

Therefore the enantiomeric signatures of monoterpenes from *Quercus ilex* originating from Spain and France appear to be distinctly different, at least for this sample set. This echoes the regiospecific enantiomeric differences noted between Tropical and Boreal forests (Williams et al., 2007; Yassaa et al., 2012) although on a much smaller geographical scale. Interestingly, it would not be possible to distinguish the French and Spanish individuals on the basis of non-enantiomerically resolved monoterpene emissions since both limonene and pinene dominated individuals exists in both groups. It is tempting to speculate whether the origin of a wood sample or individual plant may be attributed to Spain or France on the basis of the enantiomeric emissions (using Table 2), but the number of individuals sampled must be greatly increased to test this hypothesis. However, this initial screening experiment has served to demonstrate that multiple chemotypes exist within the tree species examined in this study.

3.2 Light dependence

As described in the previous Sect. 3.1, the emission rate of monoterpenes from *Quercus ilex* is controlled by light (Bertin et al., 1997; Staudt and Seufert, 1995; Staudt and Bertin, 1998). Therefore, monoterpene emission should immediately follow synthesis, as for isoprene which has been proven by isotopic labelling experiments (Loreto et al., 1996b). On the other hand, *Pinus halepensis* and *Rosmarinus officinalis* produce and store monoterpenes in the secretory organs such as resin ducts (Bracho-Nunez et al., 2011; Kesselmeier and Staudt, 1999). Figure 4 shows the normalized emission (at 800 μmol m^{-2} s^{-1} PAR) from these three different plant species and assumed two different emission types (de-novo and stored), measured at 6 different levels of light using α-pinene as an example. For *Quercus ilex*, a clear light dependent emission profile is shown with emission rates increasing with light. The dependence is strong with emissions increasing tenfold between 100 and 1000 μmol m^{-2} s^{-1} PAR. The emissions from

Pinus halepensis were much lower in magnitude than those of *Quercus ilex* with a relatively small change with light. For *Rosmarinus officinalis*, the emission rates were likewise lower than that of *Quercus ilex*, from 0.8–1.5 $\mu g\,g^{-1}\,h^{-1}$ for (+)-α-pinene, and 0.2–0.3 $\mu g\,g^{-1}\,h^{-1}$ for (−)-α-pinene. A rapid increase of emission at lower light intensity (from 0–100 PAR) and a much slower increase or decrease at higher light intensity was observed. However, it should be noted that emissions were still significant in darkness (note the different *y* scales in Fig. 4) and the apparent slight increase of the emission rate with increasing light might be due to small unresolved leaf temperature increases during the light dependence experiments. Pinene emissions from *Rosmarinus officinalis* appear to increase from 0 to 400 PAR (approximately doubling in rate) and decrease thereafter. The pinene enantiomers appear to show a similar dependency to light and no evidence for reversing of enantiomeric dominance was observed. The enantiomeric response to light for all three plant species is discussed below.

In this section we present the enantiomeric signature using the enantiomeric enrichment (i.e. (−)-enantiomer/((+)-enantiomer + (−)-enantiomer) × 100) of (−)-α-pinene. Among the replicates as a function of light, there were clear differences in the emission of the enantiomers ((−) and (+)-α-pinene between the samples). In the case of *Quercus ilex* (Fig. 5), four replicates, including two originally from Spain (QS2 and QS5) and two from France (QF7 and QF8); two limonene emitters (QS2 and QF7) and two pinene emitters (QS5 and QF8), were analyzed for light responses. The result without light is excluded due to the low emission rate detected in the darkness. Two individuals (QS2 and QF7), which are the limonene dominated chemotype, showed a clear (+)-α-pinene dominance with only 20–30 % (−)-α-pinene in the total α-pinene emission, moreover, the ratio was generally stable with light. For one pinene chemotype (QS5), the (−)-α-pinene was close to 50 % (around 60–55 %) meaning no significant enantiomeric preference. The enantiomer emission ratio was quite stable at low light intensity, but decreased slightly with increasing light levels. In contrast, the other pinene emitter, individual QF8, exhibited the reverse enantiomeric discrimination, namely that

(–)-α-pinene predominated in the emission and the (–)-enantiomer increased weakly with light, 10 % between 100 and 400 PAR.

For *Rosmarinus officinalis* (Fig. 5), the emission was dominated by (+)-α-pinene which is consistent with the only previous study (Yassaa and Williams, 2005). Individual R1, showed the strongest (+)-α-pinene predominance, but exhibited little change with light (ca. 10 % in (–)-α-pinene emission ratio) Similarly, there was about 20 % of (–)-α-pinene in the emission of individual R4, which also had a relatively stable enantiomeric emission ratio. In contrast the replicates R2 and R3 emitted much higher (–)-α-pinene than individual R1 and R4, from 39–49 % and 41–58 % respectively. These two replicates, with the higher fraction of (–)-α-pinene showed a weak increase in (–)-α-pinene emission (5–20 %) with light.

For *Pinus halepensis* (Fig. 5), individuals P1 and P2 had initially racemic α-pinene emission ratios (50 % of (–)-α-pinene) in the darkness. While P1 showed (–)-enantiomer increasing weakly (ca. 10 %) over the light range, P1 showed the reverse trend again with a relatively weak change in emission ratio. Individual P3 emitted with strong (+)-α-pinene enantiomer predominance and again showed little change in the (–)-enantiomer emission (9–11 %) with light, and individual P4 showed an (–)-enantiomer preference (60–67 %) with again little discernible change with light.

From Figs. 4 and 5 it is clear that significant differences exist between the mean enantiomeric emissions of individuals (in terms of α-pinene). For *Rosmarinus officinalis*, in almost all cases the (+)-α-pinene was shown to be predominant. For *Quercus ilex* and for *Pinus halepensis*there are individuals with (–)-α-pinene and (+)-α-pinene dominated emissions. This indicates that *Pinus halepensis* also has different chemotypes, as was shown to be the case for *Quercus ilex* in Sect. 3.1. In all cases the variation in enantiomeric emission ratio with light when present at all was weak (0–20 %).

3.3 Temperature dependence

Measured emissions of monoterpenes responded to the temperature changes imposed (see examples for normalized α-pinene emissions (at 30 °C) in Fig. 6). Highest emission rates (5–25 $\mu g\,g^{-1}\,h^{-1}$) were found from *Quercus ilex* which increased until 40 °C, and then exhibited a sharp decrease at the highest temperature of 45° C. In comparison, both of the emission rates of *Pinus halepensis* and *Rosmarinus officinalis* were very low, but a clear exponential increase with temperature, especially for rapid increasing of (–)-α-pinene at higher temperature, was observed.

In the following section the enantiomeric enrichment of (–)-α-pinene are investigated as a function of temperature. Regarding *Quercus ilex* (Fig. 7), individuals QS2 and QF7, both limonene chemotype and strong (+)-α-pinene enantiomer emitters (less than 30 % of (–)-enantiomer in total α-pinene emission) exhibited opposite albeit weak (0–15 %) enantiomeric trends in response to temperature: the (–)-enantiomer of individual QS2 was found to be decreased (more (+)-α-pinene enriched) at the highest temperatures (40–45 °C) while the individual QF7 showed an increased (–)-α-pinene enrichment at the highest temperatures. For the pinene chemotype, the ratios of individual QS5 with weak (+)-enantiomeric preference showed no clear trend in ratio with temperature. The other pinene emitter (individual QF8) in which (–)-enantiomer predominated in the emission, was found to remain stable until 35 °C and then rapidly decreased at the highest points (40–45 °C).

Figure 7 shows the enantiomeric profile of *Rosmarinus officinalis* emission. For replicates R1 and R4, there is a modest decrease in (–)-α-pinene emission in response to temperature from 25 to 45 °C. Replicate R2 shows no clear dependence on temperature varying between 44 to 50 % of (–)-α-pinene. Replicate R3 shows a weak increase in (–)-α-pinene with temperature. Thus the strongest impact on enantiomeric emission by temperature is shown for replicates R1 and R3.

In the case of *Pinus halepensis* (Fig. 7), replicate P1 shows the (–)-enantiomer emission increasing in response to temperature between 20–30 °C by ca. 10 % but thereafter decreasing (becoming more (+)-enantiomer rich) at higher temperature, the enantiomeric enrichment of replicates P2 and P3 are strongly dominated by the (+)-enantiomer with less than 20 % of (–)-enantiomer and these show no clear dependence on temperatures, and replicate P4 shows the strongest (–)-enantiomer preference in all of the four replicates (the (–)-α-pinene accounts for 60–80 % of the total (–)-α-pinene) but decreases slightly with temperature which is consistent with its change as a function of light.

Regarding *Pinus halepensis* and *Rosmarinus officinalis*, those individuals that are more enriched with (–)-enantiomer were found to be much more sensitive to temperature. This is further evidence that different chemotypes of the same species have different enzymes producing enantiomer specific monoterpenes.

3.4 Field measurements over different ecosystems

Field measurements over different forest ecosystems (oak forest, Boreal forest and temperate forest) will be discussed for comparison with the laboratory results in the following section. For all locations we examine the mixing ratios of α-pinene in terms of absolute mixing ratio and enantiomeric enrichment. The diel cycles of α-pinene are shown in Fig. 8.

A single day field measurement was performed over a *Quercus ilex* forest in Puéchabon, Southern France (43°43′ N, 3°37′ E), June 2010. The Puechabon forest is a natural forest which was previously used to produce charcoal (coppice). The last cut was about 40 yr ago. Therefore, each tree has usually several stems that result from a several hundred-year-old rootstock. Measurements were made during the daytime from 9:00 to 18:00 LT by drawing air through a cartridge in the same manner as for the laboratory experiments. The ambient temperature was from 16 to 21 °C. The temperature and light reached its maximum value at around 14:00 LT. The sampling line was placed about 2 m above the canopy top.

The mixing ratios of α-pinene increased clearly during the day over Puéchabon forest and peaked in the afternoon (14:00–15:00 LT), see Fig. 8 (oak forest). However, for α-pinene the enantiomeric enrichment of the (−)-enantiomer had a clear decrease towards noon with the lowest value around 14:00–15:00 (Fig. 9 – oak forest). That is to say that the relative abundance of the (−)-enantiomer for α-pinene decreased throughout the morning with increasing light and temperature levels, becoming (+)-enantiomer dominated at noon. For limonene enantiomers, the (−)-limonene was always predominant by a factor of circa 10. For β-pinene, the enantiomeric ratio varies but the (+)-enantiomer predominates over the forest throughout the day.

In general, the abundance of a particular monoterpene in ambient air is dependent on (1) the emission source strength from forest tree species, (2) the atmospheric removal rates (i.e. reaction with OH radical, O_3 and NO_3), and (3) the distribution of the source trees relative to the measurement location and wind direction. Limonene reacts much faster than α-pinene and β-pinene in the free troposphere (Atkinson, 2003). From a consideration of the chemical composition of the forest air with more than 70 % of pinene (α-pinene and β-pinene) and around 30 % of limonene, it appears that the forest is a mixture of *Quercus ilex* chemotypes with slightly more pinene dominant chemotype. These findings are also in agreement with the work of Staudt et al. (2001) for the same forest. Being a French forest, we would expect from the screening experiments presented above in Sect. 3.1, that (+)-α-pinene would be the predominant enantiomer. However, the enantiomeric ratio of α-pinene in the ambient data does not vary consistently with the previously measured individual identified as French pinene chemotype (chemotype IV).

For comparison, the enantiomeric enrichment diel profile over Boreal forest and temperate forest are shown in Fig. 9 as well. Chiral monoterpenes were measured over a Scot pine/Spruce forest in the Hyytiälä meteorological station in Southern Finland during the summertime of 2010 (Williams et al., 2011; Yassaa et al., 2012). During the four-week observation above the canopy, in the case of α-pinene, the (+)-enantiomer was found to be always predominant, while the (−)-enantiomer showed a distinct increase through the day peaking during the noontime (Fig. 9 – Boreal forest). From parallel cuvette measurements made on Spruce trees it can be seen that the Spruce trees at the Hyytiälä station emit strongly (−)-enantiomer enriched mixture in response to light (Yassaa et al., 2012), possibly explaining the observed variation.

The measurements in the temperate forest located at the mountain Kleiner Feldberg/Taunus, Germany was made during the summer of 2011 (July–August). The enantiomeric profile showed a quite similar trend to that over the Boreal forest (Fig. 9). This phenomenon is probably again due to the presence of Spruce trees around the site.

The initial screening shown in Fig. 3 was performed at 30 °C and 1000 PAR which corresponds approximately then ambient noon time conditions. In the ambient data, see Fig. 8, it can be seen that throughout the morning, with increasing temperature and light, the α-pinene enantiomeric ratio trends from (−)-α-pinene enrichment to racemic i.e. became more enriched in the (+)-α-pinene enantiomer. This behavior is only exhibited by the Spanish pinene chemotype (see Fig. 5). Therefore, we have to conclude that this forest is very likely a mixture of the Spanish pinene chemotype or at least we have one such individual with strong emission rates close to the measurement tower. It appears initially disappointing that the ambient measurements do not correspond more closely to the emissions of the previously measured French chemotypes. Based on the laboratory work shown here, diel variations in enantiomeric ratios could be quite different at different locations in the forest if the French chemotypes predominate elsewhere. However, it must be born in mind that the natural environment outside the laboratory contains many more stimuli than simply light and temperature and additional sources. The effect of damage by insects may have been an additional driving force on the enantiomeric emissions and significant emissions may have occurred from vegetation (grasses and shrubs) and soil in the understory.

4 Summary and conclusions

During this laboratory and field based study, conducted from 2009 to 2011, BVOCs comprising mainly of monoterpenes emissions from a dynamic leaf enclosure cuvette and in the field were measured applying GC-MS. During the two laboratory experimental sessions, two kinds of cartridges were used for the study, one with the sorbent material of Carbograph I and II and the other with Tenax and Carbograph. Both gas mixture standards and liquid ones were used as working standards. Multipoint calibrations for all reported species for both types of standard revealed a good linear dependency ($r^2 > 0.9$) of peak area to the respective compound concentration.

Quercus ilex, which is the one of the most widespread tree species in the Mediterranean basin, emits large amounts of monoterpenes. The screening experiments of *Quercus ilex* emissions under standard conditions together with the results from the light and temperature responses showed that the compositional profile of its emissions is mainly genetically controlled. Furthermore this limited sample set has provided an interesting hypothesis for regional identification according to enantiomeric chemotype.

Monoterpene emission rates from *Quercus ilex* were found to be controlled by light and temperature, while *Rosmarinus officinalis* and *Pinus halepensis* emited monoterpene depending mostly on temperature. However, the enantiomeric enrichment of the main monoterpenes, namely (−)/-α-pinene % is not strongly affected light and temperature. The biggest differentiation of the enantiomeric variations were found among individuals from the same species, for instance one *Quercus ilex* showed a clear (−)-α-pinene predominant, while another one was found to have an enantiomeric excess of (+)-α-pinene. This suggests that the enantiomeric composition is inherent for a given individual and that several enantiomeric chemotypes exist within a given plant species. These enantiomeric variations have little impact on the overall emission of the monoterpenes which respond to temperature and light according to the existing algorithms reasonably well.

There is no significant evidence for an enantiomeric trend as a function of temperature or light in the leaf emission that can be related to a leaf scale process. Yet interestingly, field data has repeatedly shown smoothly varying enantiomeric ratios throughout the diel cycle, even for campaign in which incident wind directions (and hence fetch) has varied significantly. Based on the laboratory experiments it seems that at different points in the forest the diel cycle in the enantiomeric ratios should be different, dependent on the local distribution of chemotypes. Yet, despite multiple changes in wind speed and direction experiences in these longer term Boreal/temperate campaigns the enantiomeric ratio cycle remains present and consistent. Several clear enantiomeric ratio diel cycles have been reported previously over different ecosystems, including a Mediterranean stone pine forest (Song et al., 2011), an oak forest (this study) and a Boreal forest (Yassaa et al., 2012). While the forests in Spain and Finland both show an enrichment of (−)-α-pinene at noon time (Song et al., 2011; Yassaa et al., 2012), in France the opposite was observed.

In the future, temperature is predicted to increase. From the research presented above it can be seen that the overall enantiomeric signature of the existing forest will change only weakly as a consequence. Only if evolutionary pressure is exerted on a particular chemotype is the overall signal likely to change. Such enantiomeric effects may seem subtle, however, for insects and birds that use olfactory signals for their everyday life such changes may have important consequences.

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Table 1. Overview of the measured compounds.

Compounds	Retention Time (min)	Precision (%)	Uncertainty (%)
Isoprene	1.724	10	11.2
(−)-a-Pinene	21.146	9.5	10.7
(+)-a-Pinene	21.973	12.6	13.5
Myrcene	22.607	13.5	14.4
Δ3−Carene	25.404	17.7	18.4
(+)-b-Pinene	25.851	16.6	17.3
(−)-β-Pinene	26.237	18	18.7
Ocimene	27.05	19.3	19.9
(−)-Limonene	27.472	18.6	19.2
p-Cymene	27.477	18.1	18.7
(+)-Limonene	27.81	18.6	19.3
Eucalyptol	32.874	14.1	15
(−)/(+)-Camphor	49.39	33.5	33.9

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Table 2. Chemotypes of the screened individuals.

Chemotype	Emission signature	number of individuals	Origin
I	Limonene_(−)-α-pinene	2	France
II	Limonene_(+)-α-pinene	3	Spain
III	Pinene_(−)-α-pinene	4	Spain(3), France (1)*
IV	Pinene_(+)-α-pinene	10	France

* This chemotype group contains three species from Spain and one from France.

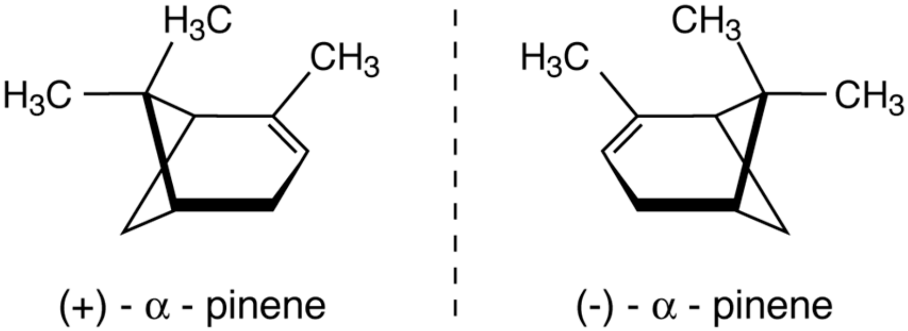

Fig. 1. Mirror image of α-pinene enantiomers.

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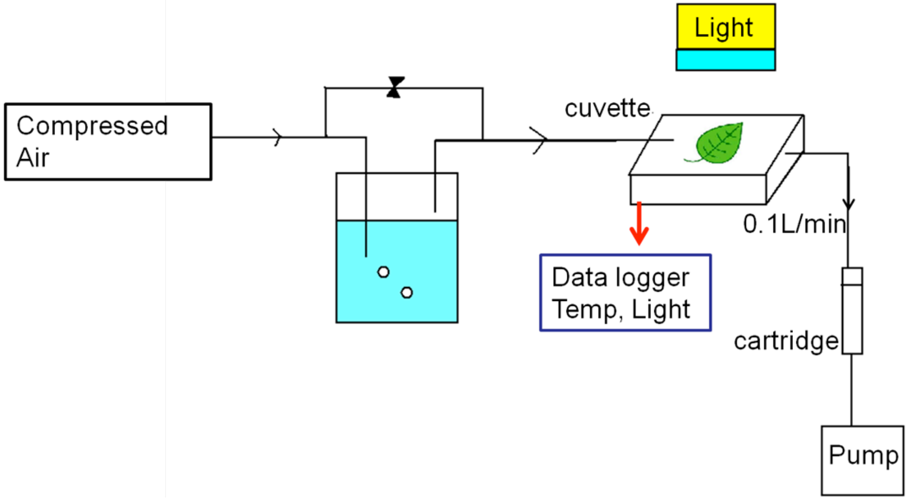

Fig. 2. A schematic of the dynamic leaves enclosure cuvette set up.

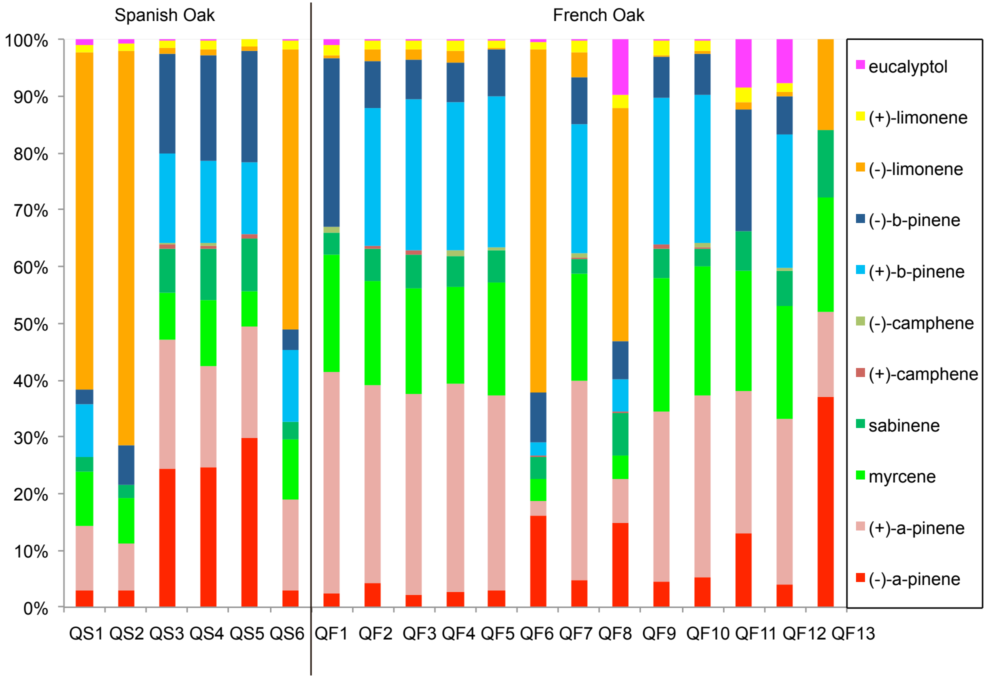

Fig. 3. Screening of *Quercus ilex* L. originally from different areas (Southern Spain and France) at standard conditions.

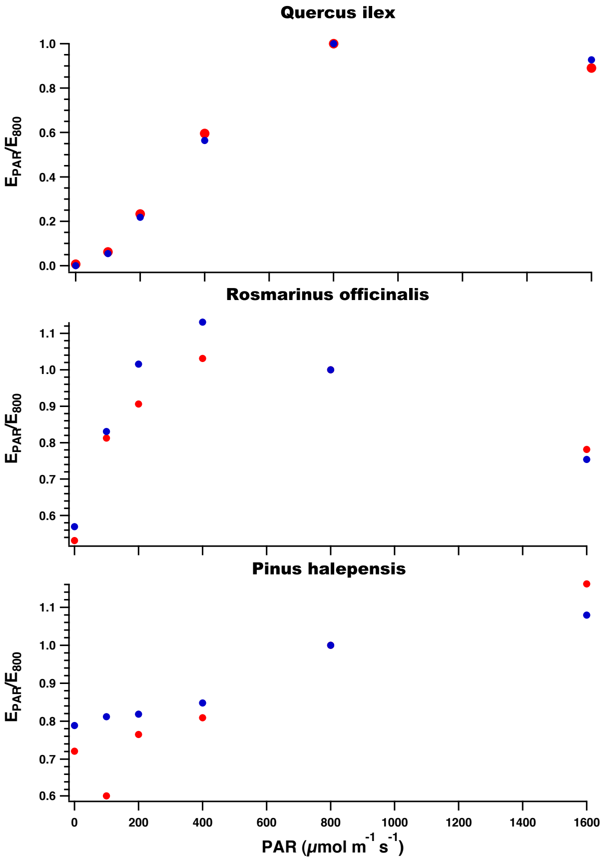

Fig. 4. An example of normalized emissions of (–)/(+)-*α*-pinene of the three selected plant species in dependency with light (at 30 °C). The red points represent normalized (–)-*α*-pinene emissions and the blue points show the normalized (+)-*α*-pinene emissions.

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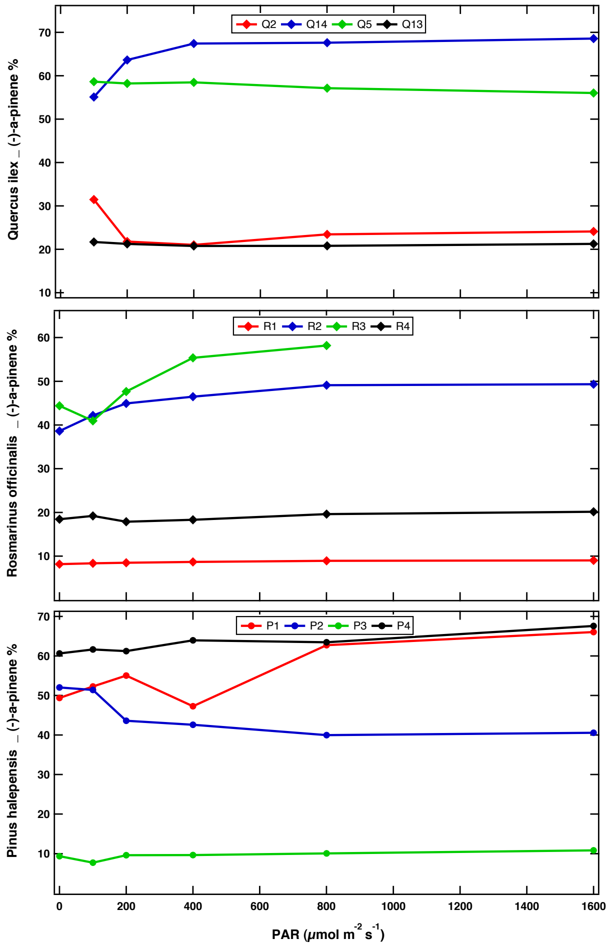

Fig. 5. Enantiomeric enrichment of (−)-α-pinene of *Quercus.ilex*, *Rosmarinus officinalis* and *Pinus halepensis* as a fuction of light.

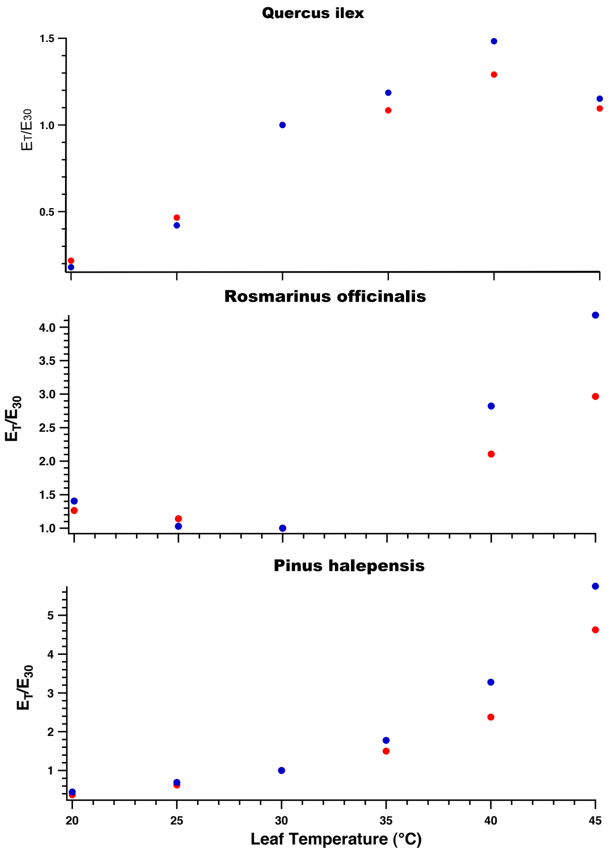

Fig. 6. An example of normalized emissions of (−)/(+)-α-pinene of different plant species in dependency with temperature. The red points represent normalized (−)-α-pinene emissions and the blue points show the normalized (+)-α-pinene emissions.

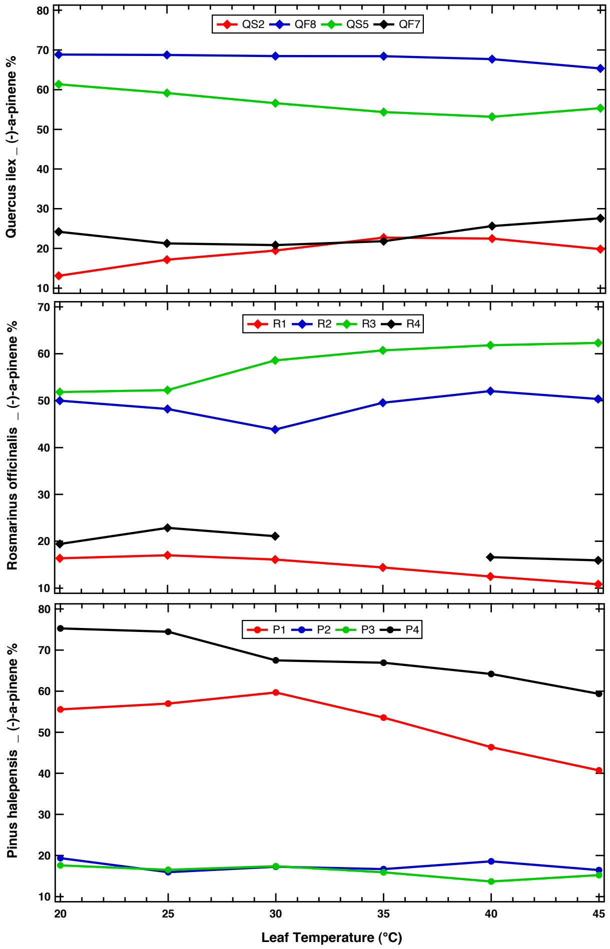

Fig. 7. Enantiomeric enrichment of (−)-α-pinene in the emission of *Quercus.ilex*, *Rosmarinus officinalis* and *Pinus halepensis* as a function of temperature.

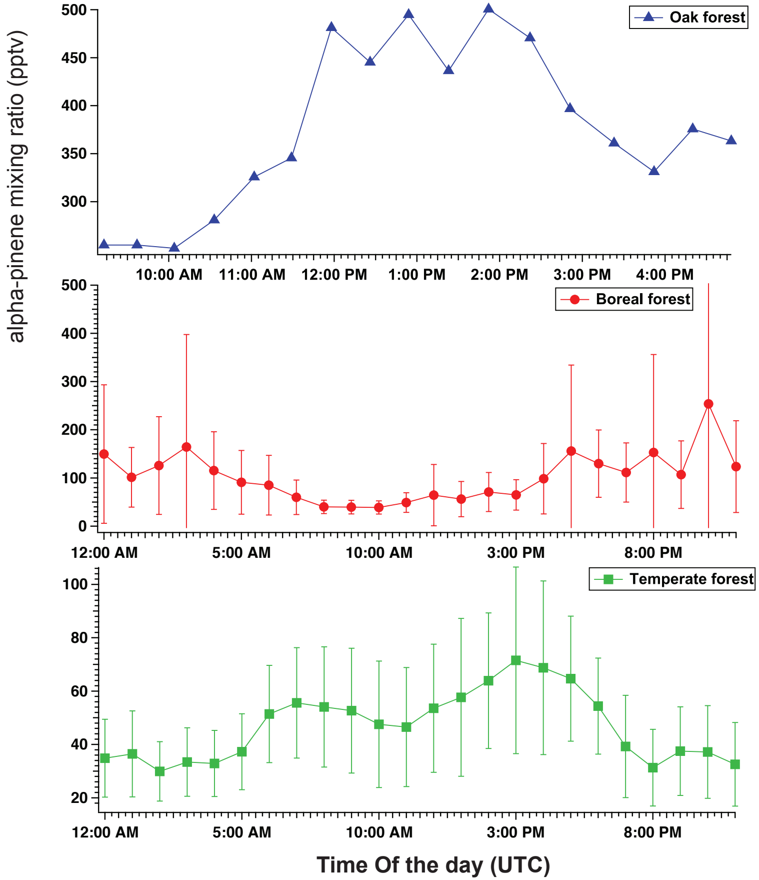

Fig. 8. Diel cycle of α-pinene mixing ratios during the measurements time. For Boreal forest and temperate forest measurements, the marks represent the mean value of every hour, the error bars were the standard deviation (Boreal forest: $n > 13$; temperate forest: $n > 12$); for oak forest measurements, the solid triangle represents the mixing ratio observed.

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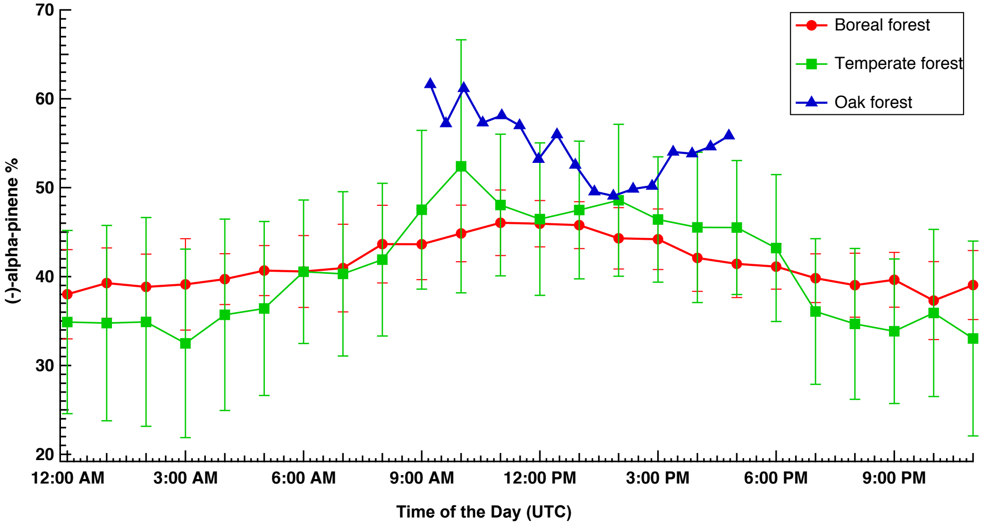

Fig. 9. Enantiomeric enrichment of (−)-α-pinene over the *Quercus ilex* forest, Boreal forest and temperate forest.