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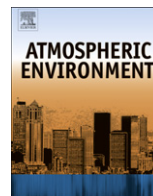Guenther, Alex
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Seasonal variations in terpene emission factors of dominant species in four ecosystems in NE Spain

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HIGHLIGHTS

► Terpene emission factors were about 15 times higher in summer than in early spring.
► The maximum emission factors were recorded around midday.
► Minimum emission rates were recorded during the night.

ARTICLE INFO

ABSTRACT

We studied the daily patterns in the rates of foliar terpene emissions by four typical species from the Mediterranean region in two days of early spring and two days of summer in 4 localities of increasing biomass cover in Northern Spain. The species studied were *Thymelaea tinctoria* (in Monegros), *Quercus coccifera* (in Garraf), *Quercus ilex* (in Prades) and *Fagus sylvatica* (in Montseny). Of the total 43 VOCs detected, 23 were monoterpenes, 5 sesquiterpenes and 15 were not terpenes. Sesquiterpenes were the main terpenes emitted from *T. tinctoria*. Total VOC emission rates were on average about 15 times higher in summer than in early spring. The maximum rates of emission were recorded around midday. Emissions nearly stopped in the dark. No significant differences were found for nocturnal total terpene emission rates between places and seasons. The seasonal variations in the rate of terpene emissions and in their chemical composition can be explained mainly by dramatic changes in emission factors (emission capacity) associated in some cases, such as for beech trees, with very different foliar ontogenical characteristics between spring and summer. The results show that temperature and light-standardised emission rates were on average about 15 times higher in summer than in early spring, which, corroborating other works, calls to attention when applying the same emission factor in modelling throughout the different seasons of the year.

1. Introduction

Many studies have shown the importance of environmental conditions, both abiotic and biotic, for the production and emission rates of VOCs (Langenheim, 1994; Takabayashi et al., 1994; Peñuelas and Llusia, 2001; Paris et al., 2010). Briefly, the main abiotic factors are irradiance and temperature (Sharkey and Loreto, 1993; Staudt and Bertin, 1998; Llusia and Peñuelas, 1999; Filella et al., 2007; Porcar-Castell et al., 2009; Peñuelas and Staudt, 2010) and the

availability of water (Ebel et al., 1995; Bertin and Staudt, 1996; Llusia et al., 2008). These abiotic factors have strong effects, especially under Mediterranean conditions that are characterized by long dry summers, coinciding with high solar irradiation and high temperatures (Di Castri, 1973; Llusia et al., 2008). In addition, seasonality (Guenther, 1997; Llusia and Peñuelas, 1998; Peñuelas and Llusia, 1999a; Llusia and Peñuelas, 2000; Niinemets et al., 2010a,b) and the daily cycle also determine the production and emission of terpenes (Niinemets et al., 2010a,b; Llusia et al., 2012).

This study is part of a campaign to understand the interactions between forests and the atmosphere within the project MONTES. The campaigns of MONTES aimed to measure the emissions of VOCs in four different terrestrial ecosystems with increasing

biomass (Peñuelas et al., 2011). The results of the measurements at the leaf level for the dominant species of each of the studied sites are here presented with the aim of providing emission factors useful for upscaling to landscape emission rates and discerning the potential need of using different emission factors for the different seasons of the year. The study also aimed to provide knowledge on seasonal and daily emission patterns including the assessment of the role of immediate temperature and PFD response relative to changes in emission factor due to phenology and ontogeny. We also conducted parallel measurements of photosynthesis and stomatal conductance. Since there was not a dominant species at the Monegros semidesertic site and for the Garraf shrubland, we measured one of the most characteristic and abundant species of the site, *Thymelaea tinctoria* in Monegros, and *Quercus coccifera* in Garraf. For Prades, a holm oak forest, the dominant species, *Quercus ilex*, was analysed and for Montseny beech forest, the dominant species, *Fagus sylvatica*, was analysed.

2. Material and methods

2.1. Study sites

2.1.1. Monegros

The region of Los Monegros is a mostly flat land ranging from the peak of the Sierra de Alcubierre (San Caprasio, 812 m) to the lowest altitude in Villanueva de Sigena (190 m). Most of the area is about 200–400 m. This great plain has a continental and arid climate that creates an extreme environment for organisms. The sampling location was near to the Santuario de Nuestra Señora de Magallón (Leciñena) at 41°48′03.22″N 0°35′17.58″W, and 531 m asl. The dominant vegetation there is composed by *T. tinctoria*, *Genista* sp., *Rosmarinus officinalis*, *Thymus vulgaris*, *Helichrysum* sp. and *Pinus halepensis*. *T. tinctoria* usually develops in degraded pine underwood, combining with *R. officinalis*, *Arctostaphylos uva-ursi*, and *T. vulgaris*.

2.1.2. Garraf

Garraf is a dry shrubland in Rosmarino-Ericion Natural Park, Barcelona, north-east Spain sampled here at 41°18′26.27″N 1°50′19.62″W at 335 m a.s.l. The climate of this site is typically Mediterranean. The site suffered big fires in the summers of 1982 and 1994. The soil is a petrocalcic calcixerept (Soil Survey Staff, 1998), thin (12–37 cm), with a loamy texture and abundant calcareous nodules. Currently the regenerating vegetation covers 50–60% with a maximum height of 70 cm. The dominant species at the study site, *Q. coccifera* (a dominant shrub species in Garraf (Folch, 1981)), *Erica multiflora* L., *Globularia alypum* L., *P. halepensis* L., *R. officinalis* L. and the less abundant *Pistacia lentiscus* L., are evergreen that typically occur on basic soils of the western Mediterranean Basin, where they are common components of the coastal shrubland.

2.1.3. Prades

The sampling location in Prades Mountains, also known as Muntanyes de Prades, was near to L'Espluga de Francolí, near to Casa del Paratge Natural, 41°21′38.84″N 1°05′26.27″W 872 asl. The Prades forest has typical Mediterranean vegetation. The forest is a holm oak forest (about 10 m tall), dominated by *Q. ilex* (the dominant plant species in Prades (Bolòs, 1983; Bolòs and Vigo, 1990)), with an important presence of other tree and shrub evergreen species (*Phillyrea latifolia*, *Arbutus unedo*, *Pinus sylvestris*, *Erica arborea*, *Juniperus oxycedrus*…) and other deciduous species such as *Sorbus torminalis* and *Acer monspessulanum*.

2.1.4. Montseny

The site of study was located within a densely forested natural park, Montseny, located about 60 km NNE of Barcelona (Catalonia, in the NE part of the Iberian Peninsula) and 25 km from the Mediterranean coast (Santa Fe del Montseny 41°46′26.37″N, 02°27′43.38″E, 1137 m above sea level). The site is highly representative of the montane forests in the Mediterranean regions of France, Italy, Greece and eastern Spain (Terradas, 1999). The site is covered by deciduous beech forest (about 20–25 m tall trees) *F. sylvatica* (Peñuelas and Boada, 2003; Bolòs, 1983; Bolòs and Vigo, 1990) with important presence of *Ilex aquifolium* and with a few patches of forest dominated by *Abies alba*.

2.2. Measurements of rates of gas exchange and sampling for leaf terpene emissions

Measurements were performed in spring 2010 (April 8 and 9 in Garraf, 14 and 15 in Monegros, 20, 21 and 22 in Prades, 27 and 28 in Montseny) and in summer 2010 (July 8 and 9 in Garraf, 14 and 15 in Monegros, 20 and 21 in Prades, 27 and 28 in Montseny). One branchlet in each plant with fully expanded, sunlit leaves of *Q. coccifera* in Garraf, *T. tinctoria* in Monegros, *Q. ilex* in Prades, and *F. sylvatica* in Montseny was used. Three or four plants were sampled five times daily for each sampling date (approximately three different of the most characteristic and abundant species of the site were sampled every 3 h (approximately one plant per hour) for 4 times daily). Measurements were conducted between 10 h and 22 h local time (7 am–18 pm solar time). Measurements of net photosynthetic rates, stomatal conductance and terpene levels were conducted using a gas-exchange system (CI-340 Hand-Held Photosynthesis System, CID, Inc., Camas, WA 98607, USA). Three to five leaves were enclosed in a 35-cm² clip-on gas-exchange cuvette. Air from the cuvette was pumped through a glass cartridge (8 cm long and 0.3 cm internal diameter) manually filled with terpene adsorbents Carbopack B, Carboxen 1003, and Carbopack Y (Supelco, Bellefonte, Pennsylvania) separated by plugs of quartz wool. Samples were taken using a Qmax air sampling pump (Supelco, Bellefonte, Pennsylvania). The hydrophobic properties of activated carbon minimized sample displacement by water. In these tubes, terpenes did not undergo chemical transformations as checked against trapped standards (α-pinene, β-pinene, camphene, myrcene, *p*-cymene, limonene, sabinene, camphor, α-humulene and dodecane). Prior to use for terpene sampling, these tubes were conditioned for 15 min at 350 °C with a stream of purified helium. The sampling time was 5–10 min, and the flow was around 450 mL min⁻¹ depending on the glass tube adsorbent and quartz wool packing. The trapping and desorption efficiency of liquid and volatilized standards such as α-pinene, β-pinene or limonene was 99%. Blank air sampling on tubes was conducted for 10 min immediately before each measurement without the plants in the cuvette. The glass tubes were stored in a portable fridge at 4 °C and taken to the laboratory. There, glass tubes were stored at −28 °C until the analysis. For calculations of the terpene emission rates, terpene concentrations measured in the blank samples without the plants were subtracted from the samples measured with the plants.

2.3. Terpene analyses

Terpene analyses were performed by a GC–MS system (Hewlett Packard HP59822B, Palo Alto, CA, USA). The monoterpenes trapped in the tubes were processed with an automatic sample processor (Combi PAL, FOCUS-ATAS GL International BV 5500 AA Veldhoven, The Netherlands) and desorbed using an OPTIC3 injector (ATAS GL International BV 5500 AA Veldhoven, The Netherlands) into a 30 m × 0.25 mm × 0.25 μm film thickness capillary column (HP-5, Crosslinked 5% pH Me Silicone; Supelco Inc.). The injector temperature (60 °C) was increased at 16 °C s⁻¹ to 300 °C. The injected
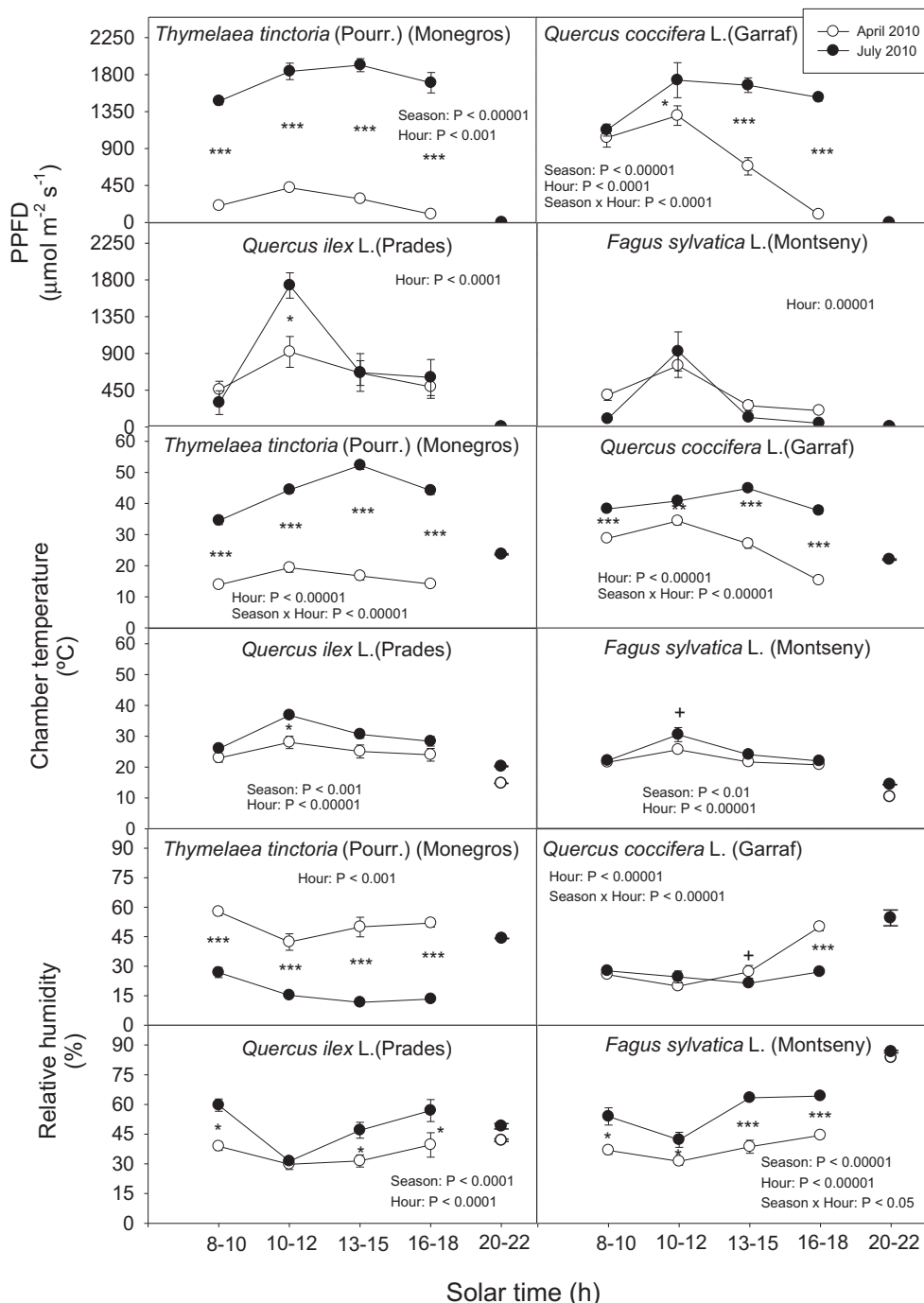
Fig. 1. Daily time averages (±S.E.) series of PPFD, cuvette air temperature and relative humidity during the sampling dates (April (○) and July (●)). Asterisks indicate significant differences between seasons (ANOVAs, +, $P < 0.01$; *, $P < 0.001$; **, $P < 0.0001$; ***, $P < 0.00001$).

sample was cryofocused at −20 °C for 2 min after which the cryotrap was heated rapidly to 250 °C. Helium flow was 0.7 ml min⁻¹. Total run time was 23 min, and the solvent delay was 4 min. The initial oven temperature (40 °C) was increased at 30 °C min⁻¹ to 60 °C and then at 10 °C min⁻¹ to 150 °C. This temperature was maintained for 3 min and then increased at 70 °C min⁻¹ to 250 °C and maintained for another 5 min.

The identification of monoterpenes was conducted by comparing the retention times with standards from Fluka (Buchs, Switzerland), and the fractionation mass spectra with standards, literature spectra, and GCD Chemstation G1074A HP and the mass

spectra library wiley7n. Terpene concentrations were determined from calibration curves. The calibration curves for common monoterpenes including α-pinene, Δ³-carene, β-pinene, β-myrcene, *p*-cymene, limonene and sabinene, and common sesquiterpenes such as α-humulene were determined once every five analyses using four different terpene concentrations (always $r^2 > 0.99$ for the relationships between the signal and terpene concentration). The other monoterpenes and sesquiterpenes were calibrated using these calibration curves of the most common mono and sesquiterpenes. The most abundant terpenes had very similar sensitivity with differences less than 5% among the

calibration factors. The quantification of the peaks was conducted using the fractionation product with mass 93.

2.4. Terpene emissions standardization

As the standardization of emission, we have considered Guenther algorithm (Guenther et al., 1993, 1995) to species that do not contain terpenes, these emitting only:

$$E = E_s C_L C_T$$

where C_L and C_T are functions of PFD and temperature, respectively:

$$C_L = \frac{\alpha\, C_{L1}\, \text{PFD}}{\sqrt{1 + \alpha^2 \text{PFD}^2}}$$

$$C_T = \frac{\exp \dfrac{C_{TI}(T - T_s)}{RT_s T}}{1 + \exp \dfrac{C_{T2}(T - T_M)}{RT_s T}}$$

where E is the emission rate in micrograms of monoterpenes per gram dry matter and hour at temperature T (in degrees kelvin, K), E_s the emission factor microprams per gram dry matter and hour at standard temperature T_s (303 K) with $R = 8.314\,\text{J K}^{-1}\,\text{mol}^{-1}$ and the empirically determined coefficients $\alpha = 0.0027$, $C_{L1} = 1.066$, $C_{T1} = 95000\,\text{J mol}^{-1}$, $C_{T2} = 230\,000\,\text{J mol}^{-1}$ and $T_M = 314\,\text{K}$.

2.5. Statistical analyses

To analyse the differences between seasons and among daily times in the variables studied, we performed ANOVAs using STA-TISTICA v.6.0 for Windows (StatSoft, Inc. Tulsa, Oklahoma). Statistical differences were also analysed with post-hoc Fisher's LSD tests. Differences were considered significant at a probability level of $P < 0.05$.

3. Results

3.1. Photosynthetic rates and stomatal conductances

PPFD (Photosynthetic Photon Flux Density), air temperature and relative humidity were significantly different between the different sites, seasons and hours (Fig. 1) ($P < 0.001$, except for seasons in RH that was not significantly different).

Photosynthetic rates (A) and stomatal conductances (g) were significantly different between the different species-sites and time of the day (Fig. 2) ($P < 0.00001$). A ranged between 1.23 ± 0.09 (average $N = 4$, $\pm$S.E.) $\mu\text{mol m}^{-2}\,\text{s}^{-1}$ in spring in *F. sylvatica* and 7.39 ± 0.6 (average $N = 4$, $\pm$S.E.) $\mu\text{mol m}^{-2}\,\text{s}^{-1}$ in spring in *Q. coccifera*. In *T. tinctoria*, the average A in spring was 3.05 ± 0.19 $\mu\text{mol m}^{-2}\,\text{s}^{-1}$ and in summer 2.14 ± 0.17 $\mu\text{mol m}^{-2}\,\text{s}^{-1}$ ($P < 0.001$, $n = 21$ and 20 respectively). In *Q. coccifera*, the average A in spring was 5.32 ± 0.33 $\mu\text{mol m}^{-2}\,\text{s}^{-1}$ and in summer 3.96 ± 0.34 $\mu\text{mol m}^{-2}\,\text{s}^{-1}$ ($P < 0.01$, $n = 25$ and 20 respectively). In *Q. ilex*, the average A in spring was 3.93 ± 0.24 $\mu\text{mol m}^{-2}\,\text{s}^{-1}$ and in summer 3.81 ± 0.32 $\mu\text{mol m}^{-2}\,\text{s}^{-1}$ (no significant differences). In *F. sylvatica*, the average A in spring was 1.19 ± 0.22 $\mu\text{mol m}^{-2}\,\text{s}^{-1}$ and in summer 3.76 ± 0.24 $\mu\text{mol m}^{-2}\,\text{s}^{-1}$ (Fig. 2) ($P < 0.00001$, $n = 22$ and 19 respectively).

In *T. tinctoria*, the average stomatal conductance (g) was 116.39 ± 8.36 $\text{mmol m}^{-2}\,\text{s}^{-1}$ in spring and 59.67 ± 7.9 $\text{mmol m}^{-2}\,\text{s}^{-1}$ in summer ($P < 0.00001$, $n = 21$ and 20 respectively) (Fig. 5). In *Q. coccifera*, the average g in spring was 213.4 ± 10.85 $\text{mmol m}^{-2}\,\text{s}^{-1}$ and in summer 89.15 ± 11.33 $\text{mmol m}^{-2}\,\text{s}^{-1}$ ($P < 0.00001$, $n = 25$

and 20 respectively) (Fig. 2). In *Q. ilex*, the average g in spring was 143.15 ± 7.65 $\text{mmol m}^{-2}\,\text{s}^{-1}$ and in summer 96.50 ± 10.23 $\text{mmol m}^{-2}\,\text{s}^{-1}$ ($P < 0.001$, $n = 27$ and 15 respectively) (Fig. 5). In *F. sylvatica*, the average g in spring was 71.59 ± 7.85 $\text{mmol m}^{-2}\,\text{s}^{-1}$ and in summer 179.27 ± 8.6 $\text{mmol m}^{-2}\,\text{s}^{-1}$ ($P < 0.00001$, $n = 22$ and 19 respectively) (Fig. 5).

In Monegros and Garraf photosynthetic rates and stomatal conductances of *T. tinctoria* and *Q. coccifera* were thus higher in spring than in summer. In Prades those of *Q. ilex* were similar while in Montseny the contrary behaviour was observed where *F. sylvatica* were much higher in summer with well developed leaves than for the very young developing spring leaves.

3.2. Terpene emissions

No significant differences were found for nocturnal total terpene emission rates between site-species and seasons (Table 1 and Fig. 3). Table 1 shows the emission rates of all individual monoterpenes and sesquiterpenes. Only in *T. tinctoria* and only in summer the sesqui-terpene emission rates were higher than the monoterpene emission rates (5.34 ± 0.84 vs 2.77 ± 0.23 $\mu\text{g g}^{-1}\,\text{dw h}^{-1}$).

Total terpene emission rates were always much larger in summer than in spring in all species and sites and were, on average, about 15 times higher in summer than in spring ($n = 184$, $P < 0.0001$) (Fig. 3). Terpene emission ranged between 0.034 ± 0.006 $\mu\text{g g}^{-1}\,\text{h}^{-1}$ in spring in *Q. coccifera* ($n = 25$) and 106.8 ± 12.74 $\mu\text{g g}^{-1}\,\text{h}^{-1}$ in summer in *Q. ilex* ($n = 27$) (Table S1 and Fig. 3), depending on the time of day and season (Table 1 and Fig. 3). Then, *T. tinctoria* emitted 12.47 ± 4.15 $\mu\text{g g}^{-1}\,\text{h}^{-1}$ at 15 h ($n = 5$), *Q. coccifera* emitted 60.92 ± 17.74 $\mu\text{g g}^{-1}\,\text{h}^{-1}$ at 15 h ($n = 6$), *Q. ilex* emitted 106.80 ± 12.73 $\mu\text{g g}^{-1}\,\text{h}^{-1}$ at 12 h ($n = 6$) and *F. sylvatica* emitted 40.14 ± 4.22 $\mu\text{g g}^{-1}\,\text{h}^{-1}$ at 12 h ($n = 6$) in Summer (Table 1 and Fig. 3). The average of terpene emissions was 1.88 ± 1.44 $\mu\text{g g}^{-1}\,\text{h}^{-1}$ in spring and 27.55 ± 1.63 $\mu\text{g g}^{-1}\,\text{h}^{-1}$ in summer ($P < 0.00001$, $n = 95$ and 74 respectively) (Fig. 3). In *T. tinctoria*, the average of terpene emissions was 0.18 ± 0.95 $\mu\text{g g}^{-1}\,\text{h}^{-1}$ in spring and 8.12 ± 0.90 $\mu\text{g g}^{-1}\,\text{h}^{-1}$ in summer ($P < 0.00001$, $n = 21$ and 20 respectively) (Fig. 3). In *Q. coccifera*, the average of terpene emissions was 0.24 ± 4.72 $\mu\text{g g}^{-1}\,\text{h}^{-1}$ and in spring 38.32 ± 4.93 $\mu\text{g g}^{-1}\,\text{h}^{-1}$ in summer ($P < 0.00001$, $n = 25$ and 20 respectively) (Fig. 3). In *Q. ilex*, the average of terpene emissions was 6.6 ± 2.36 in spring and 42.29 ± 3.15 $\mu\text{g g}^{-1}\,\text{h}^{-1}$ in summer ($P < 0.00001$, $n = 27$ and 15 respectively) (Fig. 3). In *F. sylvatica*, the average of terpene emissions was 0.49 ± 1.78 in spring and 21.48 ± 1.95 $\mu\text{g g}^{-1}\,\text{h}^{-1}$ in summer ($P < 0.00001$, $n = 22$ and 19 respectively) (Fig. 3).

Emissions strongly decreased in the dark both (average for *Q. ilex* and *F. sylvatica* 0.31 ± 7.55 $\mu\text{g g}^{-1}\,\text{dw h}^{-1}$, $n = 4$) in spring and (average for all species 1.73 ± 4.0 $\mu\text{g g}^{-1}\,\text{dw h}^{-1}$, $n = 6$) in summer (Fig. 3). The maximum rates of emission were recorded at 15 h (average for all species 2.87 ± 2.64 $\mu\text{g g}^{-1}\,\text{h}^{-1}$, $n = 27$) in spring and at 12 h (average for all species 46.97 ± 2.82 $\mu\text{g g}^{-1}\,\text{dw h}^{-1}$, $n = 23$) in summer (Fig. 3, $P < 0.00001$).

Light and air temperature were well correlated with mono-terpene ($r = 0.67$ and $r = 0.73$, $P < 0.05$) and total terpene ($r = 0.66$ and $r = 0.73$, $P < 0.05$) emission rates only in summer ($n = 85$). Considering all data and species ($n = 184$), monoterpene emission rates (Table 1 and Fig. 4), although weakly, were correlated with photosynthetic rates ($r = 0.17$, $P < 0.05$). In spring ($n = 99$) monoterpene and total terpene emissions were correlated with leaf temperature ($r = 0.34$ and $r = 0.34$ respectively with $P < 0.05$), whereas in summer they were correlated with photosynthetic rates ($r = 0.39$ and $r = 0.36$ respectively with $P < 0.05$).

In *T. tinctoria*, in spring only sesquiterpene emission rates were correlated with PPFD and air temperature ($r = 0.46$ and $r = 0.54$, $n = 21$, $P < 0.05$). In summer its monoterpene emissions were
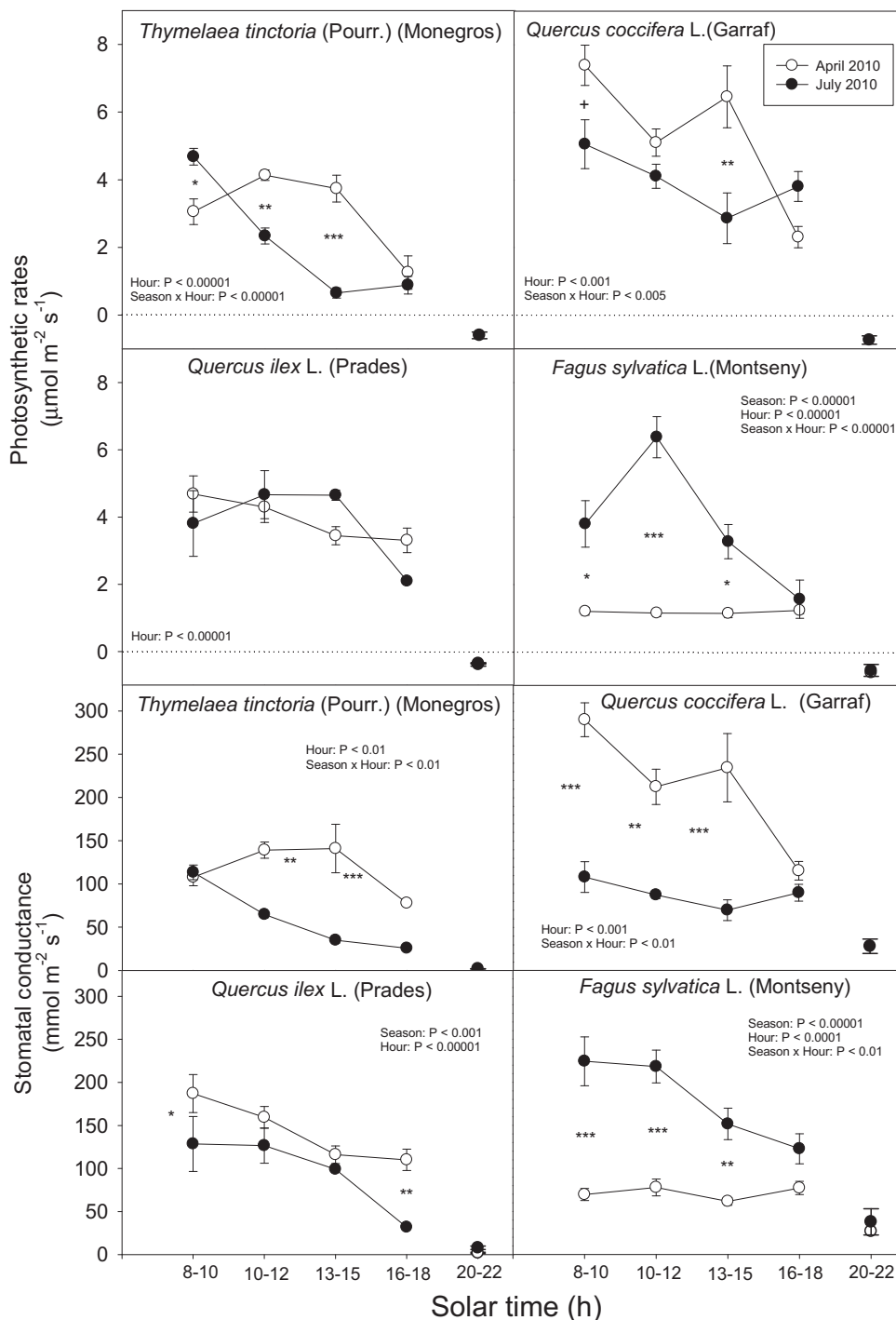
Fig. 2. Daily time averages (±S.E.) series of photosynthetic rates and stomatal conductance during the sampling dates (April (○) and July (●)). Asterisks indicate significant differences between seasons (ANOVAs, +, $P < 0.01$; ANOVAs, *, $P < 0.001$; **, $P < 0.0001$; ***, $P < 0.00001$).

Table 1
Daily averages (±S.E.) of standardized (at 30° C and 1000 µmol m^{-2} s^{-1}) total terpene emission rates during the sampling dates.

	Spring	Summer
Thymelaea tinctoria	2.50 ± 0.57	9.59 ± 3.31
Quercus coccifera	0.44 ± 0.11	25.10 ± 4.34
Quercus ilex	15.57 ± 4.51	51.50 ± 4.78
Fagus sylvatica	2.24 ± 0.43	115.18 ± 25.40

correlated with photosynthetic rates and stomatal conductances ($r = 0.51$ and $r = 0.62$, $n = 20$, $P < 0.05$) and anti-correlated correlated with leaf temperature ($r = -0.54$, $P < 0.05$). *Q. coccifera*, presented significant correlations of its monoterpene and total terpene emission rates with PPFD only in summer ($r = 0.51$, $n = 20$, $P < 0.05$). In *Q. ilex*, the monoterpene and total terpene emission rates were correlated with leaf temperature (0.54 and 0.54, $n = 27$, $P < 0.05$) in spring. In summer monoterpenes and total terpenes were correlated with photosynthetic rates ($r = 0.61$, $n = 15$, $P < 0.05$), stomatal conductance ($r = 0.58$, $P < 0.05$), PPFD ($r = 0.89$,
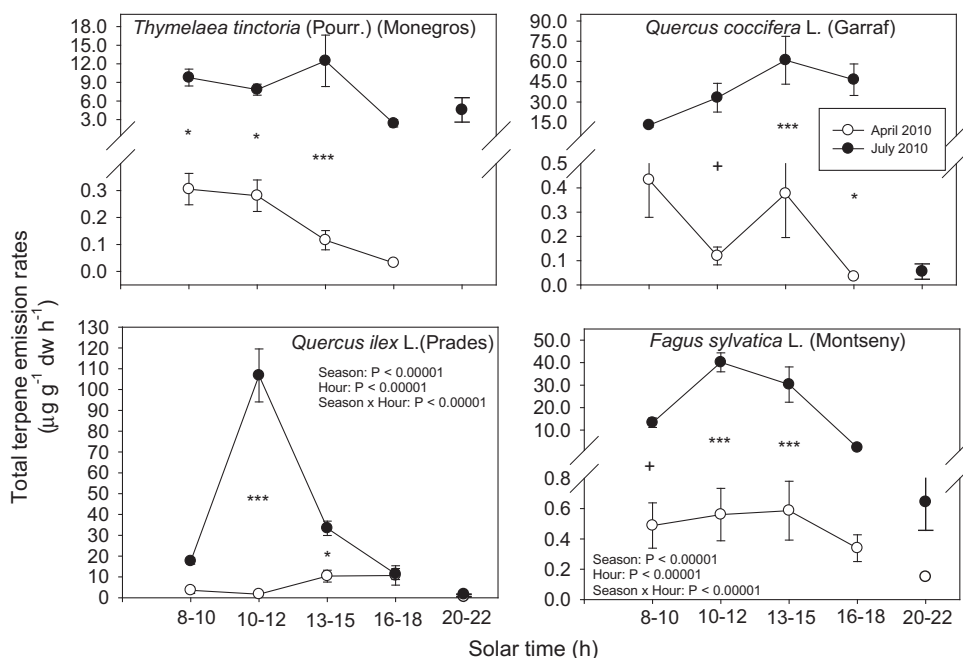
Fig. 3. Daily time averages (±S.E.) series of total terpene emission rates during the sampling dates (April (○) and July (●)). Asterisks indicate significant differences between seasons (ANOVAs, +, $P < 0.01$; ANOVAs, *, $P < 0.001$; ***, $P < 0.00001$).

$P < 0.05$) and leaf temperature ($r = 0.86$, $P < 0.05$). In *F. sylvatica*, monoterpene and total terpene emission rates were significant correlated only with photosynthetic rates ($r = 0.62$, $n = 19$, $P < 0.05$ and $r = 0.61$, $P < 0.05$), stomatal conductance ($r = 0.51$, $r = 0.51$, $P < 0.05$), PPFD ($r = 0.67$ and $r = 0.66$, $P < 0.05$) and leaf temperature ($r = 0.73$ and $r = 0.73$, $P < 0.05$) in summer.

Fig. 5 shows the percentage of terpenes emitted during the day for each sampling time in both seasons sampled. In *T. tinctoria* in April, the emitted terpenoids were mostly the monoterpenes α-pinene, Δ³-carene, limonene, camphene and β-pinene (see also Table S1) and the sesquiterpenes longifolene (see also Table S1). In July the emitted terpenoids were mostly the monoterpenes α-pinene, limonene, β-myrcene, β-pinene, camphene (see also Table S1) and the sesquiterpenes longifolene and α-humulene (Table S1). In *Q. coccifera* in April, the most emitted terpenoids were the monoterpenes camphene, limonene, α-pinene, β-pinene and β-myrcene (see also Table S1) and the sesquiterpenes longifolene, β-caryophyllene and α-humulene (see also Table S1), and in July the monoterpenes limonene, α-pinene, β-pinene, β-myrcene, camphene, α-terpinolene and γ-terpinene and the sesquiterpene β-caryophyllene (see also Table S1). In *Q. ilex* in April, the most emitted terpenoids were the monoterpenes α-pinene, β-pinene, camphene, limonene, β-myrcene (see also Table S1) and in July the monoterpenes limonene, α-pinene, β-pinene, β-myrcene, camphene, cis-ocimene, *p*-cymene, α-terpinene and γ-terpinene and the sesquiterpenes longifolene and β-caryophyllene, (see also Table S1). In *F. sylvatica* in April, the most emitted terpenoids were the monoterpenes α-pinene, limonene, β-pinene, and camphene (see also Table 1), and in July the monoterpenes sabinene, α-pinene, limonene, Δ³-carene, camphene and the sesquiterpenes longifolene and α-humulene (see also Table S1).

4. Discussion

The species studied in this work at each sampling site were different, as well as different were the environmental conditions inherent in each place. However, differences in seasonal emission rates are very significant in all species. The most obvious abiotic

factor changing seasonally is the temperature and daily is the PFD (Guenther, 1997; Llusia and Peñuelas, 1998; Peñuelas and Llusia, 1999a; Llusia and Peñuelas, 2000; Niinemets et al., 2010a,b), but they do not completely explain the large differences. Within species ontogeny–phenology must thus play a key role accounting for such differences in the emission factors between early spring and summer (Fuentes et al., 1996; Hakola et al., 1998).

The Thymeleaceae family has been reported to emit a variety of terpenoids (Knudsen et al., 2006 and references therein (*Daphne* sp)) that only coincides with β-myrcene in our study species. In this study the monoterpenes and sesquiterpenes from *T. tinctoria* were emitted in similar amounts, but slightly more sesqui than mono and in summer than in spring. The higher terpene emission rates in summer than in spring were explained mostly by a large change in emission factor (capacity). *T. tinctoria* was the lowest emitter among the species studied, and it was negatively correlated with leaf temperature ($r = -0.54$) may be due to the highest environmental stress conditions in Monegros than in the other studied sites (Fig. 1) (Loreto et al., 1996; Peñuelas and Llusià, 1999b). The *T. tinctoria* monoterpene emission rates were 19.1 (1.5 for standardised values) times higher in summer than in spring. This species emitted more sesquiterpenes than monoterpenes in the middle of the day likely following a circadian rhythm (between 10 h and 16 h) (Jakobsen and Olsen, 1994) (Fig. 5). This could be explained by the existence of flowers on the twigs sampled, in July when the flowers were in full maturity or close to senescence (Castroviejo et al., 1996). Curiously, emissions were detected at night, mainly the sesquiterpene α-humulene (Fig. 5).

Q. coccifera is a shrubby species known to be a strong emitter of terpenes (Llusia and Peñuelas, 2000; Owen et al., 2001). The average terpene emissions in *Q. coccifera* for the two sampling periods were 19.28 μg g⁻¹ h⁻¹. This is slightly higher than previously reported 1.54 μg g⁻¹ h⁻¹ in April 1997 and 7.96 μg g⁻¹ h⁻¹ in July 1997 (Llusia and Peñuelas, 2000), 18.7 μg g⁻¹ h⁻¹ (Hansen and Seufert, 1996) or between 2 μg g⁻¹ h⁻¹–6.5 μg g⁻¹ h⁻¹ (standardised values) (Ormeño et al., 2007) or about 1.4–20 μg g⁻¹ h⁻¹ (Owen et al., 2001). The *Q. coccifera* monoterpene emission rates were 201 (57 for standardised values) times higher
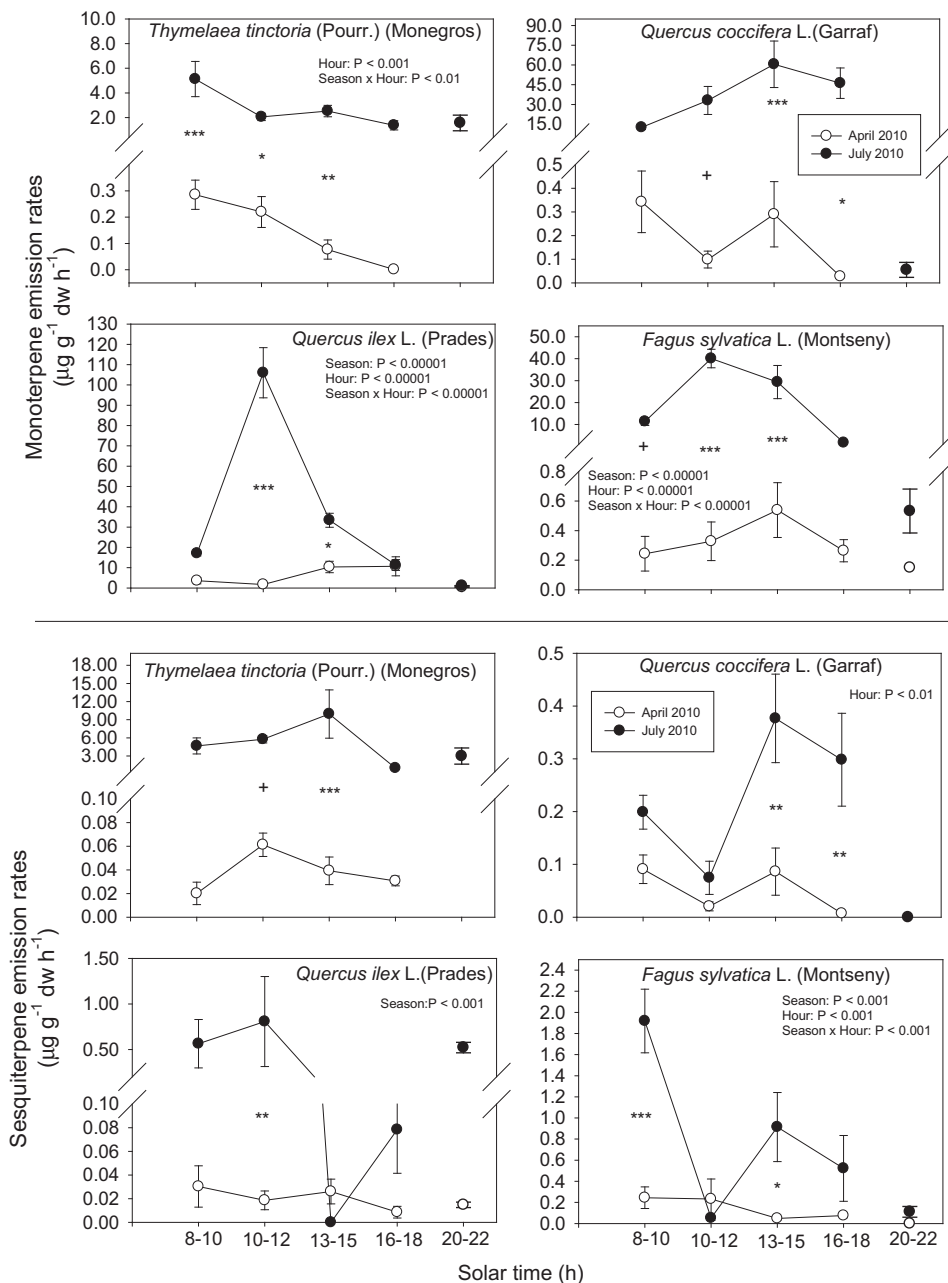
Fig. 4. Daily time averages (±S.E.) series of monoterpene and sesquiterpene emission rates during the sampling dates (April (○) and July (●)). Asterisks indicate significant differences between seasons (ANOVAs, +, $P < 0.01$; ANOVAs, *, $P < 0.001$; **, $P < 0.0001$; ***, $P < 0.00001$).

in summer than in spring. The higher terpene emission rates in summer than in spring were also explained mostly by a large change in emission factor (capacity).

Q. ilex is a strong emitter of terpenes, especially in warm summer conditions (Staudt and Seufert, 1995; Kesselmeier et al., 1996; Street et al., 1997; Holzinger et al., 2000; Llusia and Peñuelas, 2000; Loreto et al., 2001; Owen et al., 2001; Staudt et al., 2002; Plaza et al., 2005; Llusia et al., 2011). The *Q. ilex* monoterpene emission rates were 6.4 (3.7 for standardised values) times higher in summer than in spring, similar to reports of Llusia et al. (2011) in a atypically warm and dry summer. This is slightly lower than previously reported by Owen et al. (2001) in Castelporziano between June 1993 and May, October 1994 (about 2.4–83 μg g⁻¹ h⁻¹) (standardised values). Moreover, Seco et al. (2011) reported one order of magnitude higher terpene air concentrations in summer than in

winter in their PTR-MS analyses in Montseny, highlighting the importance of local biogenic summer emissions for air quality in these Mediterranean forested areas. Both mono and sesquiterpenes nocturnal emissions were detected.

F. sylvatica is also a terpene emitter with rates that depend on the period of the year, and the time of the day (Holzke et al., 2006) (1.31–8.5 μg g⁻¹ h⁻¹) and are temperature and light dependent (Schuh et al., 1997; Dindorf et al., 2005; Demarcke et al., 2010) which agrees with the high correlations with temperature and light found here. The *F. sylvatica* monoterpene emission rates were 60.1 (65 for standardised values) times higher in summer than in spring. The range of standardized monoterpene emissions (30 °C and 1000 μmol m⁻² s⁻¹ PPFD) for the European beech for the same ambient conditions of this work ranged between 65 and 125 μg g⁻¹ h⁻¹ (Moukhtara et al., 2005). At the Montseny site, the
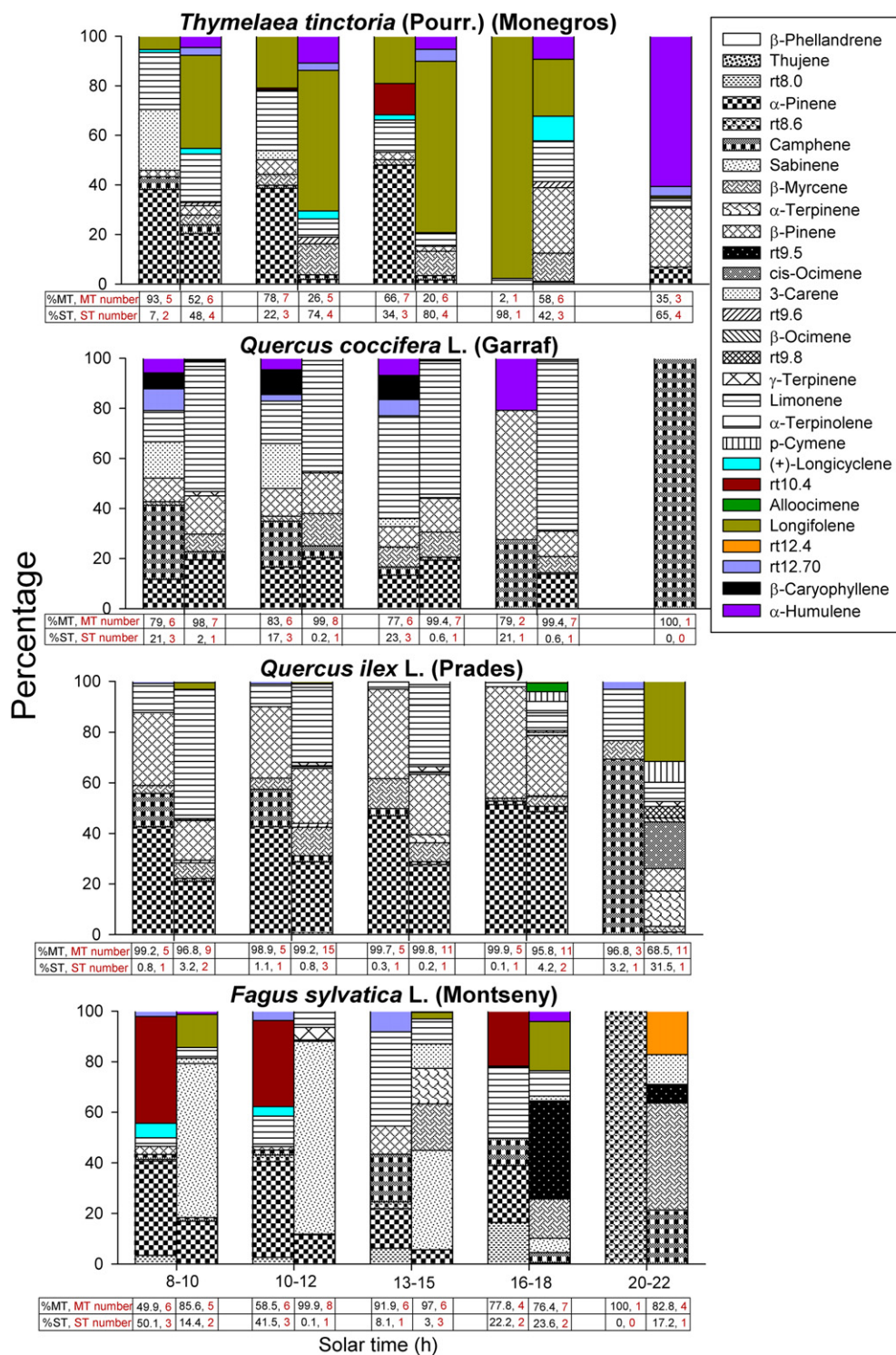
Fig. 5. Daily time series of percentage of terpene (monoterpenes and sesquiterpenes) emission rates during the sampling dates (April (first column) and July (second column)). Numbers at bottom of each graph indicate the percentage of monoterpenes (%MT) and sesquiterpenes (%ST) relative to the total terpenes emitted. The red number after comma indicates the number of compounds found. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

leaves sampled in spring were in the initial stages of development (LMA 25.3 ± 2.7 vs 115.2 ± 5) which explains their low emission rates in spring (Holzke et al., 2006).

The percentage of emission rates of mono and sesquiterpenes varied for each species and sampling period (Fig. 5). *T. tinctoria* and *F. sylvatica* were the species emitting more sesquiterpene followed by *Q. coccifera*, but close to *Q. ilex. T. tinctoria* emitted a higher

sesquiterpenes percentage than monoterpenes in July in the middle of the day (between 13 h and 16 h) (see references above) (Fig. 5).

In conclusion, clear differences in VOC emissions from spring to summer were observed in all species studied (Fuentes et al., 1996; Hakola et al., 1998), being overall 15 times higher in summer than in spring. These differences can be explained mainly by changing

emission factors with only a minor effect of climatic differences between these two seasons.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atmosenv.2013.01.005.

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