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DOI: 0.1016/j.chemosphere.2009.12.047

Environmental control of terpene emissions from *Cistus monspeliensis* L. in natural Mediterranean shrublands

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Abstract

The large amount of volatile organic compound (VOC) emitted by vegetation modifies air quality contributing to both tropospheric ozone and secondary organic aerosol production. A better understanding of the factors controlling VOC emissions by vegetation is mandatory in order to improve emission estimates derived from tropospheric chemistry models. Although the Mediterranean shrublands are particularly abundant and rich in emitting species, their emission potential is poorly known. Focusing on a VOC-emitting shrub species widespread in the Mediterranean area (*Cistus monspeliensis* L.), we measured and analysed its emissions of terpenes taking into account the age of individuals, the season of sampling and the soil type. Sampling was done under natural environmental conditions. Species of the genus *Cistus* are frequently reported to be storing species, although we found only one stored monoterpene and three sesquiterpenes in very low amount. Major emitted compounds were α-pinene and β-myrcene. Total terpene emissions were not influenced by plant age but emission of some individual terpenes was positively correlated with age. A strong seasonal effect was evidenced. A larger amount of terpenes was emitted during spring and summer than during fall and winter. Summer emission rates were nearly 70 times higher than winter emission rates. Total and individual terpene emissions were influenced by soil type; emissions on siliceous substrate were *ca*. 7 times higher than those on calcareous substrate. In conclusion, it appears clearly that environmental factors such as soil nature and season should be taken into account in order to achieve improved modelling of terpene emissions by shrub species.

Keywords:

Cistus monspeliensis L., terpene emissions, soil nature, age, season

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1. Introduction

The chemical breakdown of BVOC in the atmosphere affects air quality and climate forcing by influencing the formation and life time of greenhouse gases and air pollutants such as ozone and methane as well as the formation and growth of secondary organic aerosols (Monson and Holland, 2001). The currently available air chemistry models are unable to reproduce some pollution events and one of the main problems seems to be related to the uncertainties linked to the biogenic emission inventory (Moukhtar et al., 2005). Annual global emissions of non-methane VOC are estimated at around 1.3 Pg (10^{15} g) of carbon and about half of this total is associated with emissions from terrestrial vegetation (Guenther, 2002). The large amount of VOC emitted from vegetation modifies air quality contributing to both ozone (Fehsenfeld et al., 1992) and secondary organic aerosol production (Guenther, 2002).

Among the large variety of VOC synthesised and emitted by plants, terpenes are a dominant class that plays a role in several ecological processes. They are involved in inter and/or intra-species communication (e.g. attraction of pollinators and defence against herbivores) as well as in protection mechanisms against oxidative stress (Laothawornkitkul et al., 2009). Although our understanding of the sources, controls and effects of VOC has increased significantly over the past few decades, prediction of them still remains uncertain (Laothawornkitkul et al., 2009).

Terpene emissions are species-specific and influenced by various biotic and abiotic factors, in particular light and temperature. They show qualitative and quantitative variability according to seasons with generally maximum emissions in spring and summer (Llusia and Peñuelas, 2000; Angelopoulou et al., 2002). The range of this variability differs from one species to another and therefore has to be integrated in a specific way into biogenic emission inventories.

Whereas the effect of climate-related factors on emissions is well-documented, other factors such as plant age and soil nature constraints have been less thoroughly studied. It has been reported that plant age is related to terpene emission rate but the literature still remains inconclusive on this subject: whether in favour of a positive correlation (Street et al., 1997; Kim et al., 2005) or a negative

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correlation (Kim, 2001; Funk et al., 2006). In addition, many species occur on a wide range of substrate types with different chemical and structural properties. These soils, which differ in their pH and nutrient contents, can modify emissions, also in a species-specific way (Ormeño et al., 2008). Identifying such factors inducing variations in emission capacity is necessary to improve emission estimates and tropospheric chemistry modelling.

In the French Mediterranean region, strong anthrogenic NOx sources (Cros et al., 2004) combined with the abundance of vegetation with high terpene emission rates favour fast ozone accumulation (Simon et al., 2006). Moreover cloudless sky conditions, intense solar radiation and high temperatures amplify this phenomenon. Although emissions of Mediterranean tree species are well documented (*Pinus halepensis* L. (Ormeño et al., 2007b), *Quercus ilex* L. and *Quercus suber* L. (Staudt et al., 2004)), shrub emissions have been less widely studied. Yet, in the South of France, shrublands are the second most extensive land cover type (23% of landscape covered) after forest areas (31% of landscape covered).

This study focused on a widespread shrub species *Cistus monspeliensis* L. In spite of its abundance among Mediterranean vegetation, only few authors have studied this species under experimental conditions and their conclusions were inconsistent: Llusia and Peñuelas (1998) recorded low monoterpene emissions from *C. monspeliensis* whereas Owen et al. (2002) classified this species as a high monoterpene emitter. Thus, in order to improve biogenic emission inventories, we characterised branch terpene emissions of this species under field conditions. Our first objective was to determine the seasonal variability range of the emissions, and the second was to test the impact of plant age and soil nature (calcareous and siliceous).

2. Material and methods

2.1. Study area and species

Our study took place in South-Eastern France, under Mediterranean climate with strong seasonality (cool winters, warm dry summers), characterised by rather

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irregular precipitation with an annual average around 800 mm and mean annual temperature around 13.8 °C (Rippert and Venetier, 2002).

Sampling plots were located in shrublands dominated by *C. monspeliensis*. The genus *Cistus* (Cistaceae) comprises 21 species, distributed primarily in the Mediterranean basin. *C. monspeliensis* is native to the Mediterranean region and has a distinct resinous fragrance with hairy, lanceolate leaves that are sticky to the touch. This species is a pioneer shrub that can colonise wide areas after fire on both calcareous and siliceous substrates, where it can establish even-aged stands and persist over long periods. *C. monspeliensis* is one of the most widespread species in the circum-Mediterranean area (Guzman and Vargas, 2009).

2.2. Experimental protocol

Terpene sampling took place every 3-4 months between 11:00 a.m. and 03:30 p.m. (solar time) from November 2007 to November 2008 on three plots to determine emission seasonality. Selected plots were located on siliceous substrate, *C. monspeliensis* being more frequent on this kind of substrate. For each date, six individuals were sampled per plot. Sampling dates and microclimate conditions are shown in Table 1.

Age and soil effect were analysed in spring which is supposed to be an optimal season for emission with no limitation due to either low temperature (Tingey et al., 1980) or drought (Lavoir et al., 2009). Age effect was studied on siliceous substrate. Sampling involved young individuals (Y) from 4 to 7 years old and old individuals (O) from 6 to 18 years old. Age determination of each individual was done following the classical method of dendrochronology (Schweingruber, 1988). Soil effect was analysed on calcareous (C) and siliceous (S) substrates, selecting only old individuals because they were more abundant on calcareous soil than younger ones.

In fine, three modalities with three replicates each were sampled: young shrubs on siliceous soil (YS_1 YS_2, YS_3,), old shrubs on siliceous soil (OS_1, OS_2, OS_3) and old shrubs on calcareous soil (OC_1, OC_2, OC_3). Characteristics of sampled plots are summarised in Table 2 (see section 2.5 for experimental details). No significant difference of elevation, aspect or slope between modalities were observed (Kruskal-

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Wallis, H = 0.36 and p = 0.837 for altitude, H = 3.47 and p = 0.177 for aspect, and H = 1.16 and p = 0.561 for slope).

2.3. Terpene emissions

2.3.1. Terpene sampling

Terpene emissions were sampled using a dynamic bag enclosure system following dynamic headspace sampling techniques (Tholl et al., 2006). Dynamic enclosure technique keeps environmental parameters relatively constant and closer to ambient level, thus we can obtained realistic emission rates (Ortega and Helmig, 2008). The bag enclosure system, made of Teflon film (FEP), was carefully applied avoiding contact with leaves. We enclosed one healthy branch per plant. Healthy state of leaves was determined on the basis of a visual check (Llusia and Peñuelas, 2000). Enclosed branch carried a range from 3 to 15 g of leaf dried weight including sun and shade exposed leaves.

The system consisted of 7 bag enclosure units (5-10 L): 6 to sample *Cistus* emissions and 1 for blank sampling with no branch. Each bag enclosure was designed with inlet and outlet air streams. For this purpose, they were continuously flushed with clean air supplied by a zero-air generator (Perma Pure, Zero-Air™ Generator) through an inert Tygon tube (Tygon® Fuel and lubricant tubing, i.d: 8 mm). After enclosing the branch within the bag system, we performed an equilibration step for 30 min during which air in the bag is renewed twice. This time made it possible to achieve 80-90% of steady state for concentrations in the enclosure according to the equation given by Ortega and Helmig (2008). For this adaptation/renewal step and sampling, inflowing air (Q_i) was kept constant at 385 ± 12 ml min^{-1} (mean ± SE). Q_i was higher than Q_o (outflowing air) which allowed the air bag to be slightly over-pressurised, preventing leaf contact with the Teflon film and outside ambient air penetration. Inflowing air (Q_i) was accurately measured with a digital mass-flow controller (Aalborg® CFC17, 0–1000 ml) and Q_o was measured with a bubble flow meter (0–280 ml min^{-1}, GPE Meterate 314-140/084), placed immediately after each glass sorbent tube. The outgoing terpenes were collected on glass sorbent tubes filled with preconditioned Tenax TA (Varian®, 20–35 mesh, 150

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mg, l: 160 mm, o.d.: 5 mm, i.d.: 3 mm) using a pumping system (KNF Neuberger® N022AT.18), placed downstream from the adsorbent tubes. Terpenes were collected at a rate of 107 ± 6 ml min^{-1} for 15 min. The sampled air volume was calculated in order to optimise the signal/threshold ratio without exceeding the breakthrough volumes of each compound. During emission sampling, external PAR (Photosynthetically Active Radiation, EMS-7 Plant Canopy Transmission Meter, Surechem), such as air temperature and relative humidity (inside and outside the enclosure bag) was monitored with a time interval of 2 minutes. Internal and external air temperature were significantly correlated ($r=0.940$ and $p<0.001$), internal temperature being higher than external temperature about 3°C. Relative humidity inside the bag ranged from 23% to 86% depending on season which is in the range observed under natural conditions. After sampling, tubes were conserved in an icebox with liquid nitrogen (maximum 6 h) until being stored at -20 °C in the laboratory (analyses were performed within a maximum of three weeks).

2.3.2. Terpene analysis

For quantitative purposes, an internal standard (dodecane) was injected in adsorbent tubes (100 ng). Terpenes adsorbed on Tenax were analysed by a gas chromatography system (GC) fitted with a Flame Ionization Detector (FID) (HP®5890 series II). Prior to thermaldesorption, a preflush phase was run (3 min, 10 ml min^{-1}, 60 °C) to allow humidity in the Tenax to be evacuated. Then, thermaldesorption (Thermal Desorption Cold Trap injector, Varian®, CP4020-TCT, model) was carried out under nitrogen flow (10 min, 50 ml min-1, 250 °C) and cryogenic concentration in a silica capillary trap, cooled with liquid nitrogen at -100 °C. Compounds were then separated in the non-polar chromatographic column (Ultra 2, 50 m x 0.2 mm x•0.25 lm) through a temperature programme from 60 °C to 220 °C, at a rate of 3 °C min-1, then 220 °C (isothermal) for 5 min (Ormeño et al., 2007a). The identity of peaks was confirmed by matching the retention time of each compound with that of authentic references (Sigma-Aldrich, one standard for each identified compound listed in table 3 and 4) and by comparison of calculated retention index with those found in the literature (Adams, 2007). Internal standard dodecane, which did not mask any terpene, together with frequent calibration with terpene standards (for every

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compounds listed in table 3 and 4), were used for quantification. Terpene calibration curves (N=4 different terpene concentrations) were always highly significant (R^2>0.98) in the relationship between signal and terpene concentration.

2.4. Terpene content

At the end of terpene emission sampling, leaves enclosed in the bag were collected and immediately plunged into liquid nitrogen. Prior to terpene extraction, leaves were lyophilized for 48 h in a freeze drier (CHRIST, alpha 1-4 LD plus), in order to express results on the basis of leaf dry mass (DM). Freeze-drying is known to induce no terpene loss (Ormeño et al., 2007b). Leaves were mechanically ground with a grinder to obtain powder. This procedure allows better extraction of terpenes than manual procedures (Ormeño et al., 2008). The extraction consisted of dissolving 0.5 g of leaf DM in 10 ml of cyclohexane, for 30 min, under constant shaking at room temperature. Tightly closed glass vials wrapped in aluminium foil were used to avoid exposure to light and oxygen. A non-isoprenoid volatile internal standard (dodecane) was added to quantify measurements. Extracts were analyzed or stored at −20 °C until analysis.

Analyses were performed on a gas chromatograph (Hewlett Packard GC6890®) coupled to a mass selective detector (MSD; HP 5973N). Compound separation was achieved on a HP-5MS capillary column (30 m x 0.25 mm x 0.25 µm, J&W Scientific), in constant flow mode. 2 µl were injected through an automatic injector (ALS 7673). Helium (99.995%) was used as carrier gas. After sample injection, the initial temperature (40 °C), maintained for 5 min, was increased up to 270 °C at a rate of 4 °C min^{-1}, then maintained for 2.5 min. Identification of terpenes was established by comparison of the retention index and the mass spectrum of detected compounds with those of authentic reference samples (Sigma-Aldrich, one standard for each compound listed in table 3 and 4) and literature (Adams, 2007). Internal standard dodecane, which did not mask any terpene, together with frequent calibration with terpene standards (for every compounds listed in table 3 and 4) were used for quantification. Terpene calibration curves (N=4 different terpene

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concentrations) were always highly significant (R^2>0.98) in the relationship between signal and terpene concentration.

The same protocol was applied to leaves of a non-enclosed branch on the same individuals. Here no significant difference between enclosed and non-enclosed leaf content was observed (data not shown). Thus we considered leaf content of enclosed branch as representative of the individual.

2.5. Soil analyses

Main soil chemical parameters were measured (Table 2). For each sampling plot, five 200g-soil samples from the A1 horizon were collected. Aqueous pH (MeterLab PHM20,1 Radiometer analytical S.A.), total nitrogen (N) and total carbon (C) content (CHN elemental analyzer, FLASH-EA1112, Thermo Fisher Scientific Inc. controlled by the Eager 300 software), were determined in the laboratory. Cation exchange capacity (CEC, Metson) and available phosphorus (PA, Joret-Hébert method) were measured by an external analysis laboratory (LCA, La Rochelle, France) with a continuous-flow colorimetric analyzer (LCA instruments).

2.6. Data treatment

Temperature and light are currently agreed to be the main factors controlling emissions from non-storing species (Staudt et al., 2000). Analysis of leaf terpene content showed that emitted terpenes are not stored in *C. monspeliensis* leaves. Therefore, all emissions data were standardised at 30 °C and 1,000 µmol s^{-1} m^{-2} using Guenther's algorithm (Guenther, 1997). The standard emission factor (E_s) was calculated as follows:

$$E_s = \frac{E}{C_L \times C_T}$$

where E is the measured emission and C_L and C_T are functions of temperature and light given by:

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$$C_L = \frac{\alpha\, C_{L1}\, PAR}{\sqrt{1+\alpha^2\, PAR^2}} \qquad C_T = \frac{\exp\frac{C_{T1}(T-T_S)}{R\,T_S\,T}}{0.961+\exp\frac{C_{T2}(T-T_M)}{R\,T_S\,T}}$$

where R (8.314 J K^{-1} mol^{-1}) is the gas constant T_S (303 K or 30 °C) is the standard temperature, T_M (314 K or 41 °C), α (0.0027), C_{L1} (1.066), C_{T1}(95000 J mol^{-1}) and C_{T2} (230000 J mol^{-1}) are empirically defined parameters.

Data were log-transformed, when necessary, to meet normal requirements for statistical analyses. i) One-way analysis of variance (ANOVA) was applied to test the effect of season on terpene emissions. ii) The effect of age on terpene emissions was analysed through correlation analysis between individual age and emissions. iii) Two-sample t-test was performed to compare emissions on siliceous and calcareous substrate. Results are presented in mean ± SE throughout the paper. Statistical analyses were performed using the statistical software Minitab® for both overall terpene emissions and each compound separately.

3. Results

3.1. Terpene content and emissions

Terpene content analysis showed that only one oxygenated monoterpene and three sesquiterpenes were stored in *C. monspeliensis* leaves: β-cyclocitral (CAS number: 432-25-7), α-copaene (CAS n°: 3856-25-5), alloaromadendrene (CAS n°: 25246-27-9) and δ-cadinene (CAS n°: 483-76-1). These compounds were found in low concentration (<0.01 mg gDM^{-1} with DM means leaf dry mass, in average for each compounds, then frequently below the quantitative threshold) regardless of soil, age or season.

C. monspeliensis emitted several compounds but in small amount (Es=2.82 ±1.41 µg g^{-1} h^{-1} averaging for all seasons and modalities). α-pinene is the major released compound from *C. monspeliensis* leaves: it represented 31% to 70% of overall terpene emissions depending on the modality considered. β-myrcene is the second major compound and ranged between 7% and 25% of the overall terpene emissions. In addition, 7 other monoterpenes, 4 oxygenated monoterpenes, one

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monoterpene derivative and one sesquiterpene were determined in lower amounts (Table 4).

3.2. Seasonal variability

Despite temperature and light standardisation, season had a strong effect on overall terpene emission rate (ANOVA, $p<0.001$, Fig. 1). The overall terpene emission rate was significantly higher in spring and summer than in autumn and winter, ranging from 0.15 µg g^{-1} h^{-1} in winter to 9.87 µg g^{-1} h^{-1} in summer.
α-pinene, β-myrcene, β-pinene, 1,8 cineole and bornyl acetate were significantly more abundantly emitted in summer than in autumn and winter, spring emission rates being intermediate (ANOVA, $p<0.05$, table 3). Camphene follows the pattern of terpenes overall and had a higher emission rate in spring and summer than in autumn and winter (ANOVA, $p<0.05$, table 3). α-phellandrene had a very low emission rate throughout the year (Es<0.02 µg.g^{-1}.h^{-1}) but spring emissions increased by a factor 20 to reach *ca*. 10% of the total emissions (ANOVA, $p<0.001$, Table 3). No significant seasonal effect was detected for α-terpinene and borneol although they were detected only in spring.

3.3. Age and soil effects

Temperature, PAR and relative humidity were not significantly different between modalities (YS, OS, and OC) during spring sampling.

Comparison of emission rate between age classes (young vs old) did not show an age effect (T-test, $p>0.05$, data not shown) although positive correlations between plant age and emission rate of β-myrcene, δ-3carene and borneol were observed (Table 4).

Overall terpene emission rates of *C. monspeliensis* were significantly higher on siliceous (Es = 5.28 ± 1.96 µg g^{-1} h^{-1}) than on calcareous (Es = 0.78 ± 0.16 µg g^{-1} h^{-1}) substrate (T-test, $p<0.05$, Table 4). Likewise, emission rates of α-pinene and α-terpineol were significantly modified by substrate (T-test, $p<0.05$, Table 4). Every

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analysed compound tended to have a higher emission rate on siliceous than on calcareous substrate.

4. Discussion

Species of the genus Cistus are generally reported to be terpene storing species (Gulz et al., 1984). They have leaf external glandular hairs allowing terpene storage (Seufert et al., 1995). Our results did not evidence a consistent pool of storing terpenes in *C. monspeliensis* leaves (0.02 mg gDM^{-1} in average): Compared to *C. albidus* (Llusia and Peñuelas, 2000; Ormeño et al., 2008), they stored 10-fold less. To our knowledge, only three studies have taken an interest in leaf terpene content in *C. monspeliensis*: Llusia and Peñuelas (1998) who extracted leaf terpenes in pentane and Robles and Garzino (2000) and Angelopoulou et al. (2002), both using a hydrodistillation extraction process which allows no comparison with our results.

In terms of composition, we only found one oxygenated monoterpene, which is consistent with the poor leaf storage of these compounds in the genus *Cistus* (Llusia and Peñuelas, 2000; Ormeño et al., 2008). This compound was never reported in *C. monspeliensis* but has been reported in essential oil of other *Cistus* species (Ogutveren and Tetik, 2004; Paolini et al., 2008). Llusia and Peñuelas (1998) reported stored α-pinene, but in very low quantity (<0.002 mg gDM^{-1}). This compound is not found here probably due to very low quantity limit of detection capacity.

Conversely, sesquiterpenes are reported to be more numerous in *Cistus* sp., although we only found three of them. This observation could also be due to the very low quantity of compounds on the verge of detection capacity (<0.01 mg gDM^{-1}). The three sesquiterpenes found here have always been reported in *C. albidus* leaf content and two (alloaromadendrene and δ-cadinene) are often considered as major compounds in this species. Although this study focused on volatile compounds, we noticed heavier compound storage, probably diterpenes. In particular, we identified manoyl oxide and its isomers (0.02 mg gDM^{-1} in average) which were quoted in previous studies on *C. monspeliensis* (Robles and Garzino, 2000; Angelopoulou et al., 2002).

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Although *C. monspeliensis* showed very few leaf stored terpenes, emissions have been detected. The major emitted compound found in this study was α-pinene which represents nearly 70% of emissions in summer. This result is consistent with Owen et al. (2002) who found that this compound represented nearly 80% of the overall emissions for the same species under standard conditions. However, the highest standard emission of α-pinene reported here (6.58 μg g^{-1} h^{-1} in summer) remains lower than that found by Owen et al. (10.30 μg g^{-1} h^{-1}). The second major compound in this study, β-myrcene, has not been previously detected. The only compound reported by Llusià and Peñuelas (1998) for *C. monspeliensis* was α-phellandrene. In our study this compound is minor except in spring which corresponds to their sample season. Like Owen et al. (2002), we found significant amounts of camphene, β-pinene and limonene but no sabinene. Other terpenes were detected here for the first time: δ-3 carene, α-terpinene, p-cymene, 1,8 cineole, camphor, borneol, α-terpineol, bornyl acetate and β-caryophyllene.

Composition variability of the emissions could be due to several causes: i) composition could be influenced by plant origin which can lead to various chemotypes (Staudt et al., 2001). ii) some compounds detected in very low amount in this study, such as camphor or β-caryophyllene, may not have been detected in other studies because of the low amount of material used. Indeed, Owen et al. (2002) used one leaf and Llusia and Peñuelas used only terminal leaves of the branch whereas we used an entire branch. iii) Season and age could also influence emissions: some compounds, such as β-myrcene**,** presented a positive correlation with age. Consequently, emissions of these compounds could have been below detection level in previous studies which focused on younger plants or different seasons. iv) Moreover the studies of Owen et al. (2002) and Llusià and Peñuelas (1998) were conducted under controlled conditions (e.g. potted plants, irrigation) which differ from those of our *in situ* study.

The aim of this study was to document the seasonal effect on terpene emissions from *C. monspeliensis*. Our results showed a strong seasonal effect, with minima in winter and emission rates nearly 70 times higher in summer. This variation is in the range observed for other Mediterranean species. Pio et al. (2005) found emissions about 100 times higher in summer than in winter for *Quercus suber*.

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Staudt et al. (2001) reported emissions about 40 times higher in August than in January for *Quercus ilex*. In this study, emissions of most of the analysed compounds follow the pattern of total emissions, increasing from winter to summer and then decreasing in autumn, even after standardisation. This pattern is influenced by long-term light and temperature effect, ontogeny and leaf age (see Grote and Niinemets 2008 for a review). Moreover, a previous work on Mediterranean species (Lavoir et al., 2009) reported a decrease of terpene emissions in summer due to severe drought. However, no drought episode was observed in summer 2008 in the study area.

Another objective of this study was to look at the impact of plant age on terpene emissions. Only few studies have paid interest to individual age effect on terpene emissions and they only concerned trees. Street et al. (1997) compared 5 and 40 year old *Pinus pinea* L. emissions growing in plantations, and Kim et al. (2005) compared 20 and 60 year old conifers growing in forests. Both reported higher total terpene emission rates from older trees. In the present study, whereas total terpene emission rate was not linked to shrub age, some individual terpene emission rates were positively correlated with this parameter. Emission rate of β-myrcene (second major compound) was approximately three times higher in old individuals than in young individuals. Increase of emission rate was particularly substantial for borneol. This compound's emission rate was very low in young individuals but was about 30 times higher in old plants. Consequently, even if age did not significantly alter the total emissions, it greatly influenced emissions of some individual terpene.

Finally, this study revealed a soil effect on *C. monspeliensis* terpene emissions: higher emission rates (individual compounds and total) were observed on siliceous substrate. This result is in agreement with the carbon/nutrient balance hypotheses (CNBH (Bryant et al., 1983)) and the growth differentiation balance hypothesis (GDBH, (Lorio, 1986)) since siliceous substrate has lower nutrient content (lower N level and lower CEC) than calcareous substrate. These theories state that when all factors are favourable for growth, growth processes predominate over secondary compound production but when a nutrient such as nitrogen is scarce, the plant will allocate proportionally more resources to carbon-based secondary compounds such as terpenes. We found a negative correlation between soil nitrogen

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content and leaf terpene emissions (r=-0.428, p=0.016). This result is in agreement with Blanch et al. (2007) who reported that fertilization induced a decrease in terpene emissions of *Pinus halepensis* Mill. However, this hypothesis could not be extended to all species. Ormeño et al. (2007a) who studied terpene emissions on calcareous and siliceous substrate in natural conditions, reported higher emissions of *Cistus albidus* on siliceous substrate but an inverse trend for *Pinus halpensis* and *Rosmarinus officinalis* L. The authors justify these differences by pointing out the different growth state of the three species at the sampling period. Likewise, Lerdau et al. (1995), working on *Pseudotsuga menziensii* Mirb., an evergreen coniferous tree, showed that higher nitrogen fertilization induced an increase in terpene emissions, except during leaf expansion when the correlation was negative. In the case of *C. monspeliensis*, leaf expansion occurs throughout the year and was only slightly slower during the cold and dry seasons (Delillis and Fontanella, 1992).

5. Conclusion

Field experimentation is essential in order to improve predictions of biogenic emissions and air quality by determining absolute and species-specific emission rates and by highlighting factors influencing terpene emissions. Terpene storage is a species-specific character which is integrated in models. Two major algorithms simulate emissions according to the occurrence of storage: the Tingey algorithm which involves only temperature is used for storing-species (Tingey et al., 1980) whereas Guenther's algorithm involving light and temperature is used for non-storing species (Guenther, 1997). In our study, we found only one monoterpene and three sesquiterpene stored in very low amount in *C. monspeliensis* leaves (total storage <0.02 mg g^{-1}), although many species of the genus *Cistus* are frequently reported to be storing species. As a conclusion, the occurrence of storage could show inter- and/or intra-specific variability, which needs to be checked as a basis for prediction at local scale.

To illustrate factors influencing terpene emissions, this study focused on seasonal variability of leaf terpene emissions from *C. monspeliensis* as well as plant age and soil nature impact on this process. Whereas no age effect on total emissions

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was observed, soil and seasonal effects showed strong variability which could be integrated in simulations. Seasonal variability of emissions was characterised by minima in winter and maxima in summer (70 times higher). These high emission rates occurred during the most favourable period for ozone pollution events, and could strengthen them. Finally, terpene emissions on siliceous substrate were *ca*. 7 times higher than on calcareous substrate, therefore soil effect should be integrated in biogenic emission models. This study highlighted a strong influence of season and substrate on terpene emissions. Thus, the integration of these factors into the simulation models of VOC emissions will increase the accuracy of predictions of pollution events.

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Acknowledgements

This work was funded by AtmoPACA (contract n°01-11-18-04), the French environmental agency (ADEME) and the PACA (Provence-Alpes-Côte d'Azur) Regional Council. We thank Sylvie Dupouyet, Samira Marir and Hussain Rizvi for assistance during the field work and sample analysis.

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References

Adams, R. P. 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th edition. Allured Publishing Corporation.

Angelopoulou, D., Demetzos, C., Perdetzoglou, D., 2002. Diurnal and seasonal variation of the essential oil labdanes and clerodanes from Cistus monspeliensis L. leaves. Biochem. Syst. Ecol. 30, 189-203.

Blanch, J.S., Penuelas, J., Llusia, J., 2007. Sensitivity of terpene emissions to drought and fertilization in terpene-storing Pinus halepensis and non-storing Quercus ilex. Physiol. Plantarum 131, 211-225.

Bryant, J., Chapin III, F.S., Klein, D., 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40.

Cros, B., Durand, P., Cachier, H., Drobinski, P., Frejafon, E., Kottmeier, C., Perros, P.E., Peuch, V.H., Ponche, J.L., Robin, D., Said, F., Toupance, G., Wortham, H., 2004. The ESCOMPTE program: an overview. Atmos. Res. 69, 241-279.

Delillis, M., Fontanella, A., 1992. Comparative Phenology and Growth in Different Species of the Mediterranean Maquis of Central Italy. Vegetatio 100, 83-96.

Fehsenfeld, F., Calvert, J., Fall, R., Goldan, P., Guenther, A., Hewitt, C.N., Lamb, B., Liu, S., Trainer, M., Westberg, H., Zimmerman, P., 1992. Emissions of volatile organic compounds from vegetation and the implications for atmospheric chemistry. Global Biogeochem. Cy. 6, 389-430.

Funk, J.L., Giardina, C.P., Knohl, A., Lerdau, M.T., 2006. Influence of nutrient availability, stand age, and canopy structure on isoprene flux in a Eucalyptus saligna experimental forest. J. Geophys. Res-Biogeo. 111.

Grote, R., Niinemets, U., 2008. Modeling volatile isoprenoid emissions - a story with split ends. Plant Biol. 10, 8-28.

Guenther, A., 1997. Seasonal and Spatial Variations in Natural Volatile Organic Compound Emissions. Ecological Applications 7, 34-45.

Guenther, A., 2002. The contribution of reactive carbon emissions from vegetation to the carbon balance of terrestrial ecosystems. Chemosphere 49, 837-844.

Gulz, P.G., Kobold, U., Michaelis, K., Vostrowsky, O., 1984. The Composition of Terpene Hydrocarbons in the Essential Oils from Leaves of 4 Cistus Species. Zeitschrift Fur Naturforschung C-a Journal of Biosciences 39, 699-704.

Guzman, B., Vargas, P., 2009. Long-distance colonization of the Western Mediterranean by Cistus ladanifer (Cistaceae) despite the absence of special dispersal mechanisms. J. Biogeogr. 36, 954-968.

Kim, J.-C., 2001. Factors controlling natural VOC emissions in a southeastern US pine forest. Atmos. Environ. 35, 3279-3292.

Kim, J.-C., Kim, K.-J., Kim, D.-S., Han, J.-S., 2005. Seasonal variations of monoterpene emissions from coniferous trees of different ages in Korea. Chemosphere 59, 1685-1696.

Laothawornkitkul, J., Taylor, J.E., Paul, N.D., Hewitt, C.N., 2009. Biogenic volatile organic compounds in the Earth system (vol 183, pg 27, 2009). New Phytol. 184, 276-276.

Lavoir, A.V., Staudt, M., Schnitzler, J.P., Landais, D., Massol, F., Rocheteau, A., Rodriguez, R., Zimmer, I., Rambal, S., 2009. Drought reduced monoterpene emissions from the evergreen Mediterranean oak Quercus ilex: results from a throughfall displacement experiment. Biogeosciences 6, 1167-1180.

Lerdau, M., Matson, P., Fall, R., Monson, R., 1995. Ecological Controls over Monoterpene Emissions from Douglas-Fir (*Pseudotsuga-Menziesii*). Ecology 76, 2640-2647.

Llusia, J., Peñuelas, J., 1998. Changes in terpene content and emission in potted Mediterranean woody plants under severe drought. Can. J. Bot. 76, 1366-1373.

Llusia, J., Peñuelas, J., 2000. Seasonal patterns of terpene content and emission from seven Mediterranean woody species in field conditions. Am. J. Bot. 87, 133-140.

Lorio, P.L., 1986. Growth-Differentiation Balance - a Basis for Understanding Southern Pine-Beetle Tree Interactions. Forest Ecol. Manage. 14, 259-273.

Monson, R.K., Holland, E.A., 2001. Biospheric trace gas fluxes and their control over tropospheric chemistry. Annual Review of Ecology and Systematics 32, 547-+.

Moukhtar, S., Bessagnet, B., Rouil, L., Simon, V., 2005. Monoterpene emissions from Beech (Fagus sylvatica) in a French forest and impact on secondary pollutants formation at regional scale. Atmos. Environ. 39, 3535-3547.

Ogutveren, M., Tetik, S.S., 2004. Composition of the essential oil of Cistus parviflorus L. from Turkey. J. Essent. Oil Res. 16, 115-116.

Ormeño, E., Baldy, V., Ballini, C., Fernandez, C., 2008. Production and diversity of volatile terpenes from plants on calcareous and siliceous soils: Effect of soil nutrients. J. Chem. Ecol. 34, 1219-1229.

Ormeño, E., Fernandez, C., Bousquet-Mélou, A., Greff, S., Morin, E., Robles, C., Vila, B., Bonin, G., 2007a. Monoterpene and sesquiterpene emissions of three Mediterranean species through calcareous and siliceous soils in natural conditions. Atmos. Environ. 41, 629-639.

Ormeño, E., Fernandez, C., Mévy, J.-P., 2007b. Plant coexistence alters terpene emission and content of Mediterranean species. Phytochemistry 68, 840-852.

Ortega, J., Helmig, D., 2008. Approaches for quantifying reactive and low-volatility biogenic organic compound emissions by vegetation enclosure techniques - Part A. Chemosphere 72, 343-364.

Owen, S.M., Harley, P., Guenther, A.B., Hewitt, C.N., 2002. Light dependency of VOC emissions from selected Mediterranean plant species. Atmos. Environ. 36, 3147-3159.

Paolini, J., Tomi, P., Bernardini, A.F., Bradesi, P., Casanova, J., Kaloustian, J., 2008. Detailed analysis of the essential oil from Cistus albidus L. by combination of GC/RI, GC/MS and C-13-NMR spectroscopy. Nat. Prod. Res. 22, 1276-1284.

Pio, C.A., Silva, P.A., Cerqueira, M.A., Nunes, T.V., 2005. Diurnal and seasonal emissions of volatile organic compounds from cork oak (*Quercus suber*) trees. Atmos. Environ. 39, 1817-1827.

Rippert, C., Venetier, M., 2002. Chapitre 2bis: Evaulation des potentialités forestières. In: Edition, C. (Ed.). Guide du forestier méditerranéen français, Aix en Provence.

Robles, C., Garzino, S., 2000. Infraspecific variability in the essential oil composition of Cistus monspeliensis leaves. Phytochemistry 53, 71-75.

Schweingruber, F.H., 1988. Tree-rings, basics and applications of dendrochronology. Reidel, D. publication company, Dordrecht.

Seufert, G., Kotzias, D., C., S., Versino, B., 1995. Volatile organics in Mediterranean shrubs and their potential role in a changing environment. In: Moreno, J.M., Oechel, W.C. (Eds.). Global Change and Mediterranean-Type Ecosystems. Springer, Berlin, pp. 343–370.

Simon, V., Dumergues, L., Ponche, J.L., Torres, L., 2006. The biogenic volatile organic compounds emission inventory in France - Application to plant ecosystems in the Berre-Marseilles area (France). Science of the Total Environment 372, 164-182.

Staudt, M., Bertin, N., Frenzel, B., Seufert, G., 2000. Seasonal variation in amount and composition of monoterpenes emitted by young Pinus pinea trees - Implications for emission modeling. J. Atmos. Chem. 35, 77-99.

Staudt, M., Mandl, N., Joffre, R., Rambal, S., 2001. Intraspecific variability of monoterpene composition emitted by Quercus ilex leaves. Can. J. Forest Res. 31, 174-180.

Staudt, M., Mir, C., Joffre, R., Rambal, S., Bonin, A., Landais, D., Lumaret, R., 2004. Isoprenoid emissions of Quercus spp. (*Q. suber* and *Q. ilex*) in mixed stands contrasting in interspecific genetic introgression. New Phytol. 163, 573-584.

Street, R.A., Owen, S., Duckham, S.C., Boissard, C., Hewitt, C.N., 1997. Effect of habitat and age on variations in volatile organic compound (VOC) emissions from Quercus ilex and Pinus pinea. Atmos. Environ. 31, 89-100.

Tholl, D., Boland, W., Hansel, A., Loreto, F., Röse, U.S.R., Schnitzler, J.-P., 2006. Practical approaches to plant volatile analysis. Plant J. 45, 540-560.

Tingey, D.T., Manning, M., Grothaus, L.C., Burns, W.F., 1980. Influence of light and temperature on monoterpene emission rates from slash pine. Plant Physiol. 65, 797-801.

Rivoal A., Fernandez C., Lavoir A.V., Olivier R., Lecareux C., Greff S., Roche P. and Vila B.
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FIGURE CAPTIONS

Fig. 1: Total terpene standard emission rate (E_S, mean ± SE in µg g^{-1} h^{-1}) for each season from *C. monspeliensis* leaves, growing on siliceous substrate. Significativity was tested by ANOVA ($F=8.43$, $p<0.001$) and Tukey test (different letters indicate statistically significant differences).

TABLE CAPTIONS

Table 1: sampling date and micro-climatic conditions out of the enclosure bag during sampling: Temp is the air temperature (°C), PAR is the Photosynthetically Active Radiation (µmol m^{-2} s^{-1}) and RH is relative humidity (%).

Table 2: Plot characteristics: YS for Young Siliceous, OS for Old Siliceous and OC for Old Calcareous. Geographical coordinates are given in the system of reference UTM31N-WGS84. Elevation in meters, aspect is given as W for west, E for east, S for south and N for north, slope in degrees, average of individual age in years, available phosphorus (P_A) in mg kg^{-1} of dry soil, cation exchange capacity (CEC) in Cmol+/kg^{-1} of soil, soil carbon and nitrogen in percentage and C/N is the carbon-nitrogen ratio. Characteristics are expressed in mean ± SE.

Table 3: standard emission rate (Es, mean and SE in µg g^{-1} h^{-1}) for each season and results of ANOVA. nd = not detected.

Table 4: standard emission rate (Es, mean and SE in µg g^{-1} h^{-1}) for old individuals growing on calcareous and siliceous substrates and for young individuals on siliceous substrate. Results of T-test for comparison of emission rates of individuals according to soil nature and results of Pearson correlation between age and emission rate on siliceous substrate. nd = not detected.

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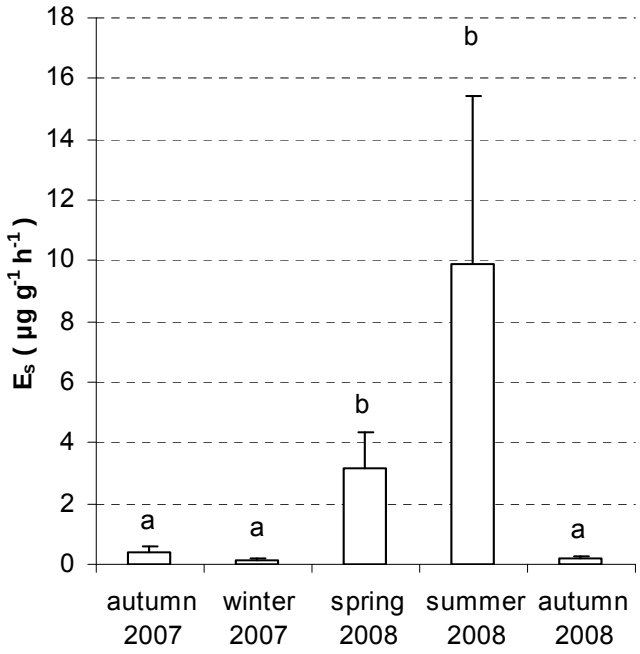

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Season	Site	Date	Temp (°C)	PAR (µmol m^{-2} s^{-1})	RH (%)
Autumn	OS1	26/11/2007	16,9	480,7	36,0
	YS1	13/11/2007	16,0	710,6	63,0
	YS2	12/11/2007	23,5	953,5	64,0
Winter	OS1	14/03/2008	24,7	358,7	43,8
	YS1	06/03/2008	10,5	242,1	52,5
	YS2	06/03/2008	16,0	321,2	51,8
Spring	YS1	21/05/2008	21,8	671,0	63,5
	YS2	03/06/2008	34,0	1319,2	49,8
	YS3	23/05/2008	34,9	1894,2	44,0
	OS1	15/05/2008	31,1	1162,1	40,9
	OS2	23/05/2008	36,8	1792,3	45,5
	OS3	03/06/2008	37,5	1411,8	91,0
	OC1	22/05/2008	39,0	1570,0	39,7
	OC2	22/05/2008	25,7	1196,5	51,2
	OC3	22/05/2008	33,0	1506,5	42,2
Summer	OS1	28/08/2008	43,5	1602,1	23,4
	YS1	29/08/2008	31,7	1528,3	31,4
	YS2	29/08/2008	41,0	1453,7	18,5
Autumn	OS1	07/11/2008	19,4	488,3	44,0
	YS1	07/11/2008	11,3	361,9	76,5
	YS2	30/10/2008	14,8	597,8	56,0

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Plot	YS_1	YS_2	YS_3	OS_1	OS_2	OS_3	OC_1	OC_2	OC_3
Geographical coordinates									
Latitude	43°18'23''	43°31'21''	43°28'07''	43°11'43''	43°33'43''	43°21'18''	43°22'50''	43°21'07''	43°13'26''
Longitude	6°27'41''	6°35'28''	6°47'38''	6°17'17''	6°47'25''	6°24'13''	5°01'57''	5°10'11''	5°30'45''
Elevation (m)	520	72	105	326	158	90	65	137	222
Aspect	NW	S	W-SW	(E)-SE	E-NE	W	E-(SE)	E	(E)-SE
Slope(degree)	14	5	7	21	4	2	3	4	8
Age (years)	5.3 ± 0.3	5.5 ± 0.5	5.3 ± 0.3	15.4 ± 0.9	11.3 ± 0.2	9.3 ± 1.0	12.5 ± 1.3	12.5 ± 1.1	12.7 ± 0.2
Soils characteristics									
Soil nature	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Calcareous	Calcareous	Calcareous
pH	5.78 ± 0.06	6.21 ± 0.07	6.22 ± 0.11	5.35 ± 0.17	6.14 ± 0.12	6.17 ± 0.08	7.74 ± 0.05	7.77 ± 0.07	7.74 ± 0.03
P_A (mg kg^{-1})	59 ± 6	24 ± 2	34 ± 3	21 ± 1	21 ± 1	20 ± 0	28 ± 5	20 ± 0	32 ± 3
CEC (Cmol+/kg^{-1})	15.8 ± 1.0	7.2 ± 2.1	9.9 ± 0.9	8.4 ± 0.5	10.4 ± 0.7	9.6 ± 0.7	17.5 ± 1.1	23.5 ± 3.2	23.5 ± 0.9
Nitrogen (%)	0.27 ± 0.04	0.09 ± 0.04	0.13 ± 0.02	0.06 ± 0.01	0.07 ± 0.02	0.07 ± 0.01	0.21 ± 0.04	0.15 ± 0.00	0.22 ± 0.02
Carbon (%)	4.52 ± 0.56	1.84 ± 0.78	2.68 ± 0.33	1.48 ± 0.26	1.08 ± 0.31	1.38 ± 0.20	3.37 ± 0.82	2.55 ± 0.11	3.69 ± 0.25
C/N	16.6 ± 0.3	18.9 ± 0.7	20. 7 ± 0.7	28.6 ± 3.7	14.8 ± 0.9	20.1 ± 1.2	15.1 ± 1.1	17.2 ± 0.7	16.9 ± 0.2

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Table 3: standard emission rate (Es, mean and SE in µg g^{-1} h^{-1}) for each season and results of ANOVA. nd = not detected.

	Autumn 2007		Winter 2007		Spring 2008		Summer 2008		Autumn 2008		ANOVA	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	F	p
Monoterpenes												
α-pinene	0.124	0.063	0.064	0.031	1.413	0.526	6.576	3.732	0.107	0.033	**6.970**	**<0.001**
camphene	0.000	0.000	0.001	0.001	0.033	0.015	0.034	0.015	0.000	0.000	**4.260**	**0.004**
β-pinene	0.013	0.007	0.001	0.001	0.118	0.043	0.597	0.396	0.007	0.003	**3.750**	**0.008**
β-myrcene	0.046	0.027	0.025	0.014	0.322	0.178	1.681	0.973	0.051	0.021	**4.860**	**0.002**
α-phellandrene	0.005	0.003	0.007	0.005	0.297	0.141	0.016	0.007	0.011	0.010	**5.850**	**<0.001**
δ-3 carene	nd		nd		0.010	0.010	nd		nd		1.130	0.349
α-terpinene	nd		nd		0.197	0.150	nd		nd		2.350	0.062
p-cymene	0.066	0.033	0.005	0.003	0.035	0.021	0.065	0.033	0.005	0.003	1.860	0.127
limonene	0.065	0.042	0.038	0.021	0.150	0.046	0.176	0.156	0.021	0.012	1.220	0.309
Oxygenated monoterpenes												
1,8 cineole	0.021	0.012	0.001	0.001	0.043	0.023	0.178	0.097	0.002	0.001	**3.420**	**0.013**
camphor	0.006	0.003	0.001	0.001	0.006	0.004	0.006	0.004	nd		1.230	0.305
borneol	nd		nd		0.299	0.296	nd		nd		1.110	0.357
α-terpineol	0.041	0.025	0.001	0.001	0.190	0.142	0.282	0.192	nd		1.970	0.108
Monoterpene derivatives												
bornyl acetate	0.012	0.005	0.001	0.001	0.072	0.033	0.260	0.152	0.002	0.001	**3.100**	**0.021**
Sesquiterpenes												
β-caryophyllene	0.009	0.004	0.006	0.006	0.007	0.003	0.001		nd		1.460	0.222
Overall terpenes	0.408	0.190	0.153	0.059	3.192	1.147	9.873	5.586	0.207	0.061	**8.430**	**<0.001**

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(2010) Environmental control of terpene emissions from Cistus monspeliensis L. in natural Mediterranean shrublands, Chemosphere, 78, 8, 942-949.
Author-produced version of the final draft post-refeering
the original publication is available at www.elsevier.com/locate/chemosphere
DOI: 0.1016/j.chemosphere.2009.12.047

Table 4: standard emission rate (Es, mean and SE in µg g^{-1} h^{-1}) for old individuals growing on calcareous and siliceous substrates and for young individuals on siliceous substrate. Results of T-test for comparison of emission rates of individuals according to soil nature and results of Pearson correlation between age and emission rate on siliceous substrate. nd = not detected.

	old - calcareous		old - siliceous		young - siliceous		T-test (soil effect)		correlation (age effect)	
	mean	SE	mean	SE	mean	SE	T	p	r	p
Monoterpenes										
α-pinene	0.336	0.106	2.422	1.073	1.139	0.492	**-2.31**	**0.035**	-0.016	0.933
camphene	0.020	0.011	0.069	0.028	0.021	0.013	-1.67	0.113	0.091	0.637
β-pinene	0.037	0.022	0.280	0.142	0.092	0.032	-1.93	0.075	0.090	0.642
β-myrcene	0.106	0.035	0.473	0.203	0.141	0.058	-1.88	0.080	**0.377**	**0.044**
α-phellandrene	0.054	0.022	0.356	0.163	0.123	0.071	-1.95	0.072	0.301	0.112
δ-3 carene	0.008	0.003	0.020	0.011	0.000	0.000	-1.10	0.289	**0.391**	**0.036**
α-terpinene	0.000	0.000	0.121	0.060	0.163	0.149	-2.03	0.063	-0.089	0.646
p-cymene	0.011	0.009	0.011	0.011	0.035	0.021	0.02	0.984	-0.072	0.711
limonene	0.074	0.020	0.454	0.217	0.138	0.040	-1.84	0.088	0.011	0.957
Oxygenated monoterpenes										
1,8 cineole	0.031	0.010	0.232	0.105	0.038	0.018	-2.09	0.057	0.083	0.669
camphor	0.012	0.005	0.021	0.019	0.012	0.006	-0.39	0.704	-0.104	0.592
borneol	0.039	0.019	0.366	0.316	0.010	0.008	-1.01	0.330	**0.422**	**0.023**
α-terpineol	0.016	0.008	0.205	0.083	0.161	0.142	**-2.52**	**0.026**	-0.028	0.887
Monoterpene derivatives										
bornyl acetate	0.025	0.012	0.212	0.090	0.041	0.018	-2.12	0.054	0.132	0.493
Sesquiterpenes										
β-caryophyllene	0.006	0.002	0.033	0.025	0.008	0.003	-1.10	0.293	0.123	0.527
Overall terpenes	0.776	0.159	5.278	1.955	2.123	0.702	**-2.55**	**0.022**	0.024	0.904