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Abstract

Little is known about terpene emissions released by plants in response to abiotic factors, except for climate-related factors. Standard emissions (E_S) of monoterpenes (E_{SM}) and sesquiterpenes (E_{SS}) o f *Rosmarinus officinalis*, *Pinus halepensis* and *Cistus albidus* in siliceous and calcareous sites were examined. Their dependency on some nutrients in these soils was also analyzed. The study was carried out in the south of France at the end of March, when *C. albidus* exhibited a leaf growth state, while the other two species exhibited a pre-budbreak state. The results revealed that E_S of all major monoterpenes released by *R. officinalis* and E_S of α-pinene and α-humulene of *P. halepensis* were higher in plants growing in calcareous soils. In contrast, for *C. albidus*, E_{SM} and E_S of β-bourbonene and α-humulene were higher in siliceous soils. E_{SM} of all species was mainly correlated with nitrogen (*N*) and available phosphorous (P_A), while dependency on Ca^{2+} or K^+ was variable. None of these nutrients was significantly correlated with E_{SS}, suggesting that sesquiterpene synthesis pathway requires different nutrient supplies. While higher soil nutrient content stimulated E_{SM} of *R. officinalis* and *P. halepensis*, it had a negative effect on E_{SM} of *C. albidus*, probably because *C. albidus* exhibited a different phenological state. Considering the soil nature, and particularly N and P_A as inputs in plant terpene inventories could hence contribute to obtain more accurate terpene estimates.

Keywords: Nitrogen; Phosphorous; Terpenes; Phenological state; Soil nature

1. Introduction

Terpenes are one of the largest and most varied groups of plant chemicals. Their emission from plants represents one of the main sources of photochemically reactive hydrocarbons in the troposphere, since they contribute to the production of different secondary pollutants such as secondary organic aerosols (SOAs) and tropospheric ozone (O_3) (Tsigaridis and Kanakidou, 2002). Terpenes have thus been demonstrated to be important inputs into photochemical models currently used

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to simulate air quality. Different terpene types have been reported to contribute in different ways to the tropospheric photochemical imbalance. Whereas sesquiterpenes are rather implicated in aerosol formation, monoterpenes play a more important role in O_3 formation (Guenther, 2002). However, while considerable effort has been devoted to the design of models capable of predicting monoterpene emissions, sesquiterpene emissions are not integrated in such models.

This effort has been particularly important in the Mediterranean area, since climatic conditions favour biogenic volatile organic compound (BVOC) emissions and especially those of terpenes (Bertin et al., 1997). These conditions include a marked seasonality, with a long summer, where the lowest precipitation rate and the highest annual irradiance coincide. This is also a highly populated area, leading to increases in anthropogenic pollutants, such as NO_x, necessary for tropospheric O_3 formation in the presence of BVOC and light. All these interacting phenomena are thereby responsible of the high tropospheric secondary pollution in the Mediterranean area. Because emissions of numerous Mediterranean species are dominated by monoterpenes (Owen et al., 2001), many studies have focused on monoterpene emission variation according to climate-related factors. Thus, light, temperature (Staudt and Bertin, 1998), drought (Hansen and Seufert, 1999) and the phenological state according to the season (Sabillon and Cremades, 2001) have been reported to be linked to monoterpene emissions. While sesquiterpenes may also characterize emissions of some Mediterranean species (e.g. *Cistus albidus*, Llusià and Peñuelas, 1998) the effect of environmental factors on these emissions is not so well documented. Considering the effect of these factors on monoterpene and sesquiterpene emissions separately could be important, not only because they do not play the same role in the troposphere, but also because they are synthesized in different cell compartments through independent synthesis pathways. Monoterpenes are synthesized through the plastidic non-mevalonate, methylerythritol-phosphate (MEP) pathway, while sesquiterpenes are mainly synthesized through the cytosolic mevalonate (MVA) pathway (Hampel et al., 2005). Moreover, after being synthesized, some species posses specialized structures (e.g. glands, resin ducts), where terpenes are accumulated prior to be released. In most cases, both monoterpenes and sesquiterpenes are accumulated (*Pinus halepensis*, Llusià and Peñuelas, 2000; *Rosmarinus officinalis*, Moretti et al., 1998), but it is possible that only sesquiterpenes are stored, even if monoterpenes contribute to emissions (*C. albidus*, Llusià and Peñuelas, 2000). Because emissions may depend on stored concentrations (Lerdau et al., 1995), factors modifying these structures could also modify terpene emissions potentially.

Apart from the climate, the Mediterranean region also largely features calcareous and siliceous soils (also commonly named alkaline and acid soils, respectively). These soils differ in their pH, permeability and nutrient status (e.g. nitrogen). While some studies have shown that terpene content in the essential oil from Mediterranean species such as *C. monspelliensis* L., (Robles and Garzino, 2000), *C. albidus* (Robles and Garzino, 1998), *Myrtus communis* L. (Flamini et al., 2004) and *R. officinalis* (Moretti et al., 1998) vary according to these soils, no information is available on terpene emission dependency on calcareous and siliceous soils. The significant role of soil nutrient availability, particularly, soil nitrogen, on terpene emissions, has been supported by the fact that monoterpene and sesquiterpene emissions have been found to be positively correlated to nitrogen supply (Gouinguene and Turlings, 2002). Examining terpene emission dependency on soils, which differ naturally in their nutrient status, could thereby provide a basis for assessing the importance of considering these soils as an input in terpene emission inventories and models. Whereas only Gouinguene and Turlings, (2002) have dealt with terpene emission variability according to nitrogen supply in soil no attention has been paid to the effect of other soil nutrients, such as phosphorous, potassium or calcium. Some lines of evidence indicate nevertheless that these nutrients could interfere in terpene emissions. These nutrients are currently linked to plant growth and carbon assimilation (Lambers et al., 1998). Moreover, under low contents of both, nitrogen and phosphorous in natural soils, Barnola and Cedeño, (2000) showed an increase in leaf terpene content.

Here we examined in situ whether monoterpene and sesquiterpene emissions of three typical Mediterranean species (i) varied between plants growing in calcareous and siliceous soils (ii) were related to nitrogen (N), available phosphorous (P_A), calcium (Ca^{2+}) and potassium (K^+) in soil.

2. Material and methods

2.1. Species, sites and sampling description

Three Mediterranean storing species were chosen: *Pinus halepensis* Mill., *Cistus albidus* L., and *Rosmarinus officinalis* L. All grew both in calcareous and siliceous soils, but were mainly widespread on calcareous soils. The study was carried out throughout the calcareous and siliceous ranges of Provence (in the PACA Region: Provence-Alpes-Côte d'Azur), in southern France, where these two soil types are geographically well separated. Sampling took place from 19 to 24 March 2004 and between 11:00 and 15:30 h (solar time). Terpene emissions of each species were analyzed at three calcareous sites (C1, C2, C3) (43°28′N–5°26′E; 43°29′N–5°18′E; 43°15′N–5°37′E, respectively) and three other siliceous sites (S1, S2, S3) (43°13′N–6°10′E; 43°12′N–6°9′E; 43°30′N–6°39′E) (approximate distance between both types of soil: 150–200 km). A supplementary siliceous site (S4) (43°20′N–6°30′E) was chosen for *R. officinalis* alone, since this species was absent from one siliceous site (S3). Four individuals per site were studied, making a total of 12 plants per species and per soil. Mean precipitation pattern of the last 5 years in March was similar at both types of sites: 30 and 37 mm in calcareous and siliceous sites, respectively (Meteo France®). Air temperature and Photosynthetically Active Radiation (PAR) during sampling were also similar for both types of soils. They ranged from 22 to 25 °C and from 750 to 960 $\mu mol\,s^{-1}\,m^{-2}$ throughout the sampling campaign.

2.2. Bag enclosure system description

For each plant, emissions from a single branch with sun and shade exposed leaves (between 3 and 7 g of leaf dried weight) were sampled. Health of leaves was visually checked. A semi-dynamic bag system was used to enclose a branch and trap terpene emissions from its leaves. Internal bag temperature, PAR (Portable photo system, plant and canopy transmission meter, Surechem©, EMS-7 Model), relative humidity (psychrometer, Jules Richard) and horizontal wind speed (Wind Speed Meter, WSC, 888 H, Huger®) were measured with a time resolution of 2 min, while emission sampling took place. The bag enclosure system used in this study consisted of a teflon bag (0.5 l). The enclosure process was done in such a way to cause little movement of leaves. After enclosing each branch within the bag system, non-polluted air was pumped into the bag. Pumping took place from the air stream inlet to the air stream outlet for 10 min at a mean flow of $120 \pm 30\,ml\,min^{-1}$. Then, gas samples were collected at a rate of $80 \pm 30\,ml\,min^{-1}$ for 12 min. A glass tube containing solid adsorbents (Tenax TA) was placed at the air stream outlet of each bag in order to trap volatile organic compounds. The flow through each glass tube was measured with a bubble flowmeter ($0–280\,ml\,min^{-1}$, GPE Meterate 314–140/084), placed immediately after each Tenax TA. The system was designed to allow 14 measurements simultaneously. A blank with no branch in the gas exchange system was sampled at each measure.

After emission measurements, each branch sampled was cut off and stored in a portable refrigerator at +4 °C until being stored at −20 °C in the laboratory. Leaves from each branch were separated and lyophilized in order to measure their dry weight (DW).

The repeatability of the method was tested for each species before the experiment took place, by placing simultaneously four Tenax TA at the air stream outlet of a single teflon bag system. No differences appeared between terpene emissions trapped in the different Tenax TA for any species.

2.3. Terpene emission analyses and standard emission (E_s) calculation

Tenax TA with adsorbed terpenes were analyzed through thermal desorption by gas chromatography (GC) fitted with a Flame ionization detector (FID) (HP®5890 series II). Previous to thermal desorption, a preflush phase was run (3 min, $10\,ml\,min^{-1}$ 60 °C) to allow humidity in Tenax to be evacuated. Thermal desorption (Thermal Desorption Cold Trap injector, Varian®, CP4020-TCT model) was carried out through nitrogen carrier gas (10 min, $50\,ml\,min^{-1}$ 250 °C) and cryogenic concentration in a silica capillary trap, cooled with liquid nitrogen at −100 °C. Then, compounds were separated in the non-polar chromatographic column (Ultra 2, l: 50 m × i.d.: 0.2 mm × f.t.: 0.25 μm).

The identity of most terpenes was confirmed by comparison with standards of high purity (Aldrich-Firminich). Calibration of monoterpene and sesquiterpene factor response, used for calculating their concentrations, was performed periodically throughout the sampling period. Calibration curves

were always highly significant ($r^2 > 0.98$). In few cases, when standards were not available, peak identification was achieved by injection of previously extracted terpenes from each species, in Tenax TA. The identity of these compounds was determined through a GC (HP®6890) coupled with a mass spectrometer (MS) (HP®5973 Network Mass Selective Detector). Their quantitative analysis was achieved by considering the average of individual response factors of compounds whose standards were available.

Standard emissions (E_S) of monoterpenes and sesquiterpenes (E_{SM} and E_{SS}, respectively) were mainly calculated following the Tingey et al. (1980) algorithm (standardisation at 30 °C), since temperature is considered to be the main parameter controlling emissions from terpene storing species. For *P. halepensis*, only emissions of linalool and myrcene were standardized following Guenther et al. (1995), (standardisation at 30 °C and 1000 $\mu mol\, m^{-2} s^{-1}$) since Simon et al. (2005) reported that these compounds were both light and temperature dependent (and not only temperature dependent).

2.4. Tree age measurement

Since age of plants may modify their emission rates (Street et al., 1997), age of each sampled plant was also assessed. The main branch of each shrub was cut off in order to collect cross sections. Meticulous sanding of cross sections was necessary in order to evidence ring limits, since ring boundaries were difficult to detect. For each cross section, rings were counted along two radii, giving an estimation of the age of each plant. For *P. halepensis,* age was calculated following classical Methods of Dendrochronology (Schweingruber, 1988). Sampling of this species involved collecting cores as low as possible. Age was determined by counting rings of cores with pith after interdatation.

2.5. Soil analyses

For each experimental site, four 200g-soil samples from the A1 horizon were collected. The main physical and chemical properties were measured (Table 1). Some of these properties were measured in the laboratory: texture, pH, total organic carbon (TOC), total N (N) and C/N ratio. Other properties were analyzed in other laboratories: Ca^{2+}, Mg^{2+}, and K^+ (Environmental and Chemical Laboratory, University of Provence, FRE 2704), available P (P_A) and total P (P) (Agriculture and Chemical Laboratory, Montpellier, France).

2.6. Statistical analyses

Results are presented in mean ± SE throughout the paper. Statistical analyses of variance (ANOVA), with site as independent factor, and post hoc Tukey tests were used to analyse intra-calcareous and intra-siliceous differences with regard to (i) physical and chemical soil properties and (ii) E_S of each species. Student test was applied to test statistical calcareous and siliceous differences regarding (i) their physical and chemical properties and (ii) E_S of each species. Heterogeneity and

Table 1
Mean ± SE (in brackets) of the physical and chemical properties of collective calcareous and siliceous sites

Soil properties	*t*	Calcareous soils	Siliceous soils
pH (H_2O)	6.75***	7.26 (0.09)	5.92 (0.15)
Sand (%) (Aubert)	4.99***	28.06 (3.61)	57.95 (4.41)
Silt (%) (Aubert)	3.78***	54.30 (4.32)	30.56 (4.30)
Clay (%) (Aubert)	2.79**	17.63 (2.09)	11.49 (1.39)
Total organic carbon (TOC) (%) (Anne)	3.76***	5.05 (0.50)	3.04 (0.27)
N (%) (Kjeldhal)	4.07***	0.74 (0.13)	0.21 (0.02)
C/N	4.04***	7.68 (1.51)	15.63 (1.11)
Total P (P) ($mg\, kg^{-1}$)	2.46*	1129.13 (44.67)	783.78 (102.22)
Available P (P_A) ($mg\, kg^{-1}$) (Olsen)	2.69*	44.67 (4.34)	32.87 (2.58)
Ca^{2+} ($mg\, kg^{-1}$)	23.23***	9332.03 (456.80)	1442.84 (86.70)
Mg^{2+} ($mg\, kg^{-1}$)	0.45 ns	106.04 (21.30)	100.99 (13.74)
K^+ ($mg\, kg^{-1}$)	5.73***	590.20 (39.80)	309.70 (31.20)

Results of statistical differences between both soils are also shown through Student test (*t*) *p*: test significance. *$0.01 < p < 0.05$, **$0.01 < p < 0.001$, ***$p < 0.001$. ns: not significant ($p > 0.05$).

homogeneity among soils of the same nature and the dichotomy between calcareous and siliceous soils were represented through a Principal components analysis (PCA). For ANOVA and Student test, data were log transformed when necessary to achieve normal distribution requirements. Relationships between E_{SM} or E_{SS} and N, P_A, K^+ and Ca^{2+} of soils were tested by linear and non-linear regression analyses. Statistical analyses were conducted using Statgraphics® 4.1 and R® 2.3.0 programs.

3. Results

3.1. Physical and chemical soil differences

Differences between calcareous and siliceous soils are mainly due to their different nutrient availability (Table 1), which explains 49% of their dichotomy (Fig. 1). In a small extent, differences are also due to their texture properties (Fig. 1B). Thus, moderately alkaline calcareous sites have a lesser permeability than acid siliceous soils and a higher nutrient availability, except for Mg^{2+} (Fig. 1B, Table 1). Moreover, even if soils of the same nature are quite homogeneous (Fig. 1) some significant differences are observed (ANOVA, $p<0.05$, Tukey test). For instance, S3 and S4 have the highest sand content (Fig. 1B).

3.2. E_S changes on calcareous and siliceous soils

Plants growing in sites with the same soil show similar E_{SS} and E_{SM} (ANOVA, $p>0.05$), except for *C. albidus,* whose E_{SM} is significantly higher in S3, than in S1 and S2 (ANOVA, $p<0.05$, Tukey test). Therefore, soil effect on terpene emissions has been tested by grouping results of sites with the same soil. Only for *C. albidus*, tests have been performed with and without S3. Moreover, for all species, the age of plants located in calcareous and siliceous soils was similar (Student test, $p>0.05$).

R. officinalis mostly releases monoterpenes (Fig. 2), which represent 85% of total emissions on average. Major released compounds are α-pinene and β-pinene (Fig. 3, Table 3). E_{SM} of this species is significantly higher when plants grow in calcareous sites ($p<0.05$; Fig. 2), where E_{SM} is 3-fold higher than E_{SM} in siliceous sites. Furthermore, E_S of all major monoterpenes is significantly higher when *R. officinalis* grows in calcareous soils ($p<0.05$; Fig. 3). N, P_A and Ca^{2+} in soil are positively correlated with E_{SM} of this species ($p<0.05$; Table 2). By contrast, E_{SS} is similar when plants grow on calcareous and siliceous sites ($p>0.05$; Fig. 2). However, when the results are expressed in percentage, E_{SS} is significantly higher when *R. officinalis* grows in siliceous sites (15%) than when it grows in calcareous soils (5%) (Student test,

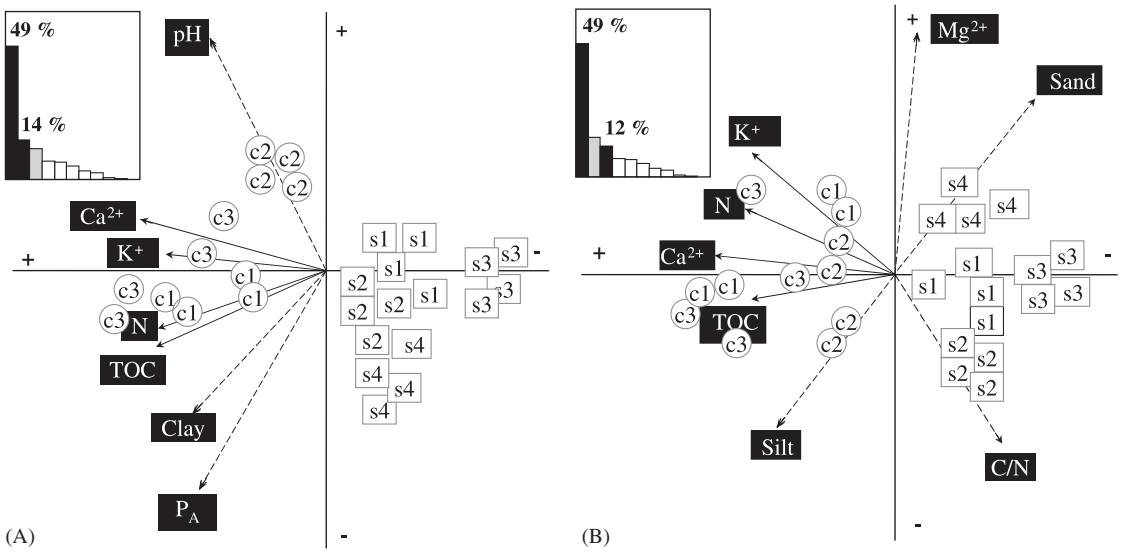

Fig. 1. Principal components analyses of the physical and chemical properties of calcareous and siliceous soils. Graphics A and B show calcareous and siliceous dichotomy according to variables explained in components 1 and 2 or components 1 and 3, respectively. Continuous arrows explain the weight of each variable on component 1 (*x*-axis). Discontinuous arrows explain the weight of component 2 or 3 (*y*-axis). Circles represent calcareous sites. Squares represent siliceous sites.

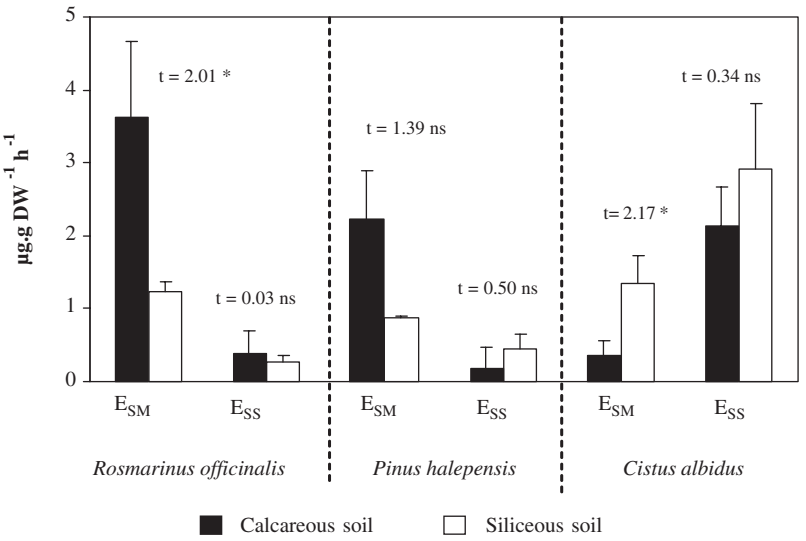

Fig. 2. Mean monoterpene and sesquiterpene standard emissions (E_{SM} and E_{SS}, respectively) of *R. officinalis*, *P. halepensis* and *C. albidus* in calcareous and siliceous soils. Bars indicate the SE, $n = 12$. Results on different emission rates between calcareous and siliceous soils through Student test (t) are also shown. p: test significance, $*0.01 < p < 0.05$. ns: not significant.

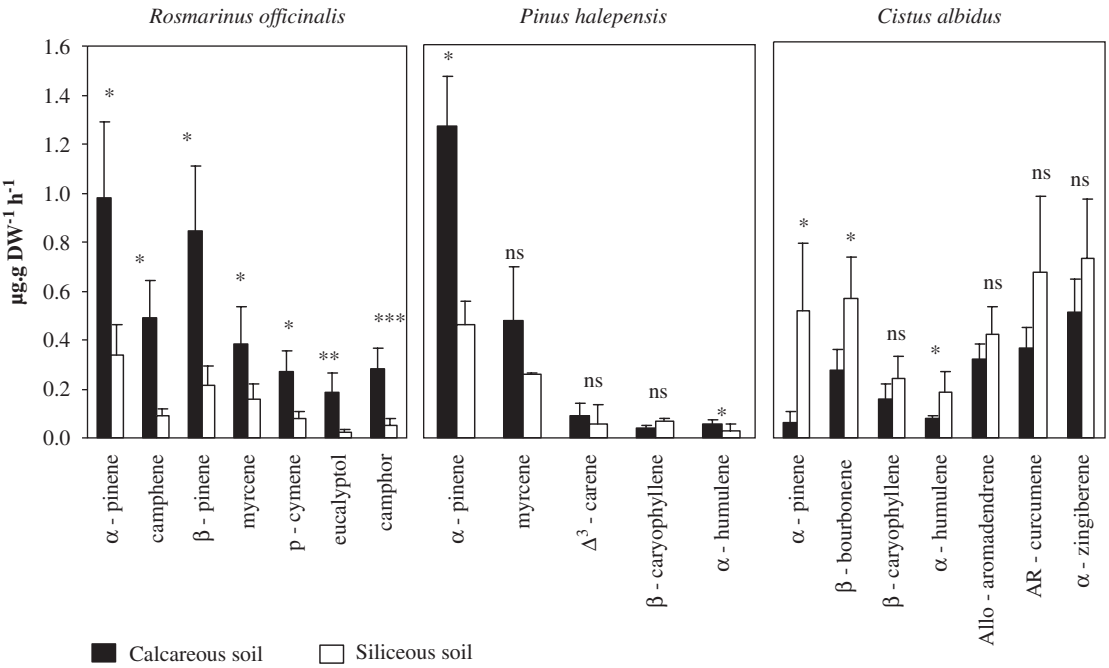

Fig. 3. Mean standard emission rates of each major compound emitted by *R. officinalis*, *P. halepensis* and *C. albidus*, in calcareous and siliceous soils. Bars indicate the SE, $n = 12$. Different emission rates are tested with Student test. p: test significance, $*0.01 < p < 0.05$, $**0.001 < p < 0.01$, $*** < 0.001$. ns: not significant ($p > 0.05$).

$p < 0.05$). No significant relationships were found between E_{SS} and soil nutrients ($p > 0.05$). Main sesquiterpenes detected for *R. officinalis* are α-humulene, α-muurolene and two unknown sesquiterpenes (Table 3).

P. halepensis mainly emits monoterpenes (Fig. 2, Table 3). They represent 82% of total emissions on average. Major monoterpenes are α-pinene and myrcene (Fig. 3). E_{SM} does not significantly vary according to soil nature ($p > 0.05$; Fig. 2). However,

Table 2
Relationship between mean standard monoterpene emissions (E_{SM}) ($\mu g\, g_{DW}^{-1}\, h^{-1}$) of *R. officinalis, P. halepensis* and *C. albidus* and total nitrogen (N) ($g\, kg^{-1}$), available phosphorous (P_A), K^+ and Ca^{2+} ($mg\, kg^{-1}$) in soil ($n = 6$)

Species	N			P_A		
	r	*P*	Eq. (*y*: E_{SM})	*r*	*p*	Eq. (*y*: E_{SM})
R. officinalis	0.94	0.005	$y = \exp(-0.26 + 0.18N)$	0.89	0.024	$y = \exp(-1.63 + 0.057P_A)$
P. halepensis	0.97	0.001	$y = 0.55 + 0.21N$	0.93	0.008	$y = -1.15 + 0.073P_A)$
C. albidus[(a)]	−0.89	0.016	$y = \exp(0.47–0.32N)$	−0.85	0.034	$y = \exp(3.00–0.11P_A)$
C. albidus[(b)]	−0.90	0.035	$y = \exp(-0.05–0.26N)$	−0.87	0.048	$y = \exp(1.87–0.08P_A)$
	K^+			Ca^{2+}		
R. officinalis		ns		0.86	0.025	$y = \exp(-0.07 + 1.3.10^{-4}Ca^{2+})$
P. halepensis	0.90	0.013	$y = \exp(-0.72 + 2.5.10^{-3}K^+)$	0.97	0.000	$y = \exp(-0.32 + 1.3.10^{-4}Ca^{2+})$
C. albidus[(a,b)]		ns			ns	

r: relationship coefficient, *p*: relationship significance. ns: not significant ($p > 0.05$). Best fitting relationship is shown. (a) Superscript indicates that correlations have been calculated with all siliceous sites. (b) Superscript indicates that correlations have been calculated without data from S3.

Table 3
Terpene emission composition of *R. officinalis* (*R.O*), *P. halepensis* (*P.H*) and *C. albidus* (*C.A*) in calcareous and siliceous soils considered together

RI	Monoterpenes	*R.O*	*P.H*	*C.A*	RI	Sesquiterpenes	*R.O*	*P.H*	*C.A*
931	α-pinene	*XXX*	*XXX*	*XX*	1382	Copaene	__	*X*(s)	*X*
948	Camphene	*XX*	tc	*X*	1406	β-bourbonene	__	*X*	*XX*
965	Sabinene	tc	*X*	*X*	1447	β-caryophyllene	tc	*X*	*XX*
970	β-pinene	*XXX*	*X*	*X*	1476	Unknown 1 ($m/z = 204$)	*X*	__	*XX*
974	Myrcene	*XX*	*XX*	*X*	1481	α-humulene	*X*	*X*	*XX*
1014	Δ^3-carene	tc	*X*	*X*(s)	1487	Unknown 2 ($m/z = 204$)	*X*	*X*	__
1036	*p*-cymene	*XX*	*X*	tc	1488	Allo-aromadendrene	__	__	*XX*
1039	Limonene	*X*	tc	__	1493	AR-curcumene	__	tc	*XXX*
1042	1,8-cineole	*XX*	tc(c)	__	1502	α-zingiberene	__	tc	*XXX*
1044	β-trans-ocimene	__	*X*	tc(s)	1519	α-muurolene	*X*	*X*	*X*
1064	γ-terpinene	tc	*X*	tc(s)	1522	Unknown 3 (γ-cadinene?) ($m/z = 204$)	__	*X*(s)	*X*
1094	Δ-terpinene	tc	tc(s)	tc					
1104	Linalool	tc	*X*	tc(s)					
1163	Camphor	*X*	*X*	__					
1183	Borneol	*X*	*X*	__					
1207	α-terpineol	*X*	*X*	__					

RI: retention index.
__: if the compound was not detected in samples. *X*: mean standard emission ($\mu g\, g_{DW}^{-1}\, h^{-1}$) both soils considered ($n = 24$). $0 < X < 0.1$, $0.1 < XX < 0.5$, $0.5 < XXX < 2.0$. (c) and (s) denote that the compound was only detected in plants growing in calcareous and siliceous sites, respectively. tc: trace concentrations.

the main released compound, α-pinene (50% of total emissions), is significantly higher when plants grow in calcareous sites ($p < 0.05$; Fig. 3). Whereas E_{SM} does not significantly vary with soil nature, it is positively correlated with all main soil nutrients examined (N, P_A, Ca^{2+} and K^+, $p < 0.05$; Table 2). *P. halepensis* also emits sesquiterpenes, which show similar emission rates in both soils ($p > 0.05$, Fig. 2). However, the percentage of all major sesquiterpenes is significantly higher in siliceous soils (Student test, $p < 0.05$), as observed for *R. officinalis*. While these compounds represent 23% of total emissions in siliceous sites they reach 6% in calcareous sites. Moreover, E_{SS} is not significantly correlated with any soil nutrients examined here ($p > 0.05$; Table 2). Main detected sesquiterpenes are α-humulene,

β-caryophyllene, α-muurolene and two unknown compounds (Table 3). α-humulene and β-caryophyllene can be considered as major compounds. Only E_S of α-humulene is significantly higher in plants occurring in calcareous soils ($p<0.05$; Fig. 3).

C. albidus shows the lowest monoterpene emission rate of the three species reported in this study (Fig. 2). E_{SM} of this species is significantly higher in siliceous soils ($p<0.05$; Fig. 2). α-pinene explains these differences ($p<0.05$; Fig. 3), since it appears as a major compound in siliceous soils alone. E_{SM} of this species is negatively correlated to N and P_A ($p<0.05$; Table 2) and neither Ca^{2+} nor K^+, are significantly correlated to E_{SM} ($p>0.05$; Table 2). When monoterpenes from S3 are excluded, results do not change and the significant negative correlation with N and P_A is maintained ($p<0.05$; Table 2). E_{SS} does not significantly vary according to the soil nature ($p<0.05$; Fig. 2) and is not significantly correlated with any of the soil nutrients selected ($p>0.05$). Numerous sesquiterpenes, such as, AR-curcumene or α-zingiberene, constitute major compounds in emissions from *C. albidus* (Table 3). In contrast to *R. officinalis* and *P. halepensis*, a similar contribution to total emissions (76%) by the main sesquiterpenes is shown in calcareous and siliceous soils (Student test, $p>0.05$). However, plants growing on siliceous sites show higher E_S of β-bourbonene and α-humulene (major compounds), than those on calcareous sites ($p<0.05$; Fig. 3).

4. Discussion

4.1. Comparison of terpene emissions of study species with bibliographical data

E_{SM} obtained in this study for *R. officinalis* was $2.38\,\mu g\,g_{DW}^{-1}\,h^{-1}$, which is within the range of results reported by Hansen et al. (1997) in spring ($\sim 2.2\,\mu g\,g_{DW}^{-1}\,h^{-1}$). These authors found similar major compounds to those reported here but in different relative amounts.

E_{SM} shown in this study for *P. halepensis* was $1.54\,\mu g\,g_{DW}^{-1}\,h^{-1}$. This is within the order of rates reported in September and June by Owen et al. (2001), but 5-fold lower than those found in April by Peñuelas and Llusia, (1999), and almost 10-fold lower than those shown by Simon et al. (2005). Major compounds reported here for this species are α-pinene and myrcene, as the two first works cited above reported. However, Simon et al. (2005) showed linalool and β-trans-ocimene as the main emitted compounds of this species, while these compounds are not the major compounds in this study (Fig. 3).

For both, *R. officinalis* and *P. halepensis* novel data are shown here with regard to their sesquiterpene emissions. This emission type had never been reported in previous studies, probably because of differences in analysis protocols and sampling techniques.

This study corroborates that *C. albidus* is mainly a sesquiterpene emitter, as reported by Llusià and Peñuelas, (1998). However, few and confusing data are available to date for this species in the Mediterranean region. Thus, Owen et al. (2001), did not find any detectable terpene emission in late spring for *C. albidus*. Moreover, Llusià and Peñuelas (2000) showed that this species had the highest emission rate in spring, with $30\,\mu g\,g_{DW}^{-1}\,h^{-1}$, that is 3-fold higher than rates reported here. These authors also found that its monoterpene emissions outstripped sesquiterpene emissions all over the seasonal cycle and among sesquiterpenes, only β-caryophyllene was detected in their study. These differences may be attributable to experimental conditions, sampling techniques, sun and shade exposed leaves as well as primary and secondary leaves. Furthermore, these authors carried out their experiment at the end of April (after budbreak) while this study was carried out in the middle to end of March (during leaf growth), suggesting that the phenological state of *C. albidus* could explain differences between both studies.

The possibility of finding a wide range of emission rates for a single species underlines the complexity of (i) integrating their standard emissions in biogenic emission inventories and (ii) comparing results from estimated emissions and experimental data.

4.2. E_{SM} and E_{SS} of study species in calcareous and siliceous soils

R. officinalis and *P. halepensis* responded similarly to soil nature. For *R. officinalis*, E_{SM} (expressed in a dry mass basis and in percentage) (Fig. 2) and E_S of major monoterpenes (e.g. α-pinene) (Fig. 3) were higher on calcareous soils. For *P. halepensis*, E_{SM} (expressed in percentage), E_S of α-pinene (50% of its total emissions) and α-humulene were shown to be higher in plants growing in calcareous soils. Because these species are mainly calcicole species, which implies that they

are mainly widespread on calcareous soils, the results found here could have important implications for the local photochemical pollution, since for instance, α-pinene has been demonstrated to be highly and rapidly reactive in the atmosphere (Yu et al., 1999). The results obtained here for *P. halepensis* could be particularly relevant since this species occupies important surfaces in the Mediterranean region, in France (241 000 ha, IFN, national forest inventory data) and in the PACA Region, where the experiment took place. In this region, *P. halepensis* is considered as the main resinous species and represents 12% of the land surface.

As shown in Table 2, while soil nutrients were never correlated to E_{SS} of these two species, they affected similarly their E_{SM}. Thus, E_{SM} of *R. officinalis* and *P. halepensis* was favored by N, P_A, Ca^{2+} or N, P_A, K^+, and Ca^{2+}, respectively. N and *P* are considered to be the most important nutrients limiting plant growth (Schukze and Chapin, 1987), they are involved in carbon assimilation (Lambers et al., 1998) and carbon allocation to defence compounds (such as terpenes) (Mihaliak and Lincoln, 1985). However, there is evidence that these two nutrients control plant growth in different ways (Portsmuth et al., 2005) and that P requirements for growth can even be higher than those of N. K^+ is considered as the most important solute in plants, which influences complex processes such as photosynthesis (Läuchili and Bieleski, 1983). Ca^{2+} plays a variety of significant roles in the biological processes of plants, such as growth (Bush, 1995) or photosynthetic system protection, under high levels of irradiance and heat (Zhao and Tan, 2005). While all these nutrients could affect terpene synthesis potentially, only soil N has been directly put into relation with leaf terpene emissions. Thus, Gouinguene and Turlings (2002) demonstrated, by supplying different doses of *N* fertilizers, that monoterpene and sesquiterpene emissions of corn plants, induced by the attack of an herbivore species, were at a minimum under lower nutrient contents. Moreover, Turtola et al. (2002) studied emission of Scots pine xylem after long-term fertilization (N, P and Ca^{2+}). They found no significant terpene variations. Therefore, no study has considered constituent (in opposition to induced) terpene emissions from leaves, under either artificial or natural N, P, K^+ and Ca^{2+} gradients.

Contrary to *P. halepensis* and *R. officinalis*, E_{SM} of *C. albidus* was higher in siliceous sites (Fig. 2) and was negatively correlated to N and P_A (Table 2). Despite the fact that E_{SM} of *C. albidus* was up to one order of magnitude higher in S3 than in S1 and S2, when data from S3 were discarded, the negative correlations with soil nutrients (N and P_A) were maintained. Moreover, it is unlikely that other environmental factor own to S3 is implicated on the greater emissions of *C. albidus* in this site, since terpene emissions of *P. halepensis* were similar in all siliceous sites. Furthermore, S4 showed similar sand content than S3 (Fig. 1), while this particularity did not result in different emission rates of *R. officinalis* in siliceous sites. Thus, it can be affirmed that *C. albidus* released higher E_{SM} on siliceous soils for the study period.

As shown for *P. halepensis* and *R. officinalis*, E_{SS} of *C. albidus* was never correlated to soil nutrients (Table 2). The fact that E_{SM} of all study species varies according to soil nutrient availability, while their E_{SS} is independent on soil nutrient content could be due to the fact that these terpenes are synthesized through different pathways. This suggests that sesquiterpene synthesis pathway maybe requires different nutrient supply.

Different monoterpene dependency on the soil nature of *C. albidus* cannot be explained by its different soil preferences, because all species were mainly installed on calcareous soils. It was hypothesized that the different phenological state exhibited by *C. albidus* (leaf growth state), in comparison to that of *R. officinalis* and *P. halepensis* (pre-budbreak state) could explain the negative monoterpene emission dependency of *C. albidus* on soil nutrient content. Two studies seem to corroborate this hypothesis. Firstly, Lerdau et al. (1995) found that leaf terpene emissions of *Pseudotsuga menziesii* Mirb., were correlated negatively to plant N content during leaf expansion exclusively, while the opposite response was observed at the pre-budbreak in spring, as this study shows for *R. officinalis* and *P. halepensis*. Secondly, Robles (1998) suggested that N uptake of *C. albidus* during leaf growth in Provence was mostly allocated to budbreak and that this allocation pattern occurred particularly in calcareous soils. Consequently, exceptionally during this phenological state, *C. albidus* leaves showed lesser nitrogen content at calcareous sites than at siliceous sites. Moreover, the fact that *C. albidus* releases monoterpenes which are not previously stored in leaf pools (Llusià and Peñuelas, 2000), as occurs in the other two species, could be linked to its different response to soil nutrient availability. Thus, while *R. officinalis* (Moretti et al., 1998), and

P. halepensis (Llusià and Peñuelas, 2000) store both monoterpenes and sesquiterpenes, only sesquiterpenes have been detected in leaves of *C. albidus* (Robles and Garzino, 1998). This implies that monoterpene emissions of *P. halepensis* and *R. officinalis* are potentially more dependent on monoterpene pools, whose size and number have been found to increase under higher nitrogen concentrations (Bjorkman et al., 1998).

Results obtained here support the idea that different emission rates of study species growing in calcareous and siliceous soils is a consequence of the different nutrient availability owing the specific nature of each kind of soil. However, the possibility that these results were linked to the occurrence of different ecotypes on calcareous and siliceous soils was examined. An ecotype consists of a population adapted genetically to particular ecological conditions (Ramade, 2002). These genetical differences explain why stands which belong to a different ecotype show often different morphological traits or different emission composition, as occurs for ecotypes of *Quercus suber* L. in different European localities (Loreto, 2002). In this study, the occurrence of different ecotypes for the same species is ruled out, since α-pinene and *β*-pinene from *R. officinalis*, α-pinene and myrcene from *P. halepensis*, and AR-curcumene and α-zingiberene from *C. albidus* were the most widely released compounds in both soils.

5. Conclusion

This study shows that the differentiation between calcareous and siliceous soils may be helpful for explaining the overall monoterpene emission variability between individuals of the same species, while for sesquiterpenes this differentiation is restricted to individual compounds. Moreover, N and P_A concentrations in soil seem to be more efficient soil properties than Ca^{2+} and K^+ for estimating E_{SM} of study species. None of these soil nutrients is efficient for assessing E_{SS}, suggesting that nutrient requirements for sesquiterpene synthesis are different and that sesquiterpenes must be rather modified by other environmental factors. It should be also borne in mind that the same species studied here could show different responses at other seasonal cycles, since the pattern of nutrient allocation to terpene synthesis has been demonstrated to vary according to the phenological state (Lerdau et al., 1995). Finally, this study highlights that further work on sesquiterpene dependency on other abiotic factors is necessary since they can constitute the most important emissions in some species.

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