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Isoprenoid emissions and physiological activities of Mediterranean macchia vegetation under field conditions.

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Abstract

The emissions of volatile organic compounds, VOC, from plants have strong relevance for plant physiology, plant ecology and atmospheric chemistry. We report here the emission rates and the ecophysiological behaviour of the Mediterranean plant species *Chamaerops humilis* L., *Pistacia lentiscus* L. and *Juniperus phoenicea* L. Emission measurements made by means of a dynamic branch enclosure under field condition indicated that *Chamaerops humilis* is a strong emitter of isoprene at a diurnal rate of 41.4 ng m^{-2} s^{-1}. Lower daily emission rates were measured for the monoterpene emitter shrubs *P. lentiscus* (13.9 ng m^{-2} s^{-1}) and *J. phoenicea* (4.1 ng m^{-2} s^{-1}) compared to *C. humilis*. A good correlation was found between isoprenoid emission rate and temperature. Monoterpene emission from the reservoir of *Pistacia* and *Juniperus* and isoprene released by Chamaerops leaves enhanced during periods of increasing irradiation and temperature in the middle of the day. The daily trends of photosynthesis showed higher values at morning and a greater CO_2 assimilation in *Pistacia lentiscus* leaves compared to the other species. For leaf transpiration we observed a slight decrease in the afternoon in *Chamaerops* leaves and a fairly constant transpiration in the other two shrubs. Xylem water potential measurements indicated that all the species were not subjected to a severe drought stress.

Abbreviations: A: net CO_2 assimilation; T: transpiration; g_s: stomatal conductance; WUE: water use efficiency; A/g_s: intrinsic water-use efficiency; PPFD: photosynthetic photon flux density

Introduction

It is well recognized at present that the vegetation of Mediterranean type ecosystems represents an important source of reactive volatile organic compounds (VOC) of the atmosphere. Among the most abundant isoprenoids, isoprene and monoterpenes are estimated to contribute 44% and 11%, respectively, to the global vegetation VOC budget of 1150 Tg C yr^{-1} (Guenther et al., 1995). These compounds, categorized as secondary metabolites, are known to play a role in allelopathy, thermal protection, chemical defence and attraction for pollinators (Kesselmeier and Staudt, 1999; Loreto and Velikova, 2001; Peñuelas and Llusià, 2004; Peñuelas et al., 2005; Sharkey 2005; Velikova and Loreto, 2005). Secondary metabolites also commonly accumulate with stress and there are indications that some stresses enhance isoprene emissions (Sharkey et al., 1991; Sharkey and Yeh, 2001). These highly reactive biogenic hydrocarbons are also involved in the formation of photochemical oxidants like ozone (Fehsenfeld et al., 1992), in the development of aerosols (Andreae and Crutzen, 1997), in the depletion of hydroxyl radicals (OH) in the troposphere and they also influence the global carbon budgets of ecosystems. Since the production and emission of monoterpenes and isoprene are species-specific, the emissions from an ecosystem are dependent on the species composition and abundance in a landscape (Baraldi et al., 2002). Different environmental and physiological factors can control the type and emission rates of these volatile hydrocarbons (Tingey et al., 1991; Staudt and Seufert, 1995; Street,1995; Ciccioli et al., 1997; Kesselmeier et al., 1996). The most important environmental variables are irradiance, temperature (Sharkey and Loreto, 1993; Staudt and Seufert, 1995; Loreto et al., 1996;

Staudt and Bertin, 1998; Guenther et al., 1993), water availability (Bertin and Staudt, 1996; Llusià and Penuelas, 1998; Penuelas and Llusià, 1999; Plaza et al., 2005) and humidity (Dement et al., 1975).

Isoprene, the most abundant biogenic VOC, is emitted immediately upon production and the emission is strongly dependent on light and temperature (Loreto and Sharkey, 1993; Monson et al., 1995; Geron et al., 2000). Monoterpenes can be or not stored in large pool in specialized structures (Seufert et al., 1995; Loreto et al., 1996; Lluisià and Penuelas, 1998; Peñuelas and Lluisià, 2001). In storing-species there is not an instantaneous light influence on the emission, but, instead, the emission rate is controlled mainly by temperature through a strong influence on the gas vapour pressure and on the resistance of emission pathway (Staudt et al., 1997; Llusià and Peñuelas, 1999; Tingey et al., 1991; Monson et al., 1995; Peñuelas and Lluisià, 2001). In the non storing-species *Quercus ilex* the high monoterpene emission rate was found to be under the short-term control of light (Seufert et al., 1995; Loreto et al., 1996; Street et al., 1997).

In this work we report the emission rates and the ecophysiological behavior of relevant species widespread in a Mediterranean area characterized by a prolonged dry summer with high air temperature and solar radiation and thus with high photochemical activity. Moreover, drought, high irradiance and temperature may strongly influence the ecophysiology of plants (Filella et al., 1998) and, by consequence, the emission.

Materials and methods

Site description

The experiment was performed in a coastal flat area located at 74 m above mean sea level in a nature reserved on the Capo Caccia peninsula (Alghero) of Sardinia Island (latitude 40° 36' 18"N; longitude 8° 09' 07"E). The 1200 ha of the reserve are characterized by typical Mediterranean climate, with 49% rainfall in the winter and 36% in autumn. This site is considered one of the most arid and hot zones of Sardinia with mean annual precipitation of 640 mm. The experimental site is populated by a typical coastal Mediterranean macchia dominated by *Juniperus phoenicea* L. (Juniper), *Pistacia lentiscus* L. (lentisk), and *Chamaerops humilis* L. (dwarf fan palm). 52.7% of the surveyed area is covered by juniper, followed in decreasing order by lentisk (22.1%), and dwarf fan palm (2.8%). The heterogeneous shrubland vegetation is distributed in patches in which *Chamaerops* is isolated. The site was an intensive study site of the MEDEFLU European project, and meteorological as well as exchange of energy, CO_2 and H_2O have been measured continuously using eddy covariance.

Isoprenoid emissions

To screen the day and night emission of the selected species, trees, shrubs and branches were selected as being visually representative of the species and in reasonable health. Emission rates were measured between 2:00 and 11:00 a.m. and 14:00 and 23:00 p.m. during two field campaigns at the beginning of June on sun-exposed branches by means of branch-enclosure technique. This system used a dynamic (open-flow) enclosure consisting of a rigid aluminum frame covered by a flexible, transparent Teflon bag. Branches were enclosed in these 12 l cuvettes that were flushed with carbon filtered air at a rate of 12 l min^{-1} measured by mass-flow meters. This flow rate prevents a large increase of the leaf temperature that could stress the branch and induce stomata closure. Only small differences (2°C) were observed between leaf and air temperature. All cuvettes were equipped with a bladed impeller to provide adequate mixing of the air inside the chamber. It was mounted in the upper face of the chamber and driven by a motor outside the chamber. Special attention has been paid to avoid leaf damage that can result in alteration of emission rate in terpene-storing species. After insertion the branches were left to equilibrate for 30 min before collecting a sample by using an aspirating pump connected to a mass flow meter. The flow rate ranged between 100 and 200 ml min^{-1} through the sampling carbon cartridges (Carbograph 1 and 2, Lida, Roma, Italy) connected directly through Teflon lines. The sampling time was 5 min for the isoprene emitter species and 15 min for the monoterpene species. A blank with no branch in the cuvette was sampled at the end of each measuring days to check isoprenoid adsorption by the materials used in the bags. The cartridges were then sealed, stored in the laboratory at 4°C before analysis. Isoprenoids were analyzed by gas-chromatography mass-spectrometry as previously described in detail (Baraldi et al*.,* 1999; Rapparini et al., 2001). Briefly, the trapped compounds were thermodesorbed at 250°C and cryofocused at –150° C (Thermal Desorption Cold Trap Injector) on a fused silica liner connected to a gas chromatograph-mass spectrometer (5890-5970 Hewlett Packard, Palo Alto, CA, USA). The desorbed sample was injected into a 60 m x 0.25 mm I.D. 0.25 µm film thickness capillary column (Hewlett Packard HP1). After sample injection, oven temperature was maintained at 40°C for 10 min and then programmed to 220°C at 5°C min^{-1}. Monoterpene identification was confirmed by comparison of retention time and mass spectra of authentic standards, while quantification was performed after calculation of standard curves and response factors for each compound, and using d_{14}-cymene as internal standards (ISTD).

After isoprenoid emission measurements, the leaves were removed from inside the cuvette and the leaf area was measured using an image analysis system giving the projected leaf area. VOC concentrations in the inflowing and outflowing air were used to obtain emission rate using air volume introduced into the chambers and referring the data per square meter of leaf. For the image acquisition a CCD camera (JVC model TK-880) was used, interfaced with a computer by an ELVIS board and Chameleon software (Sky Instr.Ltd., UK).

We followed the diurnal cycle of isoprene and monoterpene emission rate, leaf assimilation rate (A), transpiration (T), stomatal conductance (g_s), water use efficiency (WUE) and xylem water potential. Intrinsic water-use efficiency was also calculated as A/g_s.

Physiological measurements

Physiological activity of the same shrubs utilized for VOC assessment was verified by measuring leaf assimilation rate (A), transpiration (T), and stomatal conductance to water vapor (g_s) by means of a portable infrared gas analyzer (LCA2, ADC, UK) and an electronic pressure chamber. The measurements were made by enclosing in the gas-exchange cuvette a sun-exposed single leaf and replicated on at least three different leaves for each species. The hour average values of net assimilation and leaf transpiration were expressed in micromoles of carbon assimilated and millimoles of water transpirated per leaf area projected and second (µmol CO_2 m^{-2} s^{-1}, mmol H_2O m^{-2} s^{-1}), respectively. Pre-dawn and daily xylem water potential were measured with a portable pressure chamber (PMS Instruments Co, USA) (Scholander and Hammel, 1964) on five 1-year-old shoots for each species.

The water use efficiency (WUE), defined as the ratio between photosynthesis (A) and water loss in transpiration (T) (Bazzaz, 1996), was utilized to characterize the gas exchange for the different species.

Climate data were obtained from meteorological station located at 5 km from the experimental area.

Statistical analyses of variance (ANOVA) and regression were conducted using SAS (SAS System 8.1, SAS Institute Inc., Cary, NC, USA).

Results and Discussions

Meteorology

Weather during the period of measurements was sunny and warm. Mean diurnal fluctuations in PPFD and air temperature for the days of data collection are depicted in Figure 1. Daytime temperature maximum averaged 24°C over the experimental period and nighttime temperatures were mild, never falling below 18°C. Relative humidity value varied between 60% and 96% at daytime. PPFD showed a typical daily cycle with maximum values of 1700 µmol m^{-2} s^{-1} at noon.

Isoprenoid emissions

Monoterpenes were the most abundant isoprenoids emitted by *Pistacia lentiscus* and *Juniperus phoenicea* (Tab.1). These results are consistent with previous studies (Hansen et al., 1997; Owen et al., 1997). It has already been reported that *J. phoenicea* (members of the Coniferae family) and *Pistacia* (members of Anacardiaceae family) contain some volatile

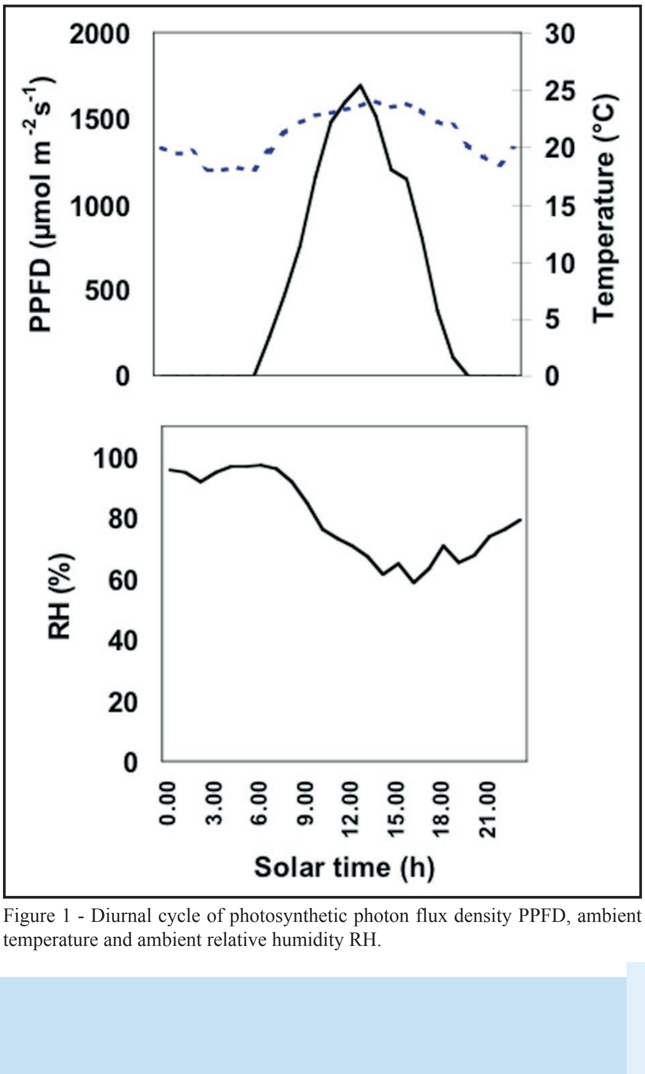

Figure 1 - Diurnal cycle of photosynthetic photon flux density PPFD, ambient temperature and ambient relative humidity RH.

Table. 1 Percent composition of isoprenoid emission.

Compounds	*Pistacia lentiscus* L.	*Juniperus phoenicea* L.	*Chamaerops humilis* L.
isoprene	1.4	3.9	100
α-pinene	54.2	43.1	
camphene	4.9	1.8	
sabinene	6.8	0.2	
β-pinene	8.8	0.7	
β-myrcene	2.0	8.0	
α-phellandrene	0.7	1.2	
Δ_3-carene	0.4	7.0	
α-terpinene	0.4	0.3	
p-cymene	8.3	5.4	
β-phellandrene	2.2	16.4	
limonene	8.9	9.7	
γ-terpinene	0.8	0.8	
α-terpinolene	0.3	1.5	

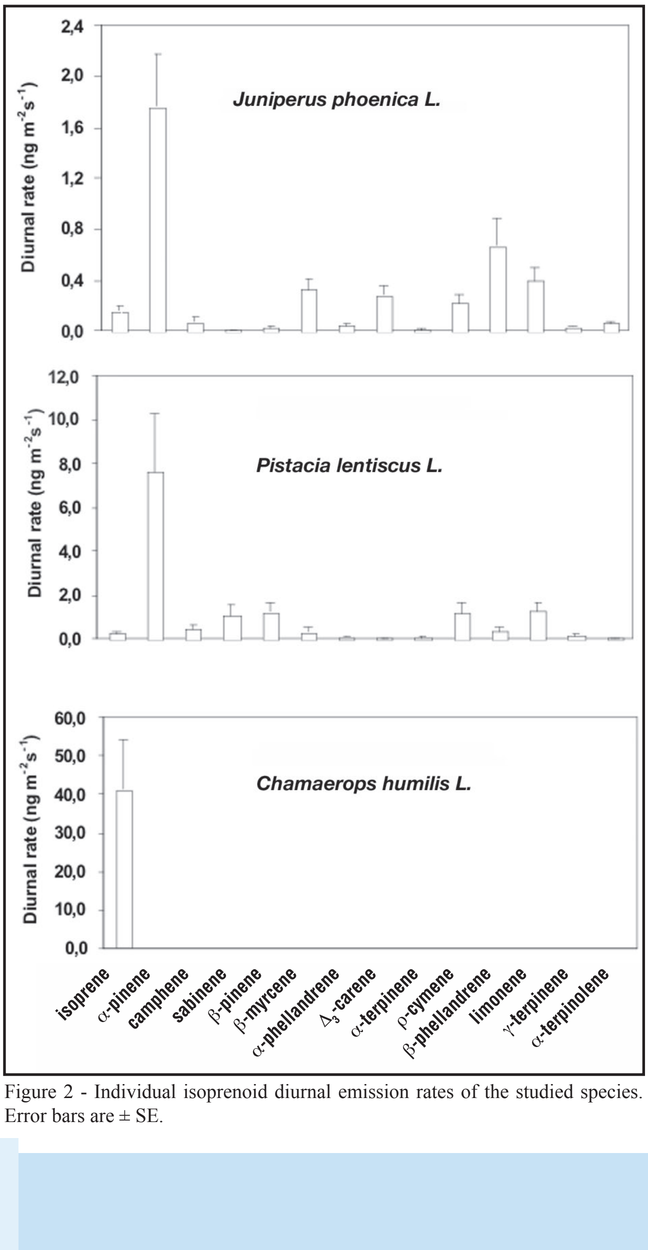

Figure 2 - Individual isoprenoid diurnal emission rates of the studied species. Error bars are ± SE.

oils in resin canals and that their emission occurs *via* diffusion through the epithelial cells and the fibres surrounding the canals (Hegnauer, 1964; Seufert et al*.,* 1995, Rossi et al., 2001; Rotondi et al*.,* 2003).

Chamaerops humilis, similarly to other Arecaceae (Palmae; Geron et al., 2002), was a strong isoprene emitter (Kesselmeier et al., 1999), while few monoterpene were released only in traces.

Thirteen monoterpenes were identified in *Pistacia lentiscus* and *Juniperus phoenicea* emissions with α-pinene being the monoterpene most emitted by both lentisk (54%) and juniper (43%), followed by limonene (8.9%), β-pinene (8.8%), ρ-cymene (8.3%) in *P. lentiscus*, and by β-phellandrene (16%), limonene (9.7%), and β-myrcene (8 %) in *J. phoenicea* (Tab. 1). Traces of isoprene were found in all species.

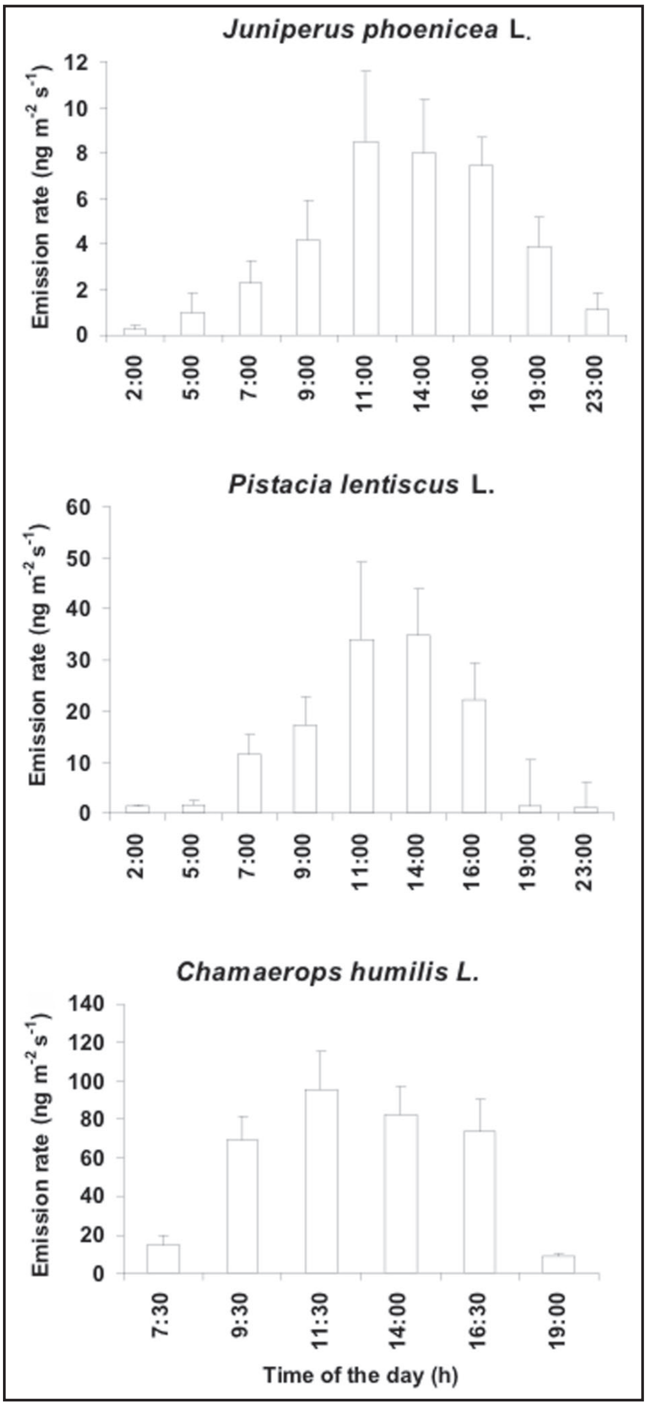

Figure 3 - Diurnal cycle of total isoprenoids. Hours are express in solar time. Error bars are ± SE.

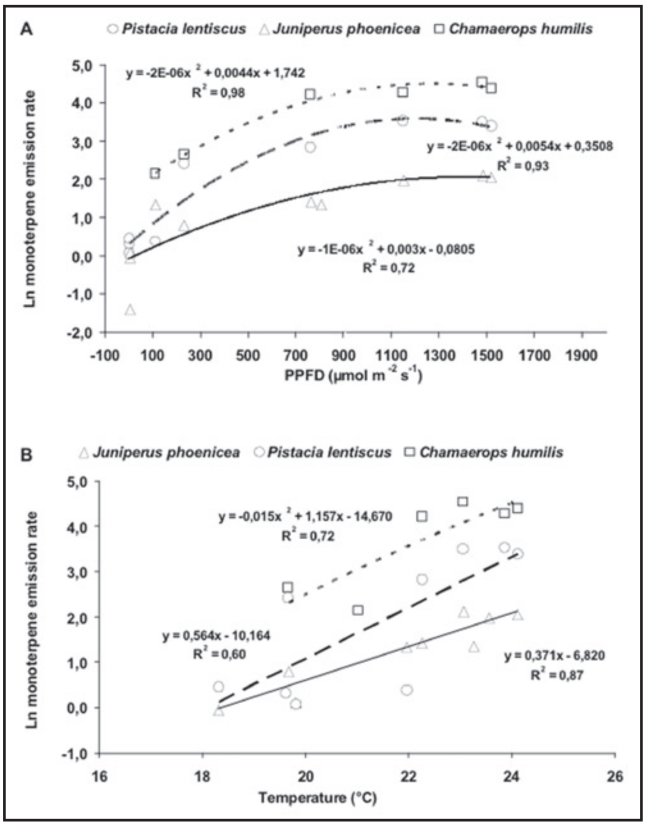

Figure 4 - Emission rates from *Juniperus phoenicea, Pistacia lentiscus* and *Chamaerops humilis* plotted against PPFD (A) and temperature (B).

Daily average monoterpene emission rates ranged between 4.1 ng $m^{-2} s^{-1}$ in juniper and 13.9 ng $m^{-2} s^{-1}$ in lentisk (Fig. 2). In *Chamaerops humilis* isoprene was emitted at a diurnal rate of 41.4 ng $m^{-2}s^{-1}$.

In all species, the emission rate showed a diurnal trend with peak emissions around midday-first afternoon and the lowest emission during the night (Fig. 3). The maximum total emission rate of juniper and lentisk was 8.0 and 34.8 ng $m^{-2} s^{-1}$, respectively, while dwarf fan palm was found to have a maximum total isoprene emission rate of 95.3 ng $m^{-2} s^{-1}$. At the time of the lowest total emission rate only α-pinene, camphene, ρ-cymene and limonene were above the detection limit in lentisk, and only β-myrcene, Δ_3-carene and β-phellandrene in juniper (data not shown).

A curvilinear relationship of isoprenoid emission rates with PPFD was found in juniper (r^2>0.72, P<0.05), lentisk (r^2>0.93, P<0.05) and dwarf fan palm (r^2>0.98, P<0.05; Fig. 4A).

Monoterpene emission of *J. phoenicea* was strongly correlated with temperature (r^2>0.87, P<0.05; Fig 4B) while the emissions from lentisk showed moderate temperature correlation (r^2>0.60, P<0.05). Isoprene emission from dwarf fan palm showed a strong correlation with temperature (r^2>0.72, P<0.05). Because temperature and PPFD are partially dependent, these correlations may also include a PPFD effect.

The percentage of carbon lost as isoprenoids by the foliage of all the tested species was less than 0.01% of the carbon fixed by photosynthesis.

Photosynthetic activity and water relations.

Pistacia lentiscus L. was characterized by a higher photosynthetic response compare to juniper and, early in the morning, also compare to *Chamaerops* (Fig. 5). The photosynthetic activity of lentisk and dwarf fan palm exhibited a

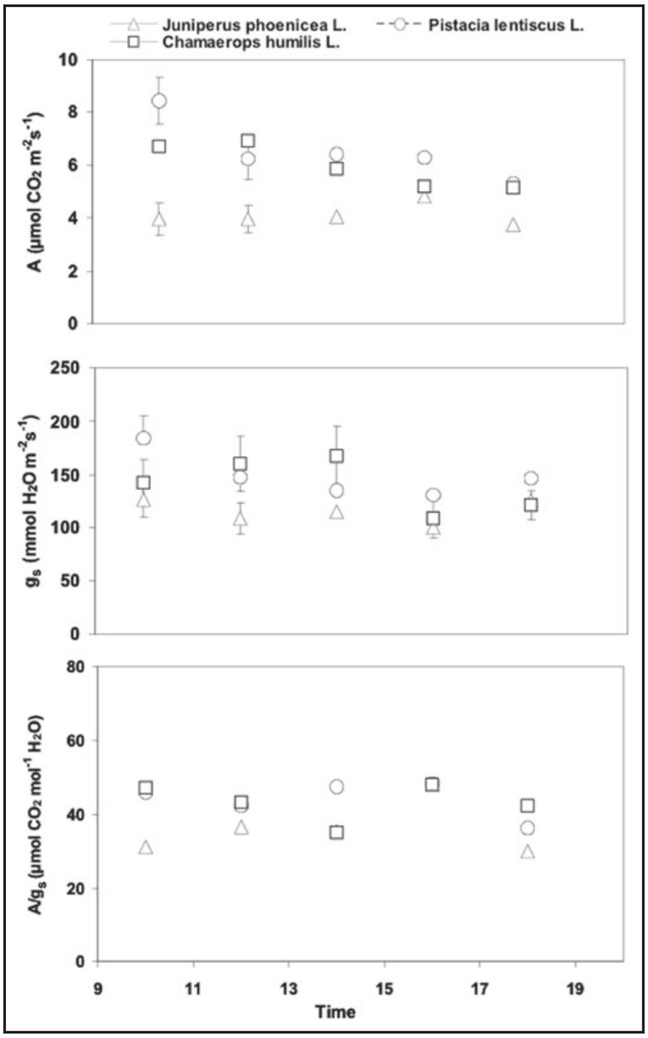

Figure 5 - Diurnal course of CO_2 assimilation (A), H_2O stomatal conductance (g_s), and intrinsic water use efficiency (A/g) measured on *Juniperus phoenicea, Pistacia lentiscus* and *Chamaerops humilis*. Hours are expressed in solar time.

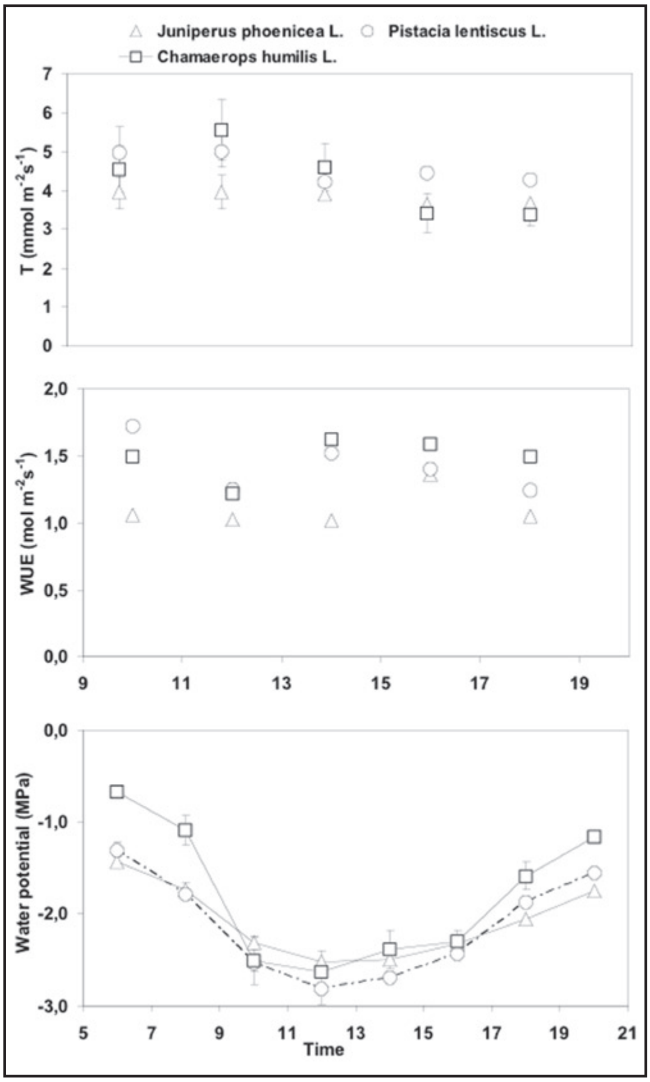

Figure 6 - Time course of changes in H_2O transpiration (T), water use efficiency (WUE) and xylem water potential measured on *Juniperus phoenicea, Pistacia lentiscus* and *Chamaerops humilis*. Hours are expressed in solar time.

typical summer Mediterranean trend with maximum rate in the morning (8.4 and 6.7 µmol m^{-2} s^{-1}) as similarly found in other Mediterranean environment (Manes et al., 1997). During the day, CO_2 assimilation was almost constant and then decreased late in the afternoon (around 5.3 µmol CO_2 m^{-2} s^{-1}). On the contrary, *J. phoenicea* exhibited only slight variations of net photosynthesis with respect to the morning values (3.9 µmol CO_2 m^{-2} s^{-1}).

Stomatal conductance of *Juniperus* leaves was quite constant through the day with values ranging between 130 mmol H_2O m^{-2} s^{-1} and 100 mmol H_2O m^{-2} s^{-1} (Fig. 5). The leaves of lentisk and *Chamaerops* were characterized by higher stomatal conductance compare to Juniperus, especially in the morning.

Leaf transpiration of juniper and lentisk shrubs showed an almost constant trend during the day with slightly greater transpiration in lentisk leaves (Fig. 6). The transpiration of dwarf fan palm leaves was maximum around 9 a.m. (5.5 mmol m^{-2} s^{-1}), while it reached the lowest values in the afternoon (3.4 mmol m^{-2} s^{-1}).

Water use efficiency reached values that were around 1.0 mol m^{-2} s^{-1} in juniper and varied between 1.7 mol m^{-2} s^{-1} and 1.2 mol m^{-2} s^{-1} in lentisk (Fig. 6). In the afternoon *Chamaerops* showed a greater water use efficiency compare to the other shrubs.

Water potential, higher at pre-dawn in *Chamaerops*, decreased in the warmest hours of the day in all the tested species down to around – 2.5 MPa (Fig. 6). Then, all plants were able to recover in a relative short time as indicated by the evening values (ranging from -1.2 and -1.7 MPa, depending on the species) that nearly reached the pre-dawn values (from 0.7 to -1.4 MPa). These data suggested that the overall water supply was sufficient for all species that were not subjected to severe drought stress.

Conclusions

Thirteen monoterpenes were identified by GC-MS in the emissions from the storing species *P.lentiscus and J. phoenicea*. The main compounds were α-pinene, β-pinene, β--myrcene, ρ-cymene, β-phellandrene and limonene. Among the detected monoterpene, α-pinene accounted for more than 40% in juniper and more than 50% in lentisk. Several other monoterpenes were detected in minor quantities or traces, namely camphene, sabinene, β-pinene, β--myrcene, α-phellandrene, Δ_3-carene, α-terpinene, ρ-cymene, β-phellandrene, limonene, γ-terpinene, and α-terpinolene.

To our knowledge, the emission of dwarf fan palm has been estimated only in relation with pollinator-attracting odors (Dufa et al*.,* 2003; Dufa et al., 2004; Caissard et al., 2004). In fact, the dioecious dwarf palm offers an interesting case since the volatile compounds, mainly monoterpenes and sesquiterpenes, that attract the specific pollinator to this species are produced by leaves, not by flower, and only during the flowering season. In this study we have estimated that, as other palmae, isoprene was the dominant volatile hydrocarbon released by the leaves of *Chamaerops humilis* at a diurnal rate of 41.4 ng $m^{-2} s^{-1}$ while few monoterpenes were present only in traces.

Lower daily emission rates were measured for the monoterpene emitter shrubs compared to dwarf palm: the maximum value was observed in *P. lentiscus* (13.9 ng $m^{-2} s^{-1}$). Emissions from all the tested species changed strongly during the day, releasing greater amounts of VOC during the warmest hours and lower amounts at night. The emission is prevalently influenced by temperature as demonstrated by the good correlation found between isoprenoid emission rate and temperature. Thus, as observed in other species that emit VOC from reservoirs and isoprene, the emission enhance during periods of increasing irradiation and temperature in the middle of the day.

Maximum daytime emission rates were about 8 and 35 times higher than at night in *J. phoenicea* and *P. lentiscus,* respectively. Day-night cycles of emissions showed changes in the relative abundance of single monoterpenes. In juniper α-pinene was the main emitted compound during the daytime while at night only traces of this monoterpene were detected. On the contrary, α-pinene still counted for more than 50% of the night emission from the foliage of lentisk. As aspected, no detectable emission of isoprene occurred at night from *Chamaerops*.

VOC emissions can make up a substantial fraction (can be up to 10%) of the assimilated carbon from the trees (Gover et al., 1995; Kesselmeier and Staudt, 1999). In our study, the percent of carbon lost as monoterpenes and isoprene was low, being less than 0.01% of photosynthetically fixed carbon, respectively. Measurements made at ecosystem level in the same experimental site indicated that greater carbon loss (1-2%) as monoterpene emission occurred in autumn when this Mediterranean ecosystem was under strong drought stresses (Baraldi et al., 2002; Miglietta and Peressotti, 1999).

For all species the xylem water potential measurements gave no indication for a severe drought stress. The ecophysiological characteristics of *Pistacia lentiscus* described in this study indicated that lentisk is one of the Mediterranean species most tolerant to drought (Tenhunen et al*.,* 1987). On the other hand the root system of this species is generally well developed and this might prevent water shortage.

The predicted changes towards warmer and drier conditions may thus not be relevant in terms of survival of all these species but, on the contrary, might affect their emission potentials and, by consequence, the potential role of biogenic VOC in future biosphere-climate-chemistry feedbacks.

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