5, 1. 2011

Trees
DOI 10.1007/s00468-010-0536-4

ORIGINAL PAPER

Daily sap flow rate as an indicator of drought avoidance mechanisms in five Mediterranean perennial species in semi-arid southeastern Spain

Esteban Chirino · Juan Bellot · Juan R. Sánchez

Author Proof

Received: 15 June 2010 / Revised: 7 November 2010 / Accepted: 21 December 2010
© Springer-Verlag 2010

UNCORRECTED PROOF

Abstract Daily sap flow rate was determined in five Mediterranean species (*Pinus halepensis, Quercus coccifera, Pistacia lentiscus, Erica multiflora,* and *Stipa tenacissima*) under two slope aspects (north- and south-facing) in a semi-arid area (Alicante, SE Spain). Sap flow velocity was measured in January, May, August and October of two consecutive years (1998 and 1999) using the stem heat balance (SHB) method. Our results have demonstrated the effects of global radiation (R_g), vapour pressure deficit (VPD) on the sap flow velocity per unit of leaf area. Mean daily sap flow rates (Q_{md}) showed values between 0.001 and 0.202 g H_2O cm^{-2} leaf area day^{-1}. Q_{md} values were higher on the south-facing slope than on the north-facing slope. In most species, the Q_{md} was higher in 1998 than in 1999 due to the higher soil water content, temperature and VPD in 1998. In all five species, a decrease in predawn leaf water potential was accompanied by a decrease in mean daily sap flow rates; nevertheless, the responses of the five species to water deficit conditions were different. In this context, we have linked the drought avoidance mechanisms of the different species through the combined use of daily sap flow rate and predawn leaf water potential under different water deficit conditions. We conclude that *Pinus halepensis, Pistacia lentiscus* and *Erica multiflora* show water-savers mechanisms to cope with drought, while *Quercus coccifera* and *Stipa tenacissima* show water-spenders mechanisms.

Keywords Drought resistance mechanisms · Water saver · Water spender · Transpiration · Ecophysiology

Introduction

Dry and semiarid ecosystems in the Mediterranean region are characterized by summers with long water deficit periods and high values of global radiation, temperature and vapour pressure deficit (Di Castri 1973). These environmental conditions affect the physiological activity of plants (Larcher 1995; Gazal et al. 2006) producing changes in plant water consumption through stomatal regulation (Salleo et al. 2000; Mediavilla and Escudero 2004) which, in turn, influence the sap flow rate (Badalotti et al. 2000; David et al. 2007; Gartner et al. 2009).

On drought stress conditions, the species have developed adaptations for escaping or resisting to drought. Drought resistance can be by means of two strategies: drought avoiding or drought tolerating. There is two distinct kinds of drought avoidance mechanisms: (1) water saver, that avoid drought by water conservation, and (2) the water spender, that avoid drought by absorbing water sufficiently rapidly to keep up with their extremely rapid water loss. These concepts and definitions exposed by Levitt (1980) have been applied in several studies with the aim of assess the drought resistance strategy of species. In this sense, previous papers have assessed some species of the present study. *Pinus halepensis*, *Erica multiflora* and *Pistacia lentiscus* have been assessed as drought avoidance

---

Communicated by M. Zwieniecki.

E. Chirino (✉)
CEAM, Department of Ecology, Mediterranean Center for Environmental Studies (Foundation CEAM),
University of Alicante, Apdo. 99, 03080 Alicante, Spain
e-mail: Esteban.Chirino@ua.es

J. Bellot · J. R. Sánchez
Department of Ecology, University of Alicante,
Apdo. 99, 03080 Alicante, Spain

Journal : Large 468	Dispatch : 31-12-2010	Pages : 14
Article No. : 536	☐ LE	☐ TYPESET
MS Code :	☑ CP	☑ DISK

Author Proof

species (Vilagrosa 2002; Ferrio et al. 2003; Lorens et al. 2003b; Baquedano and Castillo 2007); while *Quercus coccifera* has been assessed as a drought-tolerant species (Martínez-Ferri et al. 2000; Baquedano and Castillo 2006).

In semiarid areas of the Mediterranean Spain, some changes in the reforestation strategy have been observed in the last decades. In several cases, has been prioritized the introduction of native shrubs and grasses, unlike of extensive conifer reforestation (e.g. Aleppo pine) carried out during the second part of the twentieth century. In this context, knowledge about plants strategies to cope with drought, as well as the drought avoidance mechanisms of the species introduced acquires great importance; because would allow to link the selection of species for forest restoration with the soil water availability on the plantation site and the water demand of the species (Vilagrosa et al. 2003a; Vallejo and Alloza 2004).

Taking all the above into consideration, in this paper, we analyzed the daily sap flow rates in one tree species (*Pinus halepensis*), three shrub species (*Quercus coccifera, Pistacia lentiscus and Erica multiflora*), and an *alpha* grass steppe (*Stipa tenacissima)* in a semiarid ecosystem, under two slope aspects (north- and south-facing). The hypotheses of this work were: (1) Sap flow rate is affected by microclimatic conditions and soil water availability, therefore hopefully observe: (a) a common seasonal pattern in the sap flow rate by species, (b) higher sap flow rate in south-facing than north-facing slope, and (c) higher sap flow rate during the wetter year; and (2) Sap flow response of plants to cope with water deficit stress, expressed by means of predawn water potential, can be linked with drought avoidance mechanisms (water saving or water spending). To test these hypotheses, the aims of this paper were (1) evaluate the effect of microclimatic conditions and soil water content on the sap flow rate by species in the time (seasonal pattern and year) and slope aspect; and (2) establish a link between the daily sap flow of the different species as response to variations in predawn leaf water potential and drought avoidance mechanisms of plants (water saver or water spender).

Materials and methods

Site description

The research was carried out at the "El Ventós" experimental station, Alicante, Spain. Altitude 600 m asl and slopes between 23° and 26°. Loam and loamy limestone support calcareous Cambisol and Lythic Leptosol (FAO-UNESCO), with average soil depth of 20 cm, organic matter content of 4.7%, clay content between 48 and 58%, total porosity of 50.2%, field capacity of 25% and average infiltration rates measured with a double-ring infiltrometer were between 192 and 438 mm h^{-1} (Bellot et al. 2001; Chirino et al. 2006). The climate is semi-arid Mediterranean, with a very high inter-annual variability. Mean annual temperature is 18.2°C and mean annual rainfall is 275 mm (1976–2006 period). The current vegetation landscape in the study area is composed of different land cover types (patch-mosaic) which can be situated along a successional gradient of increasing structural complexity of vegetation: degraded open land or bare soil, sometimes occupied by microphytic crusts (Maestre et al. 2002); dry grassland formations of *Brachypodium retusum* Pers. Beauv., with dwarf scrubs (*Anthyllis cytisoides* L., *Helianthemum syriacum* (Jacq.) Dum.-Cours. and *Thymus vulgaris* L.); and more mature landscape patches composed of scattered thorn and sclerophyllous shrublands with *Quercus coccifera* L., *Pistacia lentiscus* L., *Erica multiflora* L., *Rhamnus lyciodes* L. and *Rosmarinus officinalis* L. (Bonet et al. 2001). Other anthropogenic components of the present landscape mosaic are afforestations with *Pinus halepensis* Mill. on dry grasslands and thorn shrublands. On south-facing slopes, the predominant vegetation is a rather interspersed mosaic of open *Stipa tenacissima* steppes and dwarf shrubland with gradual transitions between them (Bautista et al. 2007). A description of the land cover types has been presented by Chirino et al. (2006).

Experimental design for sap flow measurements and data processing

Sap flow rate measurements were carried out in sets of five consecutive days during the months of January (winter), May (spring), August (summer) and October (autumn) in the years 1998 and 1999. These measurements were taken in four species on a north-facing slope (*Pinus halepensis* Mill., *Quercus coccifera* L., *Pistacia lentiscus* L. and *Erica multiflora* L.) and four species on a south-facing slope (*Pinus halepensis* Mill., *Quercus coccifera* L., *Pistacia lentiscus* L. and *Stipa tenacissima* L.). On both slopes, Aleppo pines (*Pinus halepensis*) were planted 40 years ago, while the other species belonged to the natural succession. For each slope aspect (north- and south-facing) three individuals were selected randomly by species for the sap flow measurements. The height of the individuals is shown in Table 1. Leaf Area Index (LAI, Table 1) was measured in individuals similar to those where the sap flow was monitored. LAI was measured by means of a destructive method based on the leaf area of the shoot biomass in a rectangular prism with square base of 50 × 50 cm and a height according to the individuals sampled.

To measure the sap flow we used the stem heat balance method (SHB system, van Bavel 1984). In each of the plants selected we installed three sap flow gauges

Journal : Large 468
Article No. : 536
MS Code :
Dispatch : 31-12-2010
Pages : 14
☐ LE
☑ CP
☐ TYPESET
☑ DISK

Author Proof

Table 1 Height of the individuals selected by species for sap flow measurements

Species	Slope	Characteristics of individuals	
		Height (m)	LAI (m^2/m^{-2})
P. halepensis	North	4.57 ± 0.30	1.11 ± 0.36
	South	1.83 ± 0.12	0.87 ± 0.23
Q. coccifera	North	1.41 ± 0.12	1.01 ± 0.27
	South	0.61 ± 0.05	1.78 ± 0.32
P. lentiscus	North	1.58 ± 0.19	1.96 ± 0.03
	South	0.57 ± 0.07	0.45 ± 0.07
E. multiflora	North	0.85 ± 0.03	0.59 ± 0.04
S. tenacissima	South	0.72 ± 0.07	2.06 ± 0.10

Leaf area index (LAI) of similar individuals used for sap flow measurements (Data May 1997; $N = 3$)

(Dynagauge, Dynamax Inc., Houston, USA, models SGA2, SGA3 or SGA5) on upper third crown on branches oriented in three directions (N, SE, SW). In the case of *P. halepensis,* it was necessary to use a 5 m tall scaffolding to reach the upper third of the plant crown. Moreover, additional insulation and an exterior wrapping of tinfoil were also used to provide higher isolation from meteorological conditions. Measurements of sap flow velocity (Q; g H_2O h^{-1}) were programmed with a scan interval of 1 min and signals were averaged every 15 min. After removing the gauges, the leaf area of each branch was measured destructively to normalize sap flow velocity per unit of leaf area (Q_1; g H_2O cm^{-2} leaf area h^{-1}). Leaves were scanned with a professional scanner (Epson Expression 1680 Pro, Seiko Epson Corporation, Nagano, Japan). The images obtained were analyzed with the specific image processor WinRHIZO software (Regent Inst., Canada) to obtain leaf area (cm^2). FLOW32 software requests a time interval of reduced power, and during this time interval, the flow rate is set to zero (van Bavel, 1984). In our case, we defined the least time possible (power-down time: 00:00 AM and power-up time: 3:00 AM). Thus, the daily sap flow rate by individual (Q_d; g H_2O cm^{-2} leaf area day^{-1}) was computed by means of the Q_1 accumulated from 3:00 to 24:00. Mean daily sap flow rate (Q_{md}; g H_2O cm^{-2} leaf area day^{-1}) was computed by averaging the Q_d value obtained from three individuals selected in each sampled periods. Subsequently, we estimated the mean daily sap flow rate for each species by month (January, May, August and October), year (1998 and 1999) and slope aspect (north- and south-facing).

Meteorological conditions, soil moisture and predawn water potential

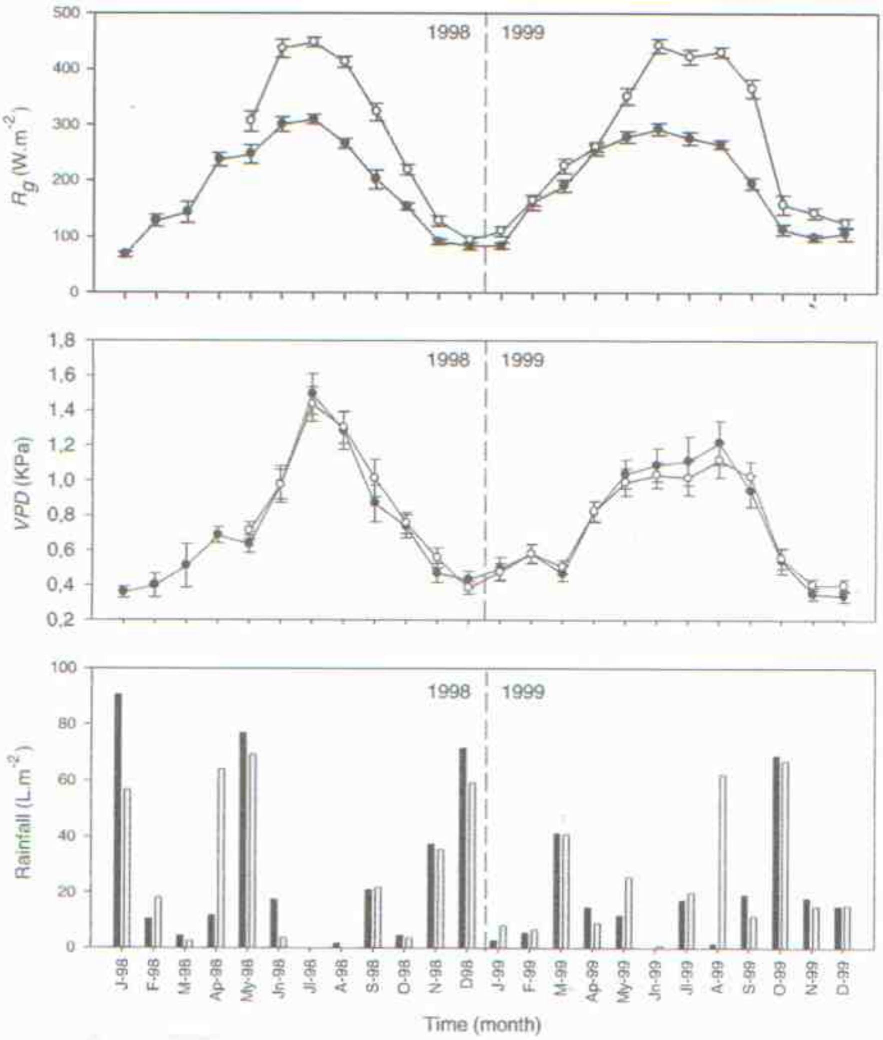
Meteorological data were monitored during 1998 and 1999. Rainfall, air temperature (T^a), air humidity (H) and global radiation (R_g) were measured continuously with an automatic weather station Campbell Scientific CR10 (Campbell Scientific Ltd. UK). The automatic weather station on north-facing slope was installed since 1995, while on the south-facing slope was installed in March, 1998. T^a, H and R_g were taken at 10 min intervals and averaged every hour. Vapour pressure deficit (VPD) was calculated from T^a and H data. Soil moisture was monitored using the Time Domain Reflectometry System (Reflectometer Tektronic 1502C, Metallic TDR cable Tester, Tektronix, Beaverton, OR, USA) from 0 to 30 cm (maximum soil depth) by means of 12 probes inserted in a patch-mosaic of three vegetation cover types: pine with dry grass and sclerophyllous shrub on the north-facing slopes and *alpha* grass steppes of *S. tenacissima* on the south-facing slopes (Chirino 2003). Relative Extractable Water (REW) in the root zone was calculated from the soil moisture monitored in these vegetation types by means of the equation $\mathrm{REW} = (\theta - \theta_{\text{mín}})/(\theta_{\text{FC}} - \theta_{\text{mín}})$ described by Granier (1987) and Bréda et al. (1995), where θ is the actual soil water content and $\theta_{\text{mín}}$ is the minimum soil water content observed during the soil moisture monitoring, and $\theta_{\text{FC}} = \theta$ at field capacity. All the variables are expressed in L m^{-2}. REW = 0.4 was identified as the threshold for soil water deficit (Granier 1987; Bréda et al. 1995). Leaf water potential before sunrise (predawn leaf water potential: Ψ_{pd}) was measured by using the Scholander pressure chamber (Soil moisture Equipment Corp., Santa Barbara, CA, USA) in three branches of three individuals selected by species. This variable was measured in all species, except in *Stipa tenacissima* due to technical difficulties.

Statistical analysis

Statistical analysis was carried out with the STATISTICA 6.0 package (Statsoft Inc., Tulsa, USA). Means monthly Q_{md} were compared using a General lineal Model (GLM) Repeated Measures by means of multi-way within-subject designs. We used the years (1998 and 1999) and monthly Q_{md} value as within-subjects factors, and slope aspect and species as between-subjects factors. Due to interactions observed in the results of GLM Repeated Measures (multi-way within-subject designs), subsequently was carried out a GLM Repeated Measures with one within-subject factor (monthly Q_{md}) and only a between-subject factor (slope aspect, year or specie). In the case of species analysis, a *post hoc Tukey HSD* was used. In order to know the differences or patterns commons between monthly Q_{md} of species, a two-factor Univariate ANOVA (year and month) was carried out, using a *post hoc Tukey HSD.* Meteorological data on the annual periods (full data on north-facing slope) were compared by means of analysis of variance (one way ANOVA). Data on T^a (°C), H (%), R_g (W m^{-2}), VPD (kPa) and rainfall by month and slope aspect were compared using nonparametric statistical methods (Mann–Whitney test and "t" test)

Author Proof

Fig. 1 Comparison between north- and south-facing slopes with respect to global radiation (R_g), vapour pressure deficit (VPD) and rainfall during 1998 and 1999. Data on R_g and VPD are mean monthly ± standard error (north-facing: *black circle* and *solid line*; south-facing: *white circle* and *solid line*). Rainfall data are monthly accumulated values (north-facing: *black bar*; south-facing: *grey bar*)



due to differences in the number of observations. The north-facing slope had full data for the years 1998 and 1999; in contrast, the south-facing slope had data only from March, 1998 to December, 1999, when the automatic weather station was installed. The relationship between Q_{md} (g H_2O cm^{-2} day^{-1}) and Ψ_{pd} (MPa) in each species was calculated by means of a exponential regression. In the case of the relationship between Q_1 (g H_2O cm^{-2} h^{-1}) and the hourly value of R_g (W m^{-2}) and VPD (kPa) throughout the day we used exponential, quadratic and linear regressions.

Results

Meteorological variables and soil moisture

Monthly global radiation (R_g) was higher on the south-facing slope than on the north-facing slope (Fig. 1; $p < 0.001$). No significant differences between slope aspects were found with respect to rainfall and VPD (Fig. 1). Nevertheless, both slope aspects showed significant differences ($p < 0.001$) between measuring months for the variables T^a, R_g, and VPD. On an annual time scale, T^a ($p = 0.028$) and VPD ($p = 0.011$) were higher in 1998 than in 1999. Annual rainfall was 1.2 times higher in 1998 (288.3 mm) than in 1999 (241.1 mm), resulting in higher soil moisture values and REW during the first year (Fig. 2). Soil moisture average on north-facing slope ranged between 18.1% in pine with dry grass and 15.2% in sclerophyllous shrub; while, on south-facing slope represented by alpha grass steppe of *S. tenacissima* the soil moisture average was 12.7% (Fig. 2, above). REW values on both slope aspects were below the threshold established for Mediterranean vegetation cover (REW = 0.4), which in our case represents a soil water content of 40% below field capacity (Fig. 2, below). These results point to the long duration of soil water deficit conditions in the study area.

Journal : Large 468
Article No. : 536
MS Code :
Dispatch : 31-12-2010
Pages : 14
☐ LE
☑ CP
☐ TYPESET
☑ DISK

Author Proof

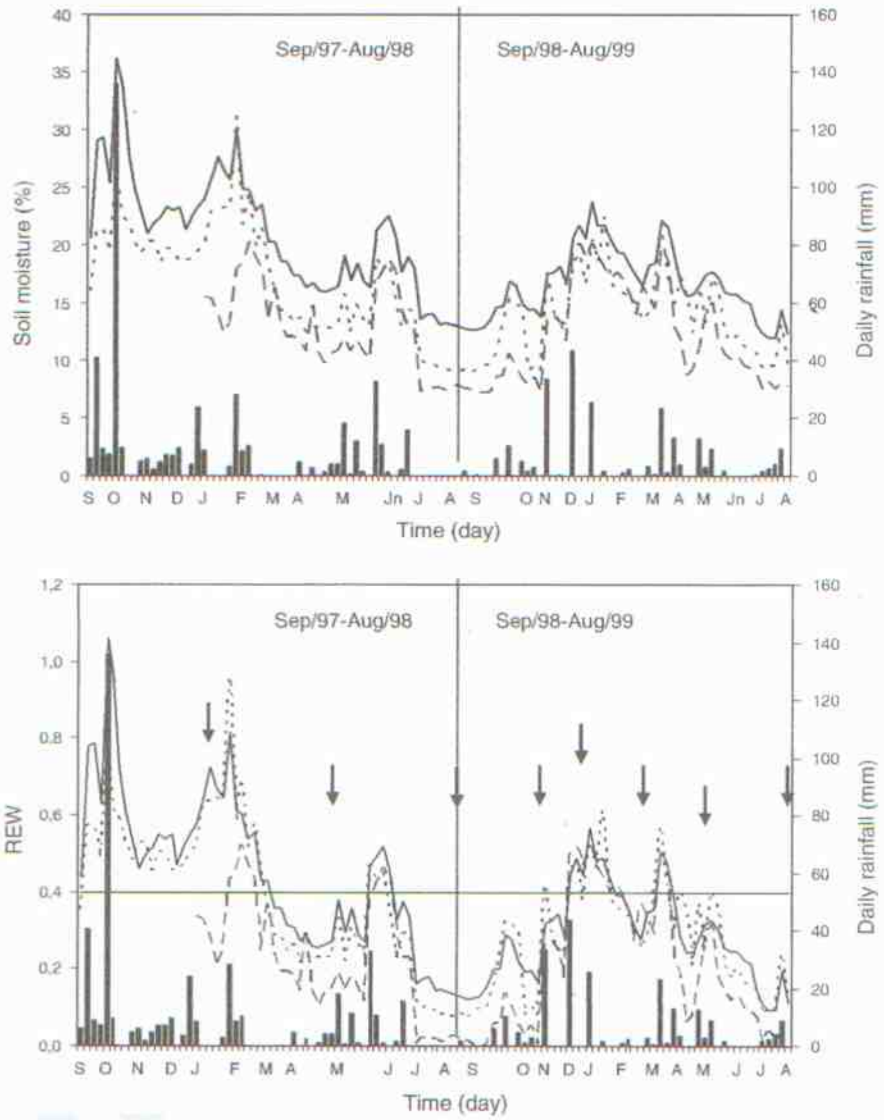


Fig. 2 Daily rainfall and soil moisture (above), and Relative Extractable Water (REW, below) in two hydrological years 1997/1998 and 1998/1999. The data showed in the x-axis correspond to daily measurements. We show label of months to improve the understanding. On north-facing slope: pine with dry grass (*solid line*) and sclerophyllous shrub (*dashed line*). On south-facing slope: alpha grass steppes of *S. tenacissima* (*long dash*). REW = 0.4 is threshold for Mediterranean species (*dotted line*). Months of sap flow measurements: *vertical arrow*

Only from September, 1997 to March, 1998 were the REW values higher than the threshold, as a result of the extraordinary rains in the autumn of 1997.

Relationship between sap flow and meteorological variables

During the course of the day, the sap flow velocity per unit of leaf area (Q_1) showed a significant relationship with R_g and VPD. As an example, we present the results obtained for *Quercus coccifera* on two different dates in 1998, with contrasting soil moisture and meteorological conditions. The first case (Fig. 3a) shows the results of the May 28th sap flow measured on the south-facing slope, with high daily soil water content (19.8%) and high daily VPD (1.22 kPa). The second case (Fig. 3b) shows the August 19th sap flow measured in north-facing conditions, with a lower daily soil water content (10.3%) and lower daily VPD (0.67 kPa). In both cases, R_g presents a significant relationship with Q_1, by means of an exponential equation $\left[Q_1 = a*\left(1 - e^{-b*R_g}\right)\right]$ where extreme values of R_g tend to produce Q_1 approaching the asymptote. VPD shows a quadratic polynomial equation $\left[Q_1 = a + b_{0*}\,\mathrm{VPD} + b_{1*}\,\mathrm{VPD}^2\right]$ on May 28th and a linear equation $\left[Q_1 = a + b_{0*}\,\mathrm{VPD}\right]$ on August 19th. These differences in the regression equations are explained by the fact that the first case presented higher values of soil water content and VPD than the second case, which favoured higher Q_1 values. However, when VPD > 1.4 kPa, Q_1 decreased. Similar results were observed in the other species (data no shown).

Journal : Large 468 Dispatch : 31-12-2010 Pages : 14
Article No. : 536 ☐ LE ☐ TYPESET
MS Code : ☑ CP ☑ DISK

Author Proof

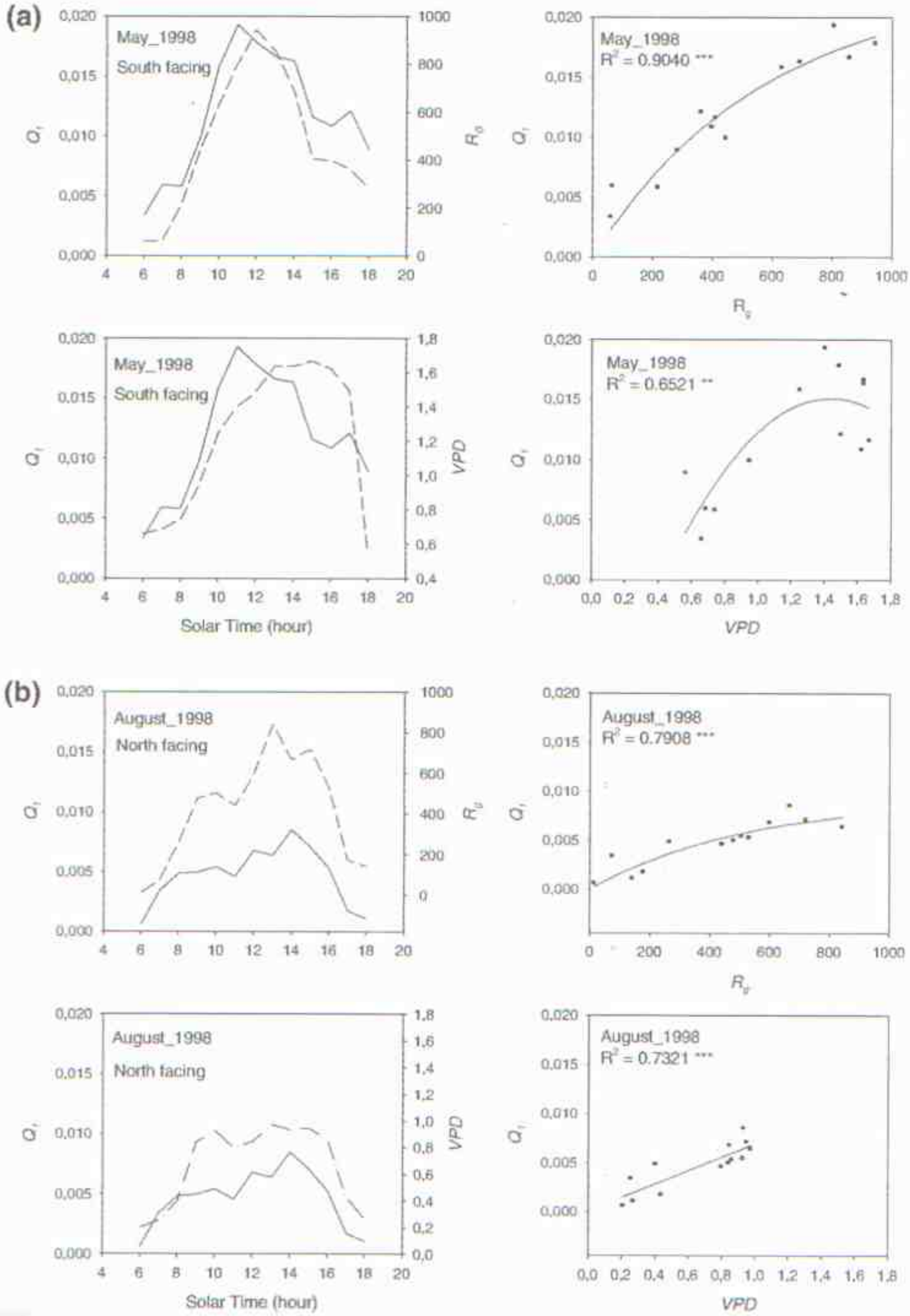


Fig. 3 Left side shows the hourly variation in sap flow velocity (Q_1: g H_2O cm^{-2} h^{-1}), global radiation (R_g: W m^{-2}) and vapour pressure deficit (VPD: kPa) in *Q. coccifera* (*solid line* Q_1, *dotted line* R_g, *dash-dot line* VPD. **a** South-facing data: May 28, 1998. **b** North-facing data: August 19, 1998. *Right side* shows the regression equations according to the variables on the left side of the figure (significance differences **$p < 0.01$ and ***$p < 0.001$)

Daily sap flow rate of each species by slope aspect, year and month

Average daily sap flow rates (Q_{md}) of the different species by months and slope aspects during 2 years, 1998 and 1999 (Fig. 4) showed values between 0.001 and 0.202 g H_2O cm^{-2} leaf area day^{-1}. General lineal Model (GLM) Repeated Measures by means of multi-way within-subject designs indicated significant differences ($p < 0.05$) in Q_{md} values between the factors: years, slope aspect and species (Table 2). In this analysis were observed interactions between all factors. Subsequent analysis using GLM Repeated Measures with only a within-subject and between-subject factor determined the effect of each factor (Table 3). *Q. coccifera* and *P. lentiscus* showed higher Q_{md} values on the south-facing slope than on the north-facing slope during

Author Proof

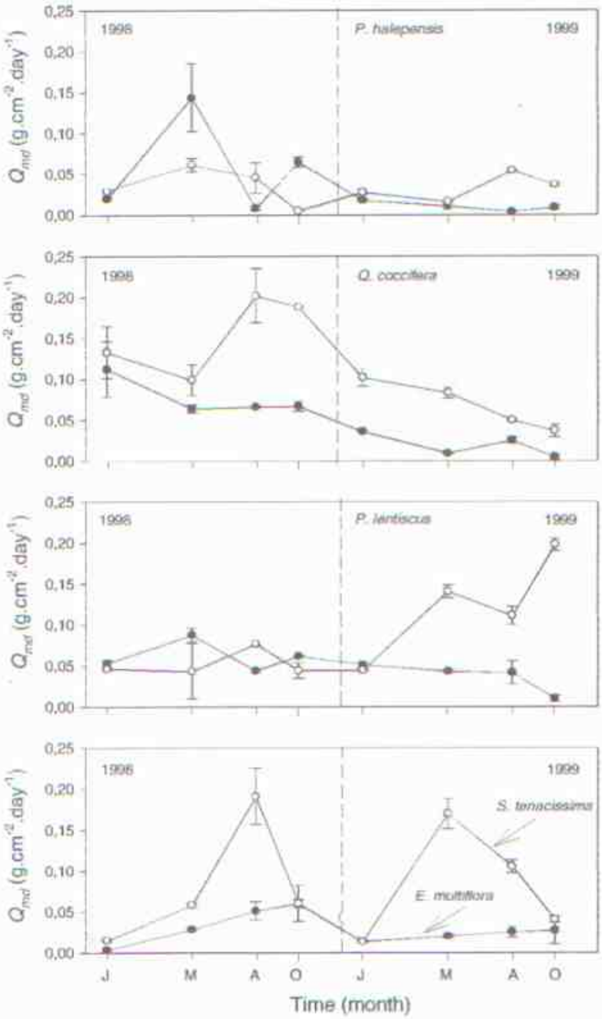


Fig. 4 Monthly sap flow rate by specie in each slope aspect (north- and south-facing) and during two years (1998 and 1999). Mean ± standard error, north-facing: *black circle* and *solid line*; south-facing: *white circle* and *solid line*. See in the last figure we show two species, *E. multiflora* from north-facing (*black circle* and *solid line*) and *S. tenacissima* from south-facing (*white circle* and *solid line*)

Table 2 Results of General lineal Model (GLM) Repeated measures by means of multi-way within-subject designs

Factor	*F* value	*p* value
Slope aspect	25,580	0.000***
Specie	8,880	0.000***
Year	8,380	0.010*
Year * Slope aspect	8,020	0.011*
Year * Specie	9,170	0.000***
Month	13,690	0.000***
Month * Slope aspect	6,000	0.001**
Month * Specie	7,400	0.000***
Year * Month	3,360	0.025*
Year * Month * Slope aspect	6,110	0.001**
Year * Month * Specie	4,500	0.000***

Significance differences * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

period of measurements (Table 3, Time factor $p < 0.05$), while *P. halepensis* showed an opposite result. A higher R_g on south-facing slope could produce stress in *P. halepensis*, induce a stomatal closure and reduce the sap flow. In the cases of *P. halepensis* and *P. lentiscus* were observed interactions between the factors time and slope aspect ($p < 0.05$). The test of between-subjects effects indicated that *Q. coccifera* and *P. lentiscus* presented two times higher Q_{md} in north- than south-facing (Table 3, slope aspect factor $p < 0.01$). *P. halepensis* not showed significance differences.

The inter-annual variability analysis on the north-facing slope indicated that Q_{md} values were higher in 1998 than in 1999 for *P. halepensis*, *P. lentiscus* and *E. multiflora* (Table 3, Time factor $p < 0.05$). Only, were observed interactions between time and years in *P. halepensis* and *P. lentiscus*. On south-facing slope *S. tenacissima* showed higher Q_{md} in 1998, while an opposite result was observed in *P. lentiscus* (Table 3, Time factor $p < 0.05$) showing Q_{md} higher in 1999 than in 1998. *P. halepensis* (on south-facing) and *Q. coccifera* (in both slope aspect) no showed significance differences in the time (Table 3, Time factor $p > 0.05$). Several interactions were observed (Table 3, time × year factor, $p < 0.05$). The year factor (test of between-subjects effects) ratified the results of previous analysis, and indicated significance differences in the annual Q_{md} in *Q. coccifera*.

Comparative analysis of Q_{md} values between species within each slope aspect indicated significant differences (Table 3, Time factor, $p < 0.001$). On the north-facing slope *Q. coccifera* and *P. lentiscus* showed higher Q_{md} than *E. multiflora*; while *P. halepensis* presented an intermediate result, no showing significance differences with the others species. On the south-facing slope *Q. coccifera* showed higher Q_{md} than *S. tenacissima* and *P. lentiscus*. In this slope aspect, *P. halepensis* showed the lowest values (Table 3, Time factor, $p < 0.001$). Both time x specie, like the specie factor showed interactions and significance differences respectively (Table 3). The analysis of sap flow by species in each slope aspect no showed a common pattern between monthly Q_{md} (Fig. 5). The month of maximum and minimum Q_{md} during the 2 years of measurements was different in each species (Fig. 5). However, on south-facing slope *P. halepensis*, *P. lentiscus* and *S. tenacissima* tended to show the highest Q_{md} values in May and August, and lowest values in January (Fig. 5). On north-facing slope, *P. halepensis* and *P. lentiscus* showed the highest Q_{md} in May and lowest values Q_{md} in August, while