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Daily time course of whole-shoot gas exchange rates in two drought-exposed Mediterranean shrubs

SALVADOR NOGUÉS,[1] SERGI MUNNÉ-BOSCH,[1] JAUME CASADESÚS,[2] MARTA LÓPEZ-CARBONELL[1] and LEONOR ALEGRE[1,3]

[1] *Departament de Biologia Vegetal, Universitat de Barcelona, Av. Diagonal 645, E-08028 Barcelona, Spain*

[2] *Servei de Camps Experimentals, Universitat de Barcelona, Av. Diagonal 645, E-08028 Barcelona, Spain*

[3] *Author to whom correspondence should be addressed*

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Summary Effects of drought on water relations, whole-shoot gas-exchange characteristics, and pigment and zeatin concentrations were investigated in the Mediterranean shrubs rosemary (*Rosmarinus officinalis* L.) and lavender (*Lavandula stoechas* L.). Two-year-old, greenhouse-grown plants were placed in a whole-shoot gas-exchange measurement system and subjected to 10 days of drought, resulting in severe water stress, and then re-watered for 5 days in order to study their recovery. Water stress resulted in a significant decline in maximum whole-shoot net CO_2 assimilation rates (A_n) for both species that was associated with reductions in leaf area and stomatal conductance. Because shoot dark respiration rate (R_d) was less sensitive to water stress than A_n, shoot R_d/A_n ratio increased from about 15 to 95% during water stress. No major changes in chlorophyll and carotenoid concentrations of rosemary leaves were observed during the experiments, but chlorophyll and carotenoid concentrations fell significantly in water-stressed lavender leaves. Zeatin concentrations were higher in rosemary leaves than in lavender leaves during water stress. After re-watering, whole-shoot A_n and R_d rapidly recovered to their pre-drought rates.

Keywords: dark respiration, drought, Lavandula stoechas, photosynthesis, Rosmarinus officinalis, zeatin.

Introduction

Photosynthetic productivity is more closely related to measurements of whole-canopy photosynthesis than to measurements of single-leaf photosynthesis, which estimate maximum potential photosynthesis if the topmost, fully expanded leaves are examined (McCree 1986*a*, Wells et al. 1986). Canopy photosynthetic measurements, on the other hand, measure CO_2 uptake of the whole stand and describe more accurately the photosynthetic activity per unit ground area by accounting for species characteristics, leaf morphology and canopy architecture (Peterson and Zelitch 1982, Wells et al. 1986, Giuliani et al. 1997). In addition, whole-canopy gas-exchange measurements allow photosynthetic parameters to be determined without the need to clamp a chamber onto a leaf and thereby disturb leaf responses, such as stomatal conductance (Donahue et al. 1997), although canopy microclimate can be altered. Furthermore, the whole-canopy approach enables repeated measurements of photosynthesis as the plants grow and are exposed to different environmental conditions. This non-destructive approach also facilities investigation of plant responses to acute stress applied during the measurement of photosynthesis (Donahue et al. 1997).

Plant canopies comprise a population of leaves with a range of developmental states and light interception profiles. Daily, or preferably weekly or monthly, canopy CO_2 exchange rate (CER) measurements provide a better estimate of photosynthetic productivity than a single instantaneous measurement (Christy and Porter 1982, Peterson and Zelitch 1982) because they account for changes in photosynthetic production occurring as a result of genotypic and environmental factors (Wells et al. 1986). Despite the advantages of long-term canopy CER measurements, this approach has rarely been used to study stress effects at the whole-plant level, especially in Mediterranean-climate sclerophyllous shrubs (Mooney 1987).

It has been estimated that 30–70% of the carbon assimilated during photosynthesis may be lost as CO_2 during respiration (Waring 1991, Amthor 2000). Whole-plant respiration rate is positively and strongly correlated to whole-plant photosynthesis and growth rates, but environmental stresses, such as drought (which greatly reduce growth and productivity), can influence respiration independently of effects on photosynthesis by causing imbalances in the production and use of photoassimilate (Amthor 2000). An objective of this study was to use long-term canopy CER measurements to assess the effects of drought on the production and use of photoassimilates in Mediterranean shrubs.

Previously, we studied variations in endogenous concentrations of abscisic acid, indole-3-acetic acid and zeatin riboside (ZR, a cytokinin) in the Mediterranean shrubs rosemary (*Rosmarinus officinalis* L.) and lavender (*Lavandula stoechas* L.) cultivated in growth chambers. When the plants were subjected to a progressive water stress (López-Carbonell et al.

1996), we found that ZR concentrations were higher in leaves of rosemary than of lavender. Because this hormone functions as a modulator of plant responses to water stress, and because plastids are targets for cytokinin (Parthier 1979), which protects chlorophyll from degradation (Hukmani and Tripathy 1994), we measured endogenous concentration of zeatins to determine whether there is a relationship between chlorophyll degradation and zeatin concentrations during water stress.

We used a system designed to perform continuous gas-exchange measurements on whole shoots for long periods of time to measure whole-shoot photosynthesis and dark respiration rates of the Mediterranean shrubs rosemary and lavender when subjected to water stress. We also characterized the effects of severe water stress on water relations and pigment and zeatin concentrations.

Materials and methods

Plant material

Experiments were conducted with two native Mediterranean evergreen shrubs (rosemary, *Rosmarinus officinalis* and lavender, *Lavandula stoechas*). Both species have narrow sclerophyllous leaves (1–5 cm length and less than 3 mm wide). Rosemary has hypostomatic leaves (with revolute margins) protected by trichomes and an adaxial epidermis with a cuticular thickening of about 2 µm covered with cuticular waxes of 5 µm thickness. Lavender has amphistomatic leaves protected by trichomes, glands and a cuticular thickening of 2–2.5 µm (Munné-Bosch et al. 1999*a*). The main stem of these shrubs is branched (5–9 branches). Both species are well adapted to survive during the periods of drought that characterize the Mediterranean climate.

Seeds of lavender were germinated on moist filter paper and the seedlings transferred to 25-l pots containing a 3:1 (v/v) soil:perlite mixture. Cuttings of rosemary were planted and rooted in soil:perlite in 25-l pots. Seedlings were grown in a greenhouse for two years. Day/night temperature and vapor pressure deficit (VPD) in the greenhouse were maintained at approximately 28/18 °C and 2.0/1.2 kPa, respectively. The plant pots were watered to saturation twice a week with Hoagland solution.

Whole-shoot gas-exchange measurements

After 2 years of growth, plants were removed from the greenhouse and placed in a specially designed whole-shoot gas-exchange measurement system built by the Servei de Camps Experimentals (Experimental Field Service) of the University of Barcelona (Casadesús 1995). This system, which is similar to the systems described by McCree (1986*b*) and Smart et al. (1994), consists of two independent Plexiglas chambers (0.125 m^3 volume; 0.5 × 0.5 × 0.5 m) whose environments (photosynthetic photon flux density (PPFD), relative humidity and temperature) can be programmed independently by an automated control mechanism (Figure 1). Light was supplied by three 500-W halogen lamps (Model 64702, OSRAM S.A., Madrid, Spain) per chamber, each supplying a PPFD of about

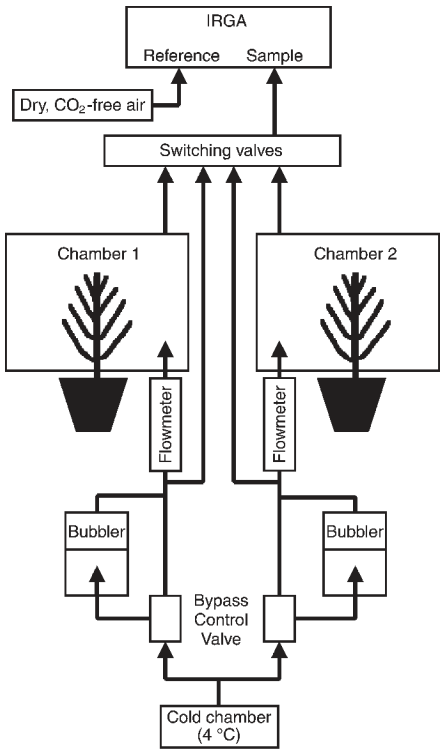

Figure 1. Schematic diagram of the system designed for continuous measurement of whole-shoot gas-exchange rates in rosemary and lavender. Air entering the chambers at about 355 ppm CO_2 was cooled to 4 °C to reduce its water content and a regulated proportion of this flow (independently determined for each chamber) was passed through a bubble tank at 18 °C before entering the chamber. For each chamber, the amount of air passed through the bubbler was regulated by a progressive valve controlled by computer. The air temperature in each chamber was automatically regulated by adjustment of the flow of water at 18 °C circulating through a heat exchanger in the chamber. At the beginning of each experiment, the flow of air entering the chambers was adjusted to between 0.06 and 0.1 mol s^{-1} depending on seedling size. This flow was measured throughout each 15-day experiment with a mass flow meter attached to each chamber. Concentrations of CO_2 and H_2O in the air streams entering and leaving each chamber were measured with an infrared gas analyzer (IRGA).

500 µmol m^{-2} s^{-1} on a plane at half the height of the shoot during a 14-h photoperiod. The lamps were placed about 30 cm above the seedlings and 7 cm of deionized water and 1 cm of Plexiglas in the container filtered the radiation. The environmental conditions during the experiments are summarized in Table 1. Air temperature and relative humidity in the chambers were measured by two HMP 112Y sensors (Vaisala Oy, Helsinki, Finland). Relative humidity in each chamber was regulated by controlling the water content of the air entering the gas exchange chamber. Air entering the chambers at about 355 ppm CO_2 was cooled at 4 °C to reduce its water content and a regulated proportion (independently determined for each chamber) passed through a bubble tank at 18 °C before entering the chamber. For each chamber, the amount of air passed through the bubbler was regulated by a progressive valve (Model VXG 41.15R, Landis & Gyr Ltd., Geneva, Swit-

Table 1. Daily environmental conditions in the Plexiglas chambers during the 15-day experiments designed to simulate Mediterranean environmental conditions during summer. Abbreviations: PPFD = photosynthetically active photon flux density, VPD = vapor pressure deficit; and T = air temperature).

Time (h)	PPFD (μmol m^{-2} s^{-1})	VPD (kPa)	T (°C)
6–8	500	1.47	23
8–11	1000	1.83	25
11–15	1500	2.03	26
15–18	1000	1.83	25
18–20	500	1.47	23
20–6	0	1.15	21

zerland) controlled by computer. The air temperature in each chamber was automatically regulated by adjusting the flow of water at 18 °C circulating through a heat exchanger in the chamber. The difference in temperature between the air and the heat exchanger prevented condensation, which would have led to errors in gas-exchange measurements.

At the beginning of each experiment, the flow of air entering the chambers was adjusted to between 0.06 and 0.1 mol s^{-1}, depending on seedling size. This flow was measured throughout each 15-day experiment with a mass flow meter (Model 5811N, Brooks Instruments B.V., Veenendaal, The Netherlands) attached to each chamber. Concentrations of CO_2 and H_2O in the air streams entering and leaving each chamber were measured with an infrared gas analyzer (IRGA) (Model LI-6262, Li-Cor, Inc., Lincoln, NE) operating in absolute mode, using a reference stream of dry, CO_2-free air. A computerized valve-tree composed of three-way valves (Model FT 8320 85, ASCO, Scherpenzeel, The Netherlands) automatically switched the analyzer stream between four samples: air entering and air within the two chambers. After changing to a new sample, a stabilization time of 3 min was used before the IRGA measurement. Gas exchange in both chambers was automatically measured every 12 minutes in an open steady-state system. The temperature difference between the air and a chosen leaf of each plant was measured with a pair of thermocouples (Model LI-6000TC, Li-Cor, Inc.). Gas-exchange parameters were calculated as described by von Caemmerer and Farquhar (1981). All CO_2 exchange rates were expressed per unit leaf area. At the beginning of every measurement, boundary layer conductance in each chamber was set at about 1800 mmol m^{-2} s^{-1} by adjusting the power of the fan, and was estimated in single leaves of shoots based on measurements of the energy balance of aluminum model leaves according to Jones (1992). Data acquisition and process control of the entire system were carried out with the aid of a computerized data logger (Model HP3412A, Hewlett Packard, Palo Alto, CA).

For each experiment, one shoot of a single plant was placed in a chamber (sealed with Terostat gum) and subjected to progressive drought by withholding water for 10 days (water-stressed (WS) plants). The root of the plant was kept outside the chamber (Figure 1). The plant was re-watered with Hoagland solution for 5 days (water-recovered (WR) plant). At the same time, a second shoot was placed in another chamber and watered every day with Hoagland solution (well-watered (WW) plant). Because the system is capable of continuous measurement of shoot gas exchange rates, we were able to compare WW and WS plants over a 15-day period. Plants were acclimated for 3 days in a chamber before an experiment began. The experiment was repeated three times.

Leaf area measurements

Total leaf area (LA) was measured by both nondestructive and destructive procedures (Long and Hällgren 1993, Welles and Cohen 1996). Total LA was estimated nondestructively at the beginning of the experiment and during the water-stress and water-recovery treatments by means of photographic silhouettes, i.e., holding up a gridded white board behind the plants and taking a photograph. At the end of the experiment, total LA was measured with a flatbed scanner (Model GT-5000, Epson, Nagano, Japan) and analyzed with an image-processing program.

Chlorophyll fluorescence measurements

At the beginning of the water-stress period (WW, Day 0), at maximum water stress (WS, Day 10), and at full recovery (WR, Day 15), steady-state modulated chlorophyll fluorescence of single leaves of apical non-woody shoots was measured fluorimetrically (Model PAM 101-102-103, Heinz Walz GmbH, Effeltrich, Germany). As well as measuring the maximum quantum efficiency of PSII photochemistry (given by F_v/F_m), the relative quantum efficiency of PSII photochemistry (ϕ_{PSII}) was estimated from $(F'_m - F'_s)/F'_m$ (Genty et al. 1989). Efficiency of excitation energy captured by open PSII reaction centers (F'_v/F'_m) and photochemical quenching (q_p) were also estimated as described by Andrews et al. (1993). Measurements of F_v/F_m were made after dark adaptation for 30 min and measurements of ϕ_{PSII}, F_v'/F_m', and q_p were made at a PPFD of about 1500 μmol m^{-2} s^{-1}.

Plant water status measurements

Water potential (Ψ_w) of apical non-woody shoots was measured at 0700 h in WW, WS and WR plants with a Scholander-type pressure chamber (Model ARIMAD-2, ARI Far Charuv-Water Supply Accessories, Ramat Hagolan, Israel). A moist paper was placed inside the chamber to avoid humidity changes during the measurements. Relative leaf water content (RWC) was determined as (FW – DW)/(TW – DW)100, where FW is fresh weight, TW is turgid weight (after rehydrating samples for 24 h) and DW is dry weight (after over-drying samples at 85 °C for 24 h) of single leaves of apical non-woody shoots (Turner 1981) collected at the same time as the shoots used for the water potential measurements.

Pigment analysis

Chlorophylls a and b and total carotenoid concentrations were estimated on apical fully expanded young leaves of WW, WS

and WR plants from both species, after extraction in 80% acetone. Absorbances at 470, 646 and 663 nm of the acetone extract were measured spectrophotometrically and chlorophyll concentrations were calculated based on the equations presented by Lichtenthaler (1987).

Zeatin determination

For zeatin analysis, mainly zeatin riboside (ZR), young, fully expanded apical leaves of WW, WS and WR plants from both species were collected, immediately frozen in liquid nitrogen, and stored at –20 °C until analyzed. The extraction and analysis of zeatins were carried out as described by López-Carbonell et al. (1998). Briefly, frozen leaves (1 g) were ground in a mortar in liquid nitrogen and extracted overnight at –20 °C in 80% methanol. The extracts were centrifuged (24,000 *g* for 15 min at 4 °C) and the supernatant was purified on two reversed-phase-C_{18} cartridges (500 mg, Waters Corp., Milford, MA) by eluting with 80% methanol. The eluates were dried under vacuum at 35 °C, rehydrated in deionized water and analyzed by enzyme-linked immunosorbent assay with ZR-specific monoclonal antibodies (Phytosciences, Chatou, France). The cross-reactivity of the antibodies used was as follows: ZR at 100%, zeatin at 88% and zeatin monophosphate at 84%. For calibration, ZR standard solutions (from 0.03 to 30 pmol per 0.1 ml of sample volume per well) were used. Standards and samples were tested in triplicate. The trends observed in this study were the same as those obtained previously in the same species when analyzing ZR by radioimmunoassay (López-Carbonell et al. 1996).

Results

Daily patterns of shoot gas exchange rates (expressed per unit leaf area) of WW and WS rosemary plants during a 15-day experiment are shown in Figure 2. During the light period, whole-shoot net photosynthesis (A_n) paralleled changes in PPFD. At the beginning of the experiment, A_n and dark respiration (R_d) of WW rosemary plants were about 3.2 and –0.7 μmol m^{-2} s^{-1}, respectively, and the corresponding values at Day 15 were 6.0 and –1.2 μmol m^{-2} s^{-1}. In WS rosemary plants, A_n declined from about 2.9 to 0.3 μmol m^{-2} s^{-1} and R_d declined from about –0.5 to –0.3 μmol m^{-2} s^{-1} during the 10-day water-stress treatment. When WS plants were re-watered, both A_n and R_d recovered within 2 days to their pre-drought values (Figure 2).

The WW and WS lavender seedlings showed similar changes in daily, shoot gas exchange rates as the corresponding rosemary plants (Figure 3). However, whole-shoot A_n and R_d of WW lavender seedlings were higher than those of rosemary plants at the beginning of the experiment (about 7.2 and –1.4 μmol m^{-2} s^{-1}, respectively). On Day 15, A_n and R_d of the WW lavender seedlings had increased to about 12.5 and –2.9 μmol m^{-2} s^{-1}, respectively. There were no major changes in A_n or R_d in lavender seedlings during the first 3 days of the WS treatment, but after 10 days, A_n decreased from about 8.7 to 0.4 μmol m^{-2} s^{-1} and R_d decreased from about –1.2 to –0.5 μmol m^{-2} s^{-1}. When WS plants were re-watered, both A_n and R_d recovered within 5 days to values similar to those at the beginning of the experiment (Figure 3). Stomatal conductance (g_s) fell by about 91% in rosemary and 77% in lavender during the 10-day water-stress treatment. After re-watering, g_s recovered to values similar to those of WW rosemary and lavender plants.

In response to water stress, total leaf area per plant decreased by about 67 and 34% in rosemary and lavender, respectively (Table 2). When WS rosemary and lavender plants were re-watered, total leaf area recovered by about 50 and 22%, respectively (Table 2). Total leaf area of WW plants increased throughout the 15-day experiment. For WW rosemary plants, total leaf area per seedling increased from about 2300 cm^2 on Day 1 to about 2770 cm^2 on Day 15. The corresponding values for WW lavender plants were 1820 and 2440 cm^2.

No significant effects of the water-stress treatment were observed on the F_v/F_m ratio in the dark or the relative quantum efficiency of PSII photochemistry (ϕ_{PSII}), photochemical quenching (q_P), or the efficiency of energy captured by open PSII reaction center F'_m/F_m at a PPFD of about 1500 μmol m^{-2} s^{-1} in WW, WS or WR rosemary or lavender plants (Table 3). These results indicate that water stress was not associated with photoinhibition of PSII or with loss of linear electron transport capacity in either of the species.

The WW plants of both species had an RWC of about 80% and a Ψ_w of about –1.3 MPa. In WS plants, RWC decreased to about 44% in rosemary and to about 30% in lavender, but increased after re-watering to the corresponding control value (Figure 4). In both species, Ψ_w of WS plants decreased to about –3.2 MPa and then recovered to the corresponding control value after re-watering (Figure 4). No major changes in Chl a and b concentrations or carotenoid concentrations of rosemary leaves were observed during the experiment. In contrast, both Chl a and b concentrations and carotenoid concentrations declined significantly in WS lavender leaves, but a complete recovery of Chl and carotenoid concentrations was observed in response to re-watering (Figure 4). Zeatin concentrations were higher in rosemary plants than in lavender seedlings. In lavender, zeatin concentrations increased simultaneously with the decrease in RWC and Ψ_w. The WS plants of rosemary and lavender had zeatin concentrations of about 24.0 and 14.1 nmol g_{DW}^{-1}, respectively. After re-watering, zeatin concentrations decreased to a value lower than that found in WW plants (Figure 4).

Discussion

In both species, whole-shoot A_n values (expressed per unit leaf area) measured in WW plants at the beginning of the experiment (Figures 2 and 3) were 30–50% lower than A_n values of apical non-woody shoots obtained with a Li-Cor LI-6200 portable measuring system (Munné-Bosch et al. 1999*a*, 1999*b*). They were also lower than values reported for small shrubs

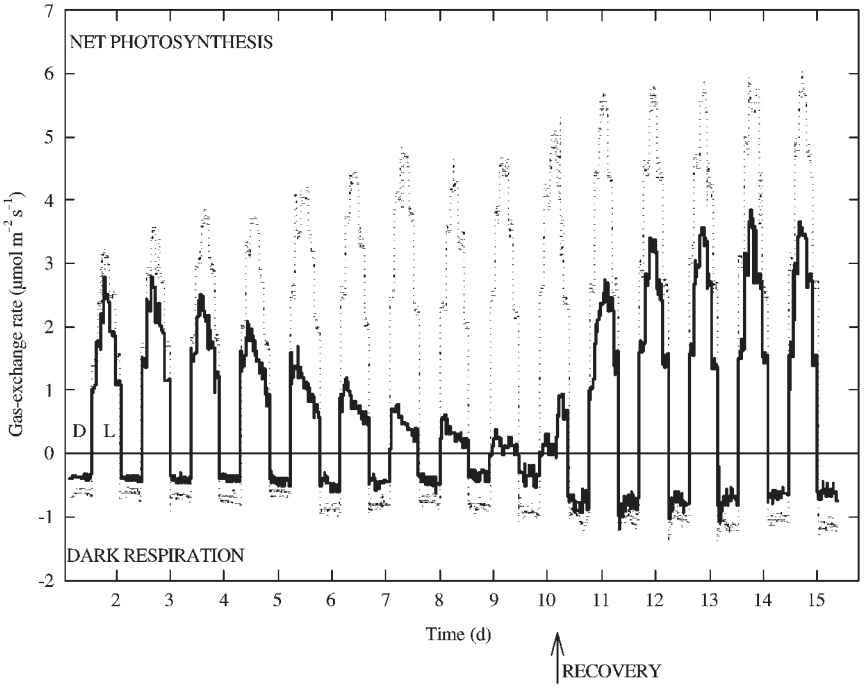

Figure 2. Daily time course of shoot gas-exchange rates (expressed per unit leaf area) of well-watered (dashed lines) and water-stressed (solid lines) rosemary plants during a 15-day experiment. Dark (D) and light (L) periods are indicated. The vertical arrow immediately under the abscissa indicates the time at which the water-stressed plant was re-watered. The kinetics of shoot gas-exchange rates are from a single well-watered plant and a single water-stressed plant, but a similar pattern was found when the experiment was repeated twice.

such as *Ceanothus thyrsiflorus* Eschsch. (Tenhunen et al. 1994).

The 10-day drought treatment resulted in severe water stress, with RWC and Ψ_w values lower than 50% and −3.0 MPa, respectively (Figure 4). During summer drought, Mediterranean vegetation often displays large reductions in RWC and Ψ_w similar to those presented in Figure 4 (Kyparissis et al. 1995, Munné-Bosch et al. 1999*a*, Tognetti et al. 1999). Water stress resulted in large decreases in A_n and R_d in both species (Figures 2 and 3). At the beginning of the water-stress treatment, only about 15% of the net CO_2 assimilated during the day was lost to shoot R_d the following night in both species (Figures 2 and 3). After 10 days of water stress, about 95% of the CO_2 assimilated during the day was released the following night in shoot R_d in both species (Figures 2 and 3). Thus, the stoichiometry between photosynthesis (and

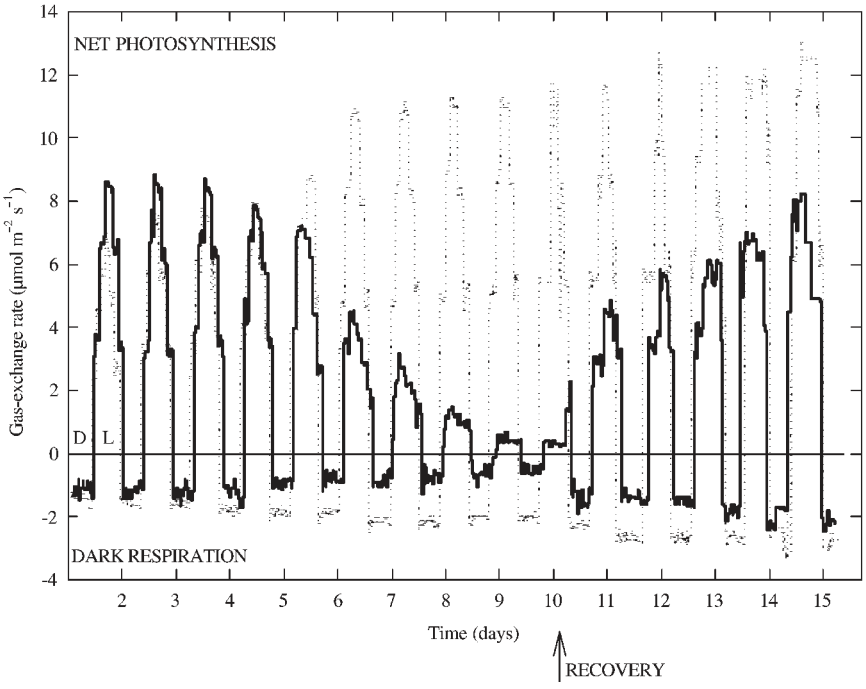

Figure 3. Daily time course of shoot gas-exchange rates (expressed per unit leaf area) of well-watered (dashed lines) and water-stressed (solid lines) lavender seedlings during a 15-day experiment. Dark (D) and light (L) periods are indicated. The vertical arrow immediately under the abscissa indicates the time at which the water-stressed seedling was re-watered. The kinetics of shoot gas-exchange rates are from a single well-watered seedling and a single water-stressed seedling, but a similar pattern was found when the experiment was repeated twice.

Table 2. Effects of a 10-day period of water stress followed by a 5-day recovery on total leaf area of rosemary and lavender plants. Data are the means of three experiments ± 1 SEM. Significant differences (*t*-test, $P < 0.05$) between water regimes for each species are indicated by an asterisk.

Parameter	Rosemary	Lavender
Total leaf area per plant at the beginning of the experiment (WW, cm^2)	2010 ± 480	1350 ± 200
Total leaf area per plant at the end of the water-stress period (WS, cm^2)	660 ± 150*	890 ± 210
Total leaf area per plant at the end of the experiment after re-watering (WR, cm^2)	1310 ± 360	1150 ± 110
Leaf area per plant lost during the period of stress (cm^2 day^{-1})	134 ± 33	46 ± 14
Leaf area per plant recovered after re-watering (cm^2 day^{-1})	128 ± 36	51 ± 12

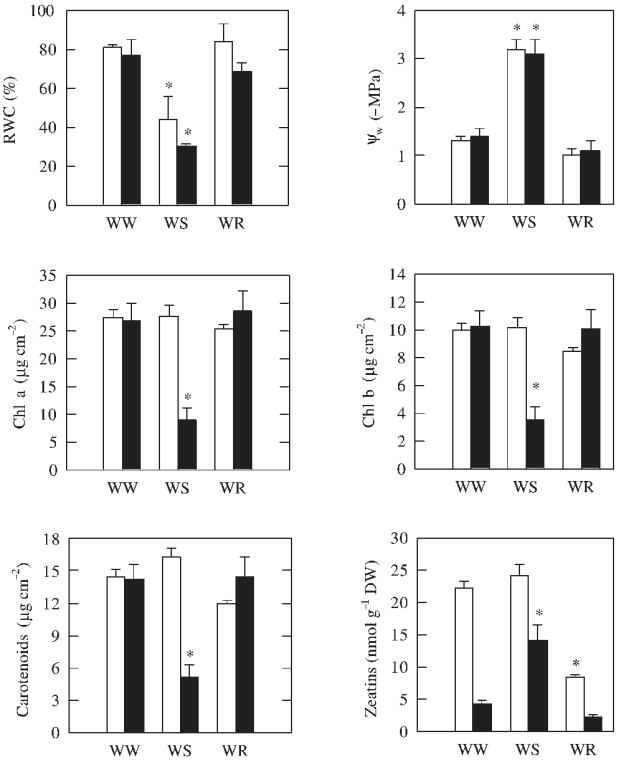

Figure 4. Changes in RWC, Ψ_w, Chl a, Chl b, total carotenoid, and zeatin concentrations of well-watered (WW), water-stressed (WS) and recovered (WR) rosemary (open bars) and lavender (solid bars) plants, measured at 0700 h. Data are the means of three experiments ± 1 SEM. Significant differences (*t*-test, $P < 0.05$) between water regimes within each species are indicated by an asterisk.

growth) and shoot respiration was altered by water stress (root respiration was not measured). Whole-plant maintenance respiration generally declines during water stress as a result of an overall slowing of metabolic activity (McCree 1986*a*, Amthor 1994). In general, R_d was less sensitive to water stress than A_n (Figures 2 and 3), reflecting the fact that photosynthesis and growth can cease for a time during severe stress, but respiration must proceed, albeit often at a reduced rate (Amthor 1994).

Whole-plant photosynthesis is the product of leaf area and photosynthetic rate per unit leaf area. In WW seedlings of both species, A_n increased over the 15-day measurement period by about 47 and 43% for rosemary and lavender, respectively (Figures 2 and 3), whereas leaf area increased by about 17 and 25% for rosemary and lavender, respectively (Table 2). This suggests that photosynthetic capacity per unit leaf area also increased in the WW seedlings throughout the 15-day experiment. However, both leaf area and photosynthetic rate per unit leaf area declined in WS plants. An early response to the development of water deficits is a reduction in leaf area, and therefore in transpiration and the amount of intercepted radiation (Pereira and Chaves 1993). Water stress decreased total leaf area (based on leaf size and leaf number) by about 67 and 34% in rosemary and lavender plants, respectively (Table 2). Water stress could reduce leaf expansion by changing turgor pressure and the wall extensibility of the leaf cells (Spollen et al. 1993), by inhibiting the proton extrusion to apoplast necessary to promote wall-loosening (van Volkenburg and Boyer 1985), or by decreasing the water potential gradient moving water into expanding cells (Nonami et al. 1997). Changes in cell expansion may be the cause of the typical leaf curling observed in Labiatae seedlings (such as rosemary and lavender)

Table 3. Changes in F_v/F_m ratio in the dark and relative quantum efficiency of PSII photochemistry (ϕ_{PSII}), photochemical quenching (q_P), and efficiency of excitation energy captured by open PSII reaction center (F_v'/F_m') at a PPFD of about 1500 μmol m^{-2} s^{-1} of well-watered (WW), water-stressed (WS) and recovered (WR) rosemary and lavender plants. Data are the means of three experiments ± 1 SEM. There were no significant differences (*t*-test, $P < 0.05$) between treatments for either species.

Parameter	Rosemary			Lavender		
	WW	WS	WR	WW	WS	WR
F_v/F_m	0.80 ± 0.04	0.76 ± 0.07	0.82 ± 0.05	0.80 ± 0.05	0.73 ± 0.04	0.81 ± 0.05
ϕ_{PSII}	0.26 ± 0.05	0.20 ± 0.04	0.24 ± 0.04	0.27 ± 0.03	0.25 ± 0.08	0.32 ± 0.05
q_P	0.60 ± 0.07	0.64 ± 0.05	0.53 ± 0.06	0.60 ± 0.02	0.71 ± 0.08	0.70 ± 0.03
F_v'/F_m'	0.42 ± 0.04	0.30 ± 0.04	0.45 ± 0.05	0.46 ± 0.05	0.34 ± 0.07	0.45 ± 0.06

and other families during water deficits (Cornic and Massacci 1996), as discussed by Nogués et al. (1998). The role of accelerated leaf senescence (especially among the lower leaves on the seedling) in the reduction of leaf area during water stress differed between the species. It was negligible in lavender, but considerable in rosemary (Table 2).

Although reduced growth in response to drought is mainly associated with a reduction in leaf surface growth (Kramer and Boyer 1995), reductions in growth under water deficits have also been ascribed to decreases in photosynthetic rate as a result of stomatal closure (Pereira and Chaves 1993, Cornic and Massacci 1996). In our study, water stress reduced stomatal conductance of both species. Furthermore, during water stress, changes in the activities of certain enzymes, e.g., ribulose-1,5-bisphospate carboxylase-oxygenase, sucrose-phosphate synthase and nitrate reductase have also been observed (Cornic and Massacci 1996). There was no significant effect of water stress on leaf PSII quantum efficiencies (Table 3), which is consistent with previous results (e.g., Cornic and Massacci 1996) demonstrating the resistance of the photosynthetic apparatus to water deficit. Upon re-watering, whole-shoot photosynthetic activity rapidly recovered to pre-drought rates (Figures 2 and 3).

In lavender leaves, the absence of photoinhibitory damage to PSII centers of apical leaves (Table 3) during water stress was accompanied by a marked decline in chlorophyll and carotenoid concentrations (Figure 4). However, there was an increase in the carotenoids/chlorophyll ratio, possibly reflecting a regulatory process that allows lavender seedlings to reduce the risk of overexcitation and photooxidative damage (Kyparissis et al. 1995). Light-induced accumulation of zeaxanthin by the transformation of violaxanthin is another photoprotective mechanism of leaves (Demming-Adams and Adams 1996). The high zeatin concentrations could be related to the maintenance of chlorophyll and carotenoid concentrations in rosemary plants during water stress, as discussed for other species (Hukmani and Tripathy 1994, Synková et al. 1997). The mechanisms underlying the zeatin (and other cytokinins) effect on plant responses to water stress remain to be elucidated.

In conclusion, water stress caused a significant decrease in whole-shoot A_n in both Mediterranean shrub species that was associated with reductions in leaf area and stomatal conductance. Shoot R_d was less sensitive to water stress than A_n. Shoot R_d/A_n ratio increased from about 15 to 95% during water stress. No major changes in the chlorophyll and carotenoid concentrations of rosemary leaves were observed in response to water stress, but both chlorophyll and carotenoid concentrations fell significantly in WS lavender leaves. Zeatin concentrations were higher in rosemary leaves than in lavender leaves during water stress. After re-watering the drought-stressed plants, whole-shoot A_n and R_d rapidly recovered to their pre-drought rates.

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