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Evaluation of water use efficiency: the contribution of structural vs. functional adaptations to drought stress

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Introduction

Mediterranean species are particularly well adapted to the seasonal fluctuations in water availability. They have developed a variety of strategies to avoid irreversible damage due to water stress, e.g. high stomatal control of transpirational water loss or structural adaptations. In this work, three species with different strategies were analysed, which correspond to three different functional groups: i) drought tolerant evergreen sclerophyll (*Quercus coccifera*), that endures water stress by high stomatal regulation of water loss and a deep root system; ii) a drought semi-deciduous shrub (*Cistus albidus*) with highly pubescent leaves; this species is seasonal dimorphic with smaller summer leaves, and avoids summer drought by reduction of the transpirational leaf area through partial leaf abscission; iii) a fast growing perennial herb (*Vinca difformis*) with mesophyllic leaves and low tolerance to water stress, normally found at more humid sites. It will be particularly important to link these structural and functional characteristics with the typical “spender” and “saver” strategy when subjected to a slow drying treatment. A significant contribution in this field was represented by the finding that carbon isotope discrimination ($\Delta^{13}\text{C}$) is negatively related to water use efficiency (WUE) in C_3 plants. Further, it has become evident that a wide range of external factors influence leaf Δ , as for example vapour pressure, temperature, light, water and nutrient supply. Additionally, life form, growth strategy or phenology should all be considered important determinants of Δ . In this work we will evaluate and compare the responses of three mediterranean species with different adaptations to drought stress, when subjected to a slow drying treatment under semi-controlled conditions. The main objective was the evaluation of different strategies of WUE through the analysis of carbon isotope composition of leaf dry matter and gas exchange measurements, and the relation with structural and functional adaptations.

Materials and methods

The drought experiment was performed under semi-controlled conditions in a green house (natural light and temperature) at the Lisbon University, Portugal, during spring 2001. *Quercus coccifera* L., *Cistus albidus* L. and *Vinca difformis* were selected. Ten plants per species were kept well watered (control), and ten were exposed to a slow

drying cycle over eleven weeks. Either than the soil moisture, the environmental conditions followed the natural climate, from a cold humid early spring to a hot dry late spring at the end of the experiment. The soil moisture was measured continuously using the sensor Theta Probe type ML1 (Delta-T Devices, Cambridge, UK). Soil moisture was maintained above $0.3 \text{ m}^3 \text{ m}^{-3}$ in control plants, and was step-wise decreased below $0.1 \text{ m}^3 \text{ m}^{-3}$ in the water stress treatment. In the case of *V. difformis* soil moisture was reduced until it reached the wilting point ($0.1 \text{ m}^3 \text{ m}^{-3}$) and maintained thereafter at this level, whereas soil moisture declined further in *C. albidus* and *Q. coccifera*. *Chlorophyll a fluorescence* was measured with a portable fluorometer (Mini-PAM, Walz, Effeltrich, Germany). The maximum quantum yield of PSII (Fv/Fm) was recorded at solar noon after 20 min dark adaptation. The effective quantum yield ($\Delta F/Fm'$) was taken at a light intensity of $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$. *Gas exchange* was measured with a $\text{CO}_2/\text{H}_2\text{O}$ Porometer (Walz, Effeltrich, Germany). Constant light conditions ($\pm 950 \mu\text{mol m}^{-2} \text{ s}^{-1}$) were provided by a fiber illuminator FL-440 (Walz, Effeltrich, Germany). Predawn and midday *water potentials* were measured with a pressure chamber (Manofrigido, Portugal). *Leaf areas* were determined from 3-6 leaves per plant. Fully developed leaves from the latest growth were used for stable isotope analysis (1-yr old leaves of *Q. coccifera* were used at the beginning of the experiment). Leaves were dried at 60°C and milled to a fine powder, followed by current standard analytical procedures in a SIRA II VG (Micromass, UK), coupled with an automatic Elemental Analyser (EuroVector, It) at the ICAT Stable Isotope Laboratory, Lisbon. All data were tested by ANOVA with Duncan-post-hoc test for statistical differences.

Results

The experiment showed a strong response of all species to the drought treatment. Results are shown of measurements taken after 10 weeks of drought, when water stress became most severe (Tab.1). Well watered and stressed plants of each species exhibited significant differences ($p < 0.005$) in all measured parameters, except leaf area and specific leaf weight (SLW) in *Q. coccifera* and SLW and $\Delta^{13}\text{C}$ -values in *C. albidus*. All water stressed plants showed a decrease in $\Delta^{13}\text{C}$, compared to well watered ones. $\Delta^{13}\text{C}$ declined from 20.5, 21.0 and 21.9‰ at the beginning of the experiment (data not shown) to 19.4, 19.1 and 18.9‰ after drought stress in *Q. coccifera*, *C. albidus* and

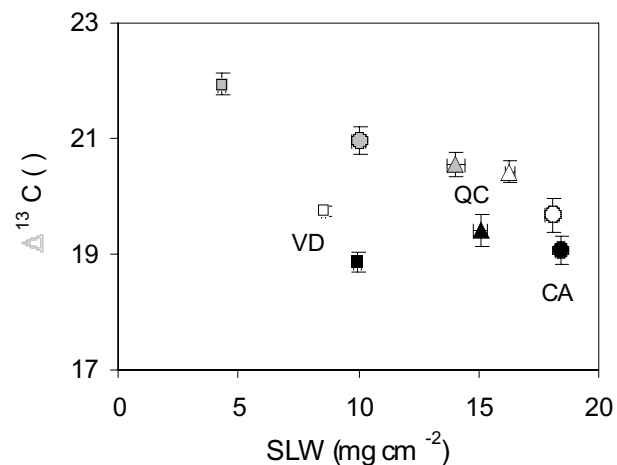


Fig. 1. Relationship between specific leaf weight (SLW) and $\Delta^{13}\text{C}$ (‰), of *Q. coccifera* (triangle, QC), *C. albidus* (circle, CA) and *V. difformis* (square, VD) at the beginning of the experiment (gray symbols, $n = 16-19$) and at the end in control (open) and water-stressed (closed symbols) plants, each $n = 8-10$, \pm SE.

V. difformis, respectively. This decline in $\Delta^{13}\text{C}$ was accompanied with an increase in SLW throughout the experiment (Fig. 1), even though differences among control and water-stressed plants were only significant in *V. difformis*. All parameters were influenced by the increase in air temperature throughout the experiment, which was reflected in the control plants. Predawn water potentials decreased to -4.1, -3.2 and -1.5 MPa in *Q. coccifera*, *C. albidus* and *V. difformis*, respectively. In *Q. coccifera* no further decline in water potentials was observed during the day, whereas *V. difformis* showed a large decrease to -3.3 MPa.

Tab. 1. Ecophysiological and structural parameters of well watered (control) and water-stressed plants after 10 weeks of drought. All parameters were measured from May 30 to June 5, except fluorescence measurements which were conducted on May 18. For the $\Delta^{13}\text{C}$ pooled data from the last two sampling dates (25.5. and 5.6) are shown to give a more representative value. Measurements were conducted on 8 to 10 plants per treatment, with 1- 6 (15) replicates per plant, \pm SD. Within each species, all data are significantly different ($p < 0.005$), except leaf area and SLW for *Q. coccifera* and SLW and $\Delta^{13}\text{C}$ -values for *C. albidus*.

	<i>Quercus coccifera</i>		<i>Cistus albidus</i>		<i>Vinca difformis</i>	
	Drought	Control	Drought	Control	Drought	Control
Predawn water potentials (MPa)	-4.1 \pm 1.1	-0.6 \pm 0.1	-3.2 \pm 0.8	-0.4 \pm 0.1	-1.5 \pm 0.9	-0.3 \pm 0.1
Midday water potentials (MPa)	-4.3 \pm 0.8	-1.9 \pm 0.3	-3.6 \pm 0.6	-1.6 \pm 0.2	-3.3 \pm 0.4	-1.6 \pm 0.3
Net photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$)	1.70 \pm 2.00	10.25 \pm 3.28	2.75 \pm 1.83	7.86 \pm 2.61	2.52 \pm 1.88	6.95 \pm 1.81
Transpiration ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$)	0.25 \pm 0.29	2.52 \pm 1.35	0.39 \pm 0.31	2.72 \pm 1.12	0.58 \pm 0.45	2.36 \pm 0.94
Maximum photochemical efficiency of PSII (Fv/Fm)	0.55 \pm 0.07	0.74 \pm 0.02	0.66 \pm 0.06	0.75 \pm 0.02	0.59 \pm 0.08	0.69 \pm 0.04
Effective quantum yield ($\Delta\text{F}/\text{Fm}'$)	0.31 \pm 0.10	0.58 \pm 0.07	0.28 \pm 0.14	0.58 \pm 0.08	0.46 \pm 0.11	0.55 \pm 0.04
Leaf area (cm^2)	3.5 \pm 0.6	3.8 \pm 0.8	2.3 \pm 0.5	4.4 \pm 0.6	2.2 \pm 0.6	6.0 \pm 0.9
Specific leaf weight (mg cm^{-2})	15.1 \pm 1.5	16.3 \pm 1.8	18.4 \pm 1.6	18.1 \pm 1.9	9.9 \pm 0.8	8.5 \pm 1.0
Isotope discrimination $\Delta^{13}\text{C}$ (‰)	19.4 \pm 0.7	20.4 \pm 0.5	19.1 \pm 0.6	19.7 \pm 0.8	18.9 \pm 0.5	19.7 \pm 0.3

Transpiration was 10, 7, 4 times lower in water-stressed plants as compared to the control, while net photosynthesis declined 6, 3, 3 times in *Q. coccifera*, *C. albidus* and *V. difformis*, respectively. These results suggest that stomatal control was highest in *Q. coccifera* and lowest in *V. difformis*. *Q. coccifera* was most susceptible to photoinhibition and Fv/Fm declined in water stressed plants to 0.55. However, even well watered plants showed a reduction in Fv/Fm as a result of the high temperatures and incident radiation intensities, which was most pronounced in *V. difformis* and least in *Q. coccifera*. The evergreen sclerophyll did also maintain the highest photosynthesis rates in control plants, inversely to the pattern found before the experiment (data not shown), indicating the high resistance of this species to increasing air temperatures. It did not adjust the transpirational area during the drought treatment, neither at the single leaf nor at the plant level, and growth occurred only at the beginning of the experiment when water stress was still moderate (data not shown). Well watered *C. albidus* developed 2 times and *V. difformis* even 3 times larger leaves in comparison to the water-stressed ones. *C. albidus* revealed enhanced leaf abscission and reduced growth (data not shown). Following the “spenders” strategy, *V. difformis* showed less effective control mechanisms of water loss. In fact, the irreversible wilting point was reached considerably faster than in the other species (data not shown).

Discussion

The drought treatment induced significant differences in nearly all measured parameters, however, WUE as measured by $\Delta^{13}\text{C}$ was strongly influenced by the growth strategy (timing and length of growing season) of the species: *Q. coccifera*, which produced new leaves during a short, flush-type growth period at the beginning of the experiment exhibited only a small increase in WUE throughout the drought treatment (Fig. 1). This relatively low WUE reflected the cooler and more humid environmental conditions in early spring when leaves emerged. Its high capacity of stomatal regulation of transpiration and carbon uptake with increasing drought (Tab. 1, Tenhunen et al. 1985) were not reflected in bulk leaf $\Delta^{13}\text{C}$. *C. albidus* and *V. difformis* exhibited lower WUE before the treatment, and a lower capacity to regulate gas exchange parameters to drought (Tab. 1). However, their extended growth period throughout the experiment allowed these adjustments to drought to be recorded in the carbon isotope signal of newly formed leaves. Both species showed structural regulation by changes of leaf area and SLW. Rapid adjustment of leaf area (both through leaf size and leaf number) and high leaf turnover is an alternative strategy to adjust the transpirational area to drought (Werner et al. 1999, 2001). A prolonged growth period allows flexible adjustment of leaf structural properties, as for example SLW and leaf pubescence, to the prevailing environmental conditions (Correia et al. 1987). These adaptations resulted in higher WUE in leaves produced at the end of the experiment as compared to the evergreen sclerophyll, which did not exhibit any structural adjustment at the leaf or plant level. In general, the differences imposed by the drought treatment were partially buffered by the strong increase in air temperature during the experiment from relative humid and cold early spring to hot dry late spring. This additional stress was also reflected in all control plants. *V. difformis* was the most responsive species, with a decline of 3‰ in $\Delta^{13}\text{C}$ throughout the experiment, also revealing the lowest tolerance to low water potentials. It was further the only species, where a decline in water potentials throughout the day exceeded that of the control plants (see Tab. 1), indicating its low ability to extract water from the dry soil and reduce water loss. *C. albidus* tolerated much lower water potentials, but still reached similar WUE as *V. difformis* at the end of the experiment.

In conclusion, even though drought stress resulted in a significant decrease in $\Delta^{13}\text{C}$, the environmental conditions during leaf formation, the length of the growing period, and leaf longevity were the major determinants of WUE. All species were highly responsive to drought stress in most ecophysiological and structural parameters, whereas only a buffered response was found in $\Delta^{13}\text{C}$ of bulk leaf material. Especially in the evergreen sclerophyll, $\Delta^{13}\text{C}$ reflected the WUE under growth conditions, rather than regulation of gas exchange to drought conditions. Hence, $\Delta^{13}\text{C}$ is not a sensitive parameter to measure WUE under stress conditions in these species with long-lived leaves and short growing period. This has important implications for the interpretation of $\Delta^{13}\text{C}$ in Mediterranean sclerophyllous species, where leaves are generally produced under favourable environmental conditions in spring, but regulation of WUE may be more important during summer drought.

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