RESEARCH PAPER

The impact of drought on leaf physiology of Quercus suber L. trees: comparison of an extreme drought event with chronic rainfall reduction

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Abstract

Understanding the responses of cork oak (Quercus suber L.) to actual and predicted summer conditions is essential to determine the future sustainability of cork oak woodlands in Iberia. Thermal imaging may provide a rapid method for monitoring the extent of stress. The ecophysiology of cork trees was studied over three years. Three treatments were applied by means of rainfall capture and irrigation, with plots receiving 120%, 100%, or 80% of natural precipitation. Despite stomatal closure, detected using both thermal imaging and porometry, leaf water potential fell during the summer, most drastically during the third year of accumulative stress. The quantum efficiency ($\Phi_{PSII}$) and the maximum efficiency ($F_{V}/F_{M}$) of photosystem II also fell more intensely over the third summer, while non-photochemical quenching (NPQ) increased. The reduced precipitation treatment sporadically further reduced leaf water potential, stomatal conductance ($g_s$), $I_G$ (an index of $g_s$ derived from thermal imaging), $\Phi_{PSIIh}$ and $F_{V}/F_{M}$, and increased leaf temperature and NPQ. It is concluded that these are very resilient trees since they were only severely affected in the third year of severe drought (the third year registering 45% less rainfall than average), and removing 20% of rainfall had a limited impact.

Key words: Climate change, cork oak, drought, non-photochemical quenching, photochemistry, photosystem II, stomatal conductance, thermography, water potential.

Introduction

Evergreen oak woodlands are silvo-pastoral ecosystems of ecological and economic importance in the western Mediterranean, especially in the Iberian Peninsula. They are characterized by a sparse tree layer, mainly dominated by species such as Quercus ilex subsp. rotundi-folia Lam. (holm oak) and Quercus suber L. (cork oak), growing over a shrub or a herbaceous layer (Bugalho et al., 2009).

Adult cork oak trees are evergreen as their leaves from the previous year usually abscise along with the spring growth flush of new leaves. A second but less intense growth flush often occurs in autumn (Pereira et al., 1987).

Abbreviations: $F_0$, initial/basal fluorescence; $F_{Mh}$, maximum fluorescence in the dark-adapted state; $F_{Ml}$, maximum fluorescence in the light-adapted state; $\Phi_{PSII}$, estimation of the quantum efficiency of non-cyclic electron transport; $F_v$, variable fluorescence; $g_s$, stomatal conductance to water; NPQ, non-photochemical quenching; PAR, photosynthetically active radiation; PSII, photosystem II; $\Psi_l$, leaf water potential; $q_v$, photochemical quenching; $T$, temperature; VPD, vapour pressure deficit of the air.

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To remain functional throughout the summer, tissue water status must be controlled. Stomatal closure is the main mechanism in evergreen Mediterranean tree species for regulating transpirational water loss and avoiding dehydration damage to cells and tissues (Martinez-Vilalta et al., 2002). Concurrent with stomatal closure, and also with a slight reversible depression of photochemical efficiency associated with short-term changes in the xanthophyll cycle (reversible de-epoxidation), Q. suber leaves undergo a midday depression of photosynthesis (Faria et al., 1996). Photosynthesis is further down-regulated over the course of the dry season (Faria et al., 1996, 1998). Shedding of leaves or even branches may occur, but only during extreme summer drought, favouring the maintenance of water in the remaining parts of the plant (Chaves et al., 2003). Such mechanisms for withstanding drought in summer are crucial for tree survival in the Mediterranean environment.

Climate change scenarios suggest an exacerbation of spring and summer moisture deficits in the Mediterranean region (IPCC-WGI, 2007). Already, spring precipitation is significantly lower, and inter-annual variability of winter precipitation and the frequency of droughts significantly higher, than in the 1970s (Luterbacher et al., 2006; Miranda et al., 2006). While it is known that under current conditions Q. suber experiences stress during the summer months, the impact of a further reduction in precipitation, as predicted in the future, is unknown. Preventing a percentage of precipitation from reaching the soil is an experimental technique currently being used in a range of studies in temperate (Osorio et al., 1998) and tropical forests (Nepstad et al., 2002; Fisher et al., 2006) to explore the impact of such a reduction in precipitation. For example, in the Amazon forest a decline in gross assimilation was observed during the two treatment years as a result of a combination of physiological responses to short-term drought (P Meir et al., unpublished results).

The impact on the leaf physiology of Q. suber in such an experiment is reported here and it is the first time this type of experiment has been conducted with this important and vulnerable species.

Stomatal closure is an indicator of plant water stress and has been useful for monitoring the response of key species to environmental change. Transpiring leaves are cooler than leaves with closed stomata (therefore without transpirational cooling). Leaf or canopy temperature can be assessed remotely by thermal imaging (Jones, 2004). Indices have been developed that relate leaf or canopy temperatures to the temperatures of selected reference surfaces under the same conditions of air temperature, radiation, and wind speed. Such indices remove the effect of influences other than stomatal conductance on leaf temperature and hence are used to indicate increases or decreases in stomatal conductance (Jones, 1999). Use of such indices has opened up the possibility of using thermal imaging for irrigation scheduling (Jones et al., 2002; Grant et al., 2007). Without a reference, an increase in the canopy temperature of a crop could relate to increased stress, or could relate to increased air temperature. Comparison with a non-water-stressed crop, however, allows calculation of a Crop Water Stress Index (Idso, 1982). Since a non-water stressed crop is not always available, much effort has been devoted to finding alternative references, with Jones (1999) using ‘wet’ and ‘dry’ leaves to calculate either a Crop Water Stress Index or an index proportional to stomatal conductance. These ‘leaves’ may be artificial, for example, wet and dry filter paper, or may be freshly cut leaves, artificially forced to show maximum (spraying with water) and minimum (covering the stomata in e.g. petroleum jelly to prevent transpiration) stomatal conductance, thus acting as wet and dry references, respectively (Jones et al., 2002). The best choice of reference depends on the crop and environment (Grant et al., 2006), and the differing properties of leaves or branches as compared with whole plant canopies can mean that reference canopies are more suitable references than cut leaves, even if the reference canopies are used as indicators of relatively high and low stomatal conductance (where it is impossible to maintain any section of the canopy with no water stress or with complete stomatal closure), rather than the extremes (Grant et al., 2007). Indeed, some authors have chosen to avoid the use of either plant canopies or leaves, or leaf-like references (e.g. filter paper of a similar size), and instead used alternatives such as the temperature of cloth in a basin of water to estimate the wet reference (Möller et al., 2007), and air temperature plus 5 °C to estimate the dry reference (Cohen et al., 2005). Where sufficient meteorological data are available, stomatal conductance can be estimated from leaf temperature using energy budget equations, without the need for any references (Leinonen et al., 2006). A completely different approach would be to use temperature variability within a canopy as an indicator of crop stress, as suggested by Fuchs (1990), the principle being that stomatal conductance has the greatest impact on leaf temperature when stomata are open, but when stomata close the differing leaf orientations in a canopy with random leaf orientation will result in a wide range of leaf temperatures within the canopy.

While much of the emphasis in applying thermal imaging under field conditions has been on irrigation scheduling, thermal imaging has also been applied to modelling regional fluxes of water (Anderson et al., 2008), and, given the close coupling of stomatal conductance and photosynthesis, holds potential for modelling carbon dioxide fluxes (Zhan and Kustas, 2001). Recently, thermal imaging has been applied to the calculation of a crop water stress index for olive trees (Ben-Gal et al., 2009). Hand-held thermal imaging, has not, however, been applied to date to monitoring responses of trees in natural or semi-natural habitats to environmental stress.

The objectives of this work were to determine whether an artificially imposed reduction in precipitation alters the physiological resilience of Q. suber trees to cumulative summer stress conditions, using hand-held thermal imaging and other measures of stomatal and photosynthetic performance.
Materials and methods

Experimental conditions
Field experiments were conducted between 2003 and 2005 on the University of Evora’s Mitra Campus (38°31′40″ N, 8°01′23″ W), 12 km south-west from Evora city in southern Portugal. The experimental site is exposed to a Mediterranean mesothermic humid climate characterized by hot and dry summers and cool and humid winters (Reis and Gonçalves, 1987). Long-term average meteorological data (1951–1990) for this area show that precipitation is mainly distributed between October and May, with average annual precipitation of 665 mm (see Otieno et al., 2006). Maximum temperatures occur in August, with an average annual maximum of 21.5 °C and annual mean of 15.4 °C. The experimental site consists of 0.26 ha (46 m × 59 m) with Q. suber, planted in 1988, as the only tree species. Twenty-seven Q. suber trees, of 5 m height on average, were selected for monitoring.

During the summer of 2003, seasonal variation in the physiology of the selected trees was determined under ambient conditions. From November 2003 onwards, in accordance with predictions of a 20% reduction in precipitation (Miranda et al., 2006), an experiment was designed to establish a ‘Dry’ treatment (preventing 20% of precipitation reaching the soil using plastic to cover 20% of the soil surface, installed at a height of 0.3–1.6 m), an ‘Ambient’ treatment (with full natural rainfall) and a ‘Wet’ treatment (adding the 20% of precipitation removed from the Dry treatment). Each treatment was replicated randomly within each of three blocks, with physiological data being recorded in three trees per treatment per block. A block design was used to take into account potential variation down the slope (a 10 m altitude difference exists within the site) on which the experimental site was located. Water collected from the Dry treatment was redistributed, along with additional water from a reservoir, onto the Wet treatment plots via irrigation pipes with drip emitters, so that the Wet treatment received 120% of ambient precipitation. These irrigation pipes were covered in similar plastic covers as used in the Dry plot to ensure that a similar effect of covering 20% of the ground area occurred in both these contrasting treatments. The autumn and winter of 2004/2005 was very dry and therefore the Wet treatment was supplied with additional water during the period July 2004 to June 2005 (126% of the rainfall occurring in the Ambient treatment).

Meteorological conditions and soil moisture content
Meteorological conditions were recorded at a meteorological station located 1 km from the study site. Volumetric soil moisture content was measured with Echo probes (Decagon Echo2 EC20, Decagon Devices Inc., Pullman, USA) in 2004 and with profile probes (PR2/6, Delta-T Devices Ltd, Burwell, Cambridge, UK) in 2005.

Leaf water potential
Leaf water potential (ΨL) of two leaves of the outer part of the canopy per tree (nine trees per treatment) was measured using a pressure chamber (model 1000, PMS Instruments, Albany, Oregon, USA) at predawn and at midday each year.

Thermal imaging and stomatal conductance
In 2003 thermal images of one leaf on a branch facing south and one leaf on a branch facing north were captured on nine trees per day, on three consecutive days (so that all 27 trees were measured). In 2004 and 2005, clusters of leaves on a south-facing branch of each tree were imaged. In 2003 and 2004 measurements were performed at 10.00 h and 14.00 h, whereas in 2005 only one series of images starting at 10.00 h was captured on any given day, with measurements repeated on two consecutive days.

Images were taken with a thermal imager (IR Snapshot 525, Infrared Solutions, Minneapolis, USA) that operates in the wave-bands 8–12 μm, has a thermal resolution of 0.1 °C, and produces pictures with a spatial resolution of 120×120 pixels. The imager was held perpendicular to the area being imaged, at a distance of approximately 1.5 m. For each series of measurements, the background temperature was determined as the temperature of a crumpled sheet of aluminium foil in a similar position to the leaves of interest. Emissivity was set at 0.96 (see review by Jones, 2004).

Dry and wet references were used to mimic leaves with fully closed and fully open stomata, respectively (Jones et al., 2002). These references consisted of a sheet of filter paper sprayed regularly with deionized water to maintain moisture (wet reference), and a leaf covered in petroleum jelly (dry reference) in 2003. In 2004 and 2005, branches were cut from a Q. suber canopy prior to measurements and placed close to the leaves of interest: a branch sprayed with water acted as the wet reference and a branch with the leaves covered in petroleum jelly on both sides acted as the dry reference (Fig. 1). The temperatures of these references were obtained (Tdry and Twet) and used in conjunction with leaf temperature (Tleaf) to calculate the thermal index Ic:

\[ I_c = (T_{dry} - T_{leaf})/(T_{leaf} - T_{wet}) \]

This index is theoretically proportional to stomatal conductance (gs) (Jones, 1999).

Thermal images were corrected for spatial calibration drift by subtracting corresponding reference images of an isothermal surface (Jones et al., 2002), and analysed in SnapView Pro software (Infrared Solutions, Minneapolis, USA).

Stomatal conductance (gs) of the leaves imaged in 2003 and of three leaves in the area imaged in 2004–2005 was measured with a steady-state porometer (Li-Cor 1600, Li-Cor, Nebraska, USA), immediately after each image was captured (2003 and 2005), or after a whole set of images (2004).

Chlorophyll a fluorescence
Chlorophyll a fluorescence parameters were measured using a PAM 2000 system (H Walz, Effeltrich, Germany) in recently matured leaves on the outside part of the tree canopy (three leaves per branch, two measurements per leaf), under ambient conditions. Measurement of basal fluorescence (F0) and maximal photochemical efficiency of photosystem (PS) II (Fv/Fm) was performed before dawn. Photochemical quenching (qs) and non-photochemical quenching (NPQ), estimation of the quantum yield of photosynthetic non-cyclic electron transport (ΦPSII), and PSII efficiency of energy conversion (Fv′/Fm′) were determined at 10.00 h and 14.00 h, on both north-west and south-east-facing branches until April 2005, and thereafter on south-east-facing branches only.

Data analyses and statistics
The significance of correlations was determined by Spearman correlation analysis. The effects of treatments were analysed by one-way ANOVA using a randomized block design. Analysis of variance of repeated measures (ANOVAR) was used to assess the significance of treatments over time, for example, along a season, to take into account that the same plants were measured through the year and treatment on August 2004 and August 2005 data. Mean values per plant were used in data analysis for all the physiological measurements. Data analysis was carried out using Genstat software (version 9.1, Rothamstead Experimental Station, UK).

Results

Environmental conditions
During the course of this study, summer air temperatures and vapour pressure deficits were very high, accompanied
by high photosynthetically active radiation (PAR) (Fig. 2). A heat-wave (with day-time air temperatures reaching more than 40 °C) occurred in late July and early August 2003. May to September was very dry in 2003 and 2004. There was little rainfall during the hydrological year of 2004–2005 i.e. from October 2004 to September 2005: total precipitation was 45% below the long-term mean, making it the driest episode recorded in the last 140 years in the southwestern Iberian Peninsula (García-Herrera et al., 2007).

Soil moisture fell during the spring of 2004, but was not affected by the different irrigation treatments (Fig. 3). In 2005 a significant effect of treatment was found on soil moisture content at 10 cm and 20 cm depth ($P < 0.02$; Fig. 3A, B), although not at deeper layers (40–100 cm) (Fig. 3C–F). Even at 10 cm and 20 cm depth, after the autumn rains soil moisture content in the Ambient and Dry treatments recovered to similar values seen in the Wet treatment (Fig. 3A, B). Up until that point, at 20 cm depth, soil in the Wet treatment was significantly wetter than in either of the other treatments through the entire year; at 10 cm depth this was the case from late May onwards, but earlier in the year soil in the Ambient treatment sometimes had a similar moisture content to that in the Wet treatment.

Plant water status

Predawn $Ψ_1$ fell over the course of the summer in all years (Fig. 4A), with particularly low values in late August 2005. Midday $Ψ_1$ showed little change between early and late summer in 2003, but dropped noticeably over the course of the summer in the following two years (Fig. 4B), with particularly low values being reached in August 2005. A significant effect of treatment was found at predawn only in June and July 2005 ($P = 0.038$ and 0.017, respectively), when trees in the Wet treatment had the highest $Ψ_1$. Two-way ANOVA showed a significant reduction in predawn $Ψ_1$ in August 2005 ($P < 0.001$) compared with August 2004, but no overall effect of treatment. Treatments did not have a significant effect on midday $Ψ_1$.

Stomatal conductance for a given branch orientation and time of day tended to fall between early and late summer, and was only significantly affected by treatment on 14
Thermal imaging

I\textsubscript{G} measured in the afternoon fell over the course of the summer in 2003, as did I\textsubscript{G} measured in the morning on south-facing branches (Fig. 4C, D). In 2004, little variation between dates was seen in I\textsubscript{G}. In 2005, I\textsubscript{G} differed substantially between consecutive dates in May, and again in July, but, in general, fell over the course of the summer. 2003 values of I\textsubscript{G} are less reliable than for 2004–2005, because the wet filter paper used for T\textsubscript{wet} in 2003 sometimes had a higher temperature than the leaf.

T\textsubscript{leaf} at 10.00 h on south-facing branches on 14 August 2004 was significantly affected by treatment (P=0.018), reflecting the effect of treatment on g\textsubscript{s} at that time on the same branches: leaves in the Wet treatment were significantly cooler than those in either the Ambient or Dry treatments (data not shown). This was also the case on two dates in 2005 (24 May and 18 July; P=0.006 and 0.041, respectively). On 14 August 2004 and 18 July and 15 August 2005 there was also a significant effect of treatment on I\textsubscript{G} (P=0.036, 0.046, and 0.046, respectively). On all three dates the highest I\textsubscript{G} was found for Wet treatment leaves. Two-way ANOVA showed no significant difference in I\textsubscript{G} in August 2005 compared with August 2004, and no overall effect of treatment.

g\textsubscript{s} and I\textsubscript{G} showed significant correlations for south-facing branches at 10.00 h on 23 September 2003, 30 July and 14 August 2004, and 18 July and 15 August 2005, and at 14.00 h on 9 July 2004 (all r >0.45, P <0.03; examples in Fig. 5).

On north-facing branches there were significant correlations between the two measures at 10.00 h on 23 September 2003 and at 14.00 h on 25 June, 23 July, and 19 August, 2003 (all
r > 0.4, P < 0.05). The average \( I_G \) per treatment per time-point was significantly inversely correlated with \( T_{air} \), VPD, and global radiation, and positively correlated with soil moisture content, while \( T_{leaf} \) was significantly positively correlated with \( T_{air} \), VPD, and global radiation, and inversely correlated with soil moisture content (Table 1). Neither \( T_{leaf} \) nor \( I_G \) was significantly correlated with wind speed.

Chlorophyll fluorescence

Predawn \( F_v/F_M \) always ranged between 0.81–0.85 except during July to August 2005 (Table 2), when \( F_0 \) increased substantially. The highest means of \( F_v/F_M \) at 10.00 h generally occurred in June (Fig. 6A), suggesting photosystem II was down-regulated later in the season. Values of both \( \Phi_{PSII} \) and \( F_v/F_M \) for July and August 2005 were lower than observed earlier in the summer (Fig. 6A-D), and at 10.00 h \( F_v/F_M \) was notably lower than for the previous two years in the same season. It is apparent that trends observed in \( \Phi_{PSII} \) are mainly dependent on \( F_v/F_M \) rather than on \( q_P \) which did not change much along the season (Fig. 6E, F), with the exception of a marked fall between June and August 2003 at 14.00 h (south-east-facing branches). In the late summer, \( NPQ \) tended to be much higher by the early afternoon (Fig. 6H) than at mid-morning (Fig. 6G). This corresponds with lower \( g_s \) in the early afternoon and therefore a limitation of carbon uptake. Particularly high \( NPQ \) was measured in August 2005.

Predawn \( F_v/F_M \) was not significantly lowered by the drought treatment compared with the Wet or Ambient treatment. \( F_v/F_M \) was, however, significantly reduced in the Dry treatment compared with the Wet treatment in leaves of both north-west and south-east-facing branches at 10.00 h in April 2005, and in north-west-facing branches at 14.00 h in both June 2004 and April 2005 (\( P=0.038, 0.011, 0.016, \) and 0.020, respectively; Fig. 6A, B). \( \Phi_{PSII} \) was significantly lower in the Dry and Ambient treatments compared with the Wet treatment in south-east-facing branches at 10.00 h in April 2005, and in north-west-facing branches at 14.00 h in June 2004 and April 2005 (\( P=0.001, 0.008, \) and 0.004, respectively; Fig. 6C, D). \( q_P \) was lower in the Dry treatment than either of the other treatments in April 2005 at 10.00 h for south-east-facing branches and at 14.00 h for north-west-facing branches (both \( P<0.001; \) Fig. 6E, F). \( NPQ \) was lower in the Wet treatment leaves compared with leaves in either of the other treatments on south-east-facing branches at 10.00 h in June 2004 and April 2005, and in north-west-facing branches at 14.00 h in June 2004 and April 2005 (\( P=0.002; \) Fig. 6G, H). Two-way ANOVA showed a significant reduction in predawn \( F_v/F_M \) (\( P<0.001 \)) and in day-time \( V_F/F_M \) (\( P=0.011 \)), accompanied by a significant increase in \( NPQ \) (\( P=0.001 \)) in August 2005 compared with August 2004, but no overall effect of treatment.

Discussion

Seasonal cycle of Q. suber physiology

In general, the physiology of \( Q. \) suber in this environment followed the seasonal pattern expected in a Mediterranean climate: \( \Psi_t \) and \( g_s \) both fell as conditions became more stressful during the summer. In \( Q. \) suber, new foliage develops from April to June (Pereira et al., 1987). Therefore measurements in June correspond to fully developed leaves.
which would be expected to have high rates of gas exchange. By contrast, by August the trees would normally be expected to be most stressed after the summer drought and gs would be reduced. However, the more intense decline of Ψ1 observed in August 2005, at the end of the 3 year period, occurred irrespective of irrigation treatment, suggesting that the combination of high temperatures during the summer and several months with little rainfall had a severe impact on the water status of these trees. In the summer of 2005 predawn Ψ1 fell to −3.6 MPa on average, values substantially lower than those recorded previously: minimum Ψ1 of −3.0 MPa in older Q. suber trees nearby (David et al., 2007) and −2 MPa at predawn and −3.5 MPa at midday (Otieno et al., 2006) in young trees. This suggests that stomatal control of transpiration might have been insufficient to prevent some xylem embolism (Tyree and Cochard, 1996; Cruiziat et al., 2002).

Relatively high gs in young leaves early in the summer should indicate that photosynthetic assimilation would have been high at this stage. Lower gs later in the summer would be expected to limit photosynthetic assimilation. Generally, high values of Fv/Fm suggest that no irreversible damage

Fig. 6. Seasonal variation in the maximum efficiency of PSII (Fv/Fm, A, B), estimated quantum yield of photosynthetic non–cyclic electron transport (ΦPSII, C, D), photochemical quenching (qP, E, F), and non–photochemical quenching (NPQ, G, H), for branches facing south-east (closed symbols) and north-west (open symbols). Data points represent means ±SE; n = 2 in 2003 and n = 4 in 2004 and 2005. A significant effect of irrigation treatment (P < 0.05) is indicated by an asterisk directly below (A–F) or above (G, H) the data points.
occurred to photosystem II of the leaves of these trees, despite high radiation in conjunction with a severe restriction of water availability. Nevertheless, the increased $F_o$ in summer 2005 suggests the occurrence of some energy overload at the PSII reaction centre (see Chaves et al., 2002). When the increase of $F_o$ is associated with a decrease in $F_v/F_M$, photoinhibitory impairments may take place in the capture and transfer of excitation energy due to irreversible photoinactivation of PSII centres (Franklin et al., 1992). Daas et al. (2008) found that, for $Q. suber$ saplings, the critical temperature at which a rapid rise in $F_o$ occurred was above 45 °C, while the optimal temperature for light-driven electron flux was 30–35 °C. $T_{leaf}$ on the days measured in this study did not reach the critical temperature but was frequently higher than optimal during the summers of 2004 and 2005. $\Phi_{PSII}$ and $F_v/F_M$ fell during the summer in response to increasing stress. Lower $F_v/F_M$ values usually corresponded to higher $NPQ$ values (as was particularly notable in August 2005), denoting dissipative processes that lowered the PS photochemical efficiency.

However, the fact that $F_v/F_M$ was never lowered significantly by drought, and that values generally were greater than 0.8, indicates that there was never significant damage to photosystem II, in contrast to what was reported for deciduous Mediterranean shrubs (Karavatas and Manetas, 1999; Grant and Incoll, 2005). This is consistent with previous studies indicating that Mediterranean evergreen sclerophylls are less sensitive to summer photoinhibition than drought semi-deciduous species (Karavatas and Manetas, 1999). Werner et al. (2002) defined chronic photoinhibition as the percentage reduction in predawn $F_v/F_M$ relative to the annual maximum $F_v/F_M$. In this study the highest values of $F_v/F_M$ (0.84) on both north and south-facing branches were measured in September 2004. Relating all other photoinhibition measurements to this value indicates that chronic photoinhibition never exceeded 10%, and only exceeded 5% in June–August 2005. Chronic photoinhibition was not correlated with predawn $\Psi$, as is characteristic of sclerophyllous, rather than semi-deciduous, Mediterranean vegetation (Werner et al., 2002).

**Influence of removing 20% of rainfall on the physiology of $Q. suber$**

Reducing the volume of rainfall reaching the soil had a limited effect on soil moisture content during this experiment. The trees at this site have been shown to rely on water from below 0.5 m depth and from the groundwater table during the drought period, with less stressed trees having access to deeper soil water sources (Kurz-Besson et al., 2006; Otieno et al., 2006). Other studies confirm that $Q. suber$ trees often rely on deep water sources (0.5–13 m) during the summer (David et al., 2004, 2007). Since there was no significant treatment effect on soil moisture below 0.2 m depth, a large influence of the different irrigation treatments on the physiology of $Q. suber$ would not have been expected during this study. This is reflected in the fact that no differences in $\Psi$ were found until June 2005, and then only at predawn. Nonetheless a significant effect of treatment on $g_S$ and $T_{leaf}$ and $I_G$ was found earlier, in August 2004, suggesting that the limited water available to some of the roots (in the upper layers) resulted in a reduction in stomatal conductance. Occasional reductions in $F_v/F_M$ and $\Phi_{PSII}$ in the Dry or Ambient treatments compared with the Wet treatment again suggest transitory influences of the treatments. In general, however, the impact of a dry year (2005) was greater than the impact of a 20% reduction in rainfall, with significantly lower predawn $\Psi$ and $F_v/F_M$ in August 2005 than in August 2004, accompanied by significantly lower $F_v/F_M$ and significantly higher $NPQ$. By contrast, combining data from August 2004 and August 2005, there was no evidence of any impact of the 20% reduction in rainfall.

**Monitoring of stress in $Q. suber$ using thermal imaging**

Thermal imaging was used successfully to detect the impact of reduced water availability. The index $I_G$ generally declined over the summer, reflecting falling $g_S$. Both $T_{leaf}$ and $I_G$ were successfully used to detect the effect of the Dry treatment, or of ambient conditions compared with increased irrigation. Indeed significant variation between treatments could be detected with these measurements on occasions when it was not detected with porometry. Similar greater sensitivity of thermal imaging in comparison with porometry has been reported previously in crop plants (Leinonen et al., 2006).

As might be expected, $T_{leaf}$ was affected more by $T_{air}$ than any other environmental variable, closely followed by $VPD$. $I_G$ was more weakly correlated with meteorological variables than $T_{leaf}$, but interestingly was more strongly correlated with soil moisture content, suggesting a real impact of drying soil on $g_S$ and hence $I_G$, rather than merely a tendency for drier soils during periods of higher air temperatures.

It has already been shown that thermal imaging is an effective method of detecting crop stress (Grant et al., 2007; Möller et al., 2007); here we suggest that it also has an application in environmental monitoring and ecosystem management, although in the particular environment studied here it gave less clear results than leaf water potential (see below). Apart from an assessment of the reductions in $g_S$, monitoring leaf or canopy temperature should be useful as a record of the duration for which leaves are at temperatures above optimal for photosynthesis, which can be incorporated into model predictions of productivity. At a larger scale, the ability to map evapotranspiration and moisture availability via satellite thermal sensing has important applications in monitoring drought and water use, administering irrigation projects, predicting local and regional water demand, and also in hydrological and weather forecast models (Andersen and Kustas, 2008). Satellite sensing does not, however, provide fine local resolution, and thermal cameras at key locations could enhance the application and uptake of important environmental information, for example, by regional authorities.
Wet and dry reference surfaces with different thermal properties or accidentally exposed to different conditions (e.g. if their angle towards sunlight is different) may have higher and lower temperatures, respectively, than the leaves of interest. In this case the wet filter paper did not act sufficiently similarly to leaves to be considered appropriate as a reference, and was not used after the initial year of the study (see Leinonen et al., 2006). Differences in the angle of reference leaves as compared to the leaves of interest could, however, reduce the validity of $I_G$ even when branches are used as references. There was considerable variation between trees in $I_G$ for each set of measurements. This may reflect the fact that the index is composed of three variables (the temperature of the leaves of interest as well as that of two references), each with an associated error. In addition, variation in leaf angle is also thought to have reduced the correlation coefficients between $I_G$ and $g_s$, as there will have been differences between trees in $T_{leaf}$ on account of different leaf angles which would not be associated with $g_s$.

We have previously described how differences between treatments in leaf angle can mask variation in $g_s$ when thermal imaging is used, since $T_{leaf}$ may be affected more by the angle of the leaf towards the sun than by its $g_s$ (Grant et al. 2006), especially as stomata close. Considerable variability was also seen, however, between trees in $g_s$, so, in a heterogeneous stand as studied here, predawn $\Psi_l$ may be the only suitable variable for monitoring stress, since it is not influenced by transient micro-environmental variation.

Elsewhere we hypothesized that the temperature of entire canopies or large areas of canopies is likely to be more sensitive than the temperature of individual leaves to differences in plant water status (Grant et al., 2007). In this study, it was only possible, given the density of planting, to take images quite close to the trees and therefore only to focus on clusters of leaves or small sections of branches, rather than whole trees. The Q. suber in this study varied greatly in tree size and shape, orientation of branches, and distance to the nearest neighbouring trees. It was not possible to measure fully exposed clusters of leaves with exactly the same orientation towards the sun on each tree. For such heterogeneous trees in a relatively dense stand, taking thermal images horizontal to the canopy is not ideal. If images were taken overhead from a sufficient distance to capture whole trees, variation in leaf angle at the branch scale should play a lesser role in whole-canopy temperature, although Leuzinger and Körner (2007) have shown how average leaf temperatures in forest trees are strongly dependent on the density of leaves and branching habits as well as leaf dimensions and $g_s$. Taking thermal images from about 15 m above canopies was found to be effective with olive trees (Ben-Gal et al., 2009), with the Crop Water Stress Index derived from thermal images correlating strongly with stomatal resistance and stem water potential, and more weakly with soil moisture content. It should be noted that while olives are grown as row crops, the relatively young cork trees in this project are more randomly distributed, and this, in addition to a relatively dense shrub layer, means that it would have been difficult to image each tree using a crane. More mature cork plantations, however, have fewer trees per hectare and would be well suited to imaging from above the canopy. Images of whole canopies would have the additional advantage of lending themselves more easily to automated analysis—it would be difficult to apply automated analysis to the images taken in this study since the leaves of interest would need to be distinguished from all the other leaves (on other branches and other trees) in the background of the images.

Ben-Gal et al.’s (2009) study of olive made use of a wide range of irrigation treatments to test the potential of thermal imaging to detect stress, as an alternative to more ‘traditional’ but non-remote techniques such as stomatal resistance and stem water potential. Results from only one single day, however, are reported in that study. In the case of the current work, thermal imaging was shown to detect the onset and severity of stress, but to determine fully the potential of the technique relative to other methods of monitoring stress, a wider range of water availability at any one time would be useful.

While fluorescence imaging has been successfully used as a remote tool to detect the effect of stress in laboratory studies (Meyer et al., 2001; Chaerle et al., 2003), application in the field is complicated by the need for adequate energising radiation (Chaerle et al., 2007). Nevertheless Kolber et al. (2005) have demonstrated the use of laser-induced fluorescence at distances of 50 m. Since chlorophyll fluorescence is clearly a good indicator of responses of Q. suber leaves to seasonal stress, fluorescence imaging techniques combined with thermal imaging could potentially deliver rapid visual assessment of both the photosynthetic and stomatal condition of the tree canopies.

Future studies should concentrate on overhead thermal imaging of Q. suber canopies exposed to a wide range of conditions, in order fully to develop the potential of these techniques in monitoring vulnerable ecosystems such as the oak woodlands of Iberia.

Conclusions

The leaf physiology of Q. suber was only severely affected following three years of severe drought (the third year registering 45% less rainfall than average). Removing 20% of ambient rainfall had a limited impact. These results indicate the resilience of this species. This study provides indications as to potential areas of improvement for application of thermal imaging to monitoring seasonal stress in vulnerable ecosystems.

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