RESEARCH PAPER

Seasonal photosynthetic responses of European oaks to drought and elevated daytime temperature

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Keywords
Air warming; chlorophyll fluorescence; photosynthesis; Quercus; water shortage.

ABSTRACT
Oaks are commonly considered as drought- and heat-tolerant trees that might benefit from a warmer and drier climate. Their tolerance to drought has been frequently studied in the past, whereas studies dealing with elevated temperature or its combination with drought are very limited in number. In this study we investigated seasonal photosynthetic patterns in three European oak species (Quercus robur, Q. petraea, Q. pubescens) exposed in lysimeter-based open-top chambers (OTC) to elevated daytime temperature, drought and their combination. Stomatal and non-stomatal traits of photosynthesis were followed over an entire growing season and related to changes in daytime temperature, soil moisture and pre-dawn leaf water potential (ΨPD). Elevated daytime temperature enhanced net photosynthesis (PN) in a season-dependent manner, with higher mid-summer rates than in controls exposed to ambient temperature. Drought imposed in early and mid-summer reduced the soil moisture content and caused a gradual decline in ΨPD, stomatal conductance (gs) and PN. Drought effects on ΨPD and PN were exacerbated when drought was combined with elevated daytime temperature. In general, PN tended to be more affected by low soil moisture content or low ΨPD in Q. robur than in Q. petraea and Q. pubescens. Non-stomatal limitations may have contributed to the drought-induced decline of PN in Q. robur, as indicated by a down-regulation of PSII photochemistry (Fv/Fm) and decreased chlorophyll content. Taken together, our findings show that European oaks may benefit from elevated temperature, but detrimental effects can be expected when elevated temperature occurs simultaneously with drought.

INTRODUCTION
Rising temperature and more frequent summer droughts are major climate change factors that will affect a plethora of physiological functions and metabolic processes in trees growing in temperate climate zones. While drought limits tree growth by suppressing photosynthetic carbon sequestration and through this growth and biomass allocation (Bréda et al. 2006), rising temperature is generally thought to act in an opposite way, as it facilitates carbon allocation to internal growth processes (Saxe et al. 2001). Such generalisation can however be difficult since drought and elevated temperature often occur simultaneously, and primary effects may be superimposed by additive or synergistic interactions between both climate change factors (Mittler 2006). In fact, elevated temperature may increase the level of drought due to higher evaporative demand, thus turning the positive temperature effect on trees into a negative one. Drought, in turn, may limit transpirational cooling, thus exacerbating temperature effects on leaf physiological traits. Unfortunately, this aspect has received less attention in former studies, even though it may be critical for reliable prediction of forest response to climate change.

Photosynthesis is among the most sensitive processes with respect to climate change, strongly affected by drought and to a lesser extent by elevated temperature. Drought mainly limits photosynthetic CO₂ fixation through early stomatal closure, although non-stomatal effects on photosynthetic processes may also occur under severe drought, e.g. impairment of photochemical reactions or decreased carboxylation efficiency (Ni & Pallardy 1992; Kubiske & Abrams 1993; Wilson et al. 2000; Flexas & Medrano 2002; Grassi & Magnani 2005). The strength of photosynthetic responses to drought may vary among tree species depending on their ecological requirements. Oaks, for instance, are commonly considered to be less sensitive to drought, and this ecological behaviour is partly attributable to their ability to maintain higher photosynthetic rates under drought conditions than co-occurring tree species (Abrams 1990).

Compared with drought, the effect of rising temperature on photosynthesis is less clear, because most tree species have a broad optimum temperature range for photosynthesis, and responsiveness to warming may vary between trees originating from different thermal environments (Berry & Björkman 1980; Way & Oren 2010). As reported for tree growth, photosynthesis is temperature-limited in trees from cold environments and may thus show positive responses to rising temperature, while photosynthesis in warmer environments, where trees grow near to the thermal optimum for photosynthesis, may show weaker or even negative responses (Way &
Photosynthetic responses to drought and elevated temperature

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grown for 2 years under ambient site conditions. at the Swiss Federal Research Institute WSL, Birmensdorf and

Collected seeds were sown in spring 2004 in a nursery field

three European oak species (Quercus robur, Q. petraea, and Q. pubescens) exposed in large lysimeter-based model ecosystem chambers to elevated daytime temperature, drought and their combination. The experiment was performed under semi-natural conditions with naturally fluctuating sunlight and baseline temperature. Specifically, we addressed the following questions: (i) what are the effects of drought, elevated daytime temperature and their combination on leaf photosynthesis; (ii) how does leaf photosynthesis recover from severe drought; and (iii) how do oak species differ in their photosynthetic responses to the climate treatments? In order to separate stomatal and non-stomatal effects on leaf photosynthesis, we combined instantaneous measurements of net photosynthesis (PN) and stomatal conductivity (gs) with analysis of PSII photochemistry (Fv/Fm) and chlorophyll content, and related them to variations in soil moisture, pre-dawn leaf water potential (ΨPD) and daytime temperature.

MATERIAL AND METHODS

Plant material

Seeds were collected in 2003 from five to 10 mature trees of Q. robur L., Q. petraea ([Matt.] Liebl.) and Q. pubescens Willd. in natural oak stands throughout Switzerland. The collection sites were characterised by xeric to mesic soil conditions, with annual precipitation ranging from 660 to 930 mm and annual temperature ranging from 8.1 to 9.0 °C (Table 1). Collected seeds were sown in spring 2004 in a nursery field at the Swiss Federal Research Institute WSL, Birmensdorf and grown for 2 years under ambient site conditions.

Table 1. Location and climatic characterisation of oak collection sites. Mean annual temperature (°C) and mean annual precipitation (mm) were taken from meteorological stations (SWISS METEO) located near to the collection sites.

<table>
<thead>
<tr>
<th>species</th>
<th>collection site</th>
<th>altitude</th>
<th>temperature</th>
<th>precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q. robur</td>
<td>Tägerwilen</td>
<td>510 m</td>
<td>8.7 °C</td>
<td>929 mm</td>
</tr>
<tr>
<td>Q. petraea</td>
<td>Corcelles</td>
<td>550 m</td>
<td>9.0 °C</td>
<td>893 mm</td>
</tr>
<tr>
<td>Q. pubescens</td>
<td>Leuk</td>
<td>720 m</td>
<td>8.1 °C</td>
<td>657 mm</td>
</tr>
</tbody>
</table>

Experimental design

The study was part of the interdisciplinary ‘Querco’ experiment carried out in the model ecosystem facility of the Swiss Federal Research Institute WSL, Birmensdorf, Switzerland (47°21′48″ N, 8°27′23″ E, 545 m a.s.l.). The design of the experiment is described in detail elsewhere (Arend et al. 2011; Kuster et al. 2012 in this issue). In brief, the research facility comprises 16 large open-top chambers based on lysimeters containing a natural forest soil (acidic Haplic Alisol; loamy sand; pH 4.1). Natural precipitation was excluded from the OTCs by retractable glass roofs closing automatically at the onset of rainfall.

In spring 2006, the 2-year-old oak saplings were transplanted from the nursery field into the chambers. Water was regularly supplied from May to October, by means of six sprinklers in each lysimeter. From October to March, the roofs of the chambers were left open to allow natural precipitation. The treatments, drought, warming and their combination, were applied from 2007 to 2009, with four chambers assigned for each climate treatment and for the control. In the drought and combination treatment, water supply was reduced during the growing season by temporary interruption of the irrigation. In the air warming and combination treatment, daytime air temperatures were passively elevated by reducing the opening angle of the side walls of the OTCs. All measurements reported in the present study were performed during the growing season of 2009.

Measurement of soil water status and air temperature

Volumetric soil water content was measured by time domain reflection (TDR 100; Campbell Scientific Inc., Logan, UT, USA) in each lysimeter at 0–25, 25–50, 50–75 and 75–100 cm depth at intervals of 1 week throughout the growing seasons. Air temperature was measured with shaded EL-USB-2 data loggers (Lascar Electronics Ltd, Salisbury, UK) in each growth chamber at 120 cm height at intervals of 1 h.

Determination of leaf water potential

Pre-dawn leaf water potentials were determined in one leaf per tree using a Scholander pressure chamber (M 600; Mosler Tech Support, Berlin, Germany).

Measurements of photosynthesis, chlorophyll fluorescence and chlorophyll content

Instantaneous net photosynthesis (PN) was measured using a portable photosynthesis system (LI-COR 6400; LI-COR,
late summer, \( P_N \) remained at a low level until the onset of autumn, with three selected leaves per tree.

Recordings of radial stem growth
Radial stem growth was recorded in \( Q. \) robur using automated single point dendrometers (Zweifel Consulting, Switzerland). The dendrometers were mounted on aluminium frames fixed with three stainless steel rods to the stem base at 10 cm above the ground. Readings of the dendrometers were taken at 1-min intervals, averaged every 10 min and recorded using a Campbell data logger (Campbell Scientific).

Statistical analysis
All statistical calculations were performed with SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Overall treatment effects and interactions among treatments were analysed as a two- or three-factorial design (species, temperature and/or irrigation) by analysis of variance (ANOVA and repeated measures ANOVA; GLM procedure). Treatments and interactions between treatments were considered significant when \( P < 0.05 \). All statistical calculations are based on four replicate trees per treatment and species.

RESULTS
Seasonal pattern of \( P_N \) and its response to elevated daytime temperature
Instantaneously measured net photosynthesis (\( P_N \)) displayed a pronounced seasonal pattern in all three oak species, with a gradual increase from spring to early summer, maximum rates in mid-summer and a sharp decrease in late summer, which coincided with the cessation of radial stem growth (only shown for \( Q. \) robur) (Fig. 1A–D). After its decrease in late summer, \( P_N \) remained at a low level until the onset of autumnal leaf senescence in late October. This general seasonal pattern was found in the control and the warming treatment, but with some differences. The maximum mid-summer rates in July and August were significantly higher in the warming treatment (8:00–18:00 h UTC; grey line) and weekly difference in daytime air temperature between control and air warming treatment (\( \Delta T \); black line), (B–D) \( P_N \) in control (black circles) and warming treatment (white circles) in \( Q. \) robur, \( Q. \) petraea and \( Q. \) pubescens (mean ± SE; \( n = 4 \) trees, with three measured leaves each). Timing of (1) second flush growth and subsequent leaf development, (2) cessation of radial stem growth in the control and (3) in the warming treatment.

enhancement of \( P_N \) by elevated daytime temperature coincided with the timing of second flush growth and the subsequent development of secondary leaves, which occurred in all treatments in mid-summer (M. Dobbertin, pers. comm.). Stomatal conductance (\( g_S \); Table 2) and pre-dawn leaf water potentials (\( \Psi_P \); Fig. 2A–C) were not affected by elevated daytime temperatures.

Responses of \( P_N \) and \( g_S \) to drought and re-watering
Net photosynthesis (\( P_N \)) and \( g_S \) were strongly affected by drought, declining gradually with increasing soil moisture deficit and reaching levels near to zero at the end of both drought periods in June and August (Table 2, Figs. 3A–D and 4A–D). Simultaneously, \( \Psi_P \) dropped to values ranging between −1.2 and −1.7 MPa at the end of the first drought and −2.7 and −2.9 MPa at the end of the second drought (Fig. 2A–C). \( P_N \) seemed to be more affected by decreasing soil water deficits in \( Q. \) robur than in \( Q. \) petraea and \( Q. \) pubescens, although the drought response of \( \Psi_P \) was not different among the three species (Table 2). Re-watering resulted in a rapid and complete recovery of \( \Psi_P \) and \( P_N \) to the levels of non-stressed trees in the control treatment, except in \( Q. \) robur, which showed a delayed recovery of \( P_N \) after the second drought period in August. A long-lasting
drought effect was observed for \( g_s \), which showed incomplete recovery from the first and second drought in Q. robur as well as a delayed recovery from the second drought in Q. petraea and Q. pubescens (Fig. 4A–D).

The combination of drought with elevated daytime air temperature significantly enhanced the drought effects on \( P_N \) (Table 2, Fig. 3A–D) and lowered \( W_{PD} \) to values ranging between \(-2.6 \) and \(-3.2 \) MPa at the end of the first drought and \(-3.3 \) and \(-3.7 \) MPa at the end of the second drought (Table 2, Fig. 2A–C). After re-watering, the recovery of \( P_N \) to the level of control trees was markedly delayed in Q. robur. \( W_{PD} \) recovered immediately to the levels of non-stressed oaks in the control treatment. The response of \( g_s \) to drought in combination with elevated daytime air temperature was generally similar to that observed in oaks exposed to drought alone (Fig. 4A–D).

Responses of \( F_{V/F_M} \) and chlorophyll content to drought

The chlorophyll fluorescence ratio \( F_{V/F_M} \) was used as an empirical stress indicator that reveals non-stomatal drought effects on the photochemical efficiency of PSII. \( F_{V/F_M} \) ranged between \(0.7 \) and \(0.8 \) in the control and the warming treatment where oaks did not experience drought at any time of the growing season. Prior to autumnal leaf shedding, however, \( F_{V/F_M} \) decreased to values below \(0.7 \), indicating senescence-related loss of leaf viability. A drought-specific decline of \( F_{V/F_M} \) was observed in the drought and the combination treatment in early summer (Fig. 5A,B) when \( W_{PD} \) dropped to values ranging between \(-1.2 \) and \(-1.7 \) MPa and \(-2.6 \) and \(-3.2 \) MPa, respectively (Fig. 2A–C). This decline was more evident in the combination treatment than in the drought treatment, and Q. robur was more affected than Q. petraea and Q. pubescens (Table 2). Drought in late summer had only a weak effect on \( F_{V/F_M} \), although \( W_{PD} \) dropped to values ranging between \(-2.7 \) and \(-2.9 \) MPa and \(-3.3 \) and \(-3.7 \) MPa in the drought and combination treatment, respectively, indicating stronger drought levels than in early summer.

### Table 2. Treatment effects on pre-dawn water potential (\( W_{PD} \)), net photosynthesis (\( P_N \)), stomatal conductance (\( g_s \)) and photochemical efficiency of PSII (\( F_{V/F_M} \)), as calculated with (a) repeated measures ANOVA or (b) ANOVA with temperature, drought and species as main factors. The effect of air warming on \( P_N \) was significant only for the time period from 9 to 28 July and only with air temperature and species as main factors. Significance of treatment effects on \( F_{V/F_M} \) was tested on 24 June and 20 August using ANOVA.

<table>
<thead>
<tr>
<th>leaf trait</th>
<th>warming</th>
<th>drought</th>
<th>drought × warming</th>
<th>drought × species</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Psi_{PD} ) (a)</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>n.s.</td>
</tr>
<tr>
<td>( P_N ) (a)</td>
<td>*</td>
<td>***</td>
<td>*</td>
<td>n.s.</td>
</tr>
<tr>
<td>( g_s ) (a)</td>
<td>n.s.</td>
<td>***</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>( F_{V/F_M} ) (b; 28 June)</td>
<td>n.s.</td>
<td>***</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>( F_{V/F_M} ) (b; 20 August)</td>
<td>n.s.</td>
<td>**</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Levels of significance: * \( P < 0.05 \); ** \( P < 0.01 \); *** \( P < 0.001 \); n.s., not significant.
Chlorophyll content was generally little affected by drought (Fig. 6A–C). Differences among treatments were rather attributable to stochastic effects than to drought-specific effects. Within the combination treatment, however, there was a transient decrease in chlorophyll content in *Q. robur* and *Q. pubescens* at the end of the first and second drought period in early and late summer, respectively. Neither the drought treatments nor the warming treatment had an influence on the seasonal timing of autumnal chlorophyll degradation and leaf senescence.

**DISCUSSION**

Net photosynthesis (*P_N*) showed a pronounced seasonal pattern that was common among the three oak species. The...
rates of PN increased gradually from spring to early summer, reached a broad maximum in mid-summer and decreased sharply in late summer. This seasonal pattern could not be simply explained by seasonal changes in temperature, i.e. the prevailing temperature on the day of measurement, more likely it reflected an intrinsic regulation of photosynthesis by factors associated with leaf metabolism. In fact, photosynthesis is reported as inversely correlated with leaf carbohydrate status (Turnbull et al. 2002; Ow et al. 2010), which is unlikely to undergo strong temperature-dependent variations throughout the growing season. Rather, the high carbon demand of growing tree organs determines the leaf carbohydrate status, and decreasing growth activity and sink strength of these organs may affect leaf photosynthesis via a feedback inhibition (Goldschmidt & Huber 1992; Krapp & Stitt 1995; Paul & Foyer 2001). Interestingly, the sharp decrease of PN in late summer coincided with the cessation of radial stem growth, supporting a coupling of leaf photosynthesis with the seasonal dynamic of oak growth and carbon demand, as hypothesised by Anderson & Tomlinson (1998).

Moderately elevated daytime temperature (1–2 °C) increased the rate of PN in the three oak species in a season-dependent manner, with higher mid-summer values in the warming treatment than in the control. Although this temperature effect on PN is consistent with previous studies (reviewed in Saxe et al. 2001), a season-dependent temperature response has not previously been reported. The cause of this seasonal effect remains unknown, but it can be speculated that high growth activity and carbon demand during mid-summer might have increased the susceptibility of photosynthesis to rising temperature. Circumstantial support for this assumption comes from the seasonal timing of second flush growth and the subsequent development of secondary leaves, which coincided with the observed increase of PN in the warming treatment. A similar response has already been shown for elevated CO₂, which has its greatest enhancement effect on photosynthesis during periods of high growth and high sink activity (Koch et al. 1986). Interestingly, in a previous study we observed a general stimulation of shoot growth in oaks at elevated air temperatures (Arend et al. 2011), providing some further evidence that high growth activity and carbon demand lead to increased rates of PN in the warming treatment.

Responses to drought and re-watering

Drought reduced the soil moisture content and caused a gradual decline in PN with photosynthetic rates strongly inhibited at the end of both drought periods. Tree water deficits, as indicated by pre-dawn leaf water potentials (ΨPD), reached levels comparable to those observed in mature oaks under natural drought conditions (Epron et al. 1992). The decline of PN was mainly due to stomatal limitation, as it decreased in parallel with stomatal conductance (gs). There was no significant evidence for non-stomatal limitation of PN, i.e. effects on the photochemical efficiency of PSII (Fv/Fm) or chlorophyll content.

Limitation of PN is a common response to drought that has been extensively studied in woody plant species, including European oaks (Epron et al. 1992; Epron & Dreyer 1993a,b; Vivin et al. 1993; Gallé et al. 2007; Haldimann et al. 2008). Little attention has, however, been paid to the restoration of photosynthesis after drought, although this process is critical for trees to overcome the consequences of former stress. PN in oaks recovered quickly after re-watering, irrespective of the soil moisture content, which recovered more slowly. This discrepancy is probably due to rapid water uptake through shallow roots, which formed a dense, fibrous network near to the soil surface. Moisture uptake from upper soil layers may allow rapid restoration of the tree water balance after prolonged drought, especially when precipitation is too low to re-hydrate the complete soil profile (Flanagan et al. 1992; Gebauer & Ehleringer 2000).

The rapid and almost complete recovery of PN after re-watering demonstrates that oaks are able to maintain the functional integrity of their photosynthetic apparatus during periods of severe drought. This observation is in line with former reports on drought-stressed oaks, showing rather high resistance of their photosynthetic apparatus to leaf water deficits (Epron & Dreyer 1990, 1992; Epron et al. 1992; Gallé et al. 2007). Contrasting results have been obtained with less drought-tolerant saplings of Fagus sylvatica, which showed some remaining impairment of PN after drought due to non-stomatal limitation of photosynthesis (Gallé & Feller 2007).

Responses to the combination of drought and elevated daytime temperature

Elevated daytime temperature in combination with drought slightly enhanced the drought effects on PN due to stronger
tree water deficits, i.e. lower $\Psi_{PD}$ than in the treatment with drought alone. Non-stomatal limitation may have contributed to the decline of $P_N$ in Q. robur, where both photochemical efficiency of PSII and chlorophyll content were temporally impaired by drought. Furthermore, the restoration of $P_N$ after drought was markedly delayed in Q. robur; the cause remains obscure, as we found no evidence for lasting non-stomatal limitation of photosynthesis. It cannot be excluded that the lasting decline of $g_s$ played a role in the response of Q. robur. Other factors might also have contributed to the remaining impairment of $P_N$, e.g. metabolic inhibition of photosynthesis by elevated levels of soluble carbohydrates, as suggested by Souza et al. (2004). Although the present study does not provide any data on leaf metabolites, drought-induced accumulation of soluble leaf carbohydrates has been frequently shown in previous studies (Picon et al. 1997; Arndt et al. 2001; Griffin et al. 2004; Li et al. 2012 in this issue).

Differences among species

The tendency of $P_N$ to be more affected by soil water shortage in Q. robur than in the other two oak species prompted us to investigate species-specific drought responses in more detail. Stomatal and non-stomatal traits of photosynthesis were related to internal leaf water deficits, i.e. $\Psi_{PD}$, revealing differences among the oak species, independent of differences in rooting depth and soil water access. This approach did not provide evidence for different stomatal responsiveness to leaf water deficits as $g_s$ reacted similarly to decreasing $\Psi_{PD}$ in all three oak species. Similar findings have been reported in a previous study with Q. robur, Q. petraea and Q. rubra (Vivin et al. 1993), although differences in stomatal responses to leaf water deficits may occur among tree species belonging to other genera or functional groups (Asamama & Söber 2011). Despite the lack of differences in stomatal regulation, $P_N$ showed higher responsiveness to decreasing $\Psi_{PD}$ in Q. robur than in Q. petraea and Q. pubescens. Different sensitivity of non-stomatal traits to leaf water deficits may provide a likely explanation for this observation. In fact, the photochemical efficiency of PSII ($F_V/F_M$) was markedly impaired at decreasing $\Psi_{PD}$ in Q. robur, while no such response could be observed in Q. petraea and Q. pubescens. The finding that the latter two species exhibited the same tolerance to decreasing $\Psi_{PD}$ was somewhat unexpected, as these two species have different ecological behaviour. Q. pubescens typically grows on drier sites than Q. petraea and is therefore commonly considered to be more tolerant to drought (Ellenberg 1988). Factors other than photosynthetic leaf traits may be responsible for such species-specific ecological behaviour, e.g. the ability of roots to access water from deep soil layers (Abrams 1990) or the vulnerability to drought-induced xylem dysfunction (Cochard et al. 1992).

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