Drought and the diurnal patterns of stem CO2 efflux and xylem CO2 concentration in young oak (Quercus robur)

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Summary A young potted oak (Quercus robur L.) tree was subjected to drought by interrupting the water supply for 9 days. The tree was placed in a growth chamber in which daily patterns of temperature and radiation were constant. The effects of drought on the water and carbon status of the stem were examined by measuring stem sap flow rate, stem water potential, stem diameter variations, stem CO2 efflux rate (Fco2) and xylem CO2 concentration ([CO2]x). Before and after the drought treatment, diurnal fluctuations in Fco2 and [CO2]x corresponded well with variations in stem temperature (Ts). Daytime depressions in Fco2 did not occur. During the drought treatment, Fco2 still responded to stepwise changes in temperature, but diurnal fluctuations in Fco2 were no longer correlated with diurnal fluctuations in Ts. From the moment daily growth rate of the stem became zero, diurnal fluctuations in Fco2 became closely correlated with diameter variations, exhibiting clear daytime depressions. The depressions in Fco2 were likely the result of a reduction in metabolic activity caused by the lowered daytime stem water status. Xylem [CO2]x showed clear daytime depressions in response to drought. When the tree was re-watered, Fco2 and [CO2]x exhibited sharp increases, coinciding with an increase in stem diameter. After resumption of the water supply, daytime depressions in Fco2 and [CO2]x disappeared and diurnal fluctuations in Fco2 and [CO2]x corresponded again with variations in Ts.

Keywords: diameter variations, growth, sap flow, stem respiration, stem water potential, water deficit, water reserves.

Introduction Total respiration of a tree consumes 30% to more than 80% of the daily production of photosynthates (Kozlowski and Pallardy 1997). The cost of stem respiration is estimated to be at least 5% of the total carbon uptake (Edwards et al. 2002). Hence, a detailed knowledge of stem respiration will contribute to our understanding of forest carbon budgets and is necessary for the correct estimation of CO2 fluxes from forest ecosystems.

Respiration rates of a whole plant have been treated empirically as the sum of two components, one associated with growth (synthesis of new structures) and a second with maintenance of biomass (resynthesis of degraded structure) (McCree 1970). Temperature is an important environmental factor controlling respiration rates (Amthor 1989). Growth respiration is determined by the growth rate, which depends on temperature among other factors, whereas maintenance respiration is directly related to enzymatic processes of degradation that are temperature dependent (Thornley and Johnson 1990). Respiration rates of woody tissues are commonly estimated from the CO2 efflux of that tissue. In some cases, there is a good relationship between measured CO2 efflux rates and temperature (e.g., Paembonan et al. 1991, Maier et al. 1998); however, in other cases, a relationship between measured CO2 efflux rate and temperature is less clear or absent. Kakubari (1988) observed reductions in CO2 efflux rates from Fagus sylvatica L. trees that were unrelated to fluctuations in xylem temperature or air temperature. In summer, Kaipiainen et al. (1998) found no correlation between measured CO2 efflux rates in stems of Pinus sylvestris L. and air temperature, even when accounting for the possibility that stem temperature may lag the change in air temperature.

Variation in sap flow rate may be responsible for the inconsistent relationship observed between temperature and stem CO2 efflux rates (Martin et al. 1994). In part, CO2 evolved by respiring stem tissue is dissolved in the sap and transported with the transpiration stream instead of escaping through the bark (Negisi 1972, Ryan 1990, Sprugel 1990, Teskey and McGuire 2002). Furthermore, CO2 originating from root tissue or soil microbial respiration is transported with the sap stream and released at the stem level (Levy et al. 1999, McGuire and Teskey 2004). In either case, measurement of stem CO2 efflux rate may provide an erroneous estimate of stem respiration.

Water deficit in the stem tissue may also modify the relationship between stem temperature and CO2 efflux. Wang et al. (2003) found that stem CO2 efflux rates of Larix gmelini Rupr. trees were closely correlated with stem temperature in the morning but not in the afternoon, and suggested that this was due to a greater stem water deficit in the afternoon. Lavigne (1987) observed lower stem respiration rates of Abies balsamea (L.) Mill. trees on some afternoons compared with mornings, and suggested that this was caused by low stem water content, which temporarily reduced rates of synthesis of
new structures and impaired normal maintenance processes. Hence, reduced growth respiration and maintenance respiration may at least partly explain the midday depressions in stem CO₂ efflux rates.

We are unaware of any investigations on how the diurnal pattern of CO₂ efflux from woody tissues is affected by declining tissue water content induced by a gradually decreasing soil water potential. Therefore, our main objectives were to study the impact of drought on: (1) the diurnal pattern of stem CO₂ efflux rate and xylem CO₂ concentration; (2) the relationship between stem CO₂ efflux rate and temperature; and (3) the relationship between stem CO₂ efflux rate and diurnal changes in stem diameter in 3-year-old Quercus robur L. trees.

Materials and methods

Plant material and experimental conditions
At the end of February 2005, two 3-year-old oak (Quercus robur L.) trees, T1 and T2, previously grown outdoors, were planted in 50-l containers filled with sandy loam soil. The trees were placed in a growth chamber (2 × 1.5 × 2 m; H × W × L) at Ghent University (51°3’ N, 3°43’ E), which allowed control of irradiance and air temperature. Light was supplied by fluorescent lamps, producing a photosynthetic photon flux (PPF) of 473 µmol m⁻² s⁻¹ at the top of the tree during the 12-hour daily photoperiod. Air temperature (Tₐ) was measured with a copper-constantan thermocouple (Omega, Amstelveen, Netherlands), installed at a height of 1.1 m between the trees. Relative humidity (RH) was measured with a capacitive RH sensor (Type HIH-3605-A, Honeywell, Freeport, IL) at the same height as the thermocouple. Water potential of the soil (Ψₛₒ) was measured with tensiometers (Type SWT6, Delta-T, Cambridge, U.K.) at a depth of 20 cm. The trees were watered daily and fertilized monthly with N, P and K plus micronutrients. The experiment was started on June 22, 2005 (day of the year, DOY 173) and ended on July 13, 2005 (DOY 194). At the beginning of the experiment the trees were ~1.6 m tall and stem diameters at the soil surface were 19.2 and 17.8 mm, for T1 and T2, respectively. Water supply to tree T2 was stopped on June 28, 2005, and at this point tree T2 was ~1.5 m tall and tree T1 was ~1.6 m tall. Sap flow rates (Fₛₒ) were measured at the stem baseURL with heat balance sap flow sensors (Model SGB16-WS, Dynamax Inc., Houston, TX). The sap flow sensors were shielded from radiation by several layers of aluminum foil. Sheath conductance of the gauge was recalculated daily based on minimum predawn values between 0400 and 0700 h. The value for stem thermal conductance was taken from the literature: for woody stems a value of 0.42 W m⁻¹ °C⁻¹ is considered appropriate according to Steinberg et al. (1989).

Measurement of stem water potential
The water potential of a leaf was measured with a pressure chamber (PMS, Corvallis, OR) just before the start of each photoperiod. Because sap flow rate was zero during the dark period, the measured value corresponded with predawn stem water potential (Ψₛₒ). At 1400 h, the water potential of a leaf enclosed for 2 h in an aluminum envelope was measured to provide an estimate of midday stem water potential (Ψₚₒ) (McCutchan and Shackel 1992).

Measurement of xylem CO₂ concentration
Xylem CO₂ concentration ([CO₂]; %) was measured at the base of the trees with a CO₂ microelectrode (Model MI-720, Microelectrodes Inc., Bedford, NH) (McGuire and Teskey 2002). Four-mm diameter holes were drilled 7 mm deep into the xylem at about 1 cm below the stem cuvettes. Teflon tubes (3.5 mm inner diameter and 30 mm length) were tightly fitted into the holes and the tubes were sealed to the trees with flexible adhesive putty (Terostat IX, Henkel, Heidelberg, Germany). The CO₂ microelectrode was inserted in the tube and adhesive putty was used to provide a gas-tight seal. The microelectrode measured the [CO₂] of the gas in the headspace of the hole, which is proportional to the concentration of all products of CO₂ dissolved in the xylem sap (Hari et al. 1991, Levy et al. 1999). The [CO₂] of the gas (%) was converted to total

CO₂ efflux measurements
Measurements of CO₂ exchange were performed at 0.1 m above soil level on 13-cm-long stem segments of trees T1 and T2. The stem segments were enclosed in opaque, air-tight, cylindrical (6-cm diameter) PVC cuvettes. Air from the growth chamber was pumped at a rate of 1 litre min⁻¹ through the cuvettes via a 50-l buffer tank, dried (Model CG/G 73-4, Hartmann and Braun AG, Germany gas cooler operating at 4 °C) and analyzed for CO₂ with an infrared gas analyzer (IRGA; Model Binos 100-4P, Fisher-Rosemount, Hasselroth, Germany) operated in differential configuration. The reference stem cuvette had the same dimensions as the measurement cuvette but contained no stem segment. Signals from the IRGA were logged every 10 s for 4 min for each stem segment, and mean values were recorded by a data logger (Model HP 34970A, Hewlett Packard, Palo Alto, CA). The CO₂ efflux rates (Fₐ) were expressed per unit of stem surface area.

Stem diameter measurements
Variations in stem diameter (D) were measured with linear variable displacement transducers (LVDTs) (Model LBB, 375-PA-100) and a transducer bridge (SC-35, Schaevitz, Hampton, VA), placed 2 cm below the stem cuvettes, and attached to the stem with a stainless steel holder. Changes in D can be divided into an irreversible component related to tissue growth and a reversible component related to changes in hydration of the extensible tissues (Kozlowski 1972). It is therefore possible to define daily growth (DG), corresponding to the difference between two successive daily maximum values of D (just before onset of shrinkage) and a maximum daily shrinkage (MDS), corresponding to the difference between the maximum and the minimum values of D during a day.

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dissolved carbon ([CO₂], mmol l⁻¹) by equations based on 
Henry’s law (Stumm and Morgan 1981, McGuire and Teskey 
2002). For this conversion, the pH of the xylem sap must be 
known. Therefore, sap was expressed from an excised twig of 
the tree with a pressure chamber (PMS Instruments, Corvallis, 
OR) and measured with a pH electrode (Model HI 1330, 
Hanna Instruments, Temse, Belgium). The microelectrode 
was calibrated with three air samples of known [CO₂] and an 
exponential equation was developed to convert millivolt output 
to [CO₂]. The results were compensated for temperature by 
applying the equation empirically derived by McGuire and 
Teskey (2002). Because of a malfunction of the CO₂ micro-
electrode, the [CO₂] of tree T1 was not measured after DOY 
182.

Temperature responses of FCO₂
Stem temperature (Tst) was measured with copper-constantan 
thermocouples inserted in the stem in 1-mm diameter holes 
with a depth of 7 mm, just below the stem cuvettes. To investi-
gate temperature dependence of physiological variables, Tst 
was altered stepwise (in four steps of 1 hour: 24, 21, 17 and 
21 °C) during the dark period of DOY 176, DOY 184 and DOY 
190 and during the light period of DOY 183 and DOY 190.

Values of FC0₂ (µmol m⁻² s⁻¹) were regressed against stem 
temperature Tst (°C) as:

\[ F_{CO₂} = F_{CO₂}(20) \times Q_{10}^{Tst-20} \]  

(1)

where FCO₂(20) is CO₂ efflux rate at 20 °C, and Q₁₀ is the rela-
tive increase in respiration rate with a 10 °C rise in tempera-
ture. Parameters FCO₂(20) and Q₁₀ were estimated by ordinary 
least squares with Matlab 6.5 software (The Mathworks Inc., 
Natick, MA).

Data logging
All signals from sensors and devices were logged at 10 s inter-
vals with a data acquisition system (HP 34970A, Hewlett 
Packard, Palo Alto, CA). Sensor signals were averaged over 
4 min periods.

Results

Microclimatological variables
Mean Tst during the 20-day measurement period was 21.0 °C ± 
0.3 and 20.7 ± 0.3 °C for the control tree T1 and the treat-
ment tree T2, respectively (Figure 1a). Mean Ψst of tree T1 was 
–0.004 MPa (Figure 1b). From DOY 176 onward, Ψst of tree 
T2 gradually decreased to a minimum of –0.1 MPa on DOY 
185.

Stem water potential
Both Ψst and Ψm of tree T1 remained constant during the me-
asurement period. Mean Ψst was –0.13 MPa and mean Ψm was 
–0.56 MPa. In the pre-drought period (Ψst > –0.01 MPa), Ψst 
of tree T2 showed daily variations with predawn values of 
–0.05 MPa and midday values of –0.4 MPa (Figure 1c). Dur-
ing the drought period, Ψst and Ψm decreased significantly, 
with Ψm reaching –2 MPa on DOY 184. Complete recovery 
of the water status was achieved shortly after re-watering the tree.

Sap flow rates
Daily FH₂O of the control tree T1 was more or less constant 
throughout the measurement period (Figure 1d), with a mean 
FH₂O during the light periods of 23 ± 1 g h⁻¹. In the pre-drought 
period, mean FH₂O of tree T2 during the light period was 31.1 ± 
0.8 g h⁻¹ (Figure 1d). Values of FH₂O of tree T2 gradually de-
creased from DOY 179 reaching a minimum value of 9.8 g h⁻¹ 
on DOY 184. When the tree was re-watered daily, FH₂O in-
creased, but took 5 days to reach a stable value during the light 
period. Mean FH₂O of tree T2 for the period DOY 190–194 
was 24.4 ± 0.4 g h⁻¹.

Stem diameter variations
Figure 1e shows the time courses of the stem diameter vari-
ations, D, in T1 and T2 as measured by the two LVDTs. Varia-
tions in D reflect both irreversible growth and reversible 
changes in stem hydration. Growth was characterized by DG, 
and changes in stem hydration by MDS. For tree T1, MDS of 
18 ± 2 µm was superimposed on a continuous growth curve.
Mean DG of the tree T1 was 25 ± 8 µm day⁻¹.

In the pre-drought period, tree T2 had an MDS of 21 ± 2 µm. 
During the pre-drought period, stem shrinkage was observed 
for 1 h after the lights were turned on and then D exhibited an 
increasing trend during the rest of the light period, indicating 
that irreversible growth occurred. In response to drought, 
MDS increased gradually reaching a maximum value of 
225 µm on DOY 184. From DOY 177, D showed a continuous 
decrease during the light period, indicating that, from DOY 
177, irreversible growth no longer occurred during the light 
period. After the drought, mean MDS of tree T2 was 17 µm, 
which is similar to the pre-drought value. Reversible changes 
in stem hydration showed a similar pattern after the drought 
as in the pre-drought period, i.e., stem shrinkage for 1 h after 
the lights were turned on and a constant or slightly increasing 
value of D during the rest of the light period. Mean DG of tree 
T2 in the pre-drought period was 93 ± 0.3 µm day⁻¹ and it be-
came zero on DOY 179. From DOY 179 onward, stem water 
reserves of tree T2 were no longer fully replenished during the 
dark period and, hence, a negative DG was observed. After the 
drought, mean DG was 33 ± 4 µm day⁻¹.

CO₂ efflux rate
During the pre-drought period, the mean DG of tree T1 was 
25 µm day⁻¹, versus 93 µm day⁻¹ in tree T2, and stem FC0₂ of 
tree T2 was about twice that of T1 (Figure 1f).

The FC0₂ of tree T1 showed diurnal fluctuations that closely 
corresponded with Tst (cf. Figures 1f and Figure 1a). For tree 
T2, six different periods could be distinguished in the time
course of $F_{\text{CO}_2}$ (Figure 1f). (Period 1) From DOY 173 until DOY 178, $F_{\text{CO}_2}$ of trees T2 and T1 showed a similar pattern. Diurnal fluctuations in $F_{\text{CO}_2}$ corresponded closely with fluctuations in $T_{\text{st}}$ (Figure 2a). (Period 2) For tree T2, DOY 178–179 was a transitional period in which fluctuations in $F_{\text{CO}_2}$ were poorly correlated with fluctuations in $T_{\text{st}}$ (Figure 2d). On DOY
Figure 2. Relationships between stem CO₂ efflux rate \( F_{\text{CO}_2} \) and stem temperature \( T_s \), stem diameter \( D \) and xylem CO₂ concentration \([\text{CO}_2^*]\) for tree T2 on six days in the six different periods as indicated in Figures 1f and 1g. Days are from 0000 to 0000 h, except for Period 4, where a time period from 0900 h (moment of water supply) until 0900 h the next day was chosen.
when DG was zero, there was no relationship between $F_{\text{CO}_2}$ and $T_{\text{st}}$. At that time $\Psi_{\text{st}}$ was –0.085 MPa and $\Psi_{\text{st}}$ was about –1.3 MPa. (Period 3) From DOY 180 until tree T2 was watered on DOY 185, $F_{\text{CO}_2}$ showed diurnal fluctuations that were unrelated to changes in $T_{\text{st}}$ (Figure 2g). During this period, fluctuations in $F_{\text{CO}_2}$ were more closely correlated with variations in $D$ than in $T_{\text{st}}$ (Figure 2h). (Period 4) When water was given on DOY 185, $F_{\text{CO}_2}$ sharply increased and reached a maximum value in the dark period of DOY 186. This increase coincided with a sharp increase in $D$ (Figure 2k). (Period 5) During the period DOY 186–189 there was still no clear correlation between fluctuations in $F_{\text{CO}_2}$ and $T_{\text{st}}$ (Figure 2m), and the correlation between $D$ and $F_{\text{CO}_2}$ was poor (Figure 2n). (Period 6) From DOY 190 on, fluctuations in $F_{\text{CO}_2}$ again corresponded with fluctuations in $T_{\text{st}}$ (Figure 2q); however, because $F_{\text{CO}_2}$ continued to show an overall declining trend (Figure 1f), the correspondence was less clear than during the pre-drought period (Period 1; Figure 2a).

**Temperature responses of CO$_2$ efflux rate**

In the pre-drought period (Period 1), diurnal fluctuations in stem $F_{\text{CO}_2}$ of tree T2 corresponded closely with fluctuations in $T_{\text{st}}$ (Figure 2a). Values of the parameters $F_{\text{CO}_2}(20)$ and $Q_{10}$ for T2 were estimated based on data of DOY 176, when $T_{\text{st}}$ was altered stepwise. A plot of $F_{\text{CO}_2}$ as a function of $T_{\text{st}}$ exhibited hysteresis with a counter clockwise time course. The hysteresis was not apparent when $F_{\text{CO}_2}$ was plotted against $T_{\text{st}}$ 28 min earlier (Figure 3). An exponential regression between $F_{\text{CO}_2}$ and lagged $T_{\text{st}}$ revealed a high coefficient of determination ($r^2 = 0.89$).

Table 1. Characteristics of the response of $F_{\text{CO}_2}$ to $T_{\text{st}}$. Abbreviations: $Q_{10}$ is the increase in respiration rate for a 10 °C increase in temperature; $F_{\text{CO}_2}(20)$ is the basal CO$_2$ efflux rate (see Equation 1); and $r^2$ is the coefficient of determination for the exponential regression between $F_{\text{CO}_2}$ and $T_{\text{st}}$. Standard errors of the mean are given in parentheses.

<table>
<thead>
<tr>
<th>Period</th>
<th>Lag period for best fit (min)</th>
<th>$Q_{10}$</th>
<th>$F_{\text{CO}_2}(20)$ (µmol m$^{-2}$ s$^{-1}$)</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOY 176</td>
<td>28</td>
<td>2.76 (0.12)</td>
<td>2.66 (0.019)</td>
<td>0.89</td>
</tr>
<tr>
<td>DOY 183–184</td>
<td>16</td>
<td>1.94 (0.090)</td>
<td>1.83 (0.0077)</td>
<td>0.90</td>
</tr>
<tr>
<td>DOY 190</td>
<td>36</td>
<td>2.45 (0.10)</td>
<td>2.95 (0.012)</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Figure 3. Stem CO$_2$ efflux rates ($F_{\text{CO}_2}$) from the stem segment of tree T2 on DOY 176 (dark period) versus (a) current stem temperature ($T_{\text{st}}$) and (b) lagged stem temperature. The coefficient of determination ($r^2$) for the $Q_{10}$ model (Equation 1) fit to the data is also given. Arrows show the time course of the hysteresis phenomenon.

Figure 4. Measured stem CO$_2$ efflux rates ($F_{\text{CO}_2}$) (bold line) and predicted respiration rates (thin lines) based on Equation 1 for tree T2, for the period DOY 173–193. Parameters $Q_{10}$ and $F_{\text{CO}_2}(20)$ were estimated based on data of DOY 176 for Model 1, on data of DOY 183–184 for Model 2 and on data of DOY 190 for Model 3 (Table 1). Vertical dotted lines correspond to the beginning and ending of the light periods.
During the drought (Period 3), diurnal fluctuations in $F_{CO_2}$ were strongly positively correlated with fluctuations in $D$ (Figure 2h). When $F_{CO_2}$ was plotted against $D$ for the period DOY 180–185, a clear exponential relationship ($r^2 = 0.93$) was observed (Figure 5). When tree T2 was re-watered on DOY 185 (Period 4), $F_{CO_2}$ and $D$ both increased sharply, and the variables were strongly positively correlated (Figure 2k).

After the drought, from DOY 186 on (Period 5), no correlation between $F_{CO_2}$ and $D$ was observed (Figure 2n), and from DOY 190 onward (Period 6), $F_{CO_2}$ appeared to be negatively correlated with $D$ (Figure 2l). However, $F_{CO_2}$ was normalized to a common temperature with Equation 1 ($T_a = 21 °C, Q_{10} = 2.45$), the correlation was no longer apparent.

**Xylem CO$_2$ concentration**

Xylem [CO$_2$]$_c$ of tree T1 was nearly constant, with only small variations that corresponded well with variations in $T_a$; higher values of [CO$_2$]$_c$ occurred during the light period together with higher values of $T_a$ (cf. Figures 1g and 1a). Xylem [CO$_2$]$_c$ and $F_{CO_2}$ were well correlated in tree T1 (cf. Figures 1g and 1f). For tree T2, again six periods could be distinguished (Figure 1g). (Period 1) From DOY 173 until DOY 177, the daily course of [CO$_2$]$_c$ followed a similar trend as in tree T1. Fluctuations in [CO$_2$]$_c$ corresponded closely with fluctuations in $T_a$.

(Period 2) On DOY 178, a first abrupt drop in xylem [CO$_2$]$_c$ was observed at 1900 h. The rest of the period, an increase was observed until the original value was reached. During the rest of the dark period and during part of the light period on DOY 179, fluctuations in [CO$_2$]$_c$ of T2 corresponded with fluctuations in $T_a$. (Period 3) From DOY 180 until DOY 185, no relationship between [CO$_2$]$_c$ and $T_a$ was observed. Xylem [CO$_2$]$_c$ was more closely correlated with the dark–light pattern: lights off coincided with an increase in [CO$_2$]$_c$ and the onset of illumination was followed by a decrease. In this period, the magnitude of the increase during the dark period gradually diminished: on DOY 180, [CO$_2$]$_c$ increased during the dark period, but the value at the end of the dark period was only 69% of the original mean [CO$_2$]$_c$, and on DOY 181, 182, 183 and 184, the peak was further decreased to 23, 20, 2, and 2% of the original value, respectively. (Period 4) When tree T2 was re-watered on DOY 185, [CO$_2$]$_c$ increased sharply. (Period 5) During the period DOY 186–189, [CO$_2$]$_c$ remained constant or exhibited a slightly declining trend during the light periods, whereas during the dark periods showed a decrease for 2 h and then an increase until the end of the dark period. In this period, fluctuations in [CO$_2$]$_c$ started to correspond again with fluctuations in $T_a$.

(Period 6) From DOY 190 on, diurnal fluctuations in [CO$_2$]$_c$ corresponded closely with fluctuations in $T_a$.

**Relationship between CO$_2$ efflux rate and diameter variations**

In the pre-drought period (Period 1), fluctuations in $F_{CO_2}$ appeared to be in opposite phase with fluctuations in $D$: lights off coincided with an increase in $D$ and a decrease in $F_{CO_2}$, and the onset of illumination was immediately followed by a decrease in $D$ and an increase in $F_{CO_2}$ (cf. Figures 1f and 1e). However, when $F_{CO_2}$ was normalized to a common temperature with Equation 1 ($T_a = 21 °C, Q_{10} = 2.76$), $F_{CO_2}$ showed little variation and was no longer correlated with $D$.

Under drought, from the moment DG became zero (Period 3), diurnal fluctuations in $F_{CO_2}$ were strongly positively correlated with fluctuations in $D$ (Figure 2h). When $F_{CO_2}$ was plotted against $D$ for the period DOY 180–185, a clear exponential relationship ($r^2 = 0.93$) was observed (Figure 5).

Figure 5. Stem CO$_2$ efflux rate ($F_{CO_2}$) of tree T2 as a function of stem diameter ($D$) for the period DOY 180–185. The solid line represents the least squares exponential regression of the relationship between $F_{CO_2}$ and $D$. The coefficient of determination is 0.93.

0.89). Estimated $Q_{10}$ and $F_{CO_2}(20)$ are given in Table 1. Respiration rates calculated with Equation 1 and parameters estimated from DOY 176 (Model 1, Figure 4) corresponded closely with measured $F_{CO_2}$ for the period DOY 173–177.

During the drought (Period 3), $F_{CO_2}$ showed diurnal fluctuations that were unrelated to fluctuations in $T_a$ (Figure 2g). However, when $T_a$ was altered stepwise on DOY 183–184, the fluctuations in $F_{CO_2}$ were observable (Figure 2f). For DOY 183–184, a temperature lag of 16 min provided the best fit of the model to current $F_{CO_2}$, based on the coefficient of determination. Estimated $Q_{10}$ and $F_{CO_2}(20)$ are given in Table 1. Respiration rates predicted from lagged $T_a$ with Equation 1 with parameters estimated from DOY 183–184 (Model 2) are shown in Figure 4. Although the fit was good for the period in which the temperature steps were imposed, predicted respiration rates did not correspond with measured $F_{CO_2}$ for the rest of the period DOY 180–185.

After the drought, from DOY 190 onward (Period 6), diurnal fluctuations in $F_{CO_2}$ again corresponded with fluctuations in $T_a$ (Figure 2q). On DOY 190, $T_a$ was altered stepwise. A time lag for temperature of 36 min provided the best fit (Equation 1) to the data, based on the coefficient of determination. Estimated $Q_{10}$ and $F_{CO_2}(20)$ are given in Table 1. Respiration rates calculated with Equation 1 and parameters estimated from DOY 190 (Model 3, Figure 4) followed the same diurnal trend as measured $F_{CO_2}$ for the period DOY 190–194. However, during this period, $F_{CO_2}$ exhibited an overall declining trend and therefore, measured $F_{CO_2}$ did not completely coincide with calculated respiration rates.
Discussion

Stem diameter variations

Variations in $D$ reflect both daily reversible shrinkage and swelling patterns due to variations in stem water potential (Klepper et al. 1971, Garnier and Berger 1986) and irreversible growth patterns. A cell grows when water influx is accompanied by cell wall extension. According to the most widely used model of cell expansion (Lockhart 1965), cell expansion depends on cell turgor pressure, cell wall extensibility and a threshold turgor pressure at which wall yielding occurs. Turgor pressure must be above this threshold value for the cell to grow (Henson 1982). This idea has recently been incorporated in a mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth (Steppe et al. 2006). The model demonstrates that radial stem growth of trees occurs only when turgor exceeds a wall-yielding threshold value. Consistent with the models, DG in our study showed a declining trend and became zero 3 days after irrigation of tree T2 was stopped. Besides water, carbon is also necessary for cells to grow. During a drought, the supply of carbohydrates declines because of reduced photosynthesis. Hence, cessation of growth can be a direct consequence of lowered turgor pressure or an indirect consequence of limited carbohydrate supply, or both (Daudet et al. 2005).

CO$_2$ efflux rate

Studies on the effects of soil water depletion on the respiration of woody tissues of trees are scarce. Moreover, we are unaware of any studies on how the diurnal pattern of CO$_2$ efflux rates of woody tissues is affected by a gradually decreasing soil water potential. In this study, we found a clear phase shift in the daily course of stem $F_{CO_2}$ during an imposed drought.

As long as DG was positive, diurnal fluctuations in $F_{CO_2}$ were well correlated with the diurnal pattern of $T_a$ (cf. Figures 1f and 1a), and daytime depressions in $F_{CO_2}$ did not occur. Three days after the imposition of drought, from the moment DG became zero (cessation of irreversible growth), the diurnal pattern of $F_{CO_2}$ was out of phase with the pattern of $T_a$, and $F_{CO_2}$ showed clear depressions during the daytime. One possible explanation is that, during the day when the tree is transpiring, the transpiration stream becomes a sink for respired CO$_2$, resulting in a reduction of $F_{CO_2}$ (Negisi 1972, Ryan 1990, Sprugel 1990, Teskey and McGuire 2002). If true, it follows that the higher the daily $F_{H_{1,0}}$, the greater the depression in $F_{CO_2}$. Hence, according to this interpretation, depressions in stem $F_{CO_2}$ should be greatest under pre-drought conditions, because $F_{H_{1,0}}$ was substantially higher during the pre-drought period compared with the drought period (Figure 1d). Nevertheless, daytime reductions in stem $F_{CO_2}$ were not observed during the pre-drought period. Reductions appeared only when daily $F_{H_{1,0}}$ was already decreasing. However, the possibility that the transpiration stream influenced $F_{CO_2}$ during the pre-drought period cannot be ruled out: CO$_2$ originating from root or soil respiration, or both, could be a source of CO$_2$ at the stem level and could contribute to the increase in $F_{CO_2}$ during the light period (Levy et al. 1999, McGuire and Teskey 2004). Thus, the increase in $F_{CO_2}$ during the light periods in the pre-drought period might be the result of an increase in stem tissue respiration as well as an increased transport of CO$_2$ originating from root and soil respiration.

During the drought, $F_{CO_2}$ showed nightly increases, most likely because stem tissue metabolism increased as a result of tissue rehydration during the night. This can be deduced from the finding that variations in $F_{CO_2}$ were synchronized with variations in $D$ (cf. Figures 1f and 1e). McGuire and Teskey (2004) found that when $F_{H_{1,0}}$ is low, stem $F_{CO_2}$ is primarily a function of local tissue respiration and of stored CO$_2$ in the xylem. Therefore, in our study, stem $F_{CO_2}$ was probably unaffected by sap flow during the drought.

When tree T2 was re-watered, there was a sharp increase in $F_{CO_2}$ (Figure 1f). If sap flow is a sink for CO$_2$ (Negisi 1972), it would be expected that with the sudden increase in $F_{H_{1,0}}$ (Figure 1d) the CO$_2$ accumulated within the stem would be carried away by the sap stream causing a depression in $F_{CO_2}$. However, we observed the opposite: the increase in $F_{H_{1,0}}$ coincided with a sharp increase in $F_{CO_2}$ (Figure 1f). Nevertheless, from our observations it cannot be concluded that sap flow was without effect on $F_{CO_2}$, as increased root and soil microbial metabolism after re-watering might have contributed CO$_2$ to the transpiration stream. Hence, the large pulse in $F_{CO_2}$ after re-watering may have been the result of both increased metabolism in the local stem tissue and increased transport of CO$_2$ originating from root and soil microbial metabolism.

Temperature response of CO$_2$ efflux rate

During the pre-drought period, the diurnal pattern of $F_{CO_2}$ was strongly determined by the pattern of $T_a$ (Figure 2a). When $T_a$ was altered stepwise (DOY 176), the relationship between $T_a$ and $F_{CO_2}$ had a good fit to an exponential equation when a time lag was taken into account (Figure 3). Hysteresis between $F_{CO_2}$ and $T_a$ has been reported in several studies. Lavigne et al. (1996) found that a sapwood temperature lag of 1.75 h provided the best relationship with $F_{CO_2}$ in 10–60-year-old balsam fir trees. Bosc et al. (2003) applied lags of up to 50 min to obtain a better fit between branch temperature and branch $F_{CO_2}$ in adult Pinus pinaster Aiton. trees. The justification for such a procedure is that a delay can be assumed to exist between CO$_2$ production in the living tissues and CO$_2$ efflux at the organ surface because of the high resistance of the cambium and the bark (Eklund and Lavigne 1995). The $Q_{10}$ value in the pre-drought period was 2.76, which is at the high end of the range of reported values for various tree species. For example, Edwards and Hanson (1996) found a $Q_{10}$ of 2.4 for stems of Quercus alba L. and Quercus prinus L.

Three days after the imposition of drought, when DG became zero, the diurnal pattern of $F_{CO_2}$ was no longer correlated with $T_a$ (Figure 2g). Nevertheless, from the stepwise alterations of $T_a$ on DOY 183–184, it was apparent that $F_{CO_2}$ continued to respond to changes in $T_a$. However, $Q_{10}$ was lower during the drought than during the pre-drought period (Table 1), probably reflecting reduced metabolic activity of the living
cells. The time lag during the drought was also smaller than during the pre-drought period (16 versus 28 min, Table 1). Because gas diffusion in air is 3–4 times faster than in water (Ekblad and Lavigne 1995), it is likely that with a reduction of the water content of the bark tissue, the resistance to CO₂ diffusion decreases.

Five days after re-watering, diurnal fluctuations in \( F_{\text{CO}_2} \) were again correlated with fluctuations in \( T_a \) (Figure 2q). The \( Q_{10} \) value was 2.45, which is higher than during the drought period (\( Q_{10} = 1.94 \)), but slightly lower than in the pre-drought period (\( Q_{10} = 2.76 \)). Hence, metabolic activity of the living cells was still lower than in the pre-drought period. The mean DG was lower after the drought than before (33 versus 93 µm day\(^{-1} \)), suggesting that tree T2 had not completely recovered from the drought. The applied time lag was about double that during the drought (36 min versus 16 min, Table 1), which is likely because of a higher bark water content and hence a higher resistance for CO₂ diffusion.

Relationship between CO₂ efflux rate and diameter variations

In the pre-drought period, irreversible growth was observed during both the light and dark periods (Figure 1e), indicating that stem turgor pressure was always higher than the threshold value necessary for cell growth (Lockhart 1965). During the pre-drought period, \( F_{\text{CO}_2} \) was not correlated with \( D \) (Figure 2b). Because variations in \( D \) reflect variations in stem water status, rates of growth and maintenance processes were unaffected by stem water status during the pre-drought period.

During the drought, irreversible growth was no longer observed (Figure 1e). The strong correlation of \( F_{\text{CO}_2} \) with \( D \) during the drought (Figure 5) demonstrates that, when turgor pressure was lower than the threshold value necessary for cell growth, \( F_{\text{CO}_2} \) became highly dependent on stem water status. When the tree was re-watered, a sharp increase of \( F_{\text{CO}_2} \) was observed that coincided with the sharp increase in \( D \) due to stem re-hydration. From the strong correlation between \( F_{\text{CO}_2} \) and \( D \), it can be deduced that the temperature-independent variations in \( F_{\text{CO}_2} \) during the drought and at the moment of re-watering were the result of variations in rates of growth and maintenance processes following variations in water status. Therefore, our results agree with the view that, during the day, water deficit of the stem tissue causes a reduction in the rates of growth and maintenance processes and, hence, in respiration rates (Lavigne 1987, Wang et al. 2003). When the stress is (partly) alleviated, because of replenishment of the water reserves at night or because of water supplied after a drought, \( F_{\text{CO}_2} \) increases as a result of an increase in the rate of the maintenance or growth processes, or both. Daudet et al. (2005) also found that \( F_{\text{CO}_2} \) in potted hybrid walnut (Juglans nigra × J. Regia cv. NG38) trees increased at night, and concluded that variations in \( F_{\text{CO}_2} \) reflect variations in the local respiration rate and must be attributed to metabolic changes. Similarly, in the herbaceous species Sorghum bicolor (L.) Moench, whole-plant respiration increased dramatically on the first day after re-watering (Richardson and McCree 1985). Amthor (1994) suggests that this response is due to either rapid growth and high growth respiration rates following stress alleviation or high maintenance respiration rates for rapid protein resynthesis and other cell repair processes, or both. These studies and our results indicate that tree water status has an important influence on stem respiration rates, particularly when water availability is limited.

CO₂ efflux rates versus xylem CO₂ concentration

Teskey and McGuire (2002) found that diurnal fluctuations in xylem \([\text{CO}_2]_x\) corresponded closely with diurnal changes in stem \( F_{\text{CO}_2} \). In our study, xylem \([\text{CO}_2]_x\) followed more or less the same pattern as \( F_{\text{CO}_2} \) in the pre-drought period (Figure 2c), but the correlation became less clear during the early drought period (Period 2, Figure 2f) and in the transitional period after re-watering (Period 5, Figure 2o). During the drought (Period 3, Figure 2i), \( F_{\text{CO}_2} \) and \([\text{CO}_2]_x\) were positively correlated, but a large time-lag between these variables was observed. However, it remains unclear why xylem \([\text{CO}_2]_x\) sometimes differed from \( F_{\text{CO}_2} \). It is possible that \( F_{\text{CO}_2} \) is not closely related to xylem respiration because lateral diffusion of gases in the xylem toward the atmosphere is restricted by a high resistance located in the cambium (Ekblad and Lavigne 1995). If so, \( F_{\text{CO}_2} \) is mainly determined by respiration rates of the cells located in the cambium and phloem, and sudden changes in xylem \([\text{CO}_2]_x\) might not have a direct influence on \( F_{\text{CO}_2} \). After the drought period (Period 6, Figure 2s), \( F_{\text{CO}_2} \) and \([\text{CO}_2]_x\) were again positively correlated.

Conclusions

In a well-watered oak tree, diurnal fluctuations in \( F_{\text{CO}_2} \) and \([\text{CO}_2]_x\) corresponded closely with fluctuations in \( T_a \), and daytime depressions in \( F_{\text{CO}_2} \) did not occur. Once daily growth rate of the stem became zero because of limited soil water availability, clear daytime depressions in \( F_{\text{CO}_2} \) were observed. During the drought, diurnal fluctuations in \( F_{\text{CO}_2} \) were more closely related to fluctuations in \( D \) than to fluctuations in \( T_a \); however, the relationship with stem \( D \) fluctuations became dominant, even during severe water stress. \( F_{\text{CO}_2} \) continued to respond to stepwise alterations in \( T_a \). Daytime depressions in \( F_{\text{CO}_2} \) during the drought likely resulted from the reduced rates of metabolic activity (growth and maintenance processes), which were in turn caused by the decrease in daytime stem water status. Xylem \([\text{CO}_2]_x\) also showed clear daytime depressions in response to drought. When the tree was re-watered, \( F_{\text{CO}_2} \) and \([\text{CO}_2]_x\) showed sharp increases that coincided with the increase in \( D \). After re-watering, daytime depressions in \( F_{\text{CO}_2} \) and \([\text{CO}_2]_x\) disappeared and the variables were again positively correlated with \( T_a \).

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