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Daytime Depression in Tree Stem CO_2 Efflux Rates: Is it Caused by Low Stem Turgor Pressure?

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• *Background and Aims* Daytime CO_2 efflux rates (F_{CO2}) from tree stems are often reported to be lower than expected from the exponential relationship between temperature and respiration. Explanations of daytime depression in F_{CO2} have focused on the possible role of internal CO_2 transport in the xylem. However, another possible cause that has been overlooked is the daily dynamics of the water status in the living stem tissues and its influence on stem growth rate and thus respiration. The objective of this study was to assess the daily dynamics of stem water status and growth rate and to determine the extent to which they may be linked to daily variations in stem F_{CO2}.
• *Methods* F_{CO2} of young beech and oak stems were measured under controlled conditions. Relative stem turgor pressure (Ψ_p), obtained from simulations with the 'RCGro' model, was used as an indicator of the water status in the living stem tissues. Daily dynamics of stem growth were derived from Ψ_p: growth was assumed to occur when Ψ_p exceeded a relative threshold value.
• *Key Results* There was a strong correspondence between fluctuations in F_{CO2} and simulated Ψ_p. The non-growth conditions during daytime coincided with depressions in F_{CO2}. Moreover, F_{CO2} responded to changes in Ψ_p in the absence of growth, indicating also that maintenance processes were influenced by the water status in the living stem tissues.
• *Conclusions* Daytime depressions in stem F_{CO2} correlate with the daily dynamics of turgor, as a measure of the water status in the living stem tissues: it is suggested that water status of tree stems is a potentially important determinant of stem F_{CO2}, as it influences the rate of growth and maintenance processes in the living tissues of the stem.

Key words: *Fagus sylvatica* (beech), *Quercus robur* (oak), CO_2 efflux, diameter variations, dynamic model, radial growth, plant–water relations, sap flow, stem respiration, stem turgor pressure.

INTRODUCTION

Respiration rates of woody tissues are commonly measured by enclosing the tissue in a cuvette, and measuring the rate of CO_2 efflux from the tissue with an infrared gas analyser (IRGA). It is assumed that all the CO_2 respired by the woody tissue enclosed in the cuvette diffuses laterally from the stem interior into the cuvette. Several studies (e.g. Negisi, 1975, 1978, 1982; Lavigne, 1987; Kakubari, 1988) have shown that on warm sunny days, measured stem CO_2 efflux rate (F_{CO2}) was lower than that expected from the exponential relationship between respiration and temperature (Amthor, 1989):

$$R = R(T_b) \times Q_{10}^{(T-T_b)/10} \quad (1)$$

with R the respiration rate at temperature T, $R(T_b)$ the respiration rate at a basal temperature T_b, and Q_{10} the relative increase in respiration rate with a 10 °C rise in temperature. This phenomenon is the so-called daytime depression in stem F_{CO2}. Several authors have suggested that sap flow rate (F_{H2O}) might have an influence on stem F_{CO2} (Negisi, 1979; Ryan, 1990; Sprugel, 1990; Hari *et al.*, 1991; Martin *et al.*, 1994; McGuire and Teskey, 2004; Bowman *et al.*, 2005). This explanation is based on the high solubility of CO_2, H_2CO_3 and HCO_3^- in water, so that a portion of the CO_2 evolved by the respiring cells of woody tissues might dissolve in the sap and be transported vertically in the xylem along with the transpiration stream instead of moving laterally through the stem into the cuvette (Hari *et al.*, 1991; Kaipiainen *et al.*, 1998). Hence, measured values of stem CO_2 efflux may be affected by the rate of CO_2 diffusion in xylem sap rather than by respiratory processes *per se*.

However, transport of dissolved CO_2 in the xylem is only one consequence of sap flow. According to the cohesion tension theory, water in the xylem of transpiring trees is under considerable tension as it is pulled from soil to leaves along a complex pathway of water-conducting elements, which together have a large hydraulic resistance (Irvine and Grace, 1997). When tension develops in the xylem, a water potential gradient develops between the phloem and xylem, which leads to a water flux from the phloem across the cambium towards the xylem (Garnier and Berger, 1986). Hence, water reserves of the living tissues external to the xylem are depleted during the daytime, resulting in stem diameter shrinkage. These living tissues respire to provide energy for growth and maintenance processes. When the water reserves in the living tissues external to the xylem are depleted, water deficits may occur, temporarily reducing rates of growth and maintenance processes and the respiratory processes

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that support them (Lavigne, 1987; Kakubari, 1988; Wang *et al.*, 2003; Daudet *et al.*, 2005). In particular, expansion growth may be reduced by water deficit: it is one of the most sensitive of all plant processes to drought stress (Hsiao, 1973). However, it is difficult to actually determine the occurrence of water deficit in the stem tissue. Wang *et al.* (2003) measured xylem water potential of *Larix gmelini* branches, which was greater (less negative) in the morning than afternoon, but this is not evidence that growth and/or maintenance processes in the living tissues are actually suppressed. It is not the xylem water potential, but the turgor pressure (Ψ_p) in the stem tissue that reflects the water status in the living cells of the stem (phloem, cambium and parenchyma; Bradford and Hsiao, 1982). Growth processes, such as cell formation, cell-wall expansion and deposition of new wall material are more dependent on Ψ_p and cell volume than on water potential (Boyer, 1968; Hsiao *et al.*, 1976; Ray, 1987; Proseus and Boyer, 2006). The cell turgor is in direct proportion to the water potential only if the osmotic potential of the cell remains constant. However, turgor and cell volume can be maintained by osmotic adjustment (i.e. the active accumulation of solutes in the symplast), which may serve to sustain growth (Woodruff *et al.*, 2004). Hence, it is the daily course of Ψ_p that can reveal when cell growth is likely to occur, and not the daily course of the water potential. A widely used model of cell expansion, developed by Lockhart (1965), relates relative cell expansion to cell Ψ_p, cell-wall extensibility (ϕ) and a threshold Ψ_p at which wall yielding occurs (Γ):

$$\text{relative cell expansion} = \phi(\Psi_p - \Gamma) \qquad (2)$$

Ψ_p must be above this threshold value for the cell to expand irreversibly. This idea has been incorporated into a mathematical model linking sap-flow dynamics in trees to daily fluctuations in stem diameter and radial growth (Steppe *et al.*, 2006). This model, which requires only transpiration rate of the whole tree as input variable, simulates the change of Ψ_p relative to the maximum Ψ_p, occurring at zero F_{H2O}.

In order to explain daytime depressions in F_{CO2}, previous studies have examined the link between F_{H2O} and stem F_{CO2} to test the hypothesis that sap flow directly determines internal CO_2 transport in the xylem. However, sap flow also determines the course of Ψ_p in the living stem tissues, which, as discussed, is a very important driving variable for cell growth and, consequently, for the associated energy demand. We know of no studies that have attempted to evaluate the effects of Ψ_p and cell growth on the daily course of stem F_{CO2}. Therefore, the objective of this study was to assess the daily dynamics of Ψ_p and growth, and to determine their association with daytime depressions in stem F_{CO2}. The present work does not aim at refuting previous explanations for daytime depressions in stem F_{CO2}, but introduces strong, if circumstantial evidence for the role of Ψ_p and the daily dynamics of cell growth as a cause of daytime depressions in stem F_{CO2}.

MATERIALS AND METHODS

Plant material and growth chamber

Two different tree species, a ring-porous oak (*Quercus robur* L.) and a diffuse-porous beech (*Fagus sylvatica* L.), were studied in order to examine the possible link between daytime depressions of F_{CO2} and Ψ_p. A 3-year-old beech tree, previously grown outdoors, was planted at the beginning of February 2004 in a 50-L container, filled with potting mixture. The tree was 1·55 m high and the stem diameter at the soil surface was 16·4 mm. A 3-year-old oak tree was studied in 2005; it was 1·6 m high and had a stem diameter at the soil surface of 19·2 mm. The trees were placed in a growth chamber with dimensions $2 \times 1{\cdot}5 \times 2$ m (height × width × length) in order to control radiation and air temperature. Light was from densely packed fluorescent lamps ('TL'D 80, Philips Lighting NV, Brussels, Belgium), producing a photon flux (400–700 nm) of photosynthetically active radiation (PAR) of approximately 470 μmol m^{-2} s^{-1} at the top of the trees (measured with a quantum sensor, type Li-190S, Li-COR, Lincoln, TE, USA). The trees were watered weekly and fertilized monthly with a NPK plus micronutrient mix (Substral, Sint-Niklaas, Belgium). Measurements were performed during the beginning of the growing season of 2004 for the beech tree and 2005 for the oak tree. During the measurement periods, the leaf area of the trees rapidly increased.

On one day during the experiment (day 154), from 1400 h until 1800 h, the leaves of the oak tree were enclosed in large transparent plastic bags in order to decrease the transpiration rate, while the light and temperature in the chamber were kept constant.

CO_2 efflux measurements

Gas exchange was measured on a 13-cm long segment of each stem, approximately 0·7 m above the soil surface. The lower and upper diameter of the segments were 8·3 and 8·0 mm, respectively, for the beech, and 11·0 and 10·7 mm, respectively, for the oak stem. The segments were enclosed in opaque, air-tight cylindrical PVC cuvettes with a diameter of 6 cm, which prevented all light from reaching the tissue to avoid corticular photosynthesis. To minimize short-term fluctuations in CO_2 concentration within the stem cuvettes, air from the growth chamber was pumped by a membrane pump (type N 86.KN 18, KNF Verder, Aartselaar, Belgium) at a flow rate of 1 L min^{-1} into a 50-L buffer tank before entering the cuvettes. Air leaving the cuvettes was first partially dried at 4 °C with a gas cooler (CG/G 73–4, Hartmann and Braun AG, Germany), before the CO_2 concentration was measured with an infra-red gas analyzer (IRGA; Binos 100-4P, Fisher-Rosemount, Hasselroth, Germany), as the difference between the air leaving the stem cuvette and a reference cuvette, which did not contain a stem segment. The system was automatically zeroed every 450 s and 360 s for the beech and oak tree, respectively, by passing air from the reference cuvette through the reference and measuring cells of the IRGA. Since only living cells are producing

CO_2 and the majority of living cells in small trees are located close to the surface (phloem and cambium; Stockfors and Linder, 1998), CO_2 efflux rates (F_{CO2}) were expressed per unit of stem surface area.

Stem diameter measurement

Stem diameter (D) was measured using a linear variable displacement transducer (LVDT) and transducer bridge (respectively LBB, 375-PA-100 and 8C-35, Schaevitz, Hampton, VA, USA), placed 1 cm below the cuvette. The LVDT was supported by a stainless steel holder: tests with a 12-mm diameter aluminium rod showed that no temperature correction was required.

Sap flow measurements

Sap flow rates (F_{H2O}) at the stem base and on a second-order branch (at the tree top), were measured with flow sensors based on the heat-balance principle (Models SGB16 and SGA5, Dynamax Inc., Houston, TX, USA), and installed according to the operation manual, as was calculation of F_{H2O} (van Bavel and van Bavel, 1990). The sensors were thermally insulated with several layers of aluminium foil. Sheath conductance of the gauge was recalculated daily using minimum values in darkness between 0400 h and 0700 h. The value for thermal conductance of woody stems of 0·42 W m^{-1} °C^{-1} was taken from Steinberg *et al.* (1989).

Data acquisition

All signals from sensors and devices were logged (HP 34970A, Hewlett Packard, Palo Alto, CA, USA) at 10 s intervals, and monitored continuously (Hewlett-Packard programme VEE). All sensor signals were averaged over 450 s and 360 s periods for the beech and oak tree, respectively, and recorded by a computer.

Temperature correction for CO_2 efflux rates

Air temperature (T_a) and stem temperature (T_{st}) were measured with copper–constantan thermocouples (Omega, Amstelveen, Netherlands). For T_{st} a 1-mm diameter hole, 7 mm deep was drilled in the stems into which the thermocouple was inserted. Although a constant temperature was set for the growth chamber, there were small variations, so the measured F_{CO2} was adjusted to T_{st} of 20 °C using the following equation (based on eqn 1):

$$F_{CO2}(20) = F_{CO2}/Q_{10}^{(T_{st}-20)/10} \quad (3)$$

with $F_{CO2}(20)$ the calculated CO_2 efflux rate at 20 °C (expressed in μmol m^{-2} s^{-1}). Q_{10} was estimated by ordinary least squares, based on measurements of F_{CO2} on days where T_a was altered stepwise (four temperature steps of 1 h: 24 → 21 → 17 → 21 °C) during the dark period of day 121 for beech and during the dark period of day 148 for oak. Calculation of Q_{10} was based on F_{CO2} data for the dark period, since then no sap was flowing and it was assumed that the stem tissue was fully hydrated.

Simulation of stem turgor pressure

Non-destructive measurement of Ψ_p is difficult, so the mechanistic flow and storage model 'RCGro', developed by Steppe *et al.* (2006), was applied to simulate the change of stem Ψ_p relative to the maximum Ψ_p, at zero F_{H2O} (Fig. 1). The model enables simulation of tree F_{H2O} dynamics (water transport submodel), which are directly linked to variations in stem diameter (D) (stem diameter variation submodel), using the radial flow of water (see Fig. 1) between the xylem (considered as a continuous rigid cylinder) and the stem storage compartment (i.e. the living, extensible cells external to the xylem). This radial flow causes changes in the water content of the living tissues, and hence in Ψ_p. D varies due to reversible stem shrinkage/swelling and irreversible radial stem growth. If Ψ_p is smaller than the threshold Ψ_p at which wall yielding occurs (Γ), then variations in D only reflect reversible shrinkage/swelling. If Ψ_p is larger than Γ, then irreversible radial growth occurs, in addition to shrinkage/swelling (Fig. 1). For a detailed description of the model see Steppe *et al.* (2006). Whole-tree transpiration rate, which is the input variable for the model, is normally determined by multiplying measured branch F_{H2O} (not stem F_{H2O} since this lags behind transpiration) by the ratio of total leaf area/leaf area upstream of the sap flow sensor (Steppe *et al.*, 2006). However, since the leaf area of both trees rapidly increased during the measurement period, it would have been necessary to measure total leaf area every day, which is a very laborious task. Therefore, F_{H2O} measured at the top of each tree was increased each day by multiplying by a scaling factor, namely the ratio between the daily sum of stem F_{H2O} and branch F_{H2O}. This up-scaled branch F_{H2O} was used as the input variable for the model. Stem F_{H2O} and variations in D were selected as suitable variables for model calibration, using the simplex method (Nelder and Mead, 1965) by minimizing the sum of squared errors between the simulated values and the measured data of stem F_{H2O} and variations in D (Steppe *et al.*, 2006). The model was calibrated for beech and oak separately, and allowed us to simulate relative changes in stem Ψ_p throughout the day, as an indicator of the water status in the living tissues of the stem. Simulated relative Ψ_p was continuously compared with the relative threshold value Γ (i.e. expressed as a percentage of maximum Ψ_p occurring at zero F_{H2O}) in order to estimate during which periods of the day irreversible radial stem growth occurred (see eqn 2).

RESULTS

Daytime depression in CO_2 efflux rate

Despite the higher T_{st} during the day than night, the daytime F_{CO2} of both tree stems were lower compared with night values (Fig. 2). When F_{CO2} was adjusted for variations in T_{st} using eqn (3) [$F_{CO2}(20)$], with estimated Q_{10} values of 3·2 (± 0·20 s.e.) and 2·0 (± 0·17) for

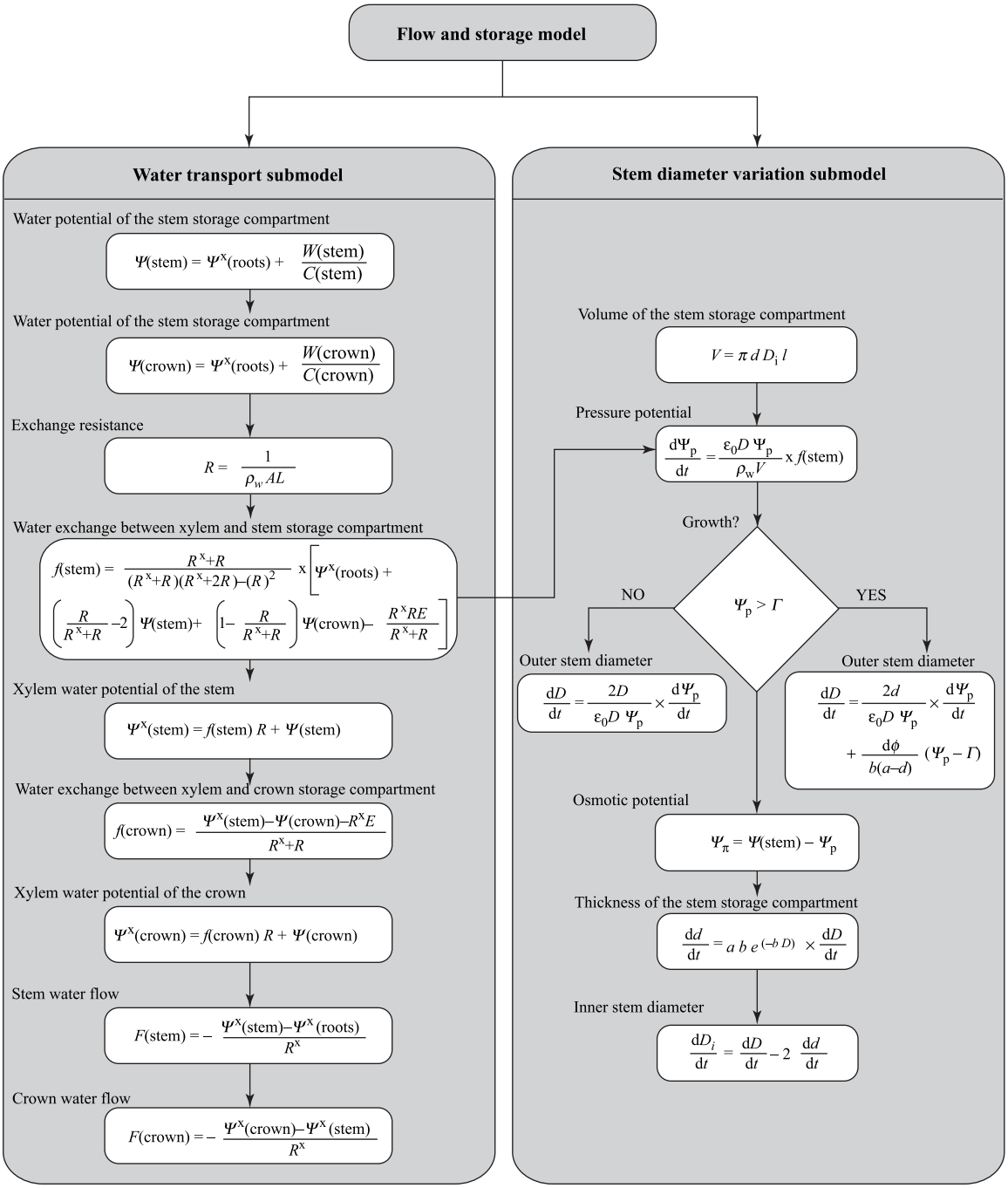

FIG. 1. Schematic of the model (algebraic and differential equations) linking the dynamics of tree sap flow and storage to changes in stem diameter and growth. The direct linkage referred to in the text is indicated by the arrow between the two submodels. Ψ = total water potential of storage compartment; Ψ^x = total water potential of xylem compartment; W = water content of storage compartment; C = capacitance of storage compartment; R = flow resistance between xylem and storage compartment; R^x = flow resistance in xylem compartment; ρ_w = density of water; A = surface area of the virtual membrane separating the stem storage compartment from the xylem compartment; L = hydraulic conductivity of the membrane; f = water flow between xylem and storage compartment; E = transpiration; F = water flow in a xylem compartment; V = volume of storage compartment; d = thickness of storage compartment; D_i = inner diameter of stem segment; D = outer diameter of stem segment; l = length of stem segment; Ψ_p = turgor pressure potential; ε_0 = proportionality constant; Γ = threshold Ψ_p at which wall-yielding occurs; ϕ = cell wall extensibility; a and b = allometric parameters; Ψ_π = osmotic potential.

beech and oak, respectively, the daytime depressions in F_{CO2} became even more pronounced (Fig. 2).

When the leaves of the oak tree were enclosed in plastic bags for 4 h on day 154, transpiration decreased substantially, although temperature and light conditions were not altered. An increment in stem $F_{CO2}(20)$ was observed (Fig. 3). Hence, factors other than temperature variations appear to exert a control over stem F_{CO2}.

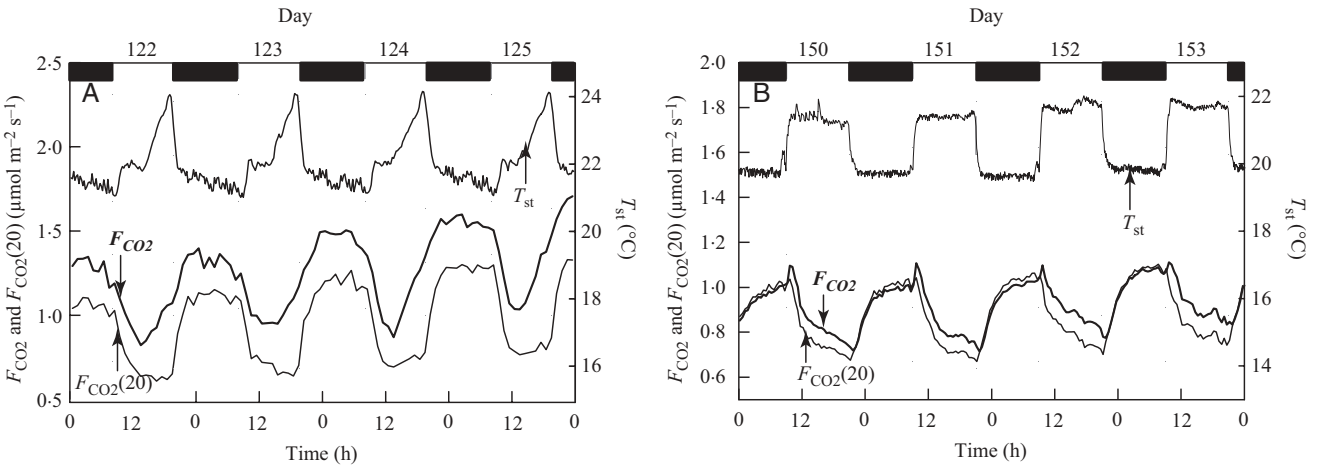

FIG. 2. Daily patterns of measured temperature (T_{st}), CO_2 efflux rate (F_{CO2}) and CO_2 efflux rate normalized to 20 °C [$F_{CO2}(20)$] for stems of (A) beech (days 122–125), and (B) oak (days 150–153). Dark periods are indicated by black boxes. Values of $F_{CO2}(20)$ were obtained using eqn (3), with estimated Q_{10} values of 3·2 and 2·0 for beech and oak, respectively.

Model input and outputs

The RCGro model used the up-scaled branch F_{H2O} as the only input variable. The values of the estimated model parameters (Table 1) were similar to those obtained by Steppe (2004), Steppe and Lemeur (2006) and Steppe *et al.* (2006) for young beech and oak trees. Figure 4 shows how well the output variables D and stem F_{H2O} of the RCGro model fit the measured values for both trees. Coefficients of determination (r^2) of the linear regression between measured and simulated data were very high for both trees.

Stem turgor pressure versus CO_2 efflux rate normalized at 20 °C

Changes in Ψ_p, simulated with the calibrated RCGro model, and $F_{CO2}(20)$ during the period day 122–125 for beech and day 150–154 for oak are shown in Fig. 5. At the onset of each light period, Ψ_p decreased sharply, due to the depletion of the water reserves in the stem tissues. This is also observed in the decrease of diameter (D; Fig, 4C, D). During the light periods, Ψ_p became lower than the critical wall-yielding threshold value needed for irreversible radial stem growth. At the beginning of each dark period, Ψ_p increased sharply due to the replenishment of the water reserves in the stem. The replenishment is also observed as an increase of D (Fig. 4C, D). During darkness, Ψ_p exceeded the wall-yielding threshold value. The diurnal variations of Ψ_p corresponded closely with the temperature-independent variations of F_{CO2}. Upon enclosure of the oak foliage in plastic bags on day 154, Ψ_p in the stem increased but remained lower than the wall-yielding threshold value necessary for irreversible growth (Fig. 5B). Nevertheless, $F_{CO2}(20)$ increased. Closer inspection of the data for $F_{CO2}(20)$ and Ψ_p (Fig. 6A, B),

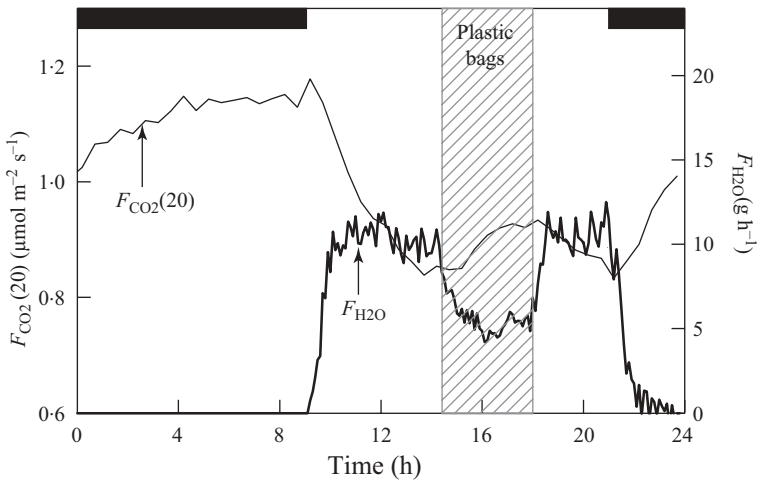

FIG. 3. Time course of CO_2 efflux rate from stems, normalized to 20 °C [$F_{CO2}(20)$], obtained using eqn (3), ($Q_{10} = 2·0$), and sap flow rate at the stem base (F_{H2O}) for the oak tree on day 154, when the leaves where enclosed in transparent plastic bags from 1400 h until 1800 h. Black boxes indicate the dark periods.

TABLE 1. *Values of the estimated model parameters for beech and oak*

Parameter	Beech	Oak
R^x (MPa s mg^{-1})	0·1478	0·1701
C(stem) (mg MPa^{-1})	638·6	1158·4
C(crown) (mg MPa^{-1})	350·3	5063·4
ϕ (MPa^{-1} s^{-1})	$6{\cdot}95 \times 10^{-7}$	$5{\cdot}16 \times 10^{-7}$
Relative Γ (% of full turgor)	92·86	95·66

R^x = flow resistance in xylem compartment; C(stem) = capacitance of stem storage compartment; C(crown) = capacitance of crown storage compartment; ϕ = cell wall extensibility; Γ = threshold Ψ_p at which wall yielding occurs.

showed that $F_{CO2}(20)$ lagged behind Ψ_p and hysteresis, following a counter-clockwise time course, is clearly visible when $F_{CO2}(20)$ is plotted as a function of Ψ_p (Fig. 6C, D).

DISCUSSION

Clear daytime depressions were observed in stem F_{CO2} compared with the night despite a higher T_{st}, suggesting that factors other than temperature controlled F_{CO2} (Fig. 2). The experiments were conducted during spring, when the tree growth rate was high, and growth respiration is the dominant contributor to total stem respiration (e.g. Stockfors and Linder, 1998). Growth processes are very sensitive to drought stress (Hsiao, 1973) and so very probably depend on the dynamics of the water status in the living stem tissues. This study used a simulation model approach to estimate Ψ_p, which is a good indicator of the water status of the living tissues. Ψ_p fluctuated diurnally due to the dynamics (depletion and replenishment) of water reserves in the stem. As a result, relative radial stem growth rate fluctuated diurnally, with the highest growth rate occurring during the night when Ψ_p exceeded the wall-yielding threshold value (Γ) necessary for cell growth (Fig. 5). Simulated and measured stem diameter patterns both illustrate that irreversible radial stem growth occurred during the night: D increased continuously (Fig. 4C, 4D). Differences in plant growth rate during day and night have been studied by several authors. Boyer (1968) observed that enlargement of leaves of a well-watered sunflower plant (*Helianthus annus*) was five to six times higher at night than during the day. Schurr *et al.* (2000) found that leaf growth rates in *Ricinus communis* peaked during the late night and were minimal in the late afternoon.

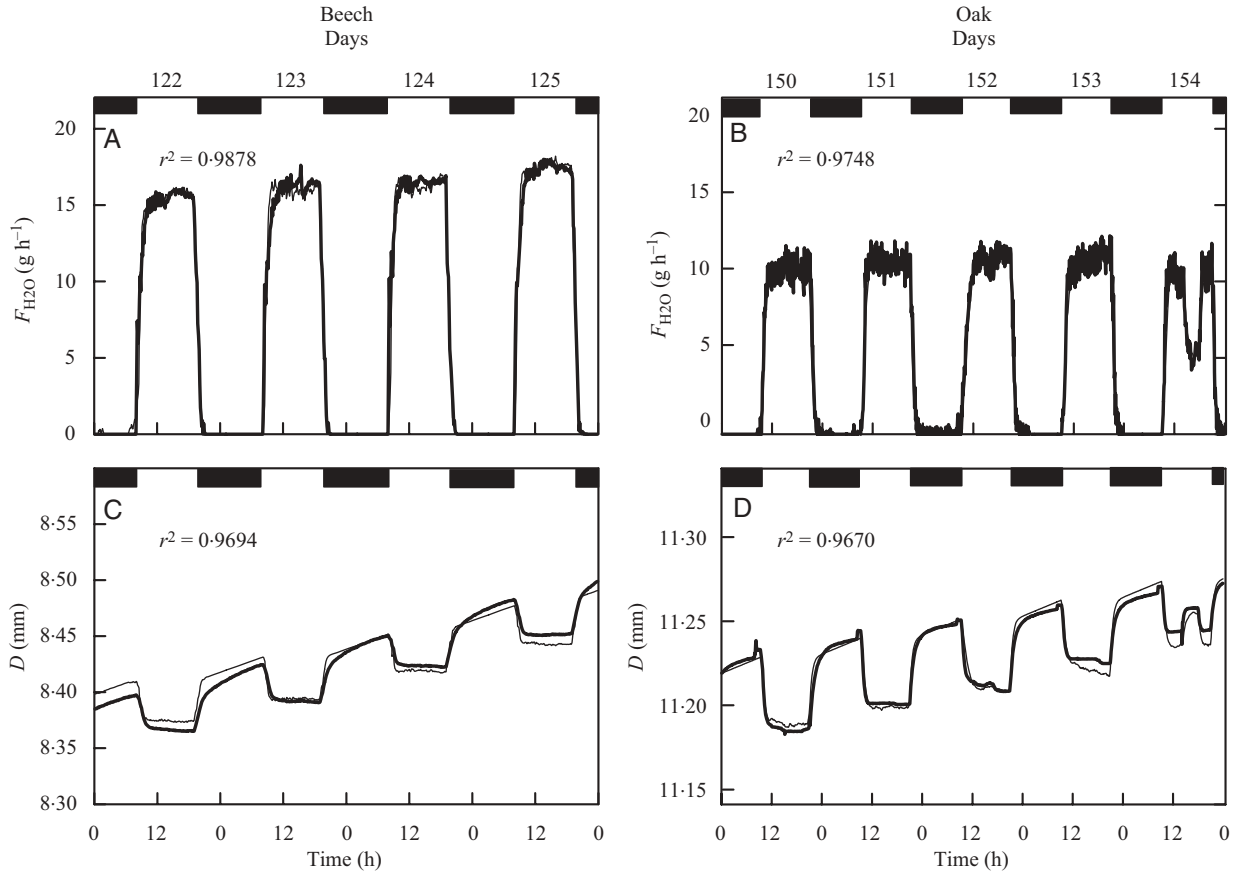

FIG. 4. Comparison between measured (thick lines) and simulated (thin lines) sap flow rates (F_{H2O}) at the stem base for (A) beech and (B) for oak, and measured and simulated stem diameter (D) for (C) beech and (D) oak. Dark periods are indicated by black boxes. The coefficients of determination (r^2) of the linear regression between measured and simulated values are given.

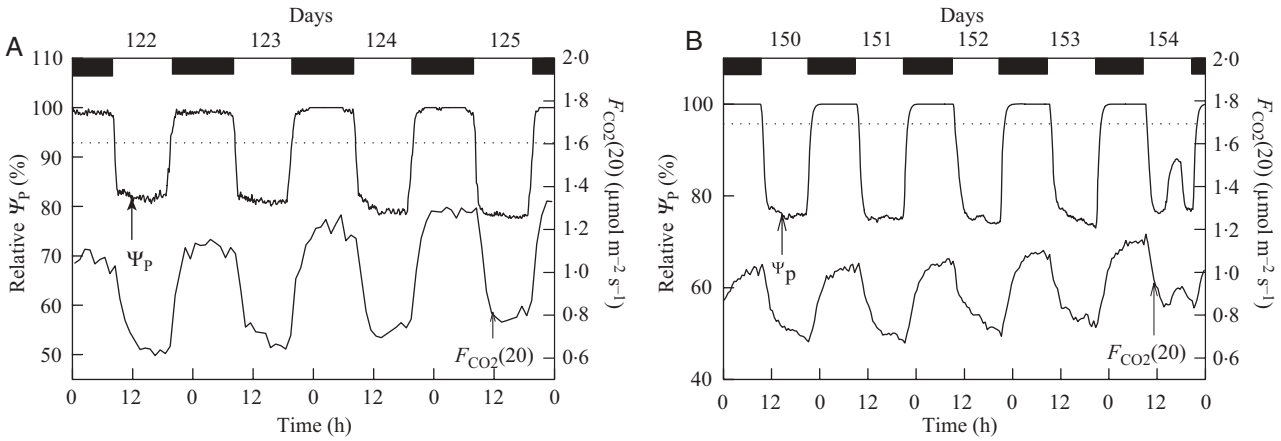

FIG. 5. Daily patterns of simulated stem turgor pressure (Ψ_p), expressed as a percentage of maximum Ψ_p occurring at zero F_{H2O}, and calculated CO_2 efflux rate normalized to 20 °C [$F_{CO2}(20)$] during the period days 122–125 for beech (A), and 150–154 for oak (B). Ψ_p was simulated with the calibrated RCGro model. Values of $F_{CO2}(20)$ were calculated using eqn (3), with a Q_{10} of 3·2 and 2·0 for beech and oak, respectively. The horizontal dotted line represents the relative wall-yielding threshold value for Ψ_p above which radial stem growth occurs. Black boxes indicate dark periods.

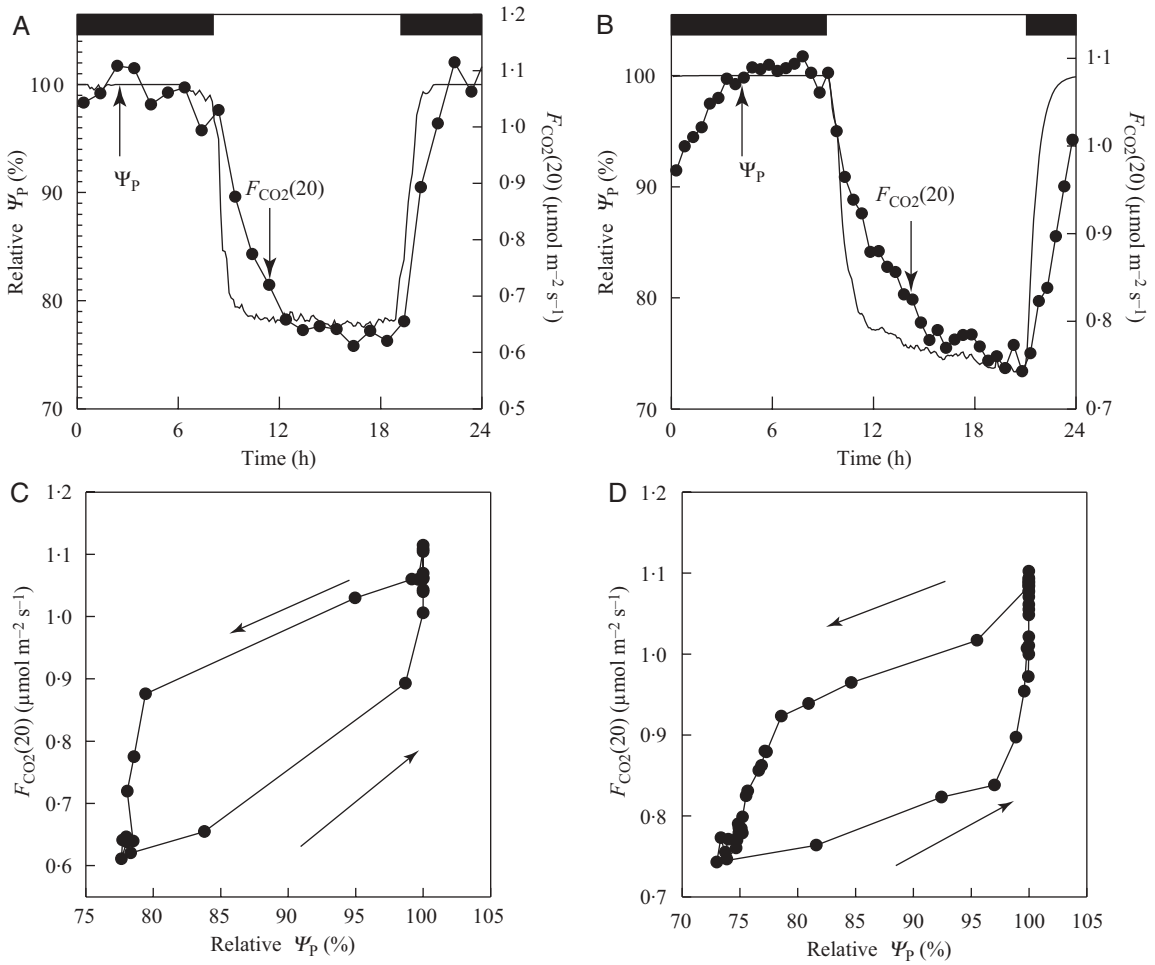

FIG. 6. Time-course of simulated stem turgor pressure (Ψ_p), expressed as a percentage of maximum Ψ_p occurring at zero F_{H2O}, and CO_2 efflux rate normalized to 20 °C [$F_{CO2}(20)$] on day 122 for beech (A), and 153 for oak (B). Values of $F_{CO2}(20)$ were calculated using eqn (3), with a Q_{10} of 3·2 and 2·0 for beech and oak, respectively. Black boxes indicate dark periods. Hysteresis occurs between Ψ_p and $F_{CO2}(20)$ for beech (C) and oak (D). Measurement symbols are connected chronologically, with the arrows indicating hysteresis.

Halter *et al.* (1996) observed that root elongation rates in *Eucalyptus nitens* and *E. pauciflora* seedlings were 60 and 67 % higher, respectively, during the night than during the day. In our study, daytime Ψ_p was lower than the wall-yielding threshold value that must be exceeded for expansion growth (Fig. 5), indicating that the stem growth rate of both trees was zero during the daytime. Measurements of stem diameter confirm this: D was constant during the day (Fig. 4C, D). Hence, the higher $F_{CO2}(20)$ during darkness compared with the light can be explained at least partially by the higher energy demand to support growth in the stem tissue.

When transpiration of the oak tree was decreased by enclosure of the leaves (Figs 3 and 5B), Ψ_p increased but did not exceed the wall-yielding threshold value. Hence, cell growth was not expected to occur during this period. Nevertheless, $F_{CO2}(20)$ increased. This indicates that F_{CO2} increased with any improvement of the water status in the living stem tissues, even when stem expansion growth did not take place. A plausible explanation is that with any increase in Ψ_p, and hence an improved water status, the rate of maintenance metabolism (such as protein turnover, membrane repair, etc.) increases. In contrast with growth, maintenance metabolism always occurs, even under stress conditions. However, when conditions become more favourable (e.g. an enhanced water status in the living stem tissues), the rate of maintenance metabolism, and hence F_{CO2}, will be enhanced. Unfortunately, we are unaware of any studies on the relationship between turgor and rates of maintenance processes. It is also important to note that cell wall expansion is not the only growth process in stems. Growth also includes processes such as cell wall deposition and assembly. Proseus and Boyer (2006) recently demonstrated that by decreasing Ψ_p, wall deposition and assembly in *Chara corallina* cells decreased. Hence, not only wall expansion is affected by changes in Ψ_p. However, it was not investigated whether a threshold Ψ_p exists for these processes to occur.

Changes in $F_{CO2}(20)$ were clearly related to simulated changes in Ψ_p. However, the response of $F_{CO2}(20)$ lagged behind changes in Ψ_p (Fig. 6C, D). A possible explanation is that when CO_2 production by the living cells increases due to improved water status, its radial diffusion through the stem into the cuvette is delayed by the large resistance in the stem. A substantial restriction to radial gas movement in *Pinus strobus* branches was demonstrated by Eklund and Lavigne (1995).

As mentioned in the Introduction, an explanation for daytime depressions in F_{CO2} is that the sap flowing in the xylem of transpiring trees transports respired CO_2 away from the stem. These studies therefore link F_{CO2} to F_{H2O}. Our study links daytime depressions in F_{CO2} to changes in Ψ_p, and hence indirectly also to F_{H2O}: changes in Ψ_p reflect changes in the water status in the living stem tissues, which result from changes in F_{H2O}. Until now, the effect of F_{H2O} on stem turgor, and hence on stem growth rate and respiration, has been ignored. With our dataset we could not distinguish between the effects of CO_2 transport in the xylem and those of turgor dynamics in the living stem tissues. Thus our work does not refute previous explanations; rather it points out that in addition to the idea that respired CO_2 is transported with the transpiration stream, the daily dynamics of Ψ_p and growth might equally well explain daytime depressions in stem F_{CO2}. Further studies need to be conducted in which the effects on stem F_{CO2} of CO_2 transport in the xylem and those of turgor and growth dynamics in the tissues external to the xylem are separated, and in which the relative importance of both processes on stem F_{CO2} are investigated. It is possible that processes in the external tissues have a larger impact on stem F_{CO2} than CO_2 transport in the xylem. Some facts support this hypothesis. The cambium is quite impermeable to gases (Hook *et al.*, 1972), which is demonstrated by the large difference in CO_2 concentrations between xylem and bark tissues: xylem CO_2 concentrations have been reported to be as high as 26 % (MacDougal and Working, 1933), while CO_2 concentrations in bark tissues are reported to be around 0·06 to 0·17 % (Cernusak and Marshall, 2000; Wittmann *et al.*, 2006). Furthermore, Maier and Clinton (2006) measured xylem CO_2 concentrations and F_{CO2} in young *Pinus taeda* tree stems during spring and found that, after partial removal of the canopy, xylem CO_2 concentrations increased but there was no apparent change in stem F_{CO2}. They assumed that during spring the cambium and phloem meristems are likely to respire at a much higher rate than the xylem parenchyma and thus would be a major source of CO_2 in the stem.

Another challenge will be to verify that respiration (i.e. not F_{CO2}) of the tissues exterior to the xylem is actually slowed during the day. One possibility might be using the method of Pruyn *et al.* (2002), who examined tissue respiratory potential by sampling stem tissues, placing them in vials and measuring the difference in CO_2 concentration after closing the vial and after an incubation period. However, an important problem with this method is that the samples lost water during the incubation period. Before their method can be used for our purpose, it needs to be improved so that water losses during sampling and incubation are completely prevented in order to maintain the Ψ_p of the living tissue at the same level.

CONCLUSIONS

This study demonstrates that the loss of turgor in the living stem tissues during daytime is quantitatively consistent with a slowing, or even cessation, of growth processes in these tissues. Since growth respiration is an important component in total stem respiration, daytime depressions in stem F_{CO2} (i.e. lower compared with what would be expected from the exponential temperature function) can at least partially be explained by the restricted growth during the daytime. However, since stem F_{CO2} also responded to changes in turgor when the turgor was still lower than the wall-yielding threshold value for growth, it was suggested that not only growth rate, but also the rate of maintenance metabolism fluctuates diurnally, due to the daily dynamics in the water status of the living stem tissues.

This work did not aim at distinguishing between the effects of turgor and the effects of transport of dissolved CO_2 in the xylem. It rather sort to demonstrate that the daily dynamics of water status in the living stem tissues might explain daytime depressions in stem F_{CO2} just as well as the idea that the transpiration stream is transporting CO_2 in the xylem. It will be a challenge in further research to separate the effects of both processes and to unravel to what extent both processes might influence stem F_{CO2}.

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