

# Intra-annual dynamics of stem CO<sub>2</sub> efflux in relation to cambial activity and xylem development in *Pinus cembra*

A. GRUBER,<sup>1,2</sup> G. WIESER<sup>3</sup> and W. OBERHUBER<sup>1</sup>

<sup>1</sup> Institute of Botany, University of Innsbruck, Sternwartestrasse 15, 6020 Innsbruck, Austria

<sup>2</sup> Corresponding author (a.gruber@uibk.ac.at)

<sup>3</sup> Federal Research and Training Center for Forests, Natural Hazards and Landscape, Rennweg 1, 6020 Innsbruck, Austria

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**Summary** The relationship between stem CO<sub>2</sub> efflux ( $E_S$ ), cambial activity and xylem production in *Pinus cembra* L. was determined at the timberline (1950 m a.s.l.) of the Central Austrian Alps, for 1 year. The  $E_S$  was measured continuously from June 2006 to August 2007 using an infrared gas-analysis system. Cambial activity and xylem production were determined by repeated microcore sampling of the developing tree ring, and radial increment was monitored using automated point dendrometers. Besides temperature, the number of living tracheids and cambial cells was predominantly responsible for  $E_S$ , and  $E_S$  normalized to 10 °C ( $E_{S10}$ ) was significantly correlated to the number of living cells throughout the year ( $r^2 = 0.574$ ;  $P < 0.001$ ). However, elevated  $E_S$  and missing correlation between  $E_{S10}$  and xylem production were detected during cambial reactivation in April and during transition from active phase to rest, which occurred in August and lasted until early September. Results of this study indicate that (i) during seasonal variations in cambial activity, nonlinearity between  $E_S$  and xylem production occurs and (ii) elevated metabolic activity during transition stages in the cambial active-dormancy cycle influences the carbon budget of *P. cembra*. Daily radial stem increment was primarily influenced by the number of enlarging cells and was not correlated to  $E_S$ .

**Keywords:** cambial reactivation, dormancy, radial stem growth, sap flow, stem respiration, xylem production.

## Introduction

Stem CO<sub>2</sub> efflux ( $E_S$ ) is an important component of the carbon balance of trees and forest stands (Sprugel et al. 1995). The  $E_S$  is commonly estimated by measuring the CO<sub>2</sub> efflux from the stem surface into chambers sealed to the tree stem. This approach assumes that CO<sub>2</sub> respired from phloem, cambium and xylem parenchyma tissues diffuses radially

from the stem into the chamber (Maier and Clinton 2006, Teskey et al. 2008) and thus reflects the actual stem respiration ( $R_S$ ) of living woody tissue cells (Saveyn et al. 2008). On the other hand there is evidence that CO<sub>2</sub> produced by respiration, instead of being released directly through the bark, is partly dissolved in xylem sap and is carried upward by the transpiration stream, while part of the CO<sub>2</sub> derived from root or soil microbial respiration is carried upward into the stem and released there (Sprugel et al. 1995, Maier and Clinton 2006, Saveyn et al. 2008, Teskey et al. 2008).

The  $E_S$  is strongly related to woody tissue temperature (cf. Lavigne 1987, Atkin and Tjoelker 2003, Larcher 2003); even after adjustment to a reference temperature,  $E_S$  still varies substantially throughout the growing season (Lavigne 1996, Lavigne and Ryan 1997, Wieser 2007) as a result of changes in phenology, above all due to growth processes (cf. Sprugel and Benecke 1991, Ryan et al. 1994, Sprugel et al. 1995, Lavigne and Ryan 1997, Wieser et al. 2009). Havranek (1981, 1985) and Lavigne et al. (2004) found a linear correlation between xylem production and  $E_S$  in *Pinus cembra* L. and *Larix decidua* Mill. during the year and in *Abies balsamea* (L.) Mill. and *Acer rubrum* L. during spring, respectively. But still phenological control over  $E_S$  during the year remains poorly understood. Therefore, monitoring of radial growth and xylogenesis is important to enlighten the  $E_S$  dynamics throughout the year. Radial stem growth is a complex process and involves cell division in the cambial zone, followed by cell enlargement and secondary wall thickening (Deslauriers et al. 2003a, Rossi et al. 2006b). Microcore sampling and histological analyses (Rossi et al. 2006a, 2006b) can thereby provide direct and detailed insight on xylem cell production, while dendrometer measurements are used to monitor radial stem increment.

In the temperate zones,  $E_S$  might not only be influenced by the radial growth but also by the cambial active-dormancy cycle (Vose and Ryan 2002). Reactivation of the cambium at the end of dormancy is a complex process

that involves changes in ultrastructure and cell biochemistry (Riding and Little 1984, Catesson 1994, Little and Pharis 1995) that cause high metabolic activity leading to elevated  $E_S$ . Lavigne et al. (2004) observed such an increase of  $E_S$  due to cambial reactivation before the onset of cell division in spring in *A. balsamea*, *A. rubrum* and *Fraxinus americana* L. Like cambial reactivation, transition to dormancy is characterized by changes in ultrastructure and cell biochemistry. Fragmentation of central vacuole and endoplasmic reticulum, as well as an increase in RNA, protein and insoluble carbohydrate in cambial cells, is required to reach cold hardiness (Riding and Little 1984, Catesson 1994, Little and Pharis 1995, Rensing and Samuels 2004). Based on the sporadic respiration measurements, Havranek (1981) assumed that apart from cambium reactivation transition to dormancy might also influence the  $E_S$  in *P. cembra*.

The purpose of this study was to determine the relation of intra-annual dynamics of  $E_S$  to radial stem growth and cambial activity in *P. cembra* throughout the year. Continuous records of  $E_S$  together with repeated wood microcore analysis allowed the determination of periods when changes in cambial activity and xylem production affected  $E_S$ .

## Materials and methods

### Study site

The study was conducted at Mt. Patscherkofel (2246 m a.s.l.) near Innsbruck, in western Austria (47°12' N and 11°27' E). Mt. Patscherkofel is located in the Central Austrian Alps within an inner-alpine dry zone. During the period 1967–2004, mean annual precipitation at the top of Mt. Patscherkofel was 890 mm with a maximum during summer (June–August: 357 mm) and minimum in winter (December–February: 147 mm). During the same period, mean annual temperature at timberline was 2.5 °C and the coldest and warmest months were February (−4.3 °C) and July (10.0 °C), respectively.

The geology of the Mt. Patscherkofel region (Tuxer Alpen as part of the Central Tyrolean Alps) is dominated by gneisses and schists. According to the World Base for Soil Resources (FAO 1998), the soil at the study site is classified as a haplic podzol, a soil type typical for the Central Austrian Alps (Neuwinger 1970).

Cembran pine (*P. cembra*) was chosen for this study because it is the dominant conifer in the study area and at the timberline in the central part of the Eastern Alps. The trees were selected on a southwest-facing slope at the timberline (1950 m a.s.l.), and they reached a height of 10–14 m.

### Determination of wood formation

Seasonal wood formation dynamics were monitored during the growing seasons 2006 and 2007 by taking small punched cores from five trees of the outermost tree rings (microcores)

with a diameter and a length of 2.5 mm and c. 2 cm, respectively (Rossi et al. 2006a). The samples were taken on the slope-parallel side of the stem following a spiral trajectory up to the stem from c. 15 cm below breast height (1.3 m) to c. 15 cm above. A distance of c. 2 cm in tangential and longitudinal direction was kept to avoid lateral influence of wound reactions on adjacent sampling positions.

Microcores were taken during June through October 2006 at weekly intervals, and in 2007 the sampling was carried out from late April to October at about 10-day intervals. The collected core samples were prepared for light microscopy. Immediately after extraction, the cores were fixed in a solution of ethanol, propionic acid and 40% formaldehyde (mixing ratio: 90/5/5), then were embedded in glycolmethacrylate (Technovit 7100, Heraeus Kulzer, Wehrheim, Germany) and polymerized after adding an accelerator. Transverse sections of c. 12 µm were cut with a microtome, stained with a water solution of 0.05% cresyl fast violet and observed under a light microscope with polarized light to differentiate the development of xylem cells, i.e., the discrimination between tracheids in enlarging and cell-wall thickening phase (Antonova and Stasova 1993, Deslauriers et al. 2003a, Rossi et al. 2006b). The number of cambial cells (i.e., fusiform cells lacking radial enlargement), radial enlarging cells, cells undergoing secondary wall thickening and mature xylem cells was counted on all the sampled cores in three radial rows. The number of living cells was defined as the sum of cambial cells, radial enlarging cells and cells undergoing secondary wall thickening. The total xylem cell number was determined by adding the number of cells in radial enlargement, in cell-wall thickening and the number of mature xylem cells (Deslauriers et al. 2003a, Rossi et al. 2006c). Values, i.e., the number of different cell types of five trees per date were averaged.

### Standardization of cell number and fitting of xylem growth

Because cell number varies within the tree circumference and hence among different samples, standardization is required (Rossi et al. 2003). The total cell number of the previous tree ring was recorded in every sample and was used for a cell number correction for each tree. The cell number in each  $j$ -sample and by each  $i$ -phase (enlarging, wall thickening and mature cells) was corrected as follows:

$$nc_{ij} = n_{ij} \times n_m / n_s,$$

where  $nc_{ij}$  is the corrected cell number,  $n_{ij}$  is the counted cell number,  $n_m$  is the mean cell number of the previous ring of all  $j$ -samples and  $n_s$  is the cell number of the previous ring for each  $j$ -sample.

Short-term variations in the measured time series of the number of tracheids were modelled with a Gompertz function using the nonlinear regression procedure included in the Origin software package (OriginLab Corporation, Northampton, MA). The Gompertz equation proved its versatility to describe limiting growth processes and to assess cell

dynamics of tree ring growth (Zeide 1993, Camarero et al. 1998, Rossi et al. 2003, 2006c, Deslauriers and Morin 2005).

Xylem cell number increases (including cells in enlarging and wall thickening phase as well as mature cells) were calculated for 2-week intervals based on developed Gompertz models.

#### *Microclimate records*

Air temperature, relative humidity (HMP45C, Vaisala, Helsinki, Finland), solar radiation (SP-Lite, Campbell Scientific, Shepshed, UK) and wind velocity (A100R, Campbell Scientific, Shepshed, UK) were monitored 10 m above ground on top of a scaffolding. On the other hand, soil temperature (107 Temperature Probe, Campbell Scientific, Shepshed, UK) and soil water potential (EQ3 Equitensiometer, Liu, Dachau, Germany) were monitored in 10 cm soil depth in the rooting zone of the tree used for respiration measurements. Stem temperatures were measured using two 1 mm type-T thermocouples inserted into the cambial zone. Measuring intervals for all sensors were 30 min. Daily mean values were calculated by averaging all daily measurements (48 values/day), and daily precipitation was recorded at a meteorological station on top of Mt. Patscherkofel (2246 m a.s.l.).

#### *Dendrometer and sap flow measurements*

Point dendrometers and sap flow sensors for measurements of radial stem increment and sap flow density ( $F$ ), respectively, were installed at the north-facing side of the tree that was used for  $E_S$  measurements and two neighbouring trees.

The dendrometers consisting of an electronic displacement sensor (linear motion potentiometer, MM10 Megatron, Putzbrunn Munich, Germany) mounted on a stainless steel rod were anchored at 1.5 m height, whereby the dead outermost layers (periderm) of the bark were slightly removed to reduce the influence of hygroscopic swelling and shrinkage of the bark on dendrometer traces and to ensure close contact with the stem (cf. Zweifel and Häslér 2001).

In 2007, sap flow density ( $F$ ) was monitored between mid-April and mid-August 2007, using custom-made flow gauges (Granier 1985, 1987) that were installed 10 cm above the dendrometers. The sensors were inserted 15 cm vertically apart into the hydroactive xylem (sapwood) and were shielded by a thermally isolating styrofoam cover. The upper probe was supplied with a constant heat of 140 mW, and the temperature difference between the heated upper and the unheated lower reference probe was used for estimating the sap flow density according to Granier (1987).

#### *Stem CO<sub>2</sub> efflux measurements*

For monitoring  $E_S$ , a stem section of 240 cm<sup>2</sup>, on the north-facing side, 1.3 m above ground of an adult tree of about 10 m height, was enclosed in a custom made clear curved Perspex chamber. The chamber was sealed to the

stem with putty (Terostat, Teroson, Ludwigsburg, Germany) and nonhardening insulating foam to ensure a gas-tight seal between the chamber and the bark.

During measurements, ambient air was continuously sucked through the chamber at a flow rate of 2 l min<sup>-1</sup>. The CO<sub>2</sub> concentrations of chamber air stream and reference air sampled 10 m above ground were measured alternately using an infrared gas analyser (Li 6262, Licor, Lincoln, NE). The corresponding flow rates were monitored and adjusted with an electronic mass flow controller (LD 20G, Walz Effeltrich, Germany). Each air stream was sampled for 60 s, whereas data were taken during the last 10 s of the interval to provide a complete flushing of the system (Wieser and Bahn 2004).

As  $E_S$  is strongly influenced by woody tissue temperature (cf. Lavigne 1987, Atkin and Tjoelker 2003, Larcher 2003), for some analyses the measured  $E_S$  was adjusted to  $E_S$  at 10 °C ( $E_{S10}$ ), using an adapted equation introduced by Lavigne et al. (2004) for the temperature normalization of  $R_s$ :

$$E_{S10} = E_S / Q_{10}^{(T-10)/10}$$

The temperature coefficient of respiration,  $Q_{10}$ , was calculated using the equation:

$$Q_{10} = 10^{[(\text{regression slope}) \times 10]}$$

The regression slope was taken from linear regression of log<sub>10</sub> of  $E_S$  versus stem temperature (Atkin et al. 2002). The  $Q_{10}$  was calculated at 2-week intervals, using half-hour mean values. Five-day mean values of  $E_{S10}$  (2 days before to 2 days after tissue sampling) were used for calculations and in figures to compensate short-term variations.

The  $E_S$  measurements and the tissue sampling started in early June 2006 and ended mid-August and mid-October 2007, respectively. After cell development was finished in October 2006, tissue sampling was interrupted until the end of March 2007, while respiration measurements were continued.

All the environmental, gas exchange, dendrometer and sap flow data were transmitted to a AM416 multiplexer (Campbell Scientific, Shepshed, UK) and recorded with a Campbell CR10X data logger (Campbell Scientific, Shepshed, UK) programmed to record 30-min mean values of measurements taken every minute.

## **Results**

### *Climate parameters*

Mean average air temperature recorded during the study was 6.4 °C and daily mean values varied between 19.1 °C during the growing season (20 July 2006) and -14.3 °C during the winter (25 January 2007) (Figure 1). The daily mean stem temperature varied between 17.4 °C (20 July 2007) and -11.2 °C (27 January 2007). The lowest daily mean soil temperature recorded was -2.6 °C (4 February 2007)

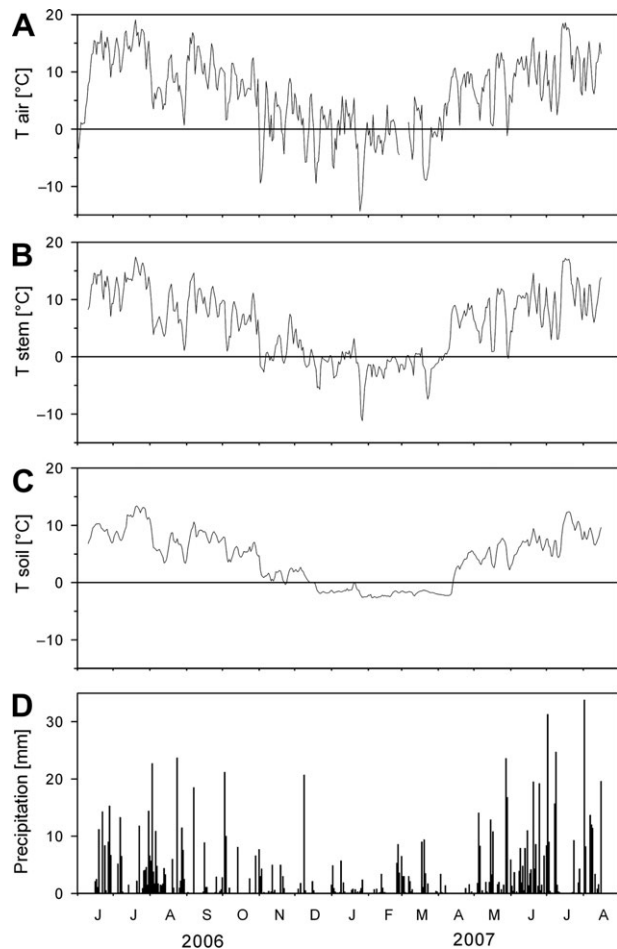


Figure 1. (A) Daily mean air temperature, (B) daily mean stem temperature in the cambial zone, (C) daily mean soil temperature at 10 cm depth and (D) daily cumulative precipitation, during the measuring period from June 2006 to August 2007.

and the highest was 13.4 °C (20 July 2006). Due to frequent precipitation in both growth periods (Figure 1), soil water potential always stayed above  $-0.08$  MPa, indicating that the trees did not experience drought stress from the soil side. From the beginning of November 2006 to the end of March 2007 precipitation primarily fell as snow. The years 2006 and 2007 showed quite different climatic conditions. Climate in 2006 was characterized by a prolonged late frost event occurring between 30 May and 8 June (minimum air temperature at timberline fell to  $-4.9$  °C), a warm June and July with mean air temperature exceeding the long-term mean (LTM) in July by 3.8 °C, followed by a sharp temperature drop in August (mean air temperature was 3.2 °C below LTM). In 2007, the growing period was extended due to the occurrence of exceptionally mild temperatures in spring (March to May, 3.4 °C above LTM).

#### *Dynamics of cambial activity and cell development*

Due to the differences in climatic conditions, dynamics of tracheid development were different during growing

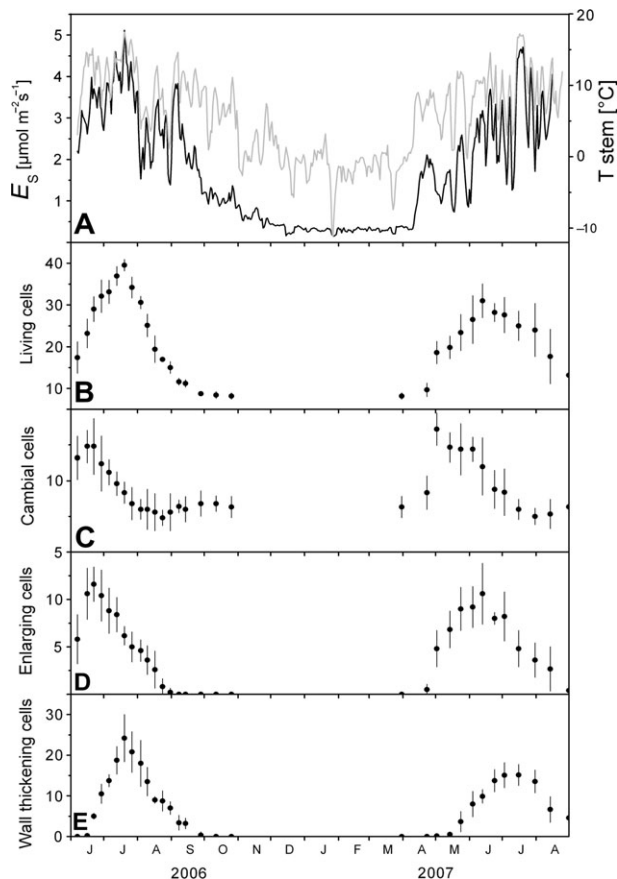


Figure 2. (A) Daily mean  $E_s$  (black line), daily mean stem temperature (grey line), (B) number of living cells, (C) cells in the cambial zone, (D) cells in radial enlargement and (E) cells in secondary wall thickening, from June 2006 to August 2007. Bars represent standard deviations.

seasons 2006 and 2007 (Figure 2) (Gruber et al. 2008). When measurements started in June 2006, the cell division had already started and the number of cambial cells reached their maximum at the end of June. Then cambial division declined and terminated at the end of July. The dormant cambium consisted of 7–8 cells. Cambial division already started at the end of April 2007, and in early May the number of cambium cells increased to about 13, and stayed on a high level until mid-June. Cambial division again terminated at about the end of July.

The dynamics of cell differentiation (enlarging and wall thickening cells) was characterized by delayed bell-shaped curves. In June 2006, cell enlarging had just started, as the first samples were taken. In 2007, in some individuals radial enlargement commenced in late April. Maximum number of cells in enlarging phase (average about 12 cells) was reached in mid-June and some enlarging cells were detected until the end of August in 2006 and 2007. In 2006 and 2007, first tracheids were undergoing wall thickening around 16 June and 2 May, respectively. Wall thickening and lignification was completed by the end of September 2006 (Figure 2E) and mid-October 2007 (data not shown).

### Dynamics of $E_S$

Changes of  $E_S$  generally followed seasonal trends in stem temperature.  $E_S$  was highest during the growing season and was reduced to the level of maintenance respiration in winter (Figure 2A). Stem temperature accounted for 68% of the variation in  $E_S$  during the growing season of 2006 and 2007 and 46% during quiescence (end of October to mid-April) (Figure 3). The temperature coefficient of respiration ( $Q_{10}$ ) varied between 2 and 2.25 during the growing seasons 2006 and 2007. During quiescence (end of November 2006 to mid-April 2007)  $Q_{10}$  decreased to about 1.81.

During the growing season,  $E_S$  at nighttimes (between 22.00 and 05.00 h) was significantly higher than that during the daytime (between 09.00 and 16.00 h) ( $P < 0.001$ ) (Figure 4). Average daytime  $E_S$  was reduced by 18% and 31% compared to nighttime values, at 10 and 13 °C, respectively.  $Q_{10}$  during the growing season was 2.37 and 2.17 at nighttimes and daytimes, respectively.

The  $E_{S10}$  was significantly correlated to the number of living cells (cambium, enlarging and wall thickening cells) throughout the whole measuring period and the number of living cells accounted for 57% of the variation in  $E_{S10}$  (Figure 5A). A detailed analysis, however, showed that the influence of the number of living cells on  $E_{S10}$  was higher during spring and early summer 2007 ( $r^2 = 0.902$ ;  $P < 0.001$ ) (Figure 5C), except for a short period at the end of the dormant season. On the other hand, from June to the end of October, the number of living cells only accounted for 52% ( $P < 0.001$ ) of  $E_{S10}$ , due to high  $E_{S10}$  value in August and the beginning of September 2006 (Figure 5B).

### Dendrometer and sap flow measurements

Stem increment measured by dendrometers was significantly correlated ( $r^2 = 0.920$ ;  $P < 0.001$ ) with the number of current year tracheids (number of enlarging, wall thick-

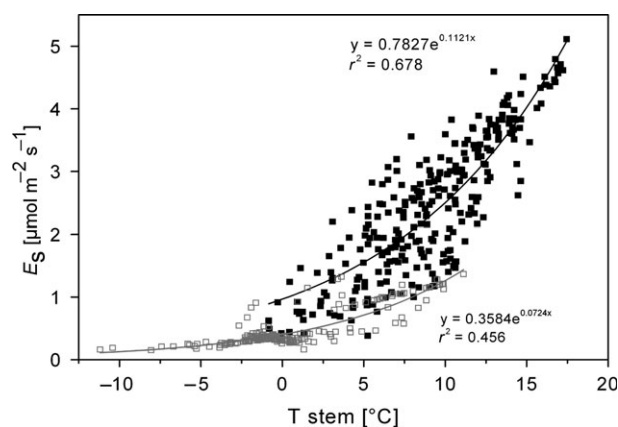


Figure 3. Relationship between daily mean  $E_S$  and daily mean stem temperature in the cambial zone during the growing seasons 2006 and 2007 (solid black symbols, black line,  $r^2 = 0.681$ ;  $P < 0.001$ ) and during dormancy (open grey symbols, grey line,  $r^2 = 0.456$ ;  $P < 0.001$ ).

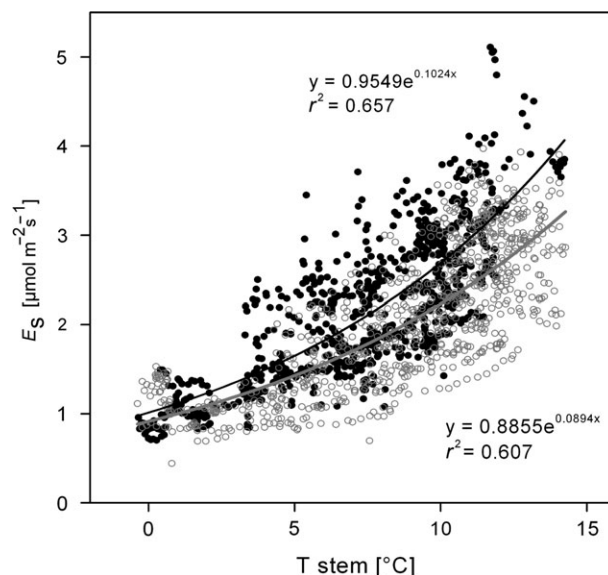


Figure 4. Relationship between half-hour mean values of  $E_S$  and stem temperatures in the cambial zone during daytime (open grey symbols, grey line,  $r^2 = 0.607$ ;  $P < 0.001$ ) and nighttime (solid black symbols, black line,  $r^2 = 0.657$ ;  $P < 0.001$ ) in May and June 2007.

ening and mature cells) in 2007 (Figure 6A). The number of enlarging cells was significantly correlated with daily change in stem increment calculated on the basis of the modelled Gompertz functions ( $r^2 = 0.960$ ;  $P < 0.001$ ) (Figure 6B). No correlation between  $E_S$  and daily increment could be detected.

Daily mean sapflow density was not significantly correlated to daily mean of  $E_{S10}$  ( $r^2 = 0.028$ ;  $P = 0.068$ ) during the growing season 2007 (Figure 7).

### Discussion

In the recent study, the stem temperature was the most significant factor influencing  $E_S$  and accounted for 68% of variations of  $E_S$  in *P. cembra* (Figure 4). This is in line with Wieser and Bahn (2004) and Maier et al. (1998) who calculated a temperature sensitivity of  $E_S$  of 71% for *P. cembra* and 61% for *Pinus taeda* L., respectively.

It has been reported that, due to diffusion resistance by cambium and bark,  $E_S$  can exhibit a lagged response to stem temperature (Ryan et al. 1995, Lavigne et al. 1996, Lavigne and Ryan 1997). This effect is usually corrected by using prior sapwood temperatures, at which the appropriate lag time is selected from the best fit to a nonlinear regression. For *P. cembra* no better fit was detected using sapwood temperatures of up to 5 h before  $E_S$  measurements (data not shown), but according to Ryan et al. (1995) the lag between  $E_S$  and stem temperature can vary between 0 and 5 h among coniferous species.

There is evidence that CO<sub>2</sub> produced by respiration, instead of being released directly through the bark into

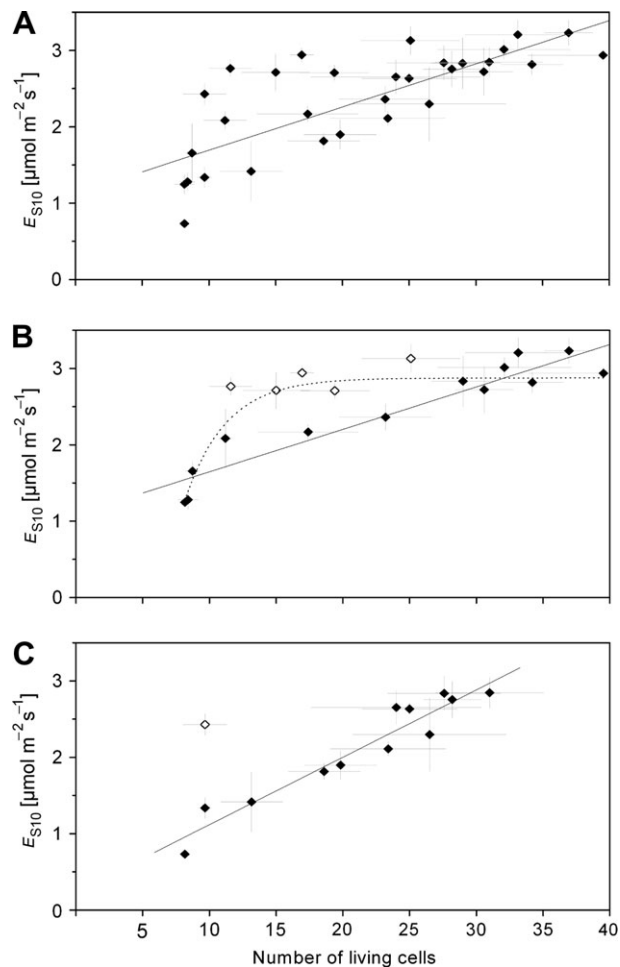


Figure 5. (A) Relationship between mean living cell number and mean  $E_{S10}$  during the growing season from June 2006 to mid-August 2007 ( $r^2 = 0.574$ ;  $P < 0.001$ ). (B) Relationship between mean living cell number and mean  $E_{S10}$  from end of June to mid-October 2006 (dotted line). Open symbols mark elevated  $E_{S10}$  values during August and early September. Solid line indicates linear correlation ignoring elevated values in August and early September ( $r^2 = 0.895$ ;  $P < 0.001$ ). (C) Relationship between mean number of living cells and mean  $E_{S10}$  from April to mid-August 2007 ( $r^2 = 0.902$ ;  $P < 0.001$ ). Open symbol marks the period of elevated  $E_{S10}$  values at the end of dormancy in April.

air or soil, may be transported by the transpiration stream. The  $\text{CO}_2$  produced by stem cells or derived from root or soil microbial respiration might be carried upward into foliage, branches and stem and be released there (Sprugel et al. 1995, Teskey and McGuire 2002, Maier and Clinton 2006, McGuire et al. 2007, Saveyn et al. 2008, Teskey et al. 2008). But the relationship between sap flow density ( $F$ ) and  $E_S$  substantially differs interspecifically (Teskey et al. 2008) and even among trees and during season (Bowman et al. 2005, Maier and Clinton 2006). Martin et al. (1994) found a weak negative correlation in *P. taeda* seedlings, and in a study Maier and Clinton (2006) suggested  $E_S$  in adult *P. taeda* to be reduced by up to 40% at maximum  $F$ . Under

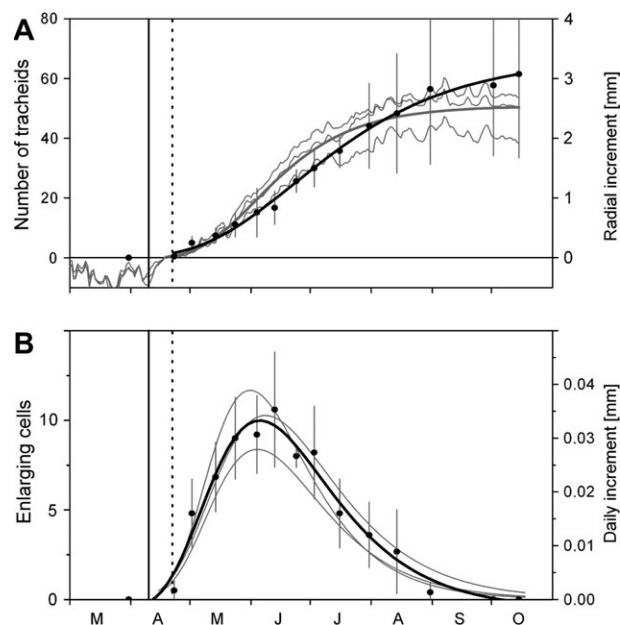


Figure 6. (A) Dynamics of tracheid development (solid symbols) and radial increment (grey lines) during the growing season 2007. Gompertz functions calculated for mean stem increment (bold grey line) and number of tracheids (bold black line). Vertical solid and dotted lines mark cambial reactivation derived from respiration measurements and date of first monitored cell division, respectively. Radial stem increments were set to zero when first dividing cells were determined. (B) Number of enlarging cells (solid symbols) and daily increments calculated on the basis of modelled Gompertz functions (grey lines). Dynamics of number of enlarging cells was modelled by Gauss function (bold black line). Vertical lines as in A.

field conditions it is difficult to separate the effect of  $\text{CO}_2$  transport by transpiration stream from other effects influencing  $E_S$ , all the more as both  $F$  and  $E_S$  are highly influenced by temperature. In our study, daily mean  $F$  and daily mean values of  $E_{S10}$  were not significantly correlated in 2007. On the other hand, during the same period,  $E_S$  at a given temperature was significantly higher at night-times (between 22.00 and 05.00 h) than during the daytimes (between 09.00 and 16.00 h) (Figure 4), which is assumed to be a function of  $F$ , as sap flow is significantly lower at night (Maier and Clinton 2006, Teskey et al. 2008). This indicates that  $\text{CO}_2$  transport by transpiration stream might nevertheless influence  $E_S$  in *P. cembra*. Lavigne et al. (1996) reported that in *A. balsamea* nighttime  $E_S$  was not higher than daytime  $E_S$  when lagged temperatures were used for correlations. This could not be confirmed for *P. cembra* (data not shown).

The present study indicates that apart from other factors, the number of living xylem cells and cambium cells (living cells) is a paramount factor influencing  $E_S$  (Havranek 1981, 1985, Ryan et al. 1994). Ryan (1990) reported that in *Pinus contorta* Dougl. ex Loud. and *Pinus engelmannii* Carr., living cells in phloem only accounted for 7% of

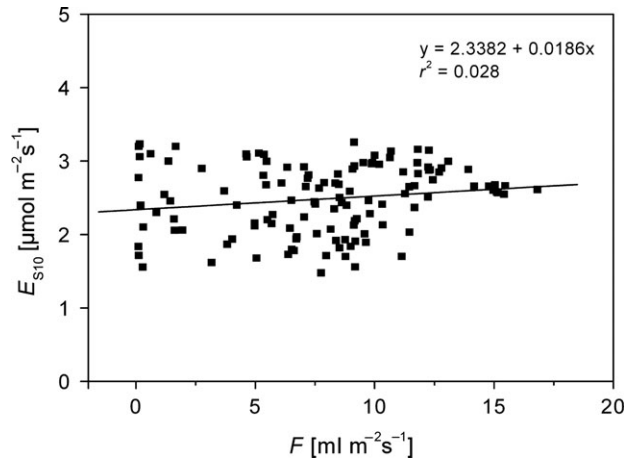


Figure 7. Relationship between the daily mean values of  $E_{S10}$  and  $F$  during the growing season 2007 ( $r^2 = 0.028$ ;  $P = 0.068$ ).

whole stem living cells and did not account for significant variations in  $E_S$  during the growing season, which also applies for *P. cembra*. A relationship between stem growth and  $E_S$  was reported previously (Ryan 1990, Maier 2001, Vose and Ryan 2002, Lavigne et al. 2004). However, we found besides growth processes there are periods when changes in cambial activity substantially influence  $E_S$ .

On 10 April, about 4 days after permanent unfreezing of the stem,  $E_S$  rapidly increased (Figure 2A), which we attribute to the onset of cambial reactivation. As soil and root zone was frozen until 14 April it is unlikely that the rise in  $E_S$  was caused by the transport of CO<sub>2</sub> in xylem sap from the rootzone as has been described by Teskey and McGuire (2007) and Teskey et al. (2008). We assume that the high metabolic activity due to changes in ultrastructure and cell biochemistry during cambial reactivation causes an increase in  $E_S$  before the onset of cell division (Riding and Little 1984, Catesson 1994, Little and Pharis 1995). Lavigne et al. (2004) proposed this rise of  $E_S$ , 2–3 weeks before the onset of cell division, as a nondestructive marker to determine the beginning of rapid xylem production.

Ignoring the elevated  $E_{S10}$  from August to early September, the correlation between the number of living cells and the  $E_{S10}$  throughout the growing season 2006 was highly significant ( $r^2 = 0.895$ ;  $P < 0.001$ ) (Figure 5B). The period of elevated  $E_{S10}$  started just as cambial division ceased at the beginning of August and the number of living cells declined rapidly, but the  $E_{S10}$  values remained at a high level. The cessation of mitosis is known to initiate the transition from active phase to rest in cambial cells (Riding and Little 1984, Catesson 1994, Rensing and Samuels 2004). Therefore, we suggest that elevated  $E_{S10}$  recorded after cessation of cell division can be attributed to elevated metabolic activity due to changes in cell ultrastructure which characterize transition to rest.

The rise of  $E_S$  at the end of dormancy in spring 2007 also marked the onset of stem diameter increase in dendrometer

measurements (Figure 6), triggered by cambial swelling due to rehydration during reactivation (Deslauriers et al. 2003b, Lavigne et al. 2004). The identification of cambial reactivation is crucial in dendrometer measurements especially at higher altitude, where frost shrinkage and thaw expansions cause high variations during the dormant period (Zweifel and Häslér 2000, Deslauriers et al. 2007). Mäkinen et al. (2003, 2008) found no correlation between cell development and dendrometer measurements, whereas Zweifel et al. (2006) state that both methods are able to detect the course of intra-annual radial growth. In our study, the radial increment measured by dendrometer and microcoring method correlated significantly ( $r^2 = 0.920$ ;  $P < 0.001$ ), even though typical fluctuations in stem radius due to changes in tree water status occurred (Zweifel et al. 2000, 2005). Only the number of enlarging cells was significantly correlated to daily increment ( $r^2 = 0.960$ ;  $P < 0.001$ ), which indicates that daily stem increment is mainly caused by cell enlarging, whereas wall thickening (Deslauriers et al. 2003b) and cambial cell division are of little account for daily increment. The fact that stem increment is not determined by the number of living cells but primarily by number of enlarging cells explains the missing correlation between  $E_S$  and daily stem increment.

We conclude that in *P. cembra*, besides temperature, the number of cambial and living xylem cells influence  $E_S$ . Variations in  $E_S$  due to changing numbers of living cells and considerably elevated  $E_S$  during transition stages in the cambial activity-dormancy cycle must be taken into account in process-based models of forest carbon cycles. Hence, our results imply that short time measurements of  $E_S$  are problematic for the calculation of forest carbon budgets.

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