INFLUENCE OF THE AIR TEMPERATURE ON THE KINETICS OF TRACHEID FORMATION IN NORWAY SPRUCE (*PICEA ABIES* [L.] KARST)

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SUMMARY

The kinetics of xylem formation of two-year-old plants of *Picea abies* [L.] Karst exposed to different air temperatures were studied in a model system. We simulated a continuous decrease of the air temperature from 26 to 2 °C (experiment 1) and an abrupt decrease of the air temperature from 10 to 5 °C (experiment 2). The kinetics of cell development were registered by high resolution laser measurements in combination with microscopical methods (accuracy: ±2 µm, spatial resolution of 12.1 to 38.43 µm, temporal resolution ≤ 1 s). In experiment 1 the offset of cambial cell divisions was induced at higher temperature (<8°C) than the offset of the subsequent steps of cell development (cell expansion (<6 °C), secondary wall formation (<5 °C), and lignification (<2 °C)). The abrupt decrease of the temperature from 10 to 5 °C (experiment 2) interrupted cell expansion, but secondary wall formation and lignification kept unaffected. From the results it is concluded that to a certain level complete tracheid formation is guaranteed at the end of the vegetation period by the differing critical temperatures for cambial cell divisions, the formation of the secondary cell wall, and the lignification. However, a fast and abrupt decrease of the temperature can interrupt developmental steps of xylem formation and can cause incomplete tracheid formation.

Key words: Cambial activity, xylem cell development, laser measurements, climate change.

INTRODUCTION

During recent years, significant changes in global climate were observed. Climate models predict an increase of extreme weather conditions in Central Europe during the next decades (Intergovernmental panel on climate change, www.ipcc.ch). In particular, climatologists assume that the number of periods of unusual low and high precipitation and temperatures will increase.

The temperature of the air is a major exogenous factor influencing wood formation in trees (Vysotskaya & Vaganov 1989; Dodd & Fox 1990). Dendroecological investigations showed a significant influence of the temperature on the rate of cambial cell divisions (Fritts 1976). Biochemical and physiological processes involved in cell expansion, the formation of the secondary cell wall, and the lignification of the cell wall are also correlated with temperature. Recent microscopical investigations of Gricar et al. (2005, 2006) elucidated the impact of an abrupt decrease of the air temperature on the structure of the cell wall of tracheids in *Abies alba* and *Picea abies*. Consequently, it is expected that changing temperature conditions related to

global climate change will have a strong impact on wood formation in trees. However, due to methodological limitations information on the development of xylem cells *in situ* under different temperature conditions is rare. In a recent methodical approach we were able to date radial cell expansion of cambium derivative cells *in situ* in a high temporal resolution using high resolution laser measurements in combination with anatomical studies (Dünisch et al. 2003, 2006). In the subsequent study we applied this method to investigate the kinetics of tracheid formation in Norway spruce (*Picea abies* [L.] Karst.), the most frequent forest tree in Central Europe. In growth chamber experiments we simulated (1) a continuous decrease of the air temperature from 26 to 2 °C and (2) an abrupt decrease of the air temperature from 10 to 5 °C.

MATERIAL AND METHODS

Plant cultivation and growth conditions

696 experimental plants were obtained from seeds from two proveniences located in the Harz mountains, Germany (seed provenineces 840-10, 840-13). The seedlings were cultivated after a two month breeding period (“Knop” nutrient solution, sand culture), in a standard soil substrate (Dünisch et al. 2006). During the first year of growth the plants were cultivated in the greenhouse (frost free, natural light; Fig. 1). In the second year of growth the plants were transferred to growth chambers one week before starting the experiments and cultivated at 26 °C and 16/8 hours day/night cycle (relative humidity of air: 60 %). The plants were watered daily. In order to study the influence of the air temperature on the formation of tracheids two experiments were carried out.

Fig. 1: Cultivation of the experimental plants (*Picea abies* [L.] Karst.) in the greenhouse.

Experiment 1: Simulating the decrease of the air temperature from summer to the end of the vegetation period in October, we decreased the air temperature in the growth chamber stepwise (one week intervals) from 26 to 2 °C during a period of 14 weeks (selected temperatures: 26, 22, 18, 14, 10, 9, 8, 7, 6, 5, 4, 3, 2 °C; Fig. 2). The experiment started at June 12, 2002.

Experiment 2: The second experiment was carried out in order to simulate an abrupt decrease of the air temperature at the end of the growing season. After the stepwise (one week intervals) decrease of the air temperature from 26 to 10 °C (according to experiment 1), we decreased the air temperature in the growth chamber from 10 to 5 °C (regulation of the air temperature in the chamber lasted approximately 30 minutes). 50 % of the plants served as controls and remained in a growth chamber with an air temperature of 10 °C. The experiment also started at June 12, 2002 (Fig. 3).

Determination of the kinetics of xylem cell development

The kinetics of xylem cell development were studied according to Dünisch et al. (2003) and Dünisch & Rühmann (2005) by high resolution laser measurements in combination with micro-

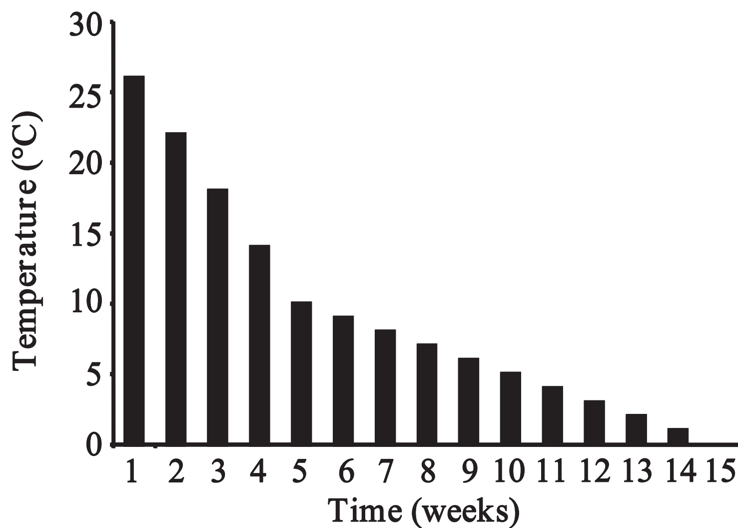

Fig. 2: Air temperature (°C) in the growth chamber (experiment 1; beginning June 12, 2002).

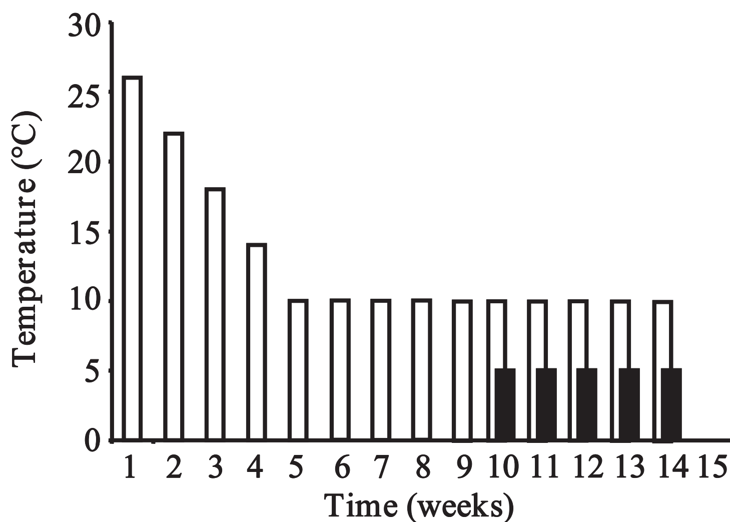

Fig. 3: Air temperature (°C) in the growth chamber (experiment 2; beginning June 12, 2002).

scopical observations. Light microscopical observations revealed no periderm formation, dilatation of rays or a collapse of sieve tubes in the phloem (Dünisch & Bauch 1994; Dünisch et al. 2003). It was therefore assumed that the increase in shoot radius detected by the measuring device was due to the formation and expansion of cambium derivatives.

Three laserscanners (MEL laserscanner M2D, MEL GmbH, Eching, Germany) were installed at the same height at an angle of 120° to each other around the shoot. Each scanner measured the distance between the scanner head and the shoot surface of a shoot segment of 120° with a spatial resolution of each laser line of approximately 1° (~150 laser lines per scanner, ~120 lines for a shoot segment of 120°, ~30 lines overlapping between two scanner heads). The corresponding optical resolution of the laser measurements along the shoot circumference was 12.1 to 38.4 µm. From the simultaneous measurement of 3 shoot segments, the profile of the shoot circumference/cross section was calculated and visualized by the MEL software package. The measuring device allows the quantification of the cross-sectional area of the shoot within a circle area of 4.52 m² as a maximum without movements of the shoot affecting the accuracy of the measurements. The data and the cross-sectional profiles were stored in a computer (i-Control, MEL GmbH) at 1 to 60 s intervals. During each measuring interval, the laser light was switched on for 20 ms only in order to avoid an impact of the laser light on the cambial activity of the plants.

The radial diameter of the phloem and xylem cells of the shoot portion analysed by the laser measurements were quantified in order to explain the significance of the increment curves of each laser line in terms of cell production and radial expansion. After harvest the samples were fixed in a FAA solution. The relevant shoot portions were embedded in polyethylene glycol (PEG 1500) with increasing concentration (PEG 1500:H_2O, 1:2, 1:1, 2:1, 1:0, 1:0). For identification and the histometrical analyses of the phloem and xylem cells, transverse sections (5 µm) were prepared from the shoot portions analysed by the laser measurements. For identification of the phases of primary wall formation, secondary wall formation, and lignification, the sections were stained with safranine (1%).

For the comparison of the laser measurements and the histometrical data the microphotographs of the sections and the shoot profiles obtained from the scanning unit were overlapped and adjusted. The histometrically determined radial diameters of the xylem and phloem cells were compared with the radius increment obtained from the laser measurements during a distinct growth period along the radial transects through the tissue using the technique of Dünisch & Bauch (1994) and Dünisch et al. (2003). Maximum conformity between the two data sets was correlated with the program SYSTAT by means of Boolean algebra. If one laser line did not fit with a radial cell row (overlap in the tangential direction < 80 %) or if during a distinct growth period the radius increment differed more than 2 µm from the radial diameter of the cell, it was not possible to time the enlargement of the individual cell.

The duration of radial cell expansion of cambium derivative cells was calculated as the histometrically determined radius increase of the derivative cells (compared to the cambium cells) per time period (obtained from the laser measurements). The period for the formation of secondary cell walls and their lignification was calculated as the time period between the date of sample collection and the date of the offset of radial cell expansion.

Measurements were carried out at three plants per treatment. The period of measurement was two days per plant.

Histometrical measurements

Transverse sections were prepared with a sliding microtome (Reichert, Austria; section thickness: 15 µm). The sections were stained with 1 % safranine. The sections were covered with coverslips. The cross sections were used to determine the radial diameter and the cell wall cross section area of the tracheids. The diameter and the cell wall cross section area were measured at microphotographs with the image analysing software package Analysis. For each treatment 10 cells with exactly dated phase of radial cell expansion were analysed.

Quantification of the lignin content of the cell wall

The lignification of the cell wall was studied by cellular UV-microspectrophotometry. Xylem samples (1 mm x 1 mm x 3 mm) were fixed for 12 hours in a 4 % solution of glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). After washing with the same buffer solution, samples were embedded after dehydration (acetone series) in Spurr´s resin (Spurr 1969). Semi-thin transverse sections (1 µm thick) were prepared by a diamond knife, placed on quartz slides and mounted in immersion oil with quartz coverslips. The UV-absorbance of the cell wall was quantified by a universal micro-spectralphotometer (UMSP 80, Carl Zeiss, Germany, software package Apamos) at a wavelength of 280 nm (Koch & Kleist 2001). Data presented for each treatment represent mean values of 10 tracheids.

Statistical analysis

The mean value and the standard deviation of each parameter were calculated. The significance of differences between species and anatomical parameters was assessed by ANOVA at $p \leq 0.05$ by *Fisher's F*-test.

RESULTS

Influence of a continuous decrease of air temperature from 26 to 2 °C on the kinetics of tracheid formation (Experiment 1)

Cambial cell divisions occurred at air temperatures between 26 and 8 °C (Fig. 4). The highest rate of cambial cell divisions was found at an air temperature of 22 °C. Between 18 and 8 °C the rate of cambial cell divisions was significantly correlated with the air temperature.

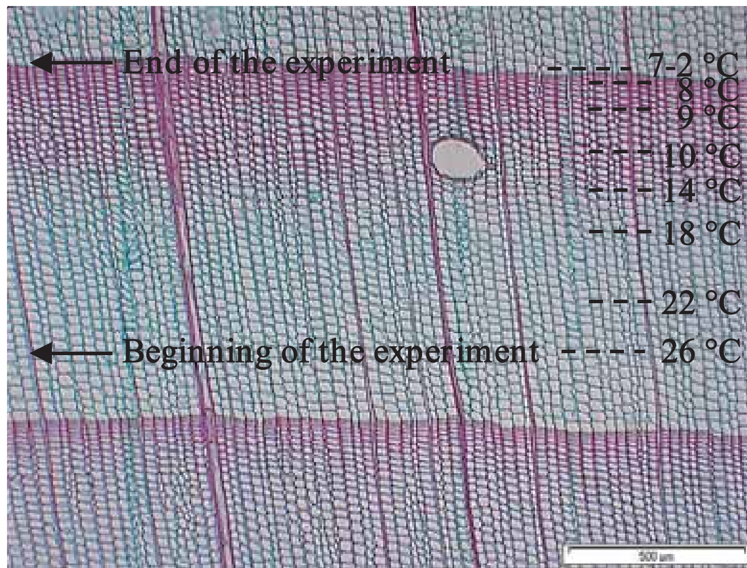

Fig. 4: Cross section of a tree ring formed in the year 2002 by a three-year-old plant (Picea abies [L.] Karst) of experiment 1. Sequence of xylem cell formation in relation to the air temperature (°C) in the growth chamber.

Radial expansion was induced in differentiating tracheids at temperatures higher than 6 °C (Fig. 5a). Radial cell expansion lasted 4.3 to 9.1 hours. The speed of radial cell expansion (Fig. 5a) as well as the radial diameter of the tracheids (Fig. 6a) were not correlated with the temperature of the air.

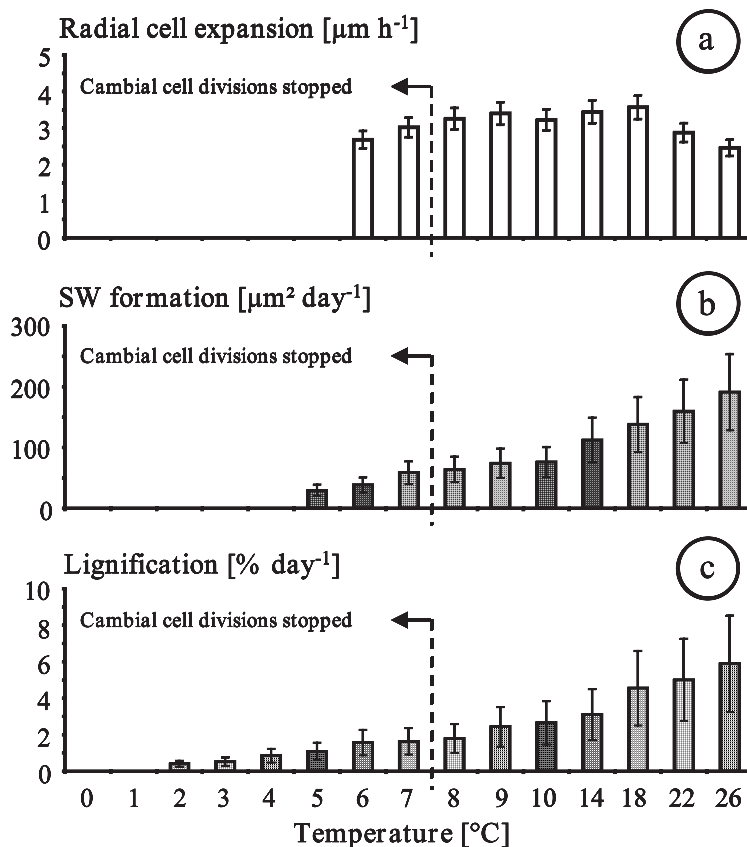

Fig. 5: Kinetics of –a: Radial cell expansion (µm h-1). –b: Formation of the secondary cell wall (µm² day-1), -c: Lignification of the cell wall (% (of total UV absorbance of mature cells) day-1) of tracheids in relation to the air temperature in the growth chamber (experiment 1). Mean values ± standard deviation .

Formation of secondary cell walls was observed in tracheids at temperatures higher than 5 °C (Fig. 5b). Microscopical observations showed that cambium derivative cells which entered the phase of cell expansion also formed complete secondary cell walls. The formation of the secondary cell wall lasted 1.9 to 6.1 days. The speed of secondary wall formation was significantly correlated with the air temperature (Fig. 5b). The cross section area of the cell walls slightly decreased at temperatures lower than 10 °C (Fig. 6b)

The lignification of the cell wall still occurred at very low temperatures, but the speed of lignification strongly decreased with decreasing air temperature (Fig. 5c). At a temperature of 26 °C the lignification of the cell wall completed after 16 days, while at a temperature of 2 °C the lignification of the cell wall lasted approximately 117 days. No correlation was found between the air temperature and the lignin content of the cell wall (Fig. 6c).

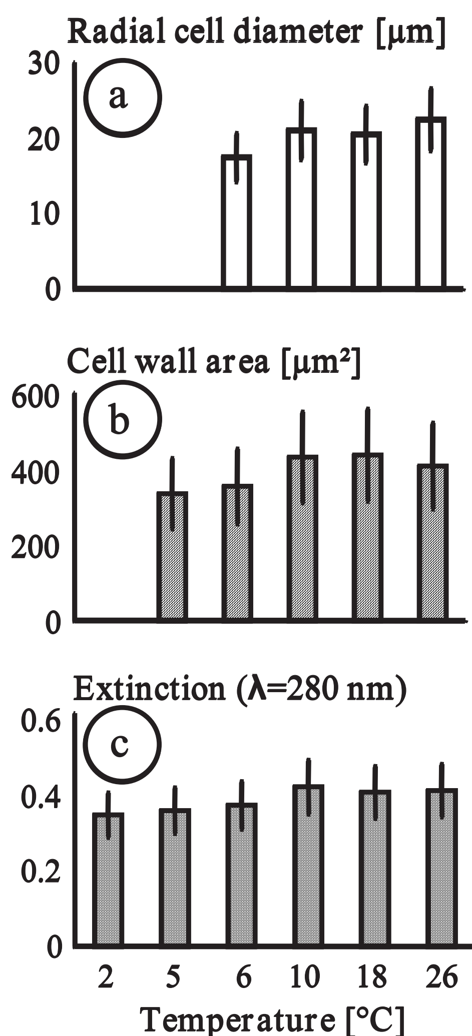

Fig. 6: a: Radial cell diameter (µm). –b: Cell wall cross section area (µm²), -c: UV absorbance (λ=280 nm) of the S2 layer of the cell wall (extinction) of tracheids in relation to the air temperature in the growth chamber (experiment 1). Mean values ± standard deviation.

Influence of an abrupt decrease of air temperature from 10 to 5 °C on the kinetics of tracheid formation (Experiment 2)

In agreement with the results obtained in experiment 1, after the decrease of the air temperature from 10 to 5 °C no further cambial cell divisions were induced. The abrupt decrease of the temperature interrupted ongoing radial expansion of tracheids during cell differentiation with a time delay of approximately 40 minutes (Fig. 7). Consequently, tracheids formed under this condition had a lower radial diameter compared to tracheids formed at a constant temperature of 10 °C (Fig. 8, Fi. 9a). The formation of the secondary cell walls also occurred at a temperature of 5 °C. Tracheids formed at 5 °C also formed complete secondary cell walls. No significant difference in cell wall area was found between tracheids formed at 10 °C and tracheids formed at a temperature of 5 °C (Fig. 9b). The lignin content of the cell walls formed at 5 °C was slightly reduced compared to the lignin content of cell walls formed at a constant temperature of 10 °C (Fig. 9c).

DISCUSSION

High resolution laser measurements in combination with microscopical studies offer a chance to study cambial activity and cell differentiation of cambium derivative cells in model systems. This methodical approach allowed three-dimensional studies especially on the kinetics of radial cell expansion. Classical methods to study cambial growth and cell differentiation like dendrometer measurements (Mitscherlich et al. 1966; Fritts 1976) and repeated sample collections for subsequent microscopical analyses of the tissue (Barnett 1992; Farrar and Evert 1997a, b) have the disadvantage of comparable low spatial and temporal resolution, respectively, while the recent technical development of high resolution laser systems gave the chance to reconstruct cell formation processes *in situ* on a cellular or tissue level in a high temporal resolution. The application of this method is restricted to young plants without secondary changes in the phloem (formation of periderm, dilatation of rays, collapse of sieve tubes; Esau 1970), which also influence the radial diameter of the shoot. Distinct differences in cell formation may occur between young plants studied in this model system and plants growing in the field, which have to be taken into consideration discussing the results. Simplified model systems have the advantage of controlled experimental conditions, but never reflect natural conditions exactly. In our experiments the temperature in the growth chamber was regulated in one week intervals of constant temperature, while other parameters kept constant. In nature, temperature will not follow this scheme exactly and intercorrelation of temperature and other factors should be considered in further studies. In particular, more drastic decreases in temperature with a stronger impact on tracheid formation at the end of the growing season are reported from field studies by Gricar et al. (2005, 2006).

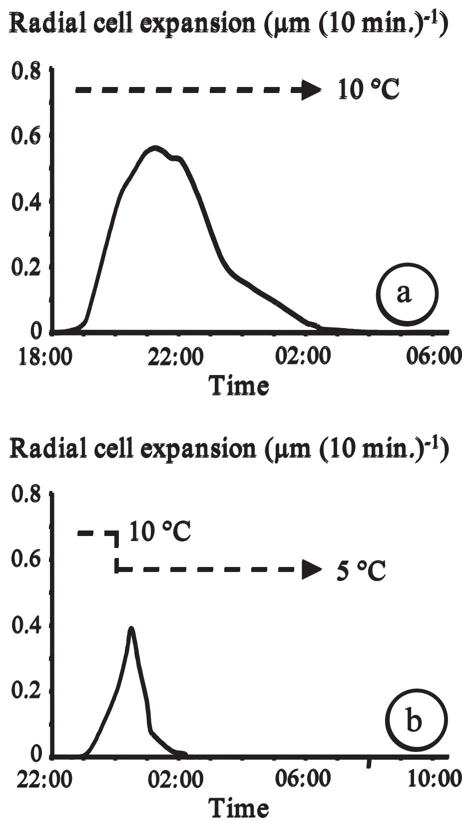

Fig. 7: Kinetics of radial cell expansion (µm (10 min.)-1) of two tracheids. –a: Cell formed under a constant air temperature of 10 °C, -b: Cell formed during the decrease of the air temperature from 10 to 5 °C (midnight).

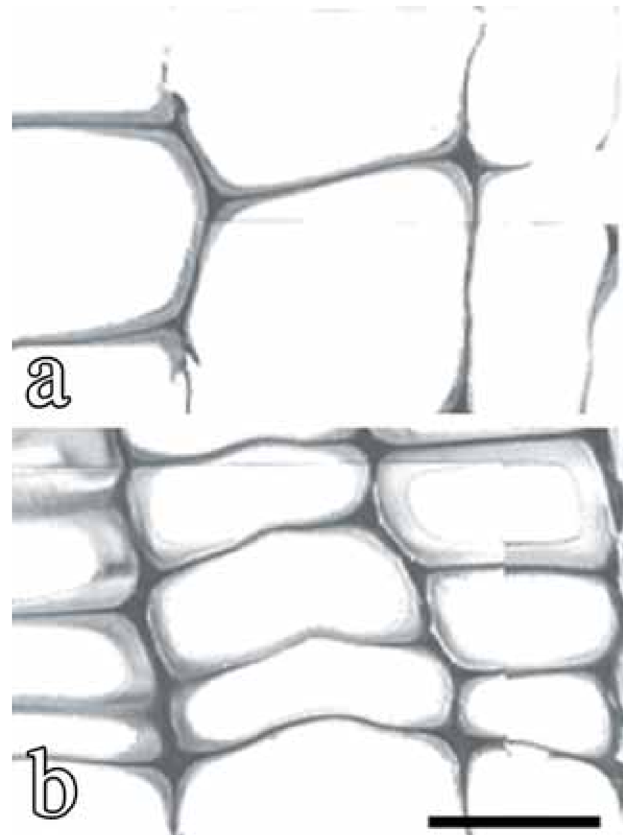

Fig. 8: Cross section of tracheids, UV microphotograph. –a: Cells formed under a constant air temperature of 10 °C, -b: Cells formed during the decrease of the air temperature from10 to 5 °C (midnight). Scale bar=10 µm.

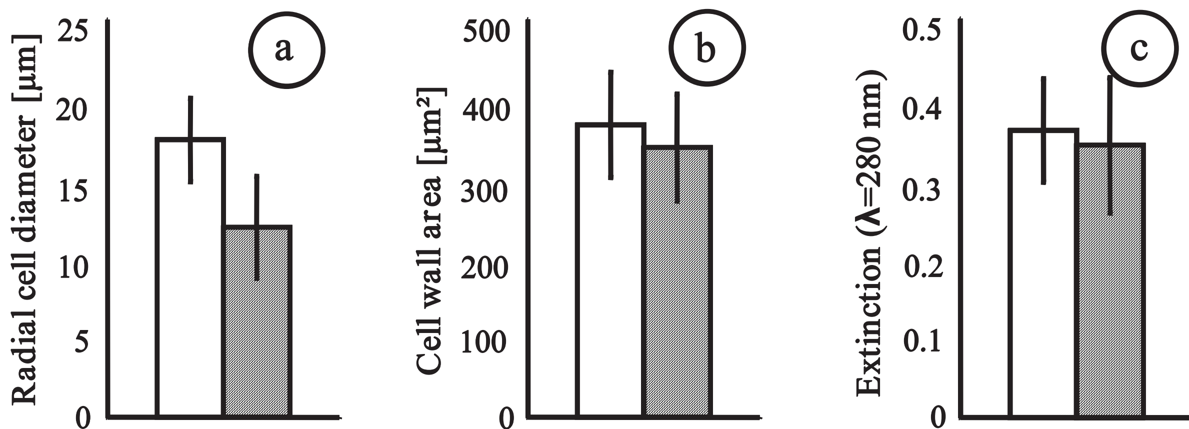

Fig. 9: a: Radial cell diameter (µm). –b: Cell wall cross section area (µm²), -c: UV absorbance (λ=280 nm) of the S2 layer of the cell wall (extinction) of tracheids formed under a constant air temperature of 10 °C (unfilled bars) and cells formed during the decrease of the air temperature from 10 to 5 °C (midnight, bars filled with criss-cross lines). Mean values ± standard deviation .

The offset of cambial cell divisions, the offset of cell expansion, the offset of secondary wall formation, and the offset of the lignification of the cell wall of tracheids were induced by low temperatures (Oribe & Kubo 1997; Oribe et al. 2001). The critical temperatures for the offset of these four developmental steps followed the sequence of xylem cell formation. The offset of cambial cell divisions (first step of xylem formation) was induced at a higher temperature (8°C) than the offset of the subsequent steps of cell development (cell expansion (6 °C), secondary wall formation (5 °C), and lignification (2 °C)). This guarantees the complete formation of xylem cells under "normal" temperature conditions at the end of the growing season. However, a

very strong decrease of the temperature from higher than 8 °C to temperatures lower than 6 °C, 5 °C, and 2 °C, respectively has a strong impact on cell expansion, secondary wall formation, and lignification, respectively. Especially, the decrease of the air temperature below 2 °C might cause severe damage of developing xylem cells and incomplete cell formation as it is reported from field studies by Gricar et al. (2006).

In agreement with other studies, the rate of cambial cell divisions as well as the speed of secondary wall formation and lignification were correlated with the air temperature. This is due to the strong relationship between the temperature and the synthesis of macromolecules (e.g. pectine, cellulose, hemicellulose, lignin; Denne & Dodd 1981). In contrast, the speed of cell expansion was not correlated with the air temperature. Several investigations showed that cell expansion predominantly depends more on the water supply and the turgor of the expanding cell (Verbelen & Vissenberg. 2007). At temperatures, which allowed complete cell development (>6 °C), the temperature had no significant influence on the final size and on the chemical composition of the tracheids. This indicates that developing cells do not need (or need to a very small portion) additional input for completing cell expansion, secondary wall formation, and lignification.

From the results it is concluded that under "normal" temperature conditions complete tracheid formation in Norway spruce is guaranteed at the end of the vegetation period by the differing critical temperatures for cambial cell divisions, the formation of the secondary cell wall, and the lignification. However, the study on the influence of an abrupt decrease of the temperature from 10 to 5 °C on cell expansion showed that a strong and fast decrease of the air temperature can interrupt developmental steps of xylem formation and can cause incomplete tracheid formation. With regard to global climate change, studies on the relationship between temperature and xylem cell formation are important for forest management and wood quality.

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