

**WALL STRUCTURE OF TERMINAL LATEWOOD TRACHEIDS OF
HEALTHY AND DECLINING SILVER FIR TREES IN
THE DINARIC REGION, SLOVENIA**

by

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SUMMARY

Fir trees (*Abies alba* Mill.) in a permanently monitored forest in the Dinaric region in Slovenia respond to crown damages by distinctly reducing their ring widths. According to transmission electron microscopy (TEM) and UV-microspectrophotometry (UMSP) of cambium-adjacent latewood tracheids of affected trees, the secondary wall formation and lignification were completed by the middle of October. In samples taken at the same date from healthy looking silver firs, the S₃ and the warty layer were not yet present in cambium-adjacent latewood tracheids. Additionally, their inner S₂ showed lower lignin deposition, whereas the compound middle lamella, S₁ and outer S₂ were distinctly lignified as revealed by TEM and UMSP. It is assumed that these youngest tracheids of healthy trees will later undergo lignification or remain less lignified. From these observations we conclude that the cambial activity at breast height ends later in healthy silver fir trees as compared to declining trees.

Key words: *Abies alba* Mill., latewood tracheids, cell wall, lignification, electron microscopy, UV-microspectrophotometry.

INTRODUCTION

Forest decline represents a severe problem in Europe as outlined in the yearly report on the forest condition prepared by the United Nations/Economic Commission for Europe and the European Commission (UNECE & EC 2000). For the southern parts of Central Europe, such as South Austria and Slovenia, this report gives data on a distinct defoliation of the main coniferous species: Scotch pine, Norway spruce, and silver fir. In contrast to pine and spruce, fir trees respond sensitively to crown damage by reducing wood formation; a close correlation between decline symptoms and narrow tree rings has been well documented for fir trees (e.g. Bauch et al. 1979; Eckstein et al. 1983; Schweingruber et al. 1983; Bauch et al. 1986; Torelli & Čufar 1986). However, since the late 1980's at some forest sites, e.g. in Slovenia (Levanic 1997), silver fir trees began

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to recover as evidenced by increasing needle densities and increasing wood formation. A low proportion of fir trees still appear affected, as observed in a permanently monitored forest stand at Ravnik in the Dinaric region in Slovenia (Torelli et al. 1999).

Duration and intensity of wood formation are influenced by various exogenous and endogenous factors (review: Savidge 1996). Besides exogenous factors like temperature and daylength, the tree's vigour affects wood formation. Several studies clearly showed that less vigorous trees had a reduced duration of wood formation with later onset and earlier cessation of cambial activity (e.g. Bauch et al. 1986). After entering cambium dormancy, the latest formed tracheids still undergo maturation, which is completed with the lignification of their innermost secondary wall layers and finally with protoplasmic autolysis (e.g. Wardrop 1965; Lewis et al. 1999; Terashima 2000). However, Gindl et al. (2000) considered that secondary wall lignification of terminal latewood tracheids of treeline spruce may be stopped at a low level because of early unfavourable cool conditions at the end of the vegetative period.

The present study focuses on the ultrastructure and lignification of the outermost latewood tracheids of healthy and declining silver firs in order to analyse whether the maturation of these tracheids is affected by tree vigour. Therefore, cambium-adjacent xylem portions from symptomless and declining silver fir trees were collected in the middle of October 1999 and investigated by means of microscopical and UV-microspectrophotometrical methods.

MATERIAL AND METHODS

Samples for light and electron microscopy as well as UV-microspectrophotometry were taken from mature silver fir trees (*Abies alba* Mill.) growing at Ravnik, Dinaric region (Fig. 1), elevation 500–700 m. The site is representative for silver fir/beech forests in

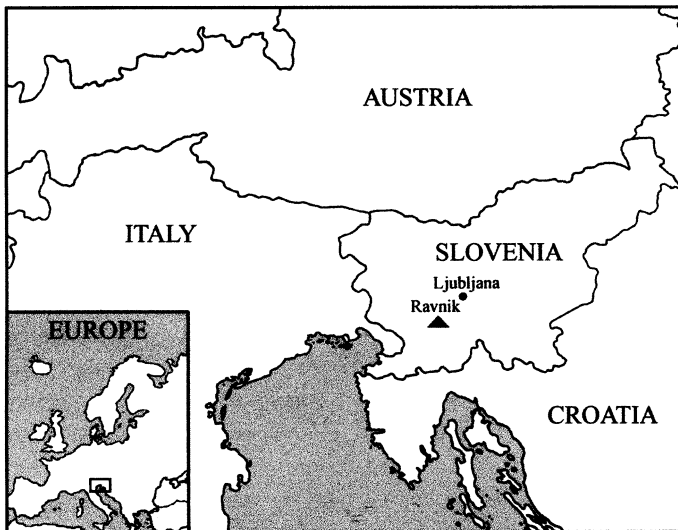


Fig. 1. Map showing the location of the experimental site at Ravnik in Slovenia.

Slovenia. The mean diameter of the stems at 1.3 m above ground was around 50 cm, the age of the experimental trees was between 150 and 180 years. Crown status index (CSI) based on progressive needle loss and necrosis was used to determine the vigour of the experimental trees (modified from Bosshard 1986). Briefly, a class 1 tree had a full, dense and green crown; a class 4 tree had a thin crown with some evidence of chlorosis, but no marked browning of the needles; a class 5 tree had distinct browning of the needles as well as a thin crown; a class 7 tree was severely diseased but retained some green needles. Class 1–4 fir trees were considered relatively healthy, but had progressively transparent crowns due to progressive needle chlorosis and shedding of older needles. Class 5–7 trees appeared distinctly diseased due to progressive needle discolouration, necrosis and increasing defoliation. Cambial electrical resistance (CER) was used to determine the cambial vitality which is significantly correlated with the number of cells produced by the cambium. CER was measured in thousands of ohms (kohm) during the first week of August 1988 and 1989. A conditionmeter (Bollmann Elektronik-Systeme, Germany) was fitted with a No. 2E Delmhorst moisture detector electrode with dual 54 mm stainless steel contact pins in a No. 552/A retainer, pin separation 11 mm. In a vertical alignment, electrode pins were driven at a stem height of 1.3 m above ground through the rhytidome, secondary phloem, cambial zone into the outer sapwood. CER was recorded following meter stabilization. The trees were rated during the growing seasons 1988 and 1989 and assigned to vitality and vigour categories as follows: the wood forming capacity or vitality of the trees was categorized by CER. High vitality means CER values ≤ 10.5 kohm (median CER of 385 sample trees), whereas low vitality means CER values > 10.5 kohm. The vigour of the same trees was categorized by CSI. High vigour means CSI = 1–4, low vigour CSI = 5–7. The trees were then classified according to combined ratings of vigour and vitality. Class A trees had high cambial vitality and high crown vigour and were considered healthy, whereas class C trees had low cambial vitality and low crown vigour and were considered unhealthy. The intermediate class B trees were subdivided into B1 with high vitality and low vigour and B2 with low vitality and high vigour (Torelli et al. 1999). For the present study 10 class A trees and 10 class C trees were selected for microscopy. Samples containing cambium and outer xylem (sample size: $2 \times 2 \times 4$ mm³) were taken at breast height from all trees on October 13, 1999, and prepared for microscopy and UV-microspectrophotometry as follows: fixation in a formaldehyde/acetic acid solution, dehydration in a graded series of ethanol, transfer into water-free acetone, and embedding in Spurr's ultra-low viscosity epoxy resin (Spurr 1969). For light microscopy, semi-thin sections of about 1 μ m thickness were prepared with an ultramicrotome and stained with a 1% toluidine blue solution. An Olympus BH-2 light microscope was used. Measurements of cell wall thicknesses (tangential walls from the middle lamella towards the cell lumen) were made of tracheids of the latest formed two cell layers adjacent to the cambium using a digital image analysis system (Olympus/SIS). Transmission electron microscopy (TEM) was carried out with sections of 80–100 nm thickness, which were mounted on copper grids, and stained with potassium permanganate according to Donaldson (1992). Examination was carried out with a Philips CM 12 TEM at accelerating voltages of 40 or 60 kV.

For UV-microspectrophotometry 1 μm thick unstained sections of the same embedded specimens were prepared, transferred to quartz microscope slides, immersed in a drop of non-UV absorbing glycerine, and covered with a quartz cover slip. Cell wall analysis was performed using a UV-microspectrophotometer (UMSP 80, Zeiss) equipped with a scanning stage which enables determining image profiles at constant wavelengths (e.g. 280 nm maximum absorbance for conifer lignin). The specimens were first conventionally investigated by point measurements with a spot size of 1 μm^2 using the programme LAMWIN® (Zeiss). The spectra were evaluated in a wavelength range from 240 to 340 nm. Additionally, specimens were scanned with a defined wavelength of 280 nm using the scan programme APAMOS® (Zeiss). The new improved scan programme digitizes rectangular fields of the tissue with a local geometrical resolution of 0.25 μm^2 and a photometrical resolution of 4096 grey scale levels which are converted in 14 basic colours to visualize the absorbance intensities (Koch & Kleist 2001).

RESULTS AND DISCUSSION

Transverse sections of the cambium and the adjacent latest formed tracheids in the stem of declining and healthy silver fir trees were examined and evaluated by light and electron microscopy. The mean width for the 1999 ring was 1.95 mm \pm 0.66 in class A trees and 0.71 mm \pm 0.34 in class C trees indicating a suppressed cambial activity in the declining trees (Fig. 2). Besides the differences in the ring width, light microscopy also revealed a distinct difference in the amount of latewood. Healthy looking class A



Fig. 2. Healthy looking class A (a) and declining class C (b) silver fir trees.

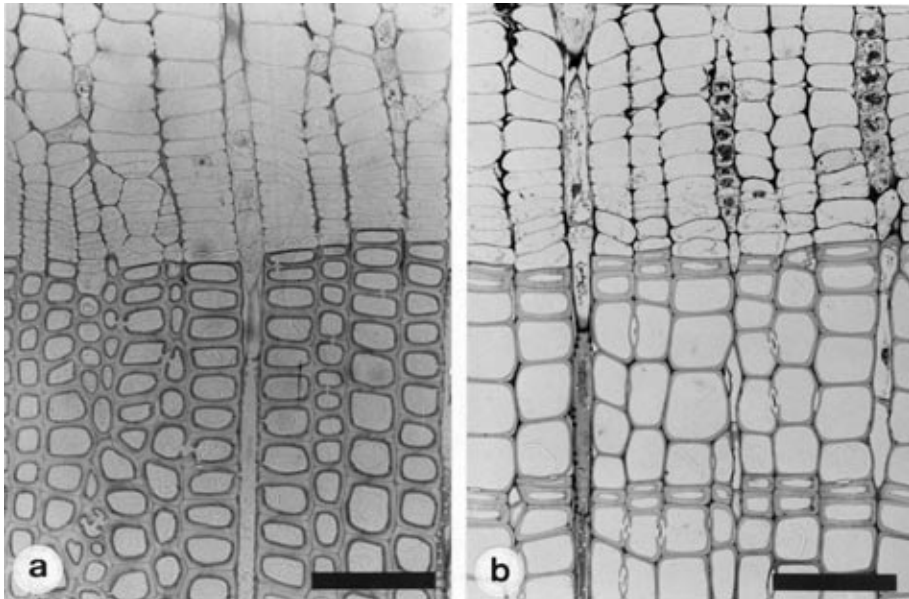


Fig. 3. Light micrographs of transverse sections of cambium-adjacent xylem. — a: Healthy looking class A fir tree with a broad latewood zone. — b: Declining class C tree with narrow tree rings and latewood zones consisting of only one or two rows of thick-walled cells. — Scale bars = $100\ \mu\text{m}$.

trees formed a broad latewood zone (Fig. 3a) with the typical gradual transition from earlywood to latewood, whereas the declining class C trees in most cases only developed one or two rows of thick-walled latewood cells (Fig. 3b). There is a tendency towards thinner cell walls of the latest formed tracheids in the healthy trees ($3.12\ \mu\text{m} \pm 1.19$) as compared to the declining trees ($3.56\ \mu\text{m} \pm 0.95$), although this difference was not significant (Fig. 4).

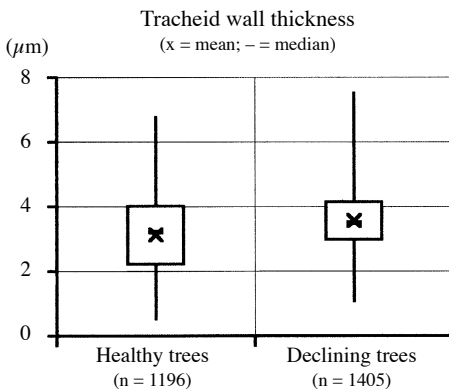


Fig. 4. Histometric measurements of tracheid wall thicknesses (from middle lamella towards lumen) of healthy and declining trees. Box and whisker plots show a tendency for thinner walls in cambium-adjacent tracheids of healthy trees.

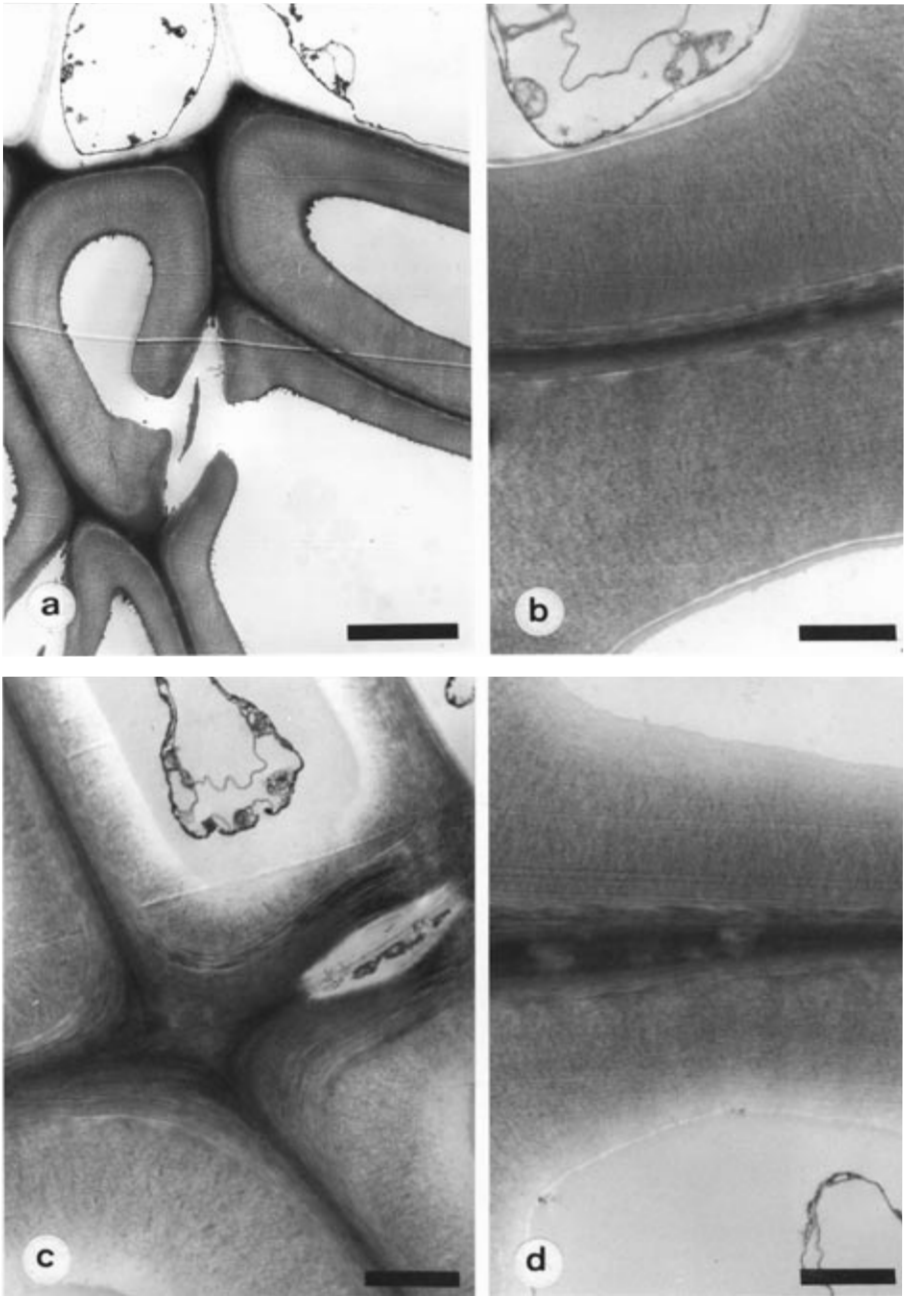


Fig. 5. TEM micrographs of transverse sections of cambium-adjacent tracheid walls, potassium permanganate staining. – a & b: Declining trees with S_3 and warty layer, inner S_2 wall distinctly stained. – c & d: Tracheid walls of healthy trees without S_3 and warty layer, inner S_2 unstained. — Scale bars = 5 μm (a), 2 μm (b, c, d).

Ultrastructural studies on the cambium-adjacent latewood tracheids of declining trees revealed the typical cell wall architecture of softwoods, with a compound middle lamella as a continuum of middle lamella and primary wall, a secondary wall consisting of a narrow S_1 , a broad S_2 , an inner, extremely narrow S_3 layer plus a warty layer (e.g. Liese 1970). Potassium permanganate staining has been used as an indicator for lignin distribution in woody tissue (e.g. Donaldson 1992; Singh et al. 1998). In declining fir trees, the latewood tracheids stained most intensely at middle lamella regions, whereas the secondary wall layers S_1 and S_2 showed less intense staining but a visible and slightly more stained transition zone between them. The warty layer and the middle lamella region have a similar electron density (Fig. 5a, b). These results show that production of cell layers as well as wall differentiation of the terminal latewood tracheids were completed prior to sampling.

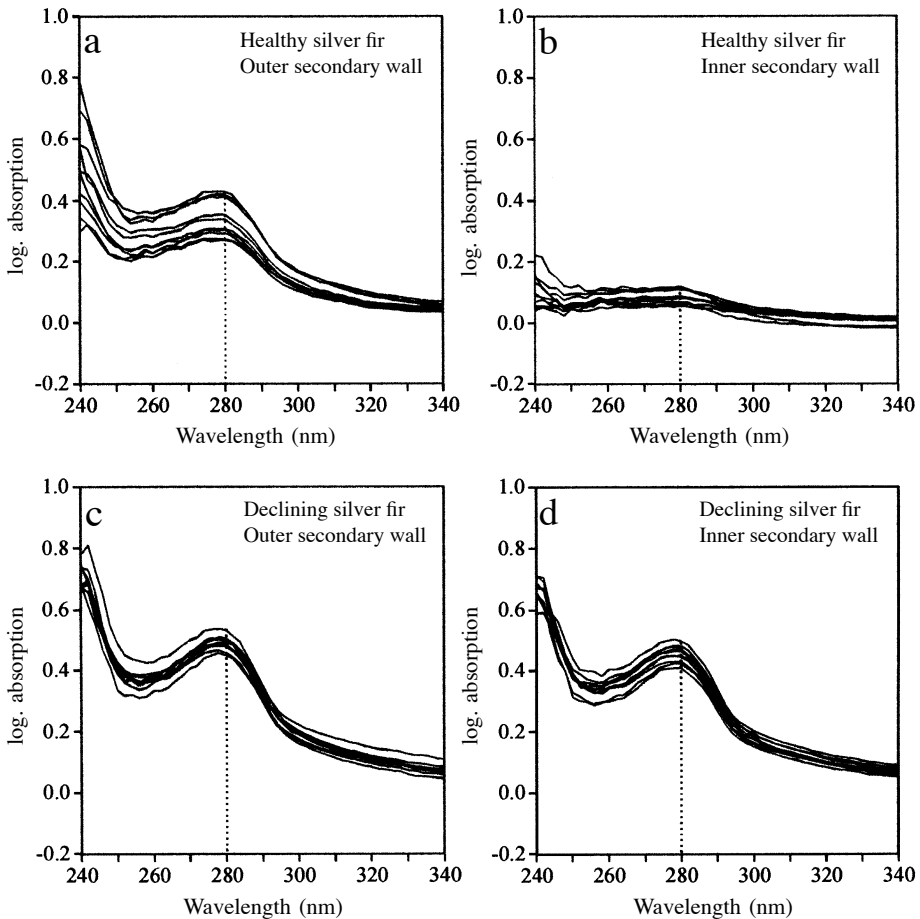


Fig. 6. UV-microspectrophotometry of secondary walls of cambium-adjacent tracheids. – a & c: Distinct absorbance values of outer secondary wall portions in healthy and declining silver fir trees. – b: Low absorbance values of inner secondary wall portions in healthy silver fir trees. – d: High absorbance values of inner secondary wall portions in declining silver fir trees.

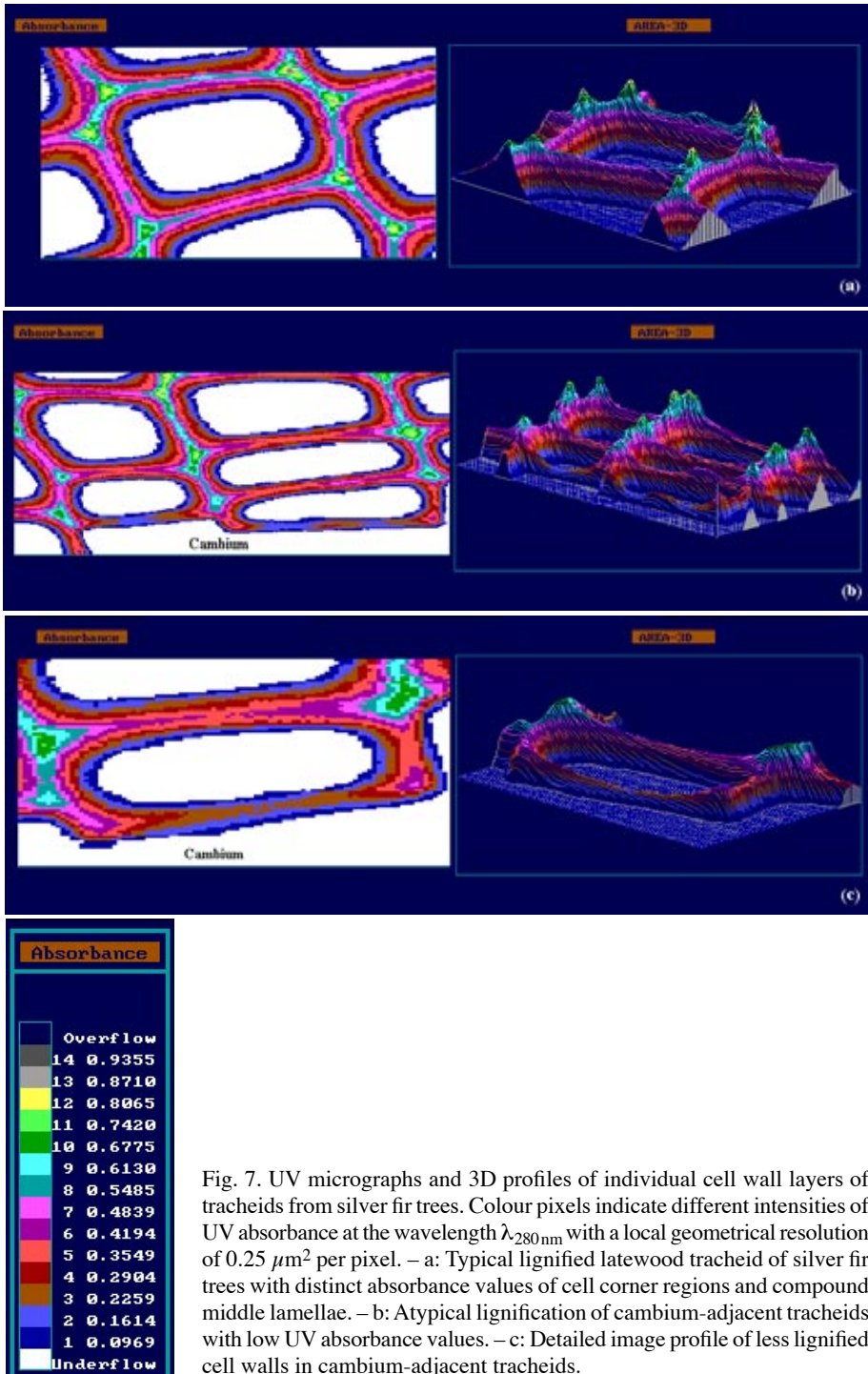


Fig. 7. UV micrographs and 3D profiles of individual cell wall layers of tracheids from silver fir trees. Colour pixels indicate different intensities of UV absorbance at the wavelength $\lambda_{280\text{nm}}$ with a local geometrical resolution of $0.25 \mu\text{m}^2$ per pixel. – a: Typical lignified latewood tracheid of silver fir trees with distinct absorbance values of cell corner regions and compound middle lamellae. – b: Atypical lignification of cambium-adjacent tracheids with low UV absorbance values. – c: Detailed image profile of less lignified cell walls in cambium-adjacent tracheids.

In healthy looking silver fir trees cambial activity also appeared to have ceased at the sampling date in the middle of October, because no early developmental stages of young tracheids were found near the cambium. However, up to three cambium-adjacent tracheid layers were still in late stages of maturation and identified as latewood according to their distinctly reduced radial dimensions and thickened walls. Regarding wall architecture, these immature tracheids exhibited completed deposition of polysaccharides of the S_1 and S_2 layers, whereas the S_3 layer was not yet present. Lignin deposition in the secondary wall, as shown by potassium permanganate staining, appeared restricted to the outer two thirds of the secondary wall, because the inner third was unstained (Fig. 5c, d). Therefore it can be assumed that polysaccharide as well as lignin deposition was not completed at the sampling date suggesting a longer duration of cambial activity in healthy trees as compared with the declining trees.

UV-microspectrophotometry, also used for semiquantitative lignin determination, confirmed the TEM results on lignin distribution in the secondary walls. According to early studies (e. g. Fergus et al. 1969; Goldschmid 1971), softwood lignin shows an absorbance maximum at the wavelength $\lambda_{280\text{nm}}$. In the present study distinct absorbance values between $\log \text{Abs}_{280\text{nm}}$ 0.4 and 0.5 were regularly found in the outer secondary wall of latewood tracheids from declining and healthy trees (Fig. 6a, c), as is typical for the S_2 layers of spruce tracheids (Koch & Kleist 2001). Structurally the outer secondary wall includes S_1 and outer S_2 . However, the point analyses taken at the inner S_2 layer of tracheids from declining silver firs revealed an absorbance of $\log \text{Abs}_{280\text{nm}}$ 0.2–0.3, whereas tracheids from healthy looking silver firs had an absorbance of less than $\log \text{Abs}_{280\text{nm}}$ 0.1 (Fig. 6b, d).

For a high resolution investigation of the lignin topochemistry in the latest formed tracheids, a specific UV-microspectrophotometric scanning device has been applied. Figure 7 shows typical two- and three-dimensional UV image profiles of lignin distributions. The colour pixels indicate different intensities of UV absorbance at the wavelength $\lambda_{280\text{nm}}$. The high resolution ($0.25 \mu\text{m}^2$ per pixel) enables a high differentiation of the UV absorbance within individual cell wall layers. The image profiles of the latewood tracheids from declining trees (Fig. 7a) are characterized by a typical high absorbance by the cell corners and compound middle lamellae ($\log \text{Abs}_{280\text{nm}}$ 0.48 to 0.67) as compared to the adjacent S_2 wall layers with a lower, slightly varying lignin distribution ($\log \text{Abs}_{280\text{nm}}$ 0.23 to 0.47). Corresponding to studies by Koch and Kleist (2001) for spruce tracheids, the average lignin content in the compound middle lamella is about twice that in the secondary wall. In contrast, the scanning of latewood tracheids of the healthy silver fir trees (Fig. 7b, c) revealed a continuous decrease of the absorbance values along a direction towards the latest formed tracheids. Especially the radially oriented cell wall layers of the latest formed tracheids showed significantly reduced absorbance values of the compound middle lamellae ($\log \text{Abs}_{280\text{nm}}$ 0.22 to 0.28) and the secondary wall ($\log \text{Abs}_{280\text{nm}}$ 0.10 to 0.21). Corresponding to the UV microspectrophotometric point analyses, only the outermost part of the secondary wall could be detected and visualized in the UV-light at $\lambda_{280\text{nm}}$.

As determined by potassium permanganate staining and UV-microspectrophotometry, the process of sequential lignin deposition from the outer to the inner wall regions was

still ongoing in healthy fir trees in the middle of October 1999. It is generally accepted that lignification of outer wall parts starts during the deposition of inner secondary wall polysaccharides (review: Lewis et al. 1999). Terashima (2000) and Terashima et al. (1998) give detailed information on the onset of lignification in cell corner and middle lamella regions with the beginning of deposition of the S_1 layer; secondary wall lignification starts after cell corners and middle lamella regions are completely lignified and when secondary wall polysaccharides are laid down to more than half of the total thickness of the secondary wall. The developmental stages of the terminal tracheids in healthy looking silver firs fit quite well into Terashima's concept. Accordingly, lignification of the S_2 layer is incomplete prior to the deposition of S_3 polysaccharides and represents an intermediate stage of cell wall differentiation. Donaldson (1992) also reported about intermediate stages of latewood lignification in *Pinus radiata* samples collected in late winter in New Zealand. Donaldson suggested that lignification of these latest formed tracheids is only interrupted for a while or continues at a highly reduced rate during winter. For the immature latewood tracheids of *A. alba* in our study it remains open whether deposition of the S_3 and the warty layer as well as lignification can be completed during winter or in early spring. However, a late lignification in mountainous regions with low temperatures in winter appears rather unlikely, especially because this phenomenon was also found in older tree rings (Gindl et al. 2000).

The reason for such an incomplete wall development might be the influence of climate during the final maturation phase. Gindl et al. (2000) found for Norway spruce sampled from the Alpine treeline a close correlation between lignification of terminal latewood tracheids and temperature. In this study it was shown for a ten-year period that the secondary wall lignin content in the youngest two tracheid layers of a tree ring was reduced when September and October temperatures were low. These distinctly reduced lignin contents therefore represent an interesting topochemical cell wall feature and also a temperature signal in the xylem that can contribute to climate reconstructions. However, as shown in this study of declining silver fir trees, narrow tree rings as the result of a premature end of wood formation are not ideally suited as indicators for early low temperature events in autumn.

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