TRACHEID LENGTH IN NORWAY SPRUCE
(*Picea abies* (L.) Karst.) ANALYSIS OF THREE DATABASES REGARDING TREE AGE, CAMBIAL AGE, TREE HEIGHT, INTER-ANNUAL VARIATION, RADIAL DISTANCE TO PITH AND LOG QUALITIES

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ABSTRACT

The study analyses three databases on tracheid length measurements in Norway spruce wood (*Picea abies* (L.) Karst.). The measurements cover a wide range of tree age from juvenile wood to very mature wood (<120 years) as well as large tree diameters (up to >60 cm). Fibre length was measured in three different tree heights separately for earlywood and the according latewood. The databases include the whole spectrum of log qualities that are classified in the Austrian grading rules (FHP 2006). Non-linear regression (NLR) models were applied to investigate the correlation between tracheid length and tree age, cambial age or radial distance from pith at different tree heights.

Trend lines of the tracheid length regarding tree age, cambial age, tree height and inter-annual variation were developed achieving high correlation coefficients up to $r^2 = 0.78$. The applied function to describe the tracheid length trend is based on a logarithmic curve (Bailey 1920). Due to the proposed additional terms the function allows for a steeper increase of fibre length in the juvenile growth phase as well a culmination/decrease at high age. The influence of log qualities (grades) on the fibre length was illustrated.

The study gives a deeper insight on tracheid cell morphology in coniferous trees and can be applied to select the most appropriate raw material for specific wood, pulp and paper products.

KEY WORDS: cambial age, log quality, juvenile / mature wood, Norway spruce (*Picea abies* (L.) Karst.), radial trend, tree age, tracheid length, tree height.
INTRODUCTION

Tracheids make up more than 90% of the Gymnosperm wood (Fengel and Wegener 1984). Therefore the characteristics of these cells dominantly determine the performances and efficiency of the xylem tissue within the tree. Due to the simplicity of the gymnosperm wood composition in terms of cell type diversity these cells have to cope with several tasks. The two main tasks are sap flow conduction and mechanical stiffness. Consequently it can be assumed that the shape and chemical composition of tracheids are a result of an evolutionary optimization process of nature to enable the tree to withstand internal and external stresses.

Numerous studies have focused on specific aspects of tracheids such as their physiological function (Kubler 1991, Sellin 1993, Burgert et al. 2004, Rosner et al. 2007), the cell morphology (Frey-Wyssling 1968, Brändström 2001, Sarén et al. 2004), the mechanical performance of single fibres (Burgert et al. 2005a, 2005b, 2005c, Burgert 2006; Gierlinger et al. 2006) or xylem tissues under various loading situations (Müller et al. 2003, Müller et al. 2004).

Sophisticated devices and analytic tools were developed to make the morphological, mechanical, and chemical properties of tracheid cell structures measurable. Devices such as SilviScan™ for example can perform accurate and rapid measurements of the tracheid cell dimensions in tangential and radial direction (cell wall thickness, lumen diameter…) on a cross section without isolating the fibres from the tissue (Evans 2000). The assessment of longitudinal characteristics such as microfibril angle (Evans et al. 1999), spiral grain (Buksnowitz et al. 2008) or the crystallinity index of the cellulose can also be performed on solid wood samples non-destructively. But for the measurement of tracheid length the cells still have to be separated by maceration techniques (Dodd 1985) or dissected into tangential longitudinal microscope sections for the method proposed by Bailey (1920), Ladell (1959) and Dodd 1985).

From previous research it is known that the characteristics of tracheid cells show a variety of trends governed by genomics, age, or growth conditions. Several studies have focused on the juvenile versus mature wood (Burdon et al. 2004), the effects of forest management such as thinnings (Herman et al. 1998a), the effect of fertilization and growth speed on the tracheid length (Lindström 1997, Dutilleul et al. 1998, Mäkinen et al. 2002a, b) the effect of genotype on the tracheid length (Sirvio and Kärenlampi 2000), the correlation between the tracheid length and cross sectional properties (Sirvio 2001) or the potential climatic signals in fibre length (Wimmer and Grabner 2000).

The present study is dedicated to the analysis of fibre length measurements of *Picea abies* L. (Karst.) from three complementing projects in order to verify known basic age trends and radial trends and reveal new aspects in terms of specific potentials.

Besides the physiological importance, the tracheid morphology and their mechanical properties are of vital importance to numerous industrial processes. The behaviour and performance of solid wood and wood-based-products is determined by specific properties on several hierarchical levels from the chemical structure to the whole tree. Fibre length determines for example the potential performance of paper and governs the production process and treatment (e.g. refining) of pulp (Evans et al. 1997; Kibblewhite and Evans 2001). Due to this significant impact on many broad scale applications and production processes (Brändström 2001) this study takes a closer look on the length of the soft woods’ tracheid cells with the following main objectives:

- Detailed analysis and illustration of the tracheid length trends of Norway spruce wood (*Picea abies* (L.) Karst.) including measurements up to a high tree age (>120 years) and large diameters (>40 cm).
- Empirically extract a mathematical description of the observed tracheid length trends from
the measurement data.
• Provide results based on an extensive data set derived from representative samples for the Alpine region and the surrounding area.
• Include different log quality grades in the analysis to representatively reflect on the practical situation in the sampling region.

Concluding, a more detailed knowledge of the potential properties and variability of tracheid cells is needed to use wood innovatively as a raw material for technical application and provided a solid basis to better understand the wood anatomical structure of coniferous trees.

MATERIAL AND METHODS

The data from three separate projects were merged to investigate the tracheid length in Norway spruce wood (Picea abies (L.) Karst.).
• 1st project: Fundamental material assessment of large diameter spruce wood (Teischinger and Müller 2006)
• 2nd project: Quality grading of resonance wood for musical instrument making (Buksnowitz 2006, Buksnowitz et al. 2007)
• 3rd project: Quality of juvenile Norway spruce wood grown in plantations (Hannrup et al. 2002, 2004)

All three projects include fibre (tracheids) length measurements and together they cover all qualities-grades of Norway spruce wood. The used grades are in accordance with the Austrian grading rules (FHP 2006). The supreme quality of knot-free logs free from defects (grade A) is represented in the 2nd project. The grades B (good average quality), C (below average) and Cx (inferior quality) are covered by the 1st project. The 3rd project does not represent a specific grade but provides information on juvenile wood from plantations.

Within the projects 1 and 2 numerous anatomical, mechanical, acoustical, optical and chemical properties were investigated on several hierarchical levels with the aim of deriving the potential of large diameter round wood in contrast to small diameter logs with a high proportion of juvenile wood. Data from the 3rd project were included in this study to cover the properties of juvenile wood grown in plantations when comparing the quality grades in terms of fibre length.

Material from the 1st project
In the "large diameter wood project" (1st project) 72 logs (36 individual trees) of Norway spruce (Picea abies (L.) Karst.) were harvested in two contrasting regions at three sites with different altitude ranges. The sample logs represented three different log-qualities and three different tree heights (butt log, middle log, crown log) (Buksnowitz et al. 2008). From each log a specimen covering the complete radius from pith to bark was taken. At several radial positions the fibre length for earlywood and the according latewood was determined separately.

Material from the 2nd project
Within the "resonance wood project" (2nd project) 84 samples from different Norway spruce (Picea abies (L.) Karst.) individuals from numerous resonance wood regions in Europe were collected. 78 of them were raw violin tops. Six non-resonance wood samples were added, which were graded as 'joinery-quality' by the retailer. These samples were A-quality (FHP 2006) and link the supreme quality with the lower qualities of the 1st project. For each sample the fibre length of
the earlywood and the latewood was determined separately at two different radial positions. The sampling positions were 10 mm from the pith-side and 10 mm from the bark-side of the 100 mm (radial) specimen (Buksnowitz 2006, Buksnowitz et al. 2007). Due to the required large diameters and the slow growth of resonance wood the samples showed up to 180 rings, not taking the juvenile part of the stem into account, which has already been discarded at the date of sampling. Therefore it was assumed that the estimated ring age of the samples exceeded 200 in a majority of cases. As a result this project extended the available data at the very high age end of the scale.

Material from the 3rd Project

The 3rd project sampled 73 clones at two different sites and a sub sample of 29 full-sib crossings from 15 parents (Hannrup et al. 2004).

In the 1st and 2nd project the wood was macerated in a solution of 2.65 g \( K_2Cr_2O_7 \) + 5 ml 65 % \( HNO_3 \) + 25 ml \( H_2O \). A detailed description of the maceration procedure is given by Jeffrey (1917). The tracheid lengths were measured on images, which were captured with a digital camera (Olympus DP 10) mounted on an incident light microscope (Olympus SZH 10 - research stereo) at a twenty-fold magnification. The obtainable accuracy is within the range of 10 µm. The image analysis program was Olympus DP Soft 3.0. For each sampling position 40 fibre length measurements were averaged.

Within the 3rd project a radial strip (approx. 3 mm wide) was prepared from each disc and cut tangentially into matchstick sized pieces. The splinters were macerated by heating in equal parts (by volume) of glacial acetic acid and hydrogen peroxide. After four hours the macerated samples were washed in distilled water and agitated vigorously to separate the tracheids. The tracheid length was determined applying a Kajaani FS-200 automated fibre length analyser. Samples were diluted in distilled water to provide a flow rate of 40-60 fibres per second. The mean tracheid length and tracheid length distribution (length weighted) was determined by measuring at least 15000 tracheids for each of the 287 samples. Applying this method the average fibre length includes also broken or crooked fibres. Therefore these results were mainly used to support the results derived from Project 1 and 2.

The statistical analysis was performed using the software package SPSS 15.0. The trend lines of tracheid lengths at different tree heights were generated by non-linear regression (NLR) models. The models used the tree age, the cambial age or the radial distance from the pith as independent variable. The NLR-coefficients were estimated applying the Levenberg-Marquardt method defining the iteration procedure.

The tree age was defined as the number of years that passed from germination of the seedling to the formation of the investigated ring. The cambial age stands for the age of the cambium in the year of the ring formation, which is equivalent to the number of annual increments from the pith to the investigated ring (Grabner and Wimmer 2006).

RESULTS AND DISCUSSION

The main part of the investigation of fibre length trends was performed using the data collected in project 1. Fibre length was analysed in three different tree heights (butt log, middle log, crown log). In combination with three independent variables (tree age, cambial age, radial distance from pith) nine scatter plots (A to I) were generated (Fig. 1). For each scatter plot the measurements were divided into earlywood and latewood. For each of the resulting 18 groups of fibre length measurements a trend line was fitted to the data points by applying the above-mentioned NLR routine (Fig. 1).
Fig. 1: Radial trends, age trends, and height trends of fibre length in Picea abies (L.) Karst. Abbreviations: EW … earlywood, LW … latewood, FL … fibre length, T … fibre length trend, n … number of measured fibres, min … minimum fibre length, max … maximum fibre length, av … average fibre length, stdev … standard deviation.

The underlying function used in the NLR models is displayed in formula. It proofed to be a suitable option to describe the trends within the data at hands.

\[ y = a \ln(x) - b x^c + \frac{d}{x^e} + f \]

\( y \) ... fibre length (dependent variable) (µm)
\( x \) ... independent variable (tree age in years (a), cambial age in years (a) or radial distance from pith (mm))
\( a, b, c, d, e \) ... coefficients estimated by the NLR
\( f \) ... offset

In accordance to Bailey (1920) or Teischinger and Müller (2006) the basic trend can be described by a natural logarithmic function “ \( y = a \ln(x) + f \) ” of the cambial age. The results displayed in Fig. 1 prove that this type of function is also highly suitable to be applied for “tree age” and “radial distance from pith” as independent variables. Two additional terms were introduced to properly describe the deviation of the fibre length trends from the plain logarithmic function. The term “ - b x^c ” allows the fibre length to decrease at high age, whereas the term “ d/x^e ” allows a steeper increase of fibre
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length in the juvenile phase and the transition phase from juvenile to mature wood.

Fig. 1 provides information on the number of fibre length measurements in each group, the tree heights in meters, the minimum/maximum/average fibre length as well as the standard deviation separately for earlywood and latewood. Additionally the correlation coefficients ($r^2$) for the trend lines are displayed besides the scatter plots. The coefficients to formula 1 are compiled in Tab. 1.

Tab. 1: Coefficients estimated by the non-linear regression models for each combination of tree height, earlywood/latewood and dependent variable (tree age, cambial age, radial distance from pith).

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In the following the aspects, which are derived from Fig. 1, are discussed. Fibre length measurements were rounded to 10 µm.

Fibre length

The longest individual latewood fibres (maximum value 7560 µm) were found in the crown logs, whereas the mean fibre length was longest in medium tree height (Fig. 1 D, E, F) with 4710 µm for earlywood and 4880 µm for latewood. The fact that the longest fibres can be found in medium tree heights (40-50 % of height) can be confirmed by previous studies (Helander 1933, Schultze-Dewitz and Götze 1973, Atmer and Thörnqvist 1982, Kucera 1994). These findings are in contrast to the results of (Mäkinen et al. 2007) who found no significant correlation between the vertical location along the stem and the fibre length.

Early wood fibres versus latewood fibres

Generally earlywood fibres (min: 1080 µm, mean: 4610 µm, max: 7460 µm) appear to be shorter than latewood fibres (min: 1340 µm, mean: 4750 µm, max: 7560 µm) of the same tree age, cambial age or radial distance from pith (Fig. 1 A - I). This confirms the studies of (Helander 1933, Vasiljevic 1955, Frimpong-Mensah 1987). A recent study on the variation of tracheid length within annuals rings of Norway spruce found (non-significant) 2-4 % longer fibres in latewood than in
earlywood with a generally high variation from ring to ring (Mäkinen et al. 2008).

The study at hands showed that earlywood fibre (standard deviation (stdev): 1230 µm) and latewood fibre (stdev: 1200 µm) lengths show approximately the same variance that increases from pith to bark, which in accordance to (Herman et al. 1998b). Although they do not increase exponentially like stated in the mentioned study.

Fibre length – age trends

After an initial steep increase of fibre length (Fig. 1 A, B, D, E) (tree age / cambial age: 0-50) the trend flattens, which can partly be explained by the theory of Bannan (1963, 1965, 1967). His studies ascribe the fibre length to the number of cambium cell that divide in a pseudo-transverse (anticlinal) manner resulting in fibres of half-length. The number of these cell divisions is inversely proportional to the tree superficies surface, explaining the initial steep increase in average fibre length. Numerous studies confirm this initial trend in fibre length (Helander 1933, Necesany 1961, Bøtulje 1968, Marton et al. 1972; Atmer and Thörnqvist 1982, Frimpong-Mensah 1987, Kucera 1994, Saranpää 1994, Herman et al. 1998a, Saranpää et al. 2000). The expansion of the circumference of the tree (Bannan 1965), the limitation of the cell elongation to approximately 9% (Bailey 1920) and the transformation of fusiform initials into ray initials (Panshin and De Zeeuw 1980) play an additional role of defining the fibre length trend.

The results of this study clearly indicate that the fibre length trend in Fig. 1 (A, B, D, E) culminates at a tree age / cambial age of around 120 years and decreases slightly beyond the age of about 130. This is in accordance to an early study of (Helander 1933), who reported a fibre length decrease near the bark in old trees. The results are in contrast to other studies reporting different radial patterns in tracheid length in mature wood. Atmer and Thörnqvist (1982) and Kucera (1994) examined a constant length after the maximum was reached, whereas another study reports of a continuous increase in tracheid length (Necesany 1961).

Fibre length – height trends

The NLR for the crown log using tree age as independent variable shows a less steep increase and a less distinct culmination of fibre length.

The NLR for the crown log using cambial age as independent variable shows an earlier culmination (cambial age: approx. 90) and earlier decrease in fibre length.

In the crown logs (Fig. 1 G, H, I) the fibre length variation is higher than in lower logs (Fig. 1 A, B, C), which is also expressed in lower r² values.

Fibre length – radial distance trends

Fibre length-measurements plotted over the radial distance from pith show different trend line behaviour (Fig. 1 C, F, I) than for tree age and cambial age. The initial increase in fibre length is less steep and turns into a flatter, almost linear, increase from a radial distance from pith of 50-100 mm. This can be explained by the known trend in tree ring width from wide rings close to pith to narrower rings close to bark. Due to this overlaying of the “annual increment width trend” in Fig. 1 C, F, I the fibre length trend has a less distinct initial increase and remains at steady slope at high diameters.

The fibre length decrease at high radial distance from pith is additionally suppressed by the fact that the majority of logs were already up to 600 mm in diameter at an age that has not reached the fibre length culmination point as shown in Fig. 1 A, B, D, E, G and H. This fibre length trend over the radial distance from pith is supported by several studies that found a negative relationship between annual ring width and tracheid length in Norway spruce (Helander 1933, Frimpong-Mensah 1987, Herman et al. 1998b, Saranpää et al. 2000, Mäkinen et al. 2007). The negative relationship between
circumferential growth rate and the fibre length (Fujiwara and Yang 2000) explain why the radial distance from pith controls the pattern of the fibre length trend (Sarén et al. 2001).

Correlation coefficients of NLR models

The models using the cambial age as independent variable (Fig. 1 B, E, H) reach slightly higher values for correlation coefficients (r²) than the ones using tree age (Fig. 1 A, D, G).

Correlation coefficients up to r² = 0.78 indicate a good fit of the NLR. A strong correlation between cambial age and fibre length was also found in a study on pulp properties (Suur-Hamari 1997). A study investigating 33 years old Norway spruce also found the tracheid length dependent on the logarithm of the cambial age, giving an r² = 0.87 (Lindström 1997). A model presented by (Mäkinen et al. 2007) predicted 82.8 % of the total variation in tracheid length using distance from pith and the site index as independent variables. Taking into account that both studies investigated only the initial phase of the fibre length trend and the latter allowed more explaining variables, the correlation coefficients of the present study are comparable.

Fibre lengh – log quality

Fig. 2 includes the fibre length measurements of project 1, 2 and 3 illustrating the tracheid length of different "grades" of Picea abies (Karst.) L. wood using box plots.

The leftmost “juvenile” group stands for the measurements performed in project 3. The juvenile samples (up to 15 years) support the trends in Fig. 1 showing consistently short fibres at low tree age.

Fig. 2: Fibre lengths in different grades ("qualities") of Picea abies (L.) Karst. wood.

Abbreviations: Cx ... inferior quality logs, C ... low quality logs (below average), B ... average to good quality logs, A ... supreme quality logs, "Juvenile"... plantation wood.

*… each sample included at minimum 15,000 fl measurements.

The groups Cx, C, and B (defined in “Materials and Methods”) are derived from project 1 following confirming the trends shown in Fig 1. The quality grades according to the Austrian grading rules (FHP 2006) are mainly defined by macroscopically visible defects and features such as knots. The older the tree the likelier is a knot free trunk surface resulting in a higher grade. Therefore the logs, which were assigned to grading class C have the tendency to be older than Cx logs.
Group A stands for fibre length measurements performed on very old clear wood samples from project 2 (resonance wood). The slightly shorter fibres in Group A support the trend of decreasing fibre length at high tree age / cambial age the NLR models in Fig. 1 shows.

The variation on fibre length within group A is lower (whiskers for the 5% and 95% percentile) as the samples did not contain juvenile wood like the groups Cx, C and B did.

CONCLUSIONS

The study clearly confirms that tracheid length (fibre length) in *Picea abies* (L.) Karst. is subjected to tree age trends, cambial age trends and trends of radial distance from the pith. In general the observed tracheid length trends for cambial age and tree age show an initial steep increase followed by a culmination and a slight decrease. Using radial distance from the pith as an independent variable the decrease of fibre length at higher distances to the pith was not observed.

The trend lines that were fitted into the tracheid length measurements and the derived non-linear regression models can serve as an indicator which fibre lengths can be expected at a certain tree age, cambial age, radial distance to the pith in a certain range of the tree height for comparable regions of origin. Due to the high correlation coefficients up to $r^2 = 0.78$ this could find a possible application in raw material choice with regional relevance.

At high age contradictory trends are reported in literature (Necesany 1961, Atmer and Thörnqvist 1982, Kucera 1994, Sirviö and Kärenlampi 2001). In contrast to them the present study observed a slight decrease in fibre length at a tree age >130 years. The behaviour of the tracheid length trends at high age is of special importance in mountainous regions like the Austrian Alps were forest management and the protective needs require large diameter trees.

It could be shown that the radial distance to pith is not the most reliable parameter to estimate the fibre length, as it does not necessarily contain consistent information on the driving factor, which seems to be the age.

When using the diameter of a log to estimate the fibre length the annual increment width has to be considered in order to take into account, whether the fibre length is still increasing, culminating or already decreasing.

It can be concluded that despite numerous other anatomical and morphological changes with age and diameter (such as cell wall thickness, cell diameter, coarseness and micro fibril angle) the position of the tracheids within the stem has to be considered, when it comes to specific applications where tracheid length is a vital parameter.

The up to 7.5 mm long fibres that were found in large diameter (old growth) Norway spruce (*Picea abies* (L.) Karst.) wood point towards innovative applications where these properties are advantageous.

The more detailed results of the present study are based on a wide data basis, give a deeper insight on tracheid cell morphology in coniferous trees and can be applied to select the most appropriate raw material for specific wood, pulp and paper products.

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