Trees (2000) 14:409–414
DOI 10.1007/s004680000057

ORIGINAL ARTICLE

Wolfgang Gindl · Michael Grabner · Rupert Wimmer

The influence of temperature on latewood lignin content in treeline Norway spruce compared with maximum density and ring width

Received: 3 January 2000 / Accepted: 31 May 2000 / Published online: 6 July 2000

Abstract The latewood lignin content, maximum density and total ring width of ten consecutive annual increments were determined in treeline Norway spruce (*Picea abies* [L.] Karst.) using ultraviolet (UV) microscopy and radiodensitometry, respectively. A positive correlation between the total ring width and the mean temperature of mid-July to August was identified, as was one between the maximum density and the temperature of August–September. Lignin content in the secondary cell wall layer of the terminal latewood tracheids was positively correlated with the temperature for the period running from the beginning of September until the third week of October. It can, therefore, be concluded that lignification of the cell wall is susceptible to the influence of climatic variability, as is the case with ring width and maximum density.

Key words Climate · Lignification · *Picea abies* · Treeline

Introduction

The growth of trees at the alpine treeline is primarily limited by temperature and the length of the growing season (Tranquillini 1979; Körner 1998). Numerous studies have shown how the radial growth and wood density of conifers varies under the influence of temperature (e.g. Hustich 1945; Larson 1964; Denne 1971; Fritts 1976; Schweingruber et al. 1979; Loris 1981; Müller 1981; Antonova and Stasova 1993,1997). A cool short growing season produces a narrow, low-density growth ring, whereas more favourable warmer conditions will lead to wider rings with higher latewood densities. Dendroclimatologists make use of this relationship to reconstruct summer temperatures of the pre-instrumental era (Eckstein and Aniol 1981; D'Arrigo et al. 1992; Briffa et al. 1998).

In addition to the influence of varying climatic conditions on ring width and ring density, their effect on lignin content is also of interest, because the latter is an important wood quality parameter, which determines the amount of chemicals to be spent in paper production (Fengel and Wegener 1984). The gross lignin content of softwoods has been shown to be negatively correlated with latitude (Kim et al. 1989). It also varies with changing mechanical stresses (Timell 1986; Okuyama et al. 1998) and situations of severe nutrient deficiency (Downes et al. 1991; Wimmer and McLaughlin 1996). However, gross lignin content is not very suitable for an investigation into the climatic effects on lignification, because the results reflect its strong reliance on wood density (Erickson and Arima 1974). Ultraviolet (UV) microscopy (Scott et al. 1969) provides a tool capable of determining the lignin content of individual cells. An exploratory study using this technique (Gindl and Grabner 2000) provided a first indication of reduced lignin content in latewood tracheids formed under abnormally low temperatures.

The present study hypothesises that cellular lignin content in trees growing at high elevations may not be only influenced by abnormal meteorological conditions but may also be systematically related to temperature changes. This argument is based on the following assumption. As with differentiating tracheids in the cell wall thickening phase, the terminal cell rows of a tree ring are characterised by a decreasing thickness of the radial cell wall towards the growth ring border (Grozdits and Ifju 1984). This pattern is most clearly seen in so-called light rings, i.e. tree rings with particularly low-density latewood, where growth is prematurely arrested due to exceptionally low temperatures in the second half of the growing season (Filion et al. 1986; Gindl 1999). The incorporation of lignin in cell walls is the process that completes the formation of a tracheid. The earlier a growing season terminates by the onset of cool temperatures, the lower the lignin content of the last formed late-

---

W. Gindl (✉) · M. Grabner · R. Wimmer
Institut für Botanik, Universität für Bodenkultur Wien,
Gregor Mendel Strasse 33, A-1180 Wien, Austria
e-mail: wgindl@edv1.boku.ac.at
Tel.: +43-1-476543190, Fax: +43-1-476544504

wood cells should be. On the other hand, conditions favourable for cell wall synthesis, potentially leading to a high lignin content, might prevail until the beginning of November (Pisek and Winkler 1958; Havranek 1981).

Using treeline Norway spruce (*Picea abies* [L.] Karst.), the relationship between lignin content in the secondary cell wall of latewood cells and temperature is investigated and compared with results obtained from measurements of maximum latewood density and total ring width.

Materials and methods

Study site and plant material

The study site was located at Mt. Kreuzkogel [47°33′N, 14°30′E, 1840 m above sea level (a.s.l.)], in the Austrian "Nördliche Kalkalpen". An area scattered with 60 to 80-year-old Norway spruce [*P. abies* (L.) Karst.] trees protruding 4–6 m from a Mountain pine (*Pinus mugo* Turra) krummholz belt was selected for sampling. The slope was oriented towards west and the soil type was Rendzina. The growth of the trees should not have been influenced by cattle grazing on nearby alpine pastures, as the area was protected by dense Mountain pine. Stem disks were collected at breast height from five spruce trees felled in August 1998. The bark was immediately removed from the samples to prevent diffusion of phenolic extractives into the xylem, which causes biased measurements in radiodensitometry (Schweingruber et al. 1978) as well as in UV microscopy (Imagawa and Fukazawa 1978). Blocks including the 1988–1997 increments and free of compression wood were taken from the disks and dissected for radiodensitometry and lignin analysis. The number of samples was limited to 50 (i.e. five trees, ten rings per tree) due to the time-consuming nature of lignin determination using UV microscopy.

Radiodensitometry

Radiodensitometric analysis was carried out at the Swiss Federal Institute for Forest, Snow and Landscape Research in Birmensdorf. The samples were air-dried and glued on wooden supports. Transverse sections, 1.2 mm thick, cut with a double bladed circular saw were placed on an X-ray sensitive film (Agfa) and exposed to an X-ray source. In order to determine density and ring width, the X-ray micrograph was analysed on a DENDRO 2003 microdensitometer (Walesch, Effretikon, Switzerland) using a cellulose acetate calibration wedge (Lenz et al. 1976; Schweingruber et al. 1978).

UV microscopic lignin analysis

UV microscopy is a method that allows the lignin content in individual cell walls to be determined. In their fundamental studies, Fergus et al. (1969), Scott et al. (1969) and Fukazawa and Imagawa (1981) demonstrated that UV absorbance of thin sections of cell walls is proportional to their lignin content. This relationship can be described using the Beer–Lambert law: $A=\varepsilon \times C \times d$, where A is the absorbance at 280 nm, ε_{280} is the respective extinction coefficient, C is the lignin concentration and d is the thickness of the section. Fergus et al. (1969) quoted a wide range of ε_{280} values of various lignin preparations given in the literature. They considered an ε_{280} value of 15.6 cm $^{-1}$ g^{-1}, determined by using sodium borohydride-reduced milled wood lignin, to be the most suitable way to describe the absorbance of softwood protolignin. Following this suggestion, the above value of ε_{280} was used in the present study. In any event, the choice of a different ε_{280} value would not have affected our results, as inter-annual changes and not absolute lignin concentrations were of interest.

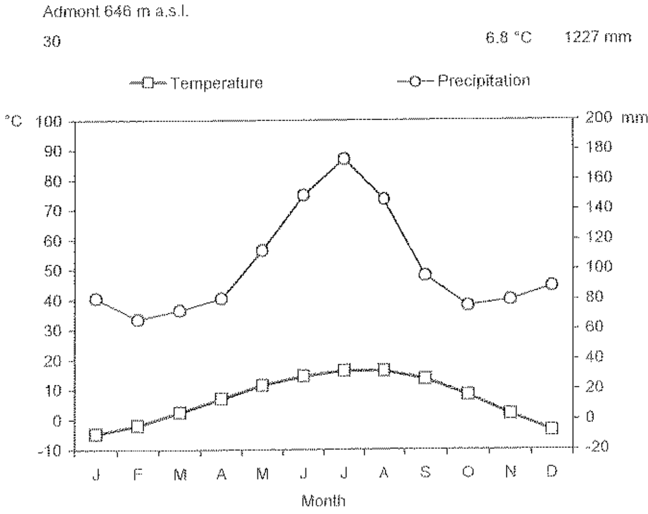


Fig. 1 Climate diagram of the Admont region according to Walter and Lieth (1960). Location of the station, its altitude and the number of years included in the record (*top left*); mean annual temperature and mean annual sum of precipitation (*top right*)

Pieces of wood, 5 mm in length and 1×1 mm^2 in cross-section, were prepared for lignin analysis with the UV photometer microscope (Zeiss). Each piece included the latewood of one of the 1988–1997 tree rings. The samples were dehydrated and extracted in a graded ethanol–acetone series and embedded in Spurr's resin (Spurr 1969; Kuhn et al. 1997). The "hard" mixture of resin components, as suggested by Spurr (1969), gave the best 1-µm-thick sections on the ultramicrotome (Leica), equipped with a diamond knife. The sections were placed on quartz slides with a platinum wire loop. The lignin analysis was carried out on a Zeiss microscope-photometer MPM800. The absorbance at 280 nm was determined in the secondary cell wall layers of the three terminal cell rows of each annual increment. A 100× Ultrafluar immersion objective (Zeiss) achieved a circular measuring spot diameter of 0.5 µm, which allowed accurate central positioning within the secondary cell walls. Scanning step width was set to 2 nm and the monochromator bandwidth to 5 nm. A set of 30 measurements (1500 in total) was completed for each ring. Measurements of absorption in the cell corner compound middle lamella (CCML) area were also attempted, but were discontinued because it was not possible to repeat them successfully. It was virtually impossible to accomplish an accurate positioning of the measuring spot in the small CCML area.

Meteorological data

A meteorological record directly from the sampling area was not available, but temperatures recorded in Admont (47°35′N, 14°30′E, 646 m a.s.l.), 4 km north of the sampling area, were provided by the Central Institute for Meteorology and Geodynamics, Vienna. Since Böhm (1992) showed that lowland and high alpine temperatures in Austria are strongly correlated (correlation coefficient r=0.92), the available record of temperatures describes the conditions at the sampled high altitude site sufficiently well. A climate diagram according to Walter and Lieth (1960), derived from mean values of the period 1961–1990 (Fig. 1), describes the general climatic characteristics of the Admont region, which, located at the northern edge of the Austrian Alps, receives plenty of precipitation during the entire growing season.

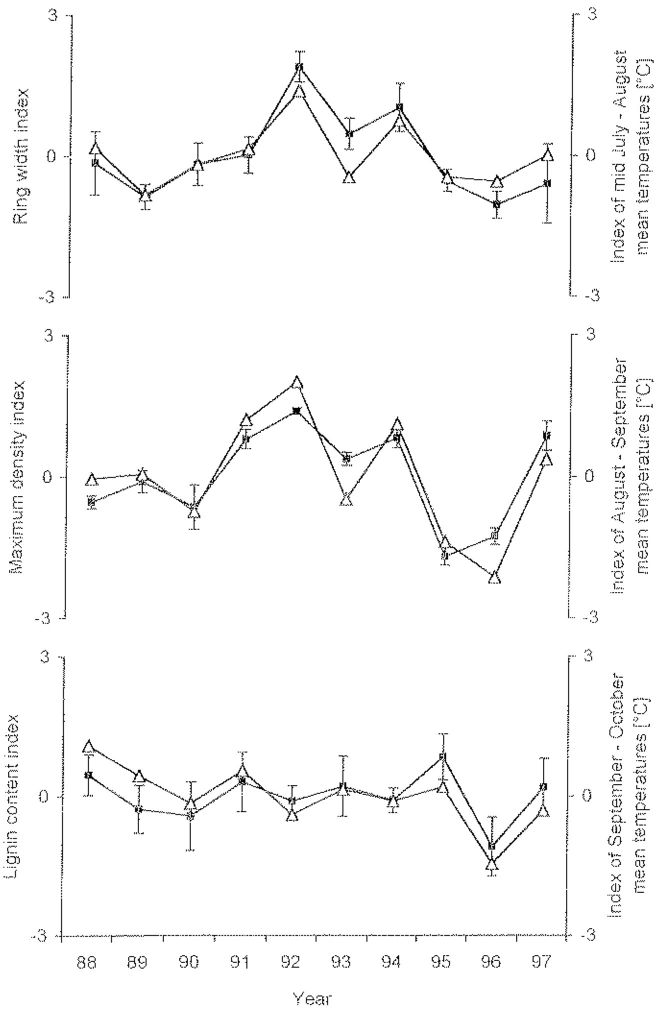


Fig. 2 Chronologies of total ring width, maximum latewood density and latewood lignin content indices (*black squares*, error bars indicate SD) compared with the mean temperature of the best correlated interval (*triangles*). Temperature is given as the departure from the mean of the entire 10-year period

Calculation of indexed chronologies

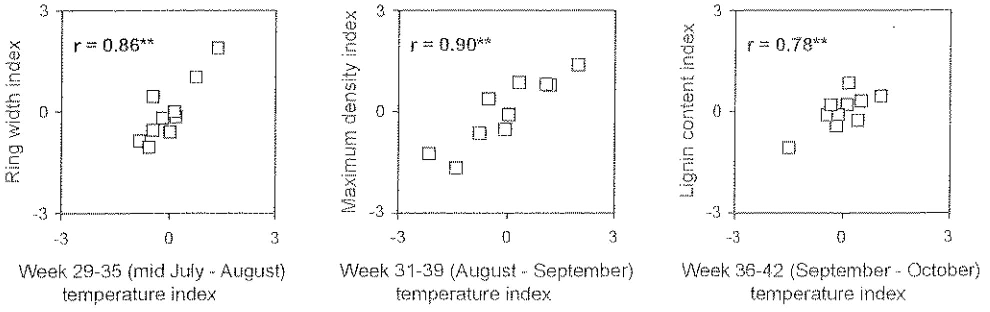
Individual series of ring width measurements were cross-dated using the nearby Johnsbach Norway spruce chronology (Gindl 1999). Single-tree chronologies of the three investigated parameters were standardised, applying the formula $x'=(x_i-x_m)/SD$, where x' is the standardised variable, x_i is the initial measurement, x_m is the mean of the period 1988–1997, and SD is the standard deviation.

Results

Three chronologies resulting from measurements of ring width, maximum density and lignin content are displayed in Fig. 2. In contrast to the ring width and maximum density, the inter-annual variability of lignin content is low, with the exception of the interval 1995–1997, where lignin content decreases to the minimum value of the 10-year period in 1996. Indexed maximum density values show the lowest mean SD (0.214) compared with ring width (SD=0.431) and lignin content (SD=0.532). All three chronologies are normal distributed (Kolmogorof–Smirnoff test, $P<0.1$).

Through analysis of monthly mean temperatures, it was found that a significant relationship existed between the temperature and total ring width, and also between the temperature and latewood density. However, this did not apply to latewood lignin content. As a second step, mean temperatures of pairs of months, e.g. June–July, July–August and so on, were used as independent variables. This resulted in significant positive relationships between the July–August temperatures and ring width, between the August–September temperatures and maximum density, and between the September–October temperatures and lignin content. In order to determine the most strongly correlated interval more precisely, weeks were added to and/or subtracted from the period obtained above until optimum correlation coefficients were achieved (Fig. 3). The optimum period extends from mid-July to August (weeks 29–35) for total ring width, from August to September (weeks 31–39) for maximum latewood density and from September until the third week of October (weeks 36–42) for lignin content in the secondary cell wall of terminal latewood tracheids (Fig. 4). In Fig. 2, the three chronologies are plotted together against the temperatures of the intervals that show the strongest correlation. An analysis of the correlation between the tree ring parameters ring width, maximum density and lignin content, respectively, showed that lignin content is not related to any of the other parameters, whereas total ring width is correlated with latewood maximum density (Fig. 5).

Fig. 3 Correlation of total ring width, maximum latewood density and latewood lignin content chronologies with the mean temperature of the respective intervals (**$P<0.01$)



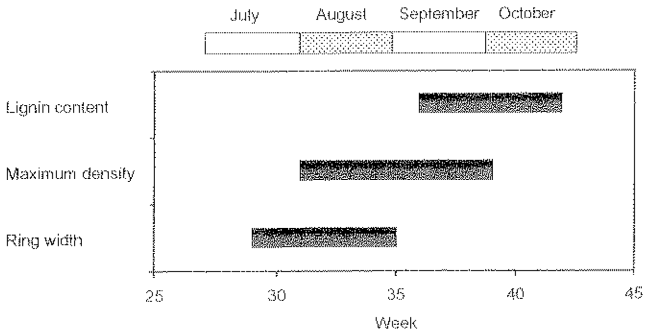


Fig. 4 Growing season intervals exhibiting best correlations between temperature and respective tree ring properties

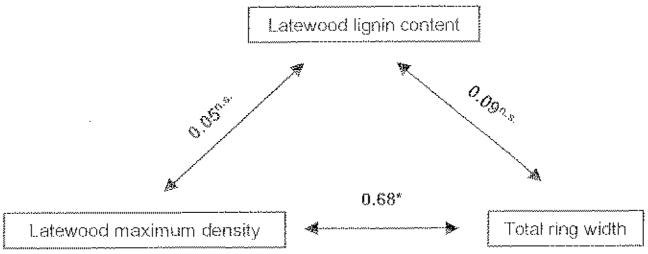


Fig. 5 Correlation between total ring width, latewood maximum density and latewood lignin content (*$P<0.05$, *n.s.* not significant)

Discussion

After a wood cell has been produced by cell division in the cambium, it undergoes three processes of differentiation (Wardrop 1965; Wodzicki 1971; Grozdits and Ifju 1984). First, a cell grows in the radial and longitudinal directions, then a secondary wall is formed and finally lignification of the cell wall completes the maturation of a tracheid. In temperate zones it takes 2–3 weeks for the formation of an earlywood tracheid and about 2 months for a latewood tracheid (Whitmore and Zahner 1966; Skene 1969; Wodzicki 1971; Kutscha et al. 1975). This study demonstrates that, not only are cell size and wall thickness subjected to the influence of climatic variability, but also lignification of the cell wall.

Total ring width is a measure of the sum of a growing season's cell division and cell enlargement activity in the direction of the xylem. Its positive correlation with the mean temperature of the period from mid-July to August (Fig. 3) agrees well with the findings of Müller (1981), who found that radial growth in alpine treeline stands starts in June and continues until August, when the last latewood cells are formed. After the cessation of radial expansion, secondary cell wall formation persists for several weeks in latewood (Whitmore and Zahner 1966; Skene 1969; Wodzicki 1971; Kutscha et al. 1975), explaining the influence of August and September temperatures on maximum latewood density (Fig. 3). The latter is a measure of the cell wall mass per unit volume and is highly correlated with cell wall thickness (Yasue et al. 1996,2000) as well as the percentage of cell wall area (Park and Telewski 1993). The period of time which influences latewood lignin content extends from September to the third week of October (Fig. 3). Thus, lignification of latewood cells in spruce persists long after the radial expansion and cell wall thickening phases have been completed. The time course of wood formation, starting with cell division and enlargement, followed by cell wall thickening and termination by lignification, is surprisingly well reproduced through the obtained relationships (Fig. 4).

A comparison of the chronologies with the best-correlated temperature curves (Fig. 2) reveals a strong year-to-year agreement between the interval trends. It can be seen that the inter-annual variability of July–August as well as August–September temperatures over the 10-year period exceeds the September–October temperature variability, with the exception of the interval 1995–1997. In 1996, lignin content reached the minimum value of the entire 10-year period, concurring with minimum temperatures.

Maximum density determined by using X-ray densitometry occurs at the end of an annual increment (Schweingruber et al. 1978), but it is not correlated with the lignin content of terminal latewood cells (Fig. 5). The percentage of cell wall area, which is highly correlated with density (Park and Telewski 1993; Wimmer 1995), reaches a maximum in the latewood and decreases in the last two cells of a tree ring (Yasue et al. 1996). Hence, maximum density might not be located exactly in the terminal cell row of a tree ring. Furthermore, lignin is one of three main polymer constituents of the wood cell wall (Fengel and Wegener 1984) and the small changes found might not have been sufficiently large to overcome the control of cell wall thickness on density. Therefore, the influence of September temperatures on latewood lignin content and maximum latewood density does not necessarily result in a correlation between them. Conversely, the influence of summer temperatures on total ring width and maximum latewood density (Eckstein and Aniol 1981; D'Arrigo et al. 1992; Briffa et al. 1998) results in a poor but statistically significant correlation between these two tree ring properties.

It could be demonstrated that lignification of the secondary cell wall in the terminal latewood tracheids of treeline Norway spruce is subjected to the influence of climate, similar to ring width and maximum latewood density. The influence of these small variations on the gross lignin content of Norway spruce is presumably minor, but it raises the question as to whether a general relationship between temperature and gross lignin content exists. Comparing the low lignin content of temperate zone tree species to highly lignified tropical trees, Trendelenburg (1939) hypothesised that low temperatures induce low gross lignin content. Referring to the positive relationship between cell wall thickness and temperature in the temperate zone, Kollmann (1951) suggested that a negative relationship between gross lignin content and temperature exists owing to the influence of the highly lignified middle lamella, which retains a uniform thickness (Fengel and Stoll 1973) and which might become more important in thin-walled cells. As lignin has to be removed in the pulping process and is thus a major cost factor, the question of a general relationship between temperature and gross lignin content, and a possible altitudinal gradient of gross lignin content, is of great economic interest and will be the subject of future research.

Acknowledgements The help of P. Cherubini, M.T. Hauser and P. Rooney in preparing the manuscript is gratefully acknowledged. Funds for this research were provided by the Austrian National Bank (PN 6019 and 6654). R. Wimmer received support by the Austrian Academy of Science, APART program.

References

Antonova GF, Stasova VV (1993) Effects of environmental factors on wood formation in Scots pine stems. Trees 7:214–219

Antonova GF, Stasova VV (1997) Effects of environmental factors on wood formation in larch (*Larix sibirica* Ldb.). Trees 11: 462–468

Böhm R (1992) Lufttemperaturschwankungen in Österreich seit 1775. Zentralanstalt für Meteorologie und Geodynamik, Vienna

Briffa KR, Jones PD, Schweingruber FH, Osborn TJ (1998) Influence of volcanic eruptions on Northern Hemisphere summer temperature over the past 600 years. Nature 393:450–455

D'Arrigo RD, Jacoby GC, Free RM (1992) Tree-ring width and maximum latewood density at the North American tree line: parameters of climatic change. Can J For Res 22:1290–1296

Denne MP (1971) Temperature and tracheid development in *Pinus sylvestris* seedlings. J Exp Bot 22:362–370

Downes GM, Ward JV, Turvey ND (1991) Lignin distribution across tracheid cell walls of poorly lignified wood from deformed copper deficient *Pinus radiata* (D. Don). Wood Sci Technol 25:7–14

Eckstein D, Aniol RW (1981) Dendroclimatological reconstruction of the summer temperatures for an alpine region. Mitt Forstl Bundesversuchsanst Wien 142:391–398

Erickson HD, Arima T (1974) Douglas-fir wood quality studies part II: effects of age and stimulated growth on fibril angle and chemical constituents. Wood Sci Technol 8:255–265

Fengel D, Stoll M (1973) Über die Veränderung des Zellquerschnitts, der Dicke der Zellwand und der Wandschichten von Fichtenholz-Tracheiden innerhalb eines Jahrrings. Holzforschung 27:1–7

Fengel D, Wegener G (1984) Wood. Chemistry, ultrastructure, reactions. De Gruyter, Berlin

Fergus BJ, Procter AR, Scott JAN, Goring DAI (1969) The distribution of lignin in sprucewood as determined by ultraviolet microscopy. Wood Sci Technol 3:117–138

Filion I, Payette S, Gauthier L, Boutin Y (1986) Light rings in subarctic conifers as a dendrochronological tool. Quaternary Res 26:272–279

Fritts HC (1976) Tree rings and climate. Academic Press, London

Fukazawa K, Imagawa H (1981) Quantitative analysis of lignin using an UV microscopic image analyser. Variation within one growth increment. Wood Sci Technol 15:45–55

Gindl W (1999) Climatic significance of light rings in timberline spruce, *Picea abies*, Austrian alps. Arctic Antarctic Alpine Res 31:242–246

Gindl W, Grabner M (2000) Characteristics of spruce [*Picea abies* (L.) Karst] latewood formed under abnormally low temperatures. Holzforschung 54:9–11

Grozdits GA, Ifju G (1984) Differentiation of tracheids in developing secondary xylem of *Tsuga canadensis* L. Carr. Changes in morphology and cell-wall structure. Wood Fiber Sci 16: 20–36

Havranek WM (1981) Stem respiration, radial growth and photosynthesis of a cembran pine tree (*Pinus cembra* L.) at the timberline. Mitt Forstl Bundesversuchsanst Wien 142:443–467

Hustich I (1945) The radial growth of the pine at the forest limit and its dependence on the climate. Soc Sci Fenn Comm Biol 9:1–30

Imagawa H, Fukazawa K (1978) Influence of wood extractives on UV microscopy for lignin determination. Mokuzai Gakkaishi 24:583–586

Kim WJ, Campbell AG, Koch P (1989) Chemical variation in Lodgepole pine with latitude, elevation, and diameter class. For Prod J 39:7–12

Kollmann F (1951) Technologie des Holzes und der Holzwerkstoffe, erster Band. Springer, Berlin Göttingen Heidelberg

Körner C (1998) A re-assessment of high elevation treeline positions and their explanation. Oecologia 115:445–459

Kuhn AJ, Schröder WH, Bauch J (1997) On the distribution and transport of mineral elements in xylem, cambium and phloem of spruce (*Picea abies* [L.] Karst.). Holzforschung 51:487–496

Kutscha NP, Hyland F, Schwarzmann JM (1975) Certain seasonal changes in Balsam fir cambium and its derivatives. Wood Sci Technol 9:175–188

Larson PR (1964) Some indirect effects of environment on wood formation. In: Zimmermann MH (ed) The formation of wood in forest trees. Academic Press, New York, pp 345–365

Lenz O, Schär E, Schweingruber FH (1976) Methodische Probleme bei der radiographisch-densitometrischen Bestimmung der Dichte und der Jahrringbreite von Holz. Holzforschung 30: 114–123

Loris K (1981) Dickenwachstum von Zirbe, Fichte und Lärche an der alpinen Waldgrenze/Patscherkofel. Ergebnisse der Dendrometermessungen 1976–79. Mitt Forstl Bundesversuchsanst Wien 142:416–441

Müller HN (1981) Messungen zur Beziehung Klimafaktoren – Jahrringwachstum von Nadelbaumarten verschiedener waldgrenznaher Standorte. Mitt Forstl Bundesversuchsanst Wien 142: 327–355

Okuyama T, Takeda H, Yamamoto H, Yoshida M (1998) Relation between growth stress and lignin concentration in the cell wall: ultraviolet microscopic spectral analysis. J Wood Sci 44: 83–89

Park WK, Telewski FW (1993) Measuring maximum latewood density by image analysis at the cellular level. Wood Fiber Sci 25:326–332

Pisek A, Winkler E (1958) Assimilationsvermögen und Respiration der Fichte (*Picea excelsa* Link) in verschiedener Höhenlage und der Zirbe (*Pinus cembra* L.) an der alpinen Waldgrenze. Planta 51:518–543

Schweingruber FH, Fritts HC, Bräker OU, Drew LG, Schär E (1978) The x-ray technique as applied to dendroclimatology. Tree-Ring Bull 38:61–91

Schweingruber FH, Bräker OU, Schär E (1979) Dendroclimatic studies on conifers from central Europe and Great Britain. Boreas 8:427–452

Scott JAN, Procter AR, Fergus BJ, Goring DAI (1969) The application of ultraviolet microscopy to the distribution of lignin in wood. Description and validity of the technique. Wood Sci Technol 3:73–92

Skene DS (1969) The period of time taken by cambial derivatives to grow and differentiate into tracheids in *Pinus radiata* D. Don. Ann Bot 33:253–262

Spurr AR (1969) A low viscosity embedding medium for electron microscopy. Ultrastruct Res 26:31–43

Timell TE (1986) Compression wood in gymnosperms, vol 1. Springer, Berlin, Heidelberg, New York

Tranquillini W (1979) Physiological ecology of the alpine timberline. Tree existence at high altitudes with special reference to the European Alps. Springer, Berlin, Heidelberg, New York

Trendelenburg R (1939) Das Holz als Rohstoff. Lehmanns, Munich

Walter H, Lieth H (1960) Klimadiagramm-Weltatlas. Fischer, Jena

Wardrop AB (1965) Cellular differentiation in xylem. In: Coté WA Jr (ed) Cellular ultrastructure of woody plants. Syracuse University Press, New York, pp 61–97

Whitmore FW, Zahner R (1966) Development of the xylem ring in stems ox young Red pine trees. For Sci 12:198–210

Wimmer R (1995) Intra-annual cellular characteristics and their implications for modelling softwood density. Wood Fiber Sci 27:413–420

Wimmer R, McLaughlin SB (1996) Possible relationships between chemistry and mechanical properties of Red spruce xylem. In: Dean JS, Meko DM, Swetnam TW (eds) Tree rings, environment and humanity. University of Arizona, Tucson, pp 659–668

Wodzicki TJ (1971) Mechanism of xylem differentiation in *Pinus sylvestris* L. J Exp Bot 72:670–687

Yasue K, Funada R, Fukazawa K, Othani J (1996) The effect of climate on the variation in maximum density of *Picea glehnii* Mast. and associated changes in tracheid dimensions. Dendrochronologia 14:89–97

Yasue K, Funada R, Kobayashi O, Ohtani J (2000) The effects of tracheid dimensions on variations in maximum density of *Picea glehnii* and relationships to climatic factors. Trees 14:223–229