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Lignification of Scots pine trees from Arctic Circle up to timberline

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SUMMARY

Lignification is a physiological process influenced by genetic and environmental factors. The variations in the lignin content are therefore a proper parameter indicating changes or differences in the living conditions of trees caused by external factors. Scots pine (*Pinus sylvestris* L.) trees of a five-stand transect from the Arctic Circle to northern timberline in northern Finland were investigated tree ring by tree ring for lignin content, using diffuse reflectance infrared Fourier transform-spectroscopy (DRIFT). For the calculation of the degree of lignification the ratio of the aromatic skeletal vibration at ~1510 cm^{-1} and the band at ~896 cm^{-1}, which derives from the C1-H vibration in cellulose and hemicellulose, was used. The measurements brought up three major tendencies: (1) The lignin variation pattern through the stem cross-section follows a similar pattern in most of the trees, indicating "special" years or events influencing the lignification process. (2) The variations are more significant in the northern trees than in the southern ones. This indicates that the more northern the tree's location, the more intense is the stress response and the more lignin – relative to the average – is produced in certain tree rings. (3) The average lignin content of the trees as a whole is lower in the northernmost trees than in the ones growing 400 km south from them at the Arctic Circle in Rovaniemi.

Key words: diffuse reflectance, DRIFT, Fourier transform infrared spectroscopy, FTIR, influence of latitude on lignification, lignification, *Pinus sylvestris*, Scots pine.

YFIRLIT

Trénun skógarfurutrjáa upp að skógarmörkum á heimskautasvæðum

Trénun er lífeðlisfræðilegt ferli sem stýrist af erfðum og umhverfisþáttum. Breytileiki í trénisinnihaldi er þess vegna góð mælistærð til að sýna breytingar eða mismun í ytri lífskjörum trjáplantna. Á fimm stöðum í Finnlandi, allt frá heimskautabaugi norður að skógarmörkum, var trénisinnihald skógarfuruplantna (*Pinus silvestris* L.) rannsakað í árhringjum með því að nota innrautt dreifendurkast við Fourier breytis-litrófsgreiningu (Diffuse Reflectance Infrared Fourier Transform-Spectroscopy, DRIFT). Trénunarstig var reiknað sem hlutfall aromatiskrar grindarsveiflu við ~1510 cm^{-1} og ~896 cm^{-1}, sem kemur frá Cl-H sveiflunni í sellulósa og hemisellulósa. Meginniðurstöður mælinganna eru: (1) Sveiflur í tréni í þversniðinu voru svipaðar í flestum trjánna og fram komu „sérstök“ ár eða atburðir sem höfðu áhrif á trénismyndun. (2) Sveiflur eru meira áberandi í norðlægum trjám en þeim suðlægari. Þetta bendir til meiri trénismyndunar og sterkari viðbragða við álagi á norðlægari slóðum. (3) Meðaltrénisinnihald er minna í norðlægum trjám en þeim sem vaxa 400 km sunnar, við Rovaniemi nálægt heimskautabaugi.

INTRODUCTION

Wood formation is a process controlled by genetic as well as environmental factors. The adaptation of the metabolism of the trees is known to occur in the transient period between summer and winter (Shain and Mackay, 1973; Nelson, 1978). Temperature, e.g., as one of the stress releasing factors, is involved in the creation of an adapted flora. Several metabolic changes happen during the season, controlled by course of the temperature (Levitt, 1980). For example, the enzymatic activity as well as the isoenzyme pattern of peroxidase, a key molecule with regard to rapid adaptation of the whole plant to changes in the environmental conditions, is known to alter according to temperature treatments (Ebermann *et al.*, 1991). Peroxidase is assumed to catalyze the conversion of coniferyl alcohol and other p-hydroxy-cinamyl alcohols to lignin (Mäder and Füssl, 1982; Harkin and Obst, 1973). Lignin biosynthesis is known to be strongly influenced by environmental conditions causing changes in the content (Hinterstoisser and Unteregger, 1999) and maybe even in the molecular structure of the polymer. Lignin is an important component of xylem cells, and as these cells begin to develop there is a noticeable build-up of peroxidase enzymes. The lignin content is therefore a proper parameter to follow the cold hardiness of trees.

Accurate methods for the assessment of lignin content in small wood samples are available for some years. As these methods were time consuming they were not suitable for screening approaches including large numbers of samples, i.e., many trees and many measurements per tree. Spectroscopic methods, such as Fourier Transform Infrared (FTIR) spectroscopy, provide a tool for numerous applications in wood chemistry by allowing to obtain better and more reliable data (Faix, 1992). Fourier Transform Infrared (FTIR) spectroscopy has proved to be an efficient and accurate tool in wood chemistry (Rodrigues, 1998). It provides simultaneous and almost instantaneous recordings of spectra at high signal-to-noise ratios with high reliability in frequency along with easy-to-operate and user-friendly software. Special techniques have been developed to achieve further improvements. Diffuse Reflectance FTIR (DRIFT) spectroscopy is a technique that allows measurements in reflectance mode at low energy levels, even with very small sample amounts. The technique requires low quantities of a given sample and almost no chemical sample preparation (Hinterstoisser *et al.*, 1996).

MATERIALS AND METHODS

Two to five Scots pine (*Pinus sylvestris* L.) trees per site were sampled from five different sites in northern Finland, site 1 being the southernmost, located at the Arctic Circle and site 5 being the northernmost, at the northern timberline (Table 1). Disks at dbh were prepared and wood splinters were taken as samples, tree ring by tree ring, through the whole disk cross section. The specimens were dried at 105°C. The small wood specimens were extracted with ethanol/toluene (v/v=1:2) for 6 h, ethanol (95% v/v) for 4 h and additionally 1 h in hot water. Wood powder was prepared by using a diamond abrasive paper. The slip of abrasive paper together with the wood powder was put directly into the diffuse reflectance (DRIFT) unit (EasiDiff PIKE Technologies) placed in the sample compartment of a Fourier-transform infrared (FTIR) spectro-

Table 1. Location of the sites.
1. tafla. Staðsetning rannsóknasvæða.

Site no.	Name	Location	Stand age yrs	Forest site	Altitude m a.s.l.
1	Vanttauskoski, Rovaniemi	26°43'E, 66°22'N	45	Dryish	150
2	Tähtelä, Sodankylä	26°38'E, 67°22'N	65	Dry	180
3	Laanila, Inari	27°30'E, 68°30'N	45	Dryish	220
4	Kaamanen, Inari	27°15'E, 69°07'N	50	Dry	155
5	Kenesjärvi, Utsjoki	27°05'E, 69°40'N	65	Dryish	110

meter (BRUKER Equinox 55). The wood powder reflects the incident IR-radiation. Two kind of reflections occur: The specular reflection (front surface reflection), which is of no use for the measurement and secondly the diffuse reflection. The latter one contains the information about the absorption properties, and therefore about the chemistry, of the sample.

The DRIFT measurements were used to determine the relative lignin content, tree ring by tree ring. The measurements were carried out in reflectance mode with a resolution of 4 cm^{-1} and accumulation of 32 scans. The calculation was done by relating the band height at 1510 cm^{-1} (aromatic skeletal vibration) to the one at 896 cm^{-1} (C1-H vibration in cellulose and hemicellulose), giving the "FTIR-value", as a measure for lignification (Unteregger, 1998) (Figure 1).

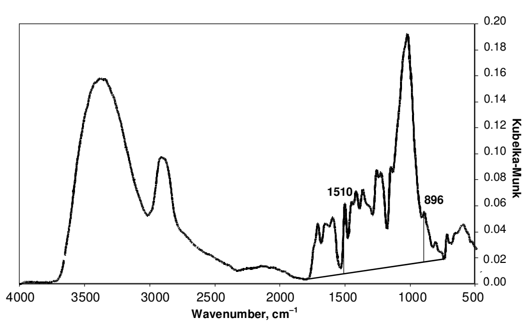

Figure 1. FTIR-spectra of wood powder on diamond abrasive paper. The ratio of the height of the aromatic skeletal vibration band around 1510 cm^{-1} divided by and the height of the band around 896 cm^{-1}, which derives from the C1-H vibration in cellulose and hemicellulose, was used to calculate the "FTIR-value", a measure for lignification.
1. mynd. FTIR-litróf viðarmjöls á pappír úr demantssvarfi. Hæð aromatisku grindarsveiflutoppsins við 1510 cm^{-1} deilt með hæðinni við 896 cm^{-1}, sem stafar frá Cl-H sveiflu í sellulósa og hemisellulósa, var notuð til að reikna út FTIR-gildi sem er mælikvarði á trénun.

RESULTS AND DISCUSSION

The performed measurements brought up three major tendencies underlining the importance of lignification in general concerning the adaptation to northern environmental conditions. When presenting the variation trends of lignin within the trees in staggered mode, distinct years can easily be recognized (Figure 2). The lignin variation pattern through the stem cross-section follows a similar pattern in most of the trees, indicating "special" years or events influencing the lignification process. The variations of the FTIR-values, which are determined by the degree of lignification of the

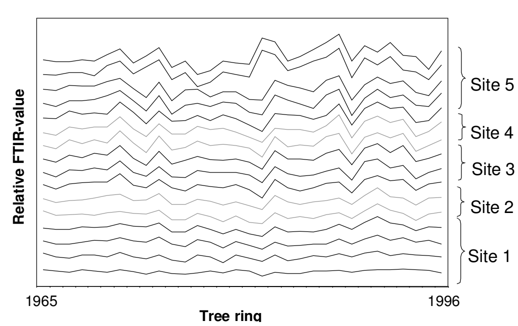

Figure 2. FTIR-values of the tree rings (1965–1996) printed in staggered mode.
2. mynd. FTIR-gildi árhringa (1965–1966) prentað á sveiflustillingu.

different tree rings, are more significant in the northernmost trees than in the southern ones (Figure 3). It indicates that the more northern the tree's location, the more intense is the stress response and the more lignin – relative to the average – is produced in certain tree rings. Average lignin content of the trees as a whole is lower in the northernmost trees than in the southern ones growing at the Arctic Circle (Figure 4). Average precipitation and average temperature during the growing season are as well decreasing from south to north. Korori (1989) found that in general the hardening process of trees depends on the adaptation of the development of plants to the climate in its seasonal variation. Temperature

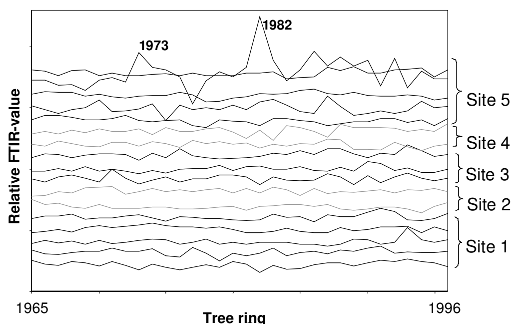

Figure 3. Variation of lignin content within the Scots pine trees increase from southern (site 1) to northern sites (site 5).
3. mynd. Sveifla í trénisinnihaldi skógarfuru vex frá suðlægari svæðum (site 1) til norðlægari svæða (site 5).

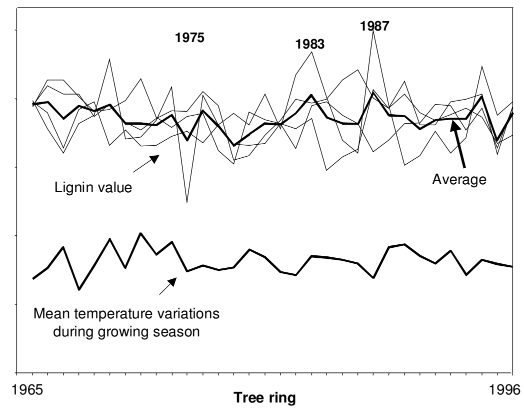

Figure 5. Comparison of average temperature and lignification pattern of four Scots pine trees at site 5 in Kenesjärvi, Utsjoki.
5. mynd. Samanburður á meðalhita og trénun fjögurra skógarfuruplantna á svæði 5 í Kenesjärvi, Utsjoki.

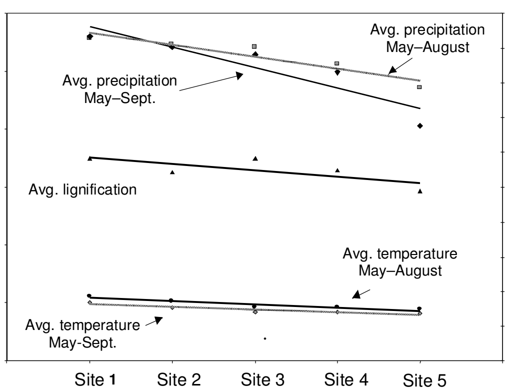

Figure 4. Average lignin content of the Scots pine trees, average precipitation and average temperature of the growing season show a decrease from south to north.
4. mynd. Meðaltrénisinnihald skógarfurutrjáa, meðalúrkoma og meðalhiti vaxtartímans sýna lækkun frá suðri til norðurs.

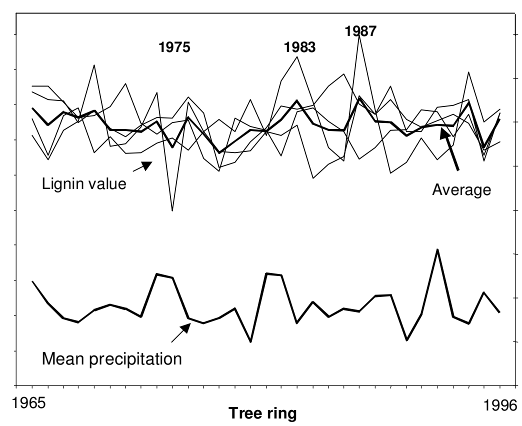

Figure 6. Comparison of average precipitation and lignification pattern of four Scots pine trees at site 5 in Kenesjärvi, Utsjoki.
6. mynd. Samanburður á meðalúrkomu og trénunarmynstri fjögurra skógarfuruplantna á svæði 5 í Kenesjärvi, Utsjoki.

seems to be an important determining factor, based on the finding that lignin content pattern correlates more with temperature variations (Figure 5) than with the precipitation curve (Figure 6). This is in agreement with Gindl and Grabner (2000). Schwarz (1968) found that the annual change in freezing tolerance depends on three factors: temperature, internal rhythm of the plant and photoperiod.

CONCLUSIONS

The amount of lignin in trees is related to the hardiness level of the tissue. Higher values indicate better adaptability to the length of the growth season. Going northwards, the growing season shortens. At the timberline it is the shortest where the trees still can grow, having the shortest time to prepare the hardiness for the winter, including the amount of lignin. Therefore the lesser amount of lignin in the northernmost trees indicates lower adaptability when comparing to the southern samples. The lignin content correlates with certain stress symptoms in trees. Chemistry and anatomy pose an effect on the mechanical integrity of wood material because of altered biochemical properties. A multifactorial phenomenon, which can rarely be related to single parameters. Stress factors act like a pulse that causes the physiological system of the trees to shift as a response.

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