Heartwood extractives in larch and effects on X-ray densitometry

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Abstract: The genus *Larix* is exceptional for its high content of extractives in the heartwood, with the dominating component arabinogalactan found abundantly in cell lumens of tracheids. On samples prepared from 100 European larch (*Larix decidua* Mill.) and hybrid larch (*L. decidua* × *Larix kaempferi* (Lamb.) Carr.) trees, extractive contents and wood density were measured using X-ray densitometry. A strong relationship between the amount of hot water extractives and the loss of density owing to the extraction process was found. Prior to extraction, increasing extractive content went hand-in-hand with higher wood density. At the heartwood–sapwood boundary, the density level dropped. After acetone and hot water extraction, the drop was no longer visible. Without proper consideration of the extractives in larch growth sites, comparisons in wood quality studies looking at wood density differences may become faulty, breeding studies could lead to incorrect selection strategies, and tree-ring studies may not deliver the expected climatic signals. Hence, hot water extractions should take place prior to radiation exposure.

Résumé : Le bois de cœur du genre *Larix* a un contenu en matières extractibles exceptionnellement élevé, surtout en arabinogalactane qui est abondamment présent dans le lumen des trachéides. Le contenu en matières extractibles et la densité du bois déterminée par radiodensitométrie ont été mesurés sur des échantillons provenant de 100 mélèzes d'Europe (*L. decidua* Mill.) et mélèzes hybrides (*L. decidua* × *Larix kaempferi* (Lamb.) Carr.). Une relation étroite a été observée entre la quantité de matières extractibles à l'eau chaude et la perte de densité due au processus d'extraction. Un contenu en matières extractibles élevé allait de pair avec une densité plus élevée du bois avant l'extraction. La densité diminuait à la limite entre le bois de cœur et le bois d'aubier. Cette diminution n'était plus apparente après une extraction à l'eau chaude et à l'acétone. Les comparaisons entre les sites où croît le mélèze dans les études sur la qualité du bois qui s'intéressent à la densité du bois peuvent s'avérer erronées, les études sur l'amélioration génétique pourraient mener à de mauvaises stratégies de sélection et les études dendrochronologiques pourraient ne pas livrer les signaux climatiques attendus si on accorde pas une attention appropriée aux matières extractibles.

[Traduit par la Rédaction]

Introduction

Wood density has an important influence on wood quality and can be seen as an indirect measure of wood properties such as mechanical strength, rate of shrinkage, workability, or even paintability (e.g., Kollmann 1951; Bosshard 1974; Panshin and De Zeeuw 1980). Wood density is an indicator of the total amount of cell wall material per unit volume and, in addition to ring width, is the most prominent param-

Received 21 January 2005. Accepted 19 July 2005. Published on the NRC Research Press Web site at http://cjfr.nrc.ca on 25 November 2005.

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eter studied in solid wood (Wimmer 1995). The variability of wood density within trees, including trends from pith to bark, from tree base to top, and from earlywood to latewood within single rings, has been studied substantially (e.g., Schweingruber 1983; Wimmer 1994, 1995; Downes et al. 1997).

For decades, X-ray densitometry has been a prominent method for determining density variation in wood samples (Downes et al. 2002), and studies have concentrated on the robustness of this method, including the effects of sample orientation, moisture content, and resinous substances as nonstructural contributions to the wood mass (Lenz et al. 1976). Resinous substances are advised to be extracted prior to X-ray densitometry with organic solvents such as acetone. However, water-soluble carbohydrates and inorganic components are also extractive substances that require an extraction with polar solvents (Fengel and Wegener 1989). Schweingruber et al. (1978) remarked that erroneous densitometric readings may occur with the heartwood of European larch (Larix decidua Mill.) if only a standard extraction in organic solvents is applied. These authors have recommended an extraction in boiling water prior to X-ray exposure.

The genus *Larix* is known for the exceptionally high content of extractives in the heartwood, with the dominating Fig. 1. X-ray images of one larch profile: control, acetone extracted, and hot-water extracted. The arrows indicate the area of comparisons: five tree-rings within the heartwood, three tree rings within the transition zone, and five tree rings within the sapwood.



component arabinogalactan usually within a wide range of 5%–30% of dry mass (Côté et al. 1966; Fengel and Wegener 1989; Dix and Roffael 1994; Gierlinger et al. 2002; Pereira et al. 2003). Arabinogalactans are heavily branched water-soluble polysaccharides that belong chemically to the hemicelluloses present in all other softwood species, contributing no more than 1% of dry mass, except *Larix* (Côté et al. 1966; Karácsonyi et al. 1984; Willför et al. 2002). Unlike all other hemicelluloses, at least 90% of the arabinogalactans in larch are located outside the cell wall (Côté et al. 1966; Sjöström and Westermark 1999), filling primarily the tracheid lumens that are near wood ray cells, which makes this component an extractive rather than a structural cell wall constituent (Grabner et al. 2005).

It has been shown that extractive contents in larch increase gradually from pith to bark, reaching the highest contents at the heartwood–sapwood boundary followed by an immediate drop to almost zero in the adjacent sapwood (Côté et al. 1966; Hillis 1971; Taylor et al. 2002; Gierlinger and Wimmer 2004). Although the effects of extractives on wood density are evident (Arganbright 1971; Parker et al. 1974; Lenz et al. 1976; Schweingruber et al. 1978; Kuo and Arganbright 1980; Pernestal and Jonsson 1992; Grabner et al. 2005), the extent to which extractives may alter wood density has not received significant study. This work presents X-ray density data from unextracted and extracted larch wood samples to quantitatively explore the effect of extractives on the microdensitometric profiles.

Materials and methods

The larch wood investigated in this work originated from the project "Towards a European Larch Wood Chain" (FAIR CT98-3354; Gierlinger et al. 2002). One hundred trees of various larch species (*L. decidua*, *L. decidua* × *Larix kaempferi* (Lamb.) Carr.) were randomly selected from a collection of over 300 trees originating from low-elevation plantation sites as well as from old-growth high-elevation alpine sites across Europe. Details about sites and sampling conditions are described elsewhere (Gierlinger et al. 2003, 2004). Data on acetone- and water-soluble extractive contents of the heartwood determined gravimetrically according to TAPPI T204 om-88 as a percentage of dry mass were already available (Gierlinger et al. 2002).

High-resolution X-ray densitometric profiles in a tangential direction were obtained on 2 mm wide and 7 mm high radial strips using the SilviScan-2TM technology available at CSIRO Australia (Downes et al. 1997; Evans et al. 2000) after the samples underwent a standard extraction in acetone. The samples were then water extracted in reaction tubes at 60 °C for 2 days followed by another densitometric scanning. The long extraction procedure, compared with the extraction of milled wood (Gierlinger et al. 2002), was necessary to remove almost all soluble substances. In addition to this procedure, densitometric profiles were also taken from a subsample of 15 trees representing the broadest range of extractive contents possible. These 15 initially unextracted slices were X-rayed in a longitudinal direction with equipment located at the BOKU after Lenz et al. (1976) followed by an acetone extraction over 24 h at 20 °C. X-ray scans were also collected prior to and after a hot water extraction lasting 5 days at 60 °C in reaction tubes. The processed X-ray radiographs were digitized with a transmission densitometer (Fig. 1). The reduction in X-ray absorption (loss of density) owing to extraction was calculated by averaging the five outermost heartwood tree rings with exclusion of the heartwood-sap-



Trees in ascending order of extractives content

Fig. 3. Wood density profiles as obtained with SilviScan-2. The thin line represents the wood density after acetone extraction; the thick line represents the wood density after acetone and hot water extraction. The heartwood–sapwood border is indicated.



Distance

wood transition zone. This transition zone was defined by a sequence of three tree rings with the outermost visible heartwood tree ring being the centre of that sequence (Fig. 1).

Results

The total extractive content varied between 4.6% and 30.9% of dry mass. The main part of the extractives was water soluble, varying between 3.1% and 27.0% of dry mass, while the acetone extract content was more stable, varying between 0.9% and 4.3% of dry mass (Fig. 2). For illustration, Fig. 3 shows five density profiles with contrasting extractive contents (A–E) representing the marked trees in Fig. 2. The

higher the hot water soluble extractives in heartwood, the clearer the effect on the X-ray densitometric profiles, expressed as loss of density. Consequently, no loss of density existed in sapwood, independent of the extractive level in heartwood (Fig. 3). Figure 1 presents X-ray exposures of a high-extractive sample, which illustrates the influence of hot water extraction. While changes seen after acetone extraction are minor, the removal of hot water soluble substances evidently results in a "clear" wood structure (Fig. 1).

For the subsample of 15 trees, mean earlywood density, mean latewood density, and mean ring density were examined (Fig. 4). Acetone extraction caused minor changes in density, and weak relationships were seen between loss of



Fig. 4. Relationships between loss of density owing to extraction and the percentage of dry mass of extractives in heartwood averaged over the five outermost heartwood tree rings plotted for earlywood density, latewood density, and mean ring density.

Loss of density due to extraction (kg/m³)

density and percentage of acetone extractives across all three density parameters. Loss of density turned out to be highest in latewood. After hot water extraction, the loss of density in heartwood rose with increasing total extractives. Loss of density owing to hot water extraction was much higher than that owing to acetone extraction and these losses are most prominent for earlywood density followed by total ring and latewood density, respectively (Fig. 4).

Discussion

Larch heartwood may have high amounts of extractives of up to 35% (Dix and Roffael 1994; Gierlinger et al. 2002; Pereira et al. 2003) and the major part is attributed to the water-soluble polysaccharide arabinogalactan (Côté et al. 1966). In Fig. 2, the major contribution of hot water soluble substances on the total amount of extractives is evident, which was already described by Côté et al. (1966). The acetone-soluble extractives reached levels of less than 5% of dry mass only; a slight upward trend is visible (Fig. 2). Slight changes in wood density owing to the extraction procedures, i.e., with hot water extraction, are possible but would affect heartwood and sapwood in the same way and are therefore not a relevant factor in this study.

Figure 3 shows five density profiles with contrasting extractive contents (A–E) representing the marked trees in Fig. 2. Looking at the densitometric profiles within the sapwood, no changes in density owing to the extraction procedure are visible. The deviation of the X-ray densitometric profiles between acetone-extracted and hot water extracted samples appears just within the heartwood of high extractive samples, while almost no deviation of the profiles is evident in the sapwood (Fig. 3).

To check the influence of the extractive fractions on wood density, a subset of 15 trees were X-rayed following Lenz et al. (1976). Mean earlywood density, mean latewood density, and mean ring density were calculated from the digitized X-ray films (Schweingruber 1983) (Fig. 4). Because of the low amounts of acetone extractives (Côté et al. 1966; Gierlinger et al. 2002), the loss of density owing to extrac-

tion was rather limited. Density loss after acetone extraction is relatively higher in latewood. Acetone only removes nonpolar substances such as resins, and the resin ducts are mainly located in latewood and in transition from earlywood to latewood, respectively (Wimmer et al. 1999; Schweingruber 2001). Loss of density after hot water extraction was much more expressed and these losses were most prominent for earlywood density owing to the extractive-filled earlywood tracheids (Côté et al. 1966).

X-ray density profiles of wood are frequently used in dendroclimatology (e.g., Polge 1977; Schweingruber et al. 1978; Briffa et al. 1998), in wood quality research (e.g., Cown et al. 1992), in studying the intratree variability of wood density (e.g., Echols 1973), in tree breeding (e.g., Cown and Parker 1979; Rozenberg et al. 2001), in studying relationships between wood density and mechanical wood properties (e.g., Rozenberg et al. 1999), and in studying the influence of wood formation on wood density (e.g., Deleuze and Houllier 1997; Wimmer et al. 2002). Our data clearly show that the high levels of hot water extractives in larch heartwood significantly alter density profiles. A careful hot water extraction procedure is therefore essential, since high extractive contents may mock high wood densities. As arabinogalactan is deposited mainly in earlywood tracheid lumens (see Fig. 4), which may go up to 30% (see Fig. 2), the highest possible density errors might be around that value, since arabinogalactan has the same density as the wooden cell wall (Côté et al. 1966). Climate signals reconstructed from maximum density remain widely unaffected by changing extractive contents because the lumen proportions of the terminal cell rows are reduced to almost zero. However, site comparisons in wood quality studies looking at wood density differences may become faulty, or breeding studies might lead to incorrect selection strategies, if water-extractable substances are not properly removed. Observed site differences without consideration of the possible differences in extractive contents could relate to significant misinterpretations of the wood density differences.

In this work, the term "wood density" is defined as the cell wall mass per unit volume. Faulty measurements are

therefore not restricted to X-ray densitometry but occur also with gravimetric determination. Grabner et al. (2005) recently showed that the buildup of extractives in tracheid lumens leads to an altered mechanical behaviour of larch wood.

Acknowledgements

This study was funded in part by the European Union, Fair project CT98-3354 "Towards a European Larch Wood Chain", coordinated by Dr. Luc Pâques, Institut national de la recherche agronomique (INRA). We are grateful to Sharee Harper, CSIRO Melbourne, who has helped with great commitment.

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