Phytochemistry 150 (2018) 31–39

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Radial distribution of wood extractives in European larch *Larix decidua* by TOF-SIMS imaging

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ARTICLE INFO

Article history:
Received 24 November 2017
Received in revised form
31 January 2018
Accepted 24 February 2018
Available online 10 March 2018

Keywords:
Mass spectrometry imaging
TOF-SIMS
European larch
Mineral nutrients
Phosphate
Triglycerides
Phytosterols
Resin acids

ABSTRACT

Wood extractives in the xylem of European larch *Larix decidua* were mapped by time-of-flight secondary ion mass spectrometry (TOF-SIMS) imaging, which allows the radial distribution of both mineral and lipophilic extractives in the xylem to be scrutinized with high spatial resolution for the first time. Results show that all the components are inhomogeneously distributed across the annual ring. Mineral nutrients including Na^+, K^+, Ca^+, and Cl^- ions exhibit no preferential localization between earlywood and latewood, whereas PO_3^- ion is exclusively present in the ray cells, indicating it may be related to acid phosphatase. Lipophilic extractives were found to be more abundant in the inner secondary xylem. Ion images with 400 nm spatial resolution reveal that fatty acids, triglycerides and phytosterols are co-localized principally in the earlywood within the first annual ring. Resin acids prove to be the main components in the resin canal of the secondary xylem and are distributed in the outer of it.

1. Introduction

Wood extractives are solvent extractable components in wood tissues, comprising of a variety of compounds such as fatty acids, sterols, phenols, and so on (Rowell et al., 2005). These extractives play an important role in decay resistance to termite, fungi, and environmental stress.

European larch (Pinaceae *Larix decidua* Mill.) is an important conifer species endemic to alpine forests in western and central Europe. In France, it is found mainly in Briançonnais, Queyras, Ubaye, Dévoluy, and Mercantour. The fast-growing nature and good durability make it a valuable timber tree of which the wood is widely used in carpentry, especially in making waterproof objects that can be used outdoors (Da Ronch et al., 2016). For example, traditional roofs in Briançonnais are made of larch shingles. It has been revealed that, as a property shared by many hardwood and softwood trees, the natural durability of larch wood is due to its decay resistance to microbial deterioration. The quantity and composition of heartwood extractives vary dramatically among different larch species, and there is a close correlation between extractive contents and natural durability (Windeisen et al., 2002; Gierlinger et al., 2004). In addition, heartwood extractives could also affect the wood quality including mechanical property (Grabner et al., 2005) and stability (Panday, 2005; Shebani et al., 2008).

Conventionally, the extractives were roughly classified into organic solvent extractives, water extractives, and phenolics, which were then determined by solvent extraction or more rapidly by Fourier transform near infrared (FTNIR) spectroscopy (Gierlinger et al., 2002). Nevertheless, precise characterization of the chemical composition of some larch species has also been performed with gas chromatography (GC) (Zule et al., 2015) and liquid chromatography (LC) (Ostroukhova et al., 2012)-mass spectrometry (MS). However, very few studies concerning the spatial distribution of the extractives have been carried out. Spatial distribution of the extractives is very important in understanding heartwood formation and it largely determines the wood quality (Taylor et al., 2002). Radial distribution of European larch extractives has been examined by Gierlinger and Wimmer (Gierlinger and Wimmer, 2004) through micro-sampling of wood blocks which were subsequently analyzed by FTIR. Their results have shown that in mature larch the amount of the wood extractives increase linearly from pith to the heartwood/sapwood boundary. Similar sampling method followed

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https://doi.org/10.1016/j.phytochem.2018.02.017
0031-9422/

by GC analyses was also applied to scrutinize the chemical compositional difference in earlywood and latewood of spruce (Bertaud and Holmbom, 2004) and radial distribution of extractives in scots pine wood (Ekeberg et al., 2006). However, this manual sampling method leads to limited spatial resolution and could not provide cellular localization of the extractives. The time consuming extraction with toxic solvents has also to be considered.

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is a surface analytical technique, which is recognized by its high spatial resolution. By dividing a certain analytical area into pixels and recording mass spectra from each pixel, TOF-SIMS provides simultaneously chemical and spatial information of the sample surface. Although traditionally employed for analysis of inorganic materials, TOF-SIMS has been gaining its reputation in biological imaging since the development of cluster ion beams (for example, Bi_3^+, Au_3^+, C_{60}^+) which has effectively improved the capability of providing direct molecular information up to *m/z* 1500 (Winograd, 2005; Brunelle et al., 2005; Bich et al., 2014). Concurrently, the number of studies using TOF-SIMS to map chemical constituents in wood tissues is also increasing. In addition to inorganic ions (Tokareva et al., 2007) and fragments of wood structural polymer lignin and polysaccharides (Saito et al., 2012; Jung et al., 2012), specific extractives such as diterpene phenol (Imai et al., 2005), hinokinin and its derivatives (Saito et al., 2008), and tryptamine (Vanbellingen et al., 2016) in various wood species have been mapped by TOF-SIMS.

In this study, TOF-SIMS was employed to investigate the spatial distribution of extractives in the branch wood of European larch (*Larix decidua* Mill.). Large area analysis of the xylem wood was performed to examine the radial distribution of wood extractives from the pith towards the secondary xylem. Meanwhile, cellular localization of the extractives in earlywood and latewood within different annual rings was revealed with a high spatial resolution of 400 nm. Different localization patterns were observed for mineral ions and organic extractives, whereas the extractive species are heterogeneously distributed in the secondary xylem.

2. Results and discussion

2.1. Large area imaging and distribution of mineral nutrients

To examine the radial distribution of wood extractives from pith towards the secondary xylem in the larch branch, a large area of 3000 μm × 500 μm was first mapped by TOF-SIMS. Although wood extractives generally refer to extractable organic components, mineral elements also constitute an important part of the extractives and different translocation patterns of mineral elements have been observed in heartwood formation (Taylor et al., 2002). Therefore, the mineral ions in the branch heartwood were also investigated in addition to the organic constituents. Fig. 1 illustrates the distributions of mineral ions in the xylem as well as in the pith. It is found that very little Cl^- and Na^+ are present in the xylem. Also interesting to note is that Cl^- is well co-localized with Na^+, indicating that Cl^- is mainly associated with Na^+ in larch wood, although K^+ turns out to be more abundant than Na^+ in the larch branch. Calcium shows similar distribution to K^+, however with lower intensity. Calcium has low mobility in plant tissues and plays an important role in physiological process and environmental response (McLaughlin, and Wimmer, 1999). Phosphate was exclusively detected in the ray cells and is probably related to acid phosphatase, which is believed to facilitate the transport of carbohydrates in wood tissues through the phosphorylation/dephosphorylation process (Sauter, 1972). The ion images demonstrate that except from the ray cell-specific phosphate, all the other minerals show heterogeneous distribution in the secondary xylem and no obvious preferential distribution between earlywood and latewood. In all the cases, minimum amount of mineral nutrients is present in the pith and primary xylem. Besides endogenous mechanisms (Chun and Hui-yi, 1992), variations in the concentration of the minerals across the annual rings may also reflect the environmental influence (Penninckx et al., 2001).

2.2. Radial distribution of lipophilic extractives

Organic extractives are more abundant than inorganic minerals in wood. They constitute an important proportion of wood chemistry and comprise a wide range of non-structural lipophilic compounds. To scrutinize the precise localization of the organic components, three different regions presenting wood tissue within different annual rings in the xylem were mapped with high spatial resolution of 400 nm (Fig. 2a). Fig. 2b shows TOF-SIMS mass spectra obtained from the three different analyzed areas in both positive and negative polarities. Similar to the inhomogeneous distribution of mineral nutrients, the comparison of spectra from the three different regions indicates the relative abundance of the organic extractive also varies dramatically throughout the xylem. The composition and radial distribution of fatty acids, glycerides, resin acids, and phytosterols will be discussed in detail in the following paragraphs. Table 1 summarizes the chemical species which are detected in the positive and negative ion mass spectra, with their measured and calculated mass-to-charge ratio, mass deviation in ppm, assignments and literature references. Assignments of fatty acids, diglycerides, and triglycerides were made according to the literature (Tokareva et al., 2007; Debois, 2008; Debois et al., 2009), and especially thanks to a study made by our lab of human liver steatosis (Debois, 2008; Debois et al., 2009). In addition, the ethyl acetate extract from the Larch sample was analyzed by LC-MS, helping the assignments of the fatty acid ion species. The corresponding chromatogram and extracted mass spectra are shown in Figures S1 to S7. Assignments of ions from resin acid and phytosterols were made according to the TOF-SIMS analyses of pure standards of abietic acid, campesterol, stigmasterol, and β-sitosterol. We did not analyze pure brassicasterol and cycloartenol, because the purchase of these was too expensive, even in very small quantities. The corresponding TOF-SIMS mass spectra are shown in Figures S8 and S9, respectively. The ion peaks at *m/z* 502.3 in the positive mass spectrum and *m/z* 501.4 in the negative spectrum were unable to be identified. In addition, the most abundant phenol compound taxifolin in the knotwood of *Larix decidua* (Kebbi-Benkeder et al., 2015) was not detected here, probably due to compositional variation in different anatomic plant parts.

2.3. Glycerides and fatty acids

Glycerides and fatty acids are common extractives present in various wood species. As shown in Fig. 2b, ion peaks corresponding to fatty acids were detected in the negative mass spectra: *m/z* 255.2 ($C_{16}H_{31}O_2^-$, palmitic acid), *m/z* 269.2 ($C_{17}H_{33}O_2^-$, heptadecanoic acid), *m/z* 277.2 ($C_{18}H_{29}O_2^-$, α-linoleic acid), *m/z* 279.2 ($C_{18}H_{31}O_2^-$, linoleic acid), and *m/z* 281.2 ($C_{18}H_{33}O_2^-$, oleic acid). Meanwhile, triglycerides were mainly detected as diglycerides fragments $[M-H_2O+H]^+$ in the positive mass spectra (Tokareva et al., 2007): *m/z* 573.5 ($C_{37}H_{65}O_4^+$, DG34:3), *m/z* 575.5 ($C_{37}H_{67}O_4^+$, DG34:2), *m/z* 577.5 ($C_{37}H_{69}O_4^+$, DG34:1), *m/z* 595.5 ($C_{39}H_{63}O_4^+$, DG36:6), *m/z* 597.5 ($C_{39}H_{65}O_4^+$, DG36:5), *m/z* 599.5 ($C_{39}H_{67}O_4^+$, DG36:4), *m/z* 601.5 ($C_{39}H_{69}O_4^+$, DG36:3), *m/z* 603.5 ($C_{39}H_{71}O_4^+$, DG36:2). Mass assignments of these fatty acid and diglyceride ion species were made thanks to previous analyses of lipids in human liver (Debois, 2008; Debois et al., 2009). Fig. 3 displays the radial distribution of fatty acids and triglycerides in the xylem (the positive and negative total