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TECHNICAL NOTE

Correlation of carbon isotope ratios in the cellulose and wood extractives of Douglas-fir

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Abstract

Cellulose is usually isolated from the other components of plant material for analysis of carbon stable isotope ratios ($\delta^{13}C$). However, many studies have shown a strong correlation between whole-wood and cellulose $\delta^{13}C$ values, prompting debate about the necessity of cellulose extraction for tree-ring studies. The $\delta^{13}C$ values were measured in whole wood, extractive-free wood, purified cellulose, acetone/water-soluble extractives and hot water-soluble extractives of Douglas-fir sapwood. Cellulose and acetone/water-soluble extractives from heartwood from the same trees also were compared. Although the various materials showed different absolute $\delta^{13}C$ values, the components of the same samples, including the extractives, were correlated. The correlations of carbon isotope ratios of cellulose, extractive-free wood and extractives, and the relatively low concentration of the extractives in the wood, suggests that extraction of purified cellulose from Douglas-fir wood samples may not be necessary for some tree-ring analyses.

Keywords: Sapwood; Heartwood; *Pseudotsuga menziesii*; $\delta^{13}C$; Extraction necessity

Introduction

Wood cell walls are composed of cellulose, hemicelluloses and lignin, which are immobile compounds, but xylem also contains extractives. These substances are soluble in organic solvents or water and are mobile, potentially allowing for movement across ring boundaries (Sjostrom, 1993). Purified cellulose, isolated from wood and other plant materials, is a standard material used in analyses of variations in carbon stable isotope ratios ($\delta^{13}C$). The analysis of a single stable compound such as cellulose is preferred to that of a whole tissue because the variation in the $\delta^{13}C$ of a single compound is likely to reflect conditions of photosynthesis during the year of formation rather than be influenced by compound mixtures with varying isotopic signals or mobile elements (Leavitt and Long, 1989; Leavitt and Danzer, 1993).

Fractionation of carbon isotopes occurs during photosynthesis in plants (O'Leary, 1993) and the isotopic signature is preserved in the products of photosynthesis and the materials derived from them. However, cellulose and other wood components such as hemicellulose and lignin made in the same year do not have the same $\delta^{13}C$ value because of differences in ^{13}C discrimination along their biosynthetic pathways (Schmidt and Gleixner, 1998). Nevertheless, carbon

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isotope values of these substances can be correlated over time (Damesin and Lelarge, 2003; Loader et al., 2003; Helle and Schleser, 2004; Scartazza et al., 2004) if the differences in discrimination along the various biosynthetic routes are consistent. Alternatively, intra-annual variations in photosynthetic conditions may lead to differences between cellulose and other wood components if these substances are formed from photosynthate at different times of the year.

Extractives in an individual wood increment may differ isotopically from the cellulose if they were translocated to that wood increment at some time after the wood was formed or if there are differences in biosynthetic pathways. Extractives in sapwood, often starch, simple sugars or lipids, are generally considered to be energy reserve materials for the tree (Hillis, 1987; Taylor et al., 2002). There is evidence that sapwood extractives are used and replenished on an annual basis in some species (e.g. Barbaroux and Breda, 2002), thus one could expect there would be little correlation between the carbon isotope ratios of cellulose and sapwood extractives. Energy reserves are absent from heartwood and heartwood extractives, such as phenols and terpenes, are thought to act as part of a passive defensive system (Hillis, 1987). Sapwood (carbohydrate/lipid) extractives are believed to be converted to defensive compounds during heartwood conversion. Additionally, heartwood extractives can be formed in part by materials translocated from the cambium (Hillis and Hasegawa, 1963). Thus, heartwood extractives could be expected to differ isotopically from the cellulose of the wood in which they are located.

Despite potential isotopic differences between wood extractives, cellulose and other cell wall components, some researchers have analyzed whole-wood samples instead of cellulose after finding a close correlation between whole wood and cellulose (Livingston and Spittlehouse, 1993, 1996; Warren et al., 2001). This correlation suggests that extractives and other compounds in whole wood have similar annual isotopic variation as the cellulose. Our objective in this study was to directly examine the isotopic composition of the extractives and to compare them with that of whole wood, extracted wood and cellulose to help researchers determine if time-consuming cellulose extraction is necessary for isotopic analysis of tree rings.

Materials and methods

Plant materials

Douglas-fir trees were sampled from one site located in the Coast Range in western Oregon, USA (latitude 44°38′N, longitude 123°12′W, elevation 75 m) designated as Site Class III (McArdle et al., 1961). The site receives about 1500 mm precipitation per year, with about 85% falling between October 15 and April 15. There is no surface water on the site. The climate is mild, with fewer than 30 freezing days in winter, and fewer than 15 days in which temperature exceeds 32 °C. Soils are of the Ritner-Price series, with gravelly clay in the top 60 cm, rooting depth about 90 cm, and Siletz River basalt as parent material. Water stress can be severe in late summer. The primary anthropogenic influence relates to large-scale wildfire in the period prior to 1860, followed by grazing until the 1930s. The sample trees were part of a study of enhancement of mature forest characteristics in second-growth Douglas-fir. The stand had been thinned at intervals in the past, including in 1992 and 2000. Following the 2000 thinning, six basal disks (30 mm thick) were removed from the randomly selected, freshly cut stumps about 300 mm above the ground line. At the time the samples were taken, the stand was 58 years old. There had been no previous tree-ring analysis of trees in the stand. The disks were air-dried in the lab. The heartwood/sapwood boundary was located by staining with alizarin red (Kutscha and Sachs, 1962). The sapwood and heartwood were analyzed separately, as described below.

Sapwood analysis

A radial strip, 50 mm wide (tangential) by 30 mm thick (longitudinal), was cut from each disk using a band saw. Working from the cambium inward, groups of three consecutive annual rings were separated from the strip using a chisel. Rings were grouped into sets of three to provide sufficient material for each of the analyses. Four sets of rings were taken, in the same manner, from each disk-comprising 12 sapwood annual rings in total, the most recent of which was formed in 2001. Wood from each three-ring set was analyzed for the carbon isotope composition of the whole-wood, acetone/water-soluble extractives (dihydroquercetin glucoside, procyanidins and pinoresinol), the hot water-soluble extractives (carbohydrates such as the simple sugars glucose and xylose), the extracted wood and the α-cellulose.

The 3-year ring samples were chiseled into match-stick-sized pieces and reduced to powder by grinding them in a ball-type tissue pulverizer mill (Kleco 4100, Garcia Manufacturing, Visalia, CA) for 2 min. Samples of the wood powder (~2 g oven-dried) were weighed and enclosed in heat-sealable polyester filter bags (mesh size 25 μm, ANKOM Technology, Macedon, NY). The bags were extracted with 25 ml per bag of a 70:30 mixture of acetone and water (Dellus et al., 1997) for 72 h on a rotary shaker table (100 rpm). The extractive solutions were not cloudy and there was no visual evidence that