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Contribution of tyloses quantification in earlywood oak vessels to archaeological charcoal analyses: Estimation of a minimum age and influences of physiological and environmental factors

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Among the main parameters contributing to the characterization of firewood exploitation modes, the age of the collected wood is very difficult to assess in anthracology. In this paper, we propose an original way to investigate the age of the exploited wood, the heartwood formation process which takes place in 20–25-year-old deciduous oak trees. The formation of tyloses in earlywood vessels is an important feature of the changeover of sapwood to heartwood. However, tylosis formation also occurs in sapwood. Therefore, the observation of the mere presence of tyloses in vessels, as routinely performed in anthracology, is not sufficient to distinguish sapwood from heartwood. The aim of this study was thus to use the proportion of vessels sealed by tyloses as a discriminating marker between sapwood and heartwood applicable to anthracology in order to characterize firewood exploitation in the past. The trunks and branches of ten deciduous oak trees ranging from 15 to 60 years old were sampled in three French sites. For an application to archaeological charcoal (tyloses are preserved until 800 °C), thresholds of less than 65% for sapwood and up to 85% for heartwood appear to be significant at the population level for deciduous oak (*Quercus petraea/robur*) in a temperate climate. Besides the indication of the minimal age (*ca.* 25 years old in the case of deciduous oak), the variability of the proportion of vessels with tyloses in sapwood is explored as a good indicator of the vitality of the wood which opens new prospects for the exploitation of dendro-anthracological parameters, such as the discrimination of branch wood.

1. Introduction

The gathering of domestic firewood is a daily activity and depends on social and economic contexts (see for example Asouti and Austin, 2005; Picornell et al., 2011; Salavert and Dufraisse, 2014). Archaeological charcoals are a reflection of firewood gathering practices and woodland management, which are partially conditioned by the environment. However, the reconstruction of woodland management is still restricted as charcoal analysis is most often limited to the study of a list of taxa and their relative frequency without exploiting the dendrological information (i.e. woodland growth conditions) contained in the anatomy of the wood. Thus, practices of wood gathering and woodland management are often discussed even though direct evidence remains rare, in particular for Pre- and Protohistoric periods (Vernet, 1992; Thiébault, 2002; Fiorentino and Magri, 2008; Badal et al., 2012; Damblon, 2013).

In the 1990s, anthracology was enhanced by the development of image analysis techniques and new approaches based on wood anatomy. Charcoal identification, based on cellular structure, was associated with measurements of growth-ring width, charcoal-pith distances (i.e. the distance between the outmost ring boundary and the missing pith, see Dufraisse et al., accepted), size and number of vessels, etc. New characteristics of the collected woods and management techniques are now discussed, such as woodland structure and density, and competition dynamics (Marguerie and Hunot,

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2007; Cabanis and Marguerie, 2013), diameter of firewood (Lundström-Baudais, 1986; Ludemann and Nelle, 2002; Dufraisse, 2006; Dufraisse and Garcia Martinez, 2011; Paradis-Grenouillet, 2012), woodland regeneration (Deforce and Haneca, 2015; Girardclos et al., accepted) and domestication of some arboreal species (Terral, 2000; Terral and Durand, 2006).

Among the main parameters contributing to the characterization of modes of wood exploitation, the age of the collected wood is still very difficult to assess in anthracology. Unlike dendrochronology, anthracological assemblages represent a fragmented and incomplete material (mass loss due to carbonization) and they represent the exploitation of numerous trees, trunks and/or branches. A way to investigate the age of the exploited wood is the heartwood formation process which takes place in mature trees. Most mature trees contain a central core of heartwood which is usually significantly darker in colour than the surrounding sapwood (Hillis, 1987 pp. 21). The light outer sapwood is the living xylem, most recently produced by the cambium; it participates in water conduction and the storage of starch and other substances. Heartwood is the oldest part of the wood and its formation is the normal consequence of xylem senescence. It results from physiological death due to cytological and physiological factors (Tyree and Zimmermann, 2010, pp. 264).

In some species the coloration of heartwood due to the deposition of lignins and polyphenols makes heartwood recognizable, but the charcoalification process which occurs during carbonization obliterates the colour difference, making colour unusable in anthracology. Fortunately, in many angiosperm genera, the formation of tyloses in earlywood vessels is an important feature of the changeover of sapwood to heartwood initiated by the loss of water in vessels. Tyloses are cells that grow in the vessel lumen from neighbouring parenchyma cells (Tyree and Zimmermann, 2010, pp. 260). They are easily detectable in anatomical analysis and preserved after carbonization at temperatures of less than 800 °C (Kim and Hanna, 2006).

However, tylosis formation also occurs in sapwood. This is one of the means by which the plant seals off injured xylem caused by frost, drought and pathogens (Cochard et al., 1992; Granier et al., 1994; Fromm et al., 2011). Thus, tylosis formation occurs in sapwood and increases with the formation of heartwood, from 0% of tyloses in the cambial region and close to 100% in the heartwood Bakour (2004). Therefore, the mere observation of the presence of tyloses in vessels, as is usual in anthracology, is not sufficient to distinguish sapwood from heartwood. To our knowledge, only one study focuses on the number of oak earlywood vessels with and without tyloses (Babos, 1993).

This study aims to use the proportion of vessels sealed by tyloses as a new dendro-anthracological parameter for decoding firewood exploitation, by establishing discriminating thresholds between sapwood and heartwood applicable to anthracology. We chose to focus this original study on deciduous oaks (*Quercus petraea/robur*), known to produce tyloses sealing the vessels during heartwood formation in 20–25-year-old trees (Lambert, 1996; Dhôte et al., 1997). These taxa were chosen for their abundance in temperate forests and their representativeness in charcoal spectra. Besides the estimation of a minimal age of the wood, the variability of the proportion of vessels sealed by tyloses in sapwood is explored in relation to physiological and ecological factors with the perspective to use it as an indicator of tree vitality.

2. Material and methods

2.1. Regional setting

The location characteristics of the sampled material are summarized in Fig. 1. The "Les Cagouillères" station is located in the Vienne department, on a limestone plateau. The studied stand is an old abandoned coppice woodland, currently undergoing conversion to high forests. The trees are about 60 years old and the number of stems per hectare amounts to 1300. The "Le Bois de l'Or" station is located in the Ardennes department, near Bogny-sur-Meuse on a brown soil on schists. This is a young stand, about 15 years old, formed by a mixture of self-seeded and coppice trees. The "Chantrans" station is located in the Doubs department on a brown limestone soil. This is an old abandoned coppice, where the 50-70-year-old trees are currently undergoing conversion to high forest.

2.2. Sampling

The reference data are made up of 3 or 4 dominant trees per station. Discs were sawn at the base of the trunk, 1.30 m from the ground, at the bottom of the trunk just below the crown, and in the branches (Fig. 2). In each disc, 2 cm side cubes were removed from each different zone (sapwood, heartwood and transition zone) in two directions, that is to say 6 cubes per disc when heartwood is formed and 2 cubes per disc when the formation of heartwood is not yet initiated. Wood tension and zones of pathogen attack were avoided. A total of 20 discs corresponding to 63 cubes were analyzed. Each tree ring was associated with a sapwood, heartwood or transition zone on the basis of the colour observed on fresh wood. The cubes were placed in aluminium, buried in sand to create anoxic conditions and then carbonized in a muffle furnace at 400 °C for 30 min. Vessels were counted on fresh fractures by observing the transversal section under a reflected light microscope, as is customary for charcoal identification.

2.3. Measurements and data treatment

The number of vessels with and without tyloses was counted for all the cubes, considering each tree ring independently and noting and measuring cambial age, width and proportion of latewood. Only the vessels that formed continuous lines in the earlywood were considered. Three sets of 15 vessels were counted in each ring to estimate the minimum number of vessels and the variability within a ring. Furthermore, for 26 cubes, all the vessels were taken into account to estimate the optimal number of vessels.

In order to estimate the minimal and optimal number of vessels to count, the proportion of vessels with tyloses was expressed using the calculation of a margin of error calculated between the final and the intermediate percentages of sealed vessels. When the margin of error reaches 0, relative frequencies are stabilized.

The selected variables for statistical analysis are the "site", "tree" and "tree-ring" descriptors. The data were processed with the programming language R (version 3.2.2, R Foundation for Statistical Computing, Vienna, Austria) using descriptive and multivariate statistics on the basis of a 5% significance level.

3. Results and discussion

3.1. Minimal number of vessels and saturation curves

The size of the archaeological charcoal fragments is often small, less than 1 cm, and generally comprised between 2 and 5 mm. In order to assess the potential application of this quantification approach on archaeological charcoal, the minimum and optimal numbers of vessels to count were first estimated.

Regarding the cubes for which 3 sets of 15 vessels were counted, the percentage of sealed vessels between each set is not significantly different (Kruskall-Wallis, p-value:< 2.2e−16) (Fig. 3). This indicates low spatial variability for the percentage of sealed vessels