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ASSESSMENT OF ANATOMICAL CHARACTERISTICS OF WOOD –BARK, SAPWOOD AND HEARTWOOD – IN HARDWOODS SPECIES OF GALICIAN OAKS BY IMAGE PROCESSING: RELATIONSHIP WITH AGE

Diaz-Maroto, I.J., Vila-Lameiro, P.
Universidad De Santiago De Compostela, Escuela Politécnica Superior
Lugo, España

Tahir, S.
École Superieure Du Bois
Nantes Cedex 3, France

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ABSTRACT

Our work on the physical properties of wood in hardwood species, *Quercus robur* L., *Q. petraea* (Matts) Liebl., and *Q. pyrenaica* Willd., for study its use in cooperage, began with the proportion appraisal of bark, sapwood, and heartwood, and its relationship with age, to know and assess the variation thereof in the Galician oaks. To properly perform our study, it was necessary to fell several *Quercus* trees within the study area (Galicia, NW Spain). In total, 45 trees were selected in 15 different stands located in the Galician provinces of Lugo and Ourense, from which we obtained 45 slices of wood at 60 cm height on the tree trunk. There are many anatomical differences between hardwood and softwood species; however wood parts of a tree system are common to both. The analysis of different proportions of bark, sapwood, and heartwood made it possible to reveal: i) the rapid growth of *Quercus pyrenaica* gives rise to the formation of large proportion of heartwood in a few years; ii) *Quercus petraea* has the largest proportion of heartwood, but the studied trees are older and their growth is slower than the other species; iii) *Quercus robur* has an intermediate growth between the other two species. Therefore, our aim was to carry out a complete description of these parts of the wood, later, in upcoming work estimate their physical properties for use in cooperage industry

KEYWORDS: *Quercus* spp., wood, features, digital images, cooperage industry.

INTRODUCTION

Hardwood trees contrast with softwood trees and they are not necessarily harder than them (Hoadley 1990, Alden 1995). Notably, this nomenclature does not necessarily reflect the actual wood properties; "balsa" (a hardwood) is much softer than average softwood (Plomion et al. 2001). There are many anatomical differences between them; however the wood parts of the tree system are common (Alden 1997, Zhang et al. 2014).

The growth of the tree trunk is achieved by two types of processes. The first is caused by the terminal bud ("apical meristem") and is responsible for the rising, common primary growth for all vascular plants. In contrast, to non-tree plants, they also have a secondary growth, which means that their trunks are becoming thicker (Ramagea et al. 2017). This growth is determined by the proliferative activity of the vascular cambium, technically speaking, the cambium is only one cell thick that produces bark on one side and wood on the other (Schweingruber 1990, University of Kentucky 1997). The young xylem is the tissue that conducts water (sapwood), and if the tissue dies and wood cells become hollow, forms the heartwood (Myburg et al. 2013). As the new rings of sapwood are laid on top, the older sapwood losses its vitality and turns into heartwood (University of Kentucky 1997, Hacke and Sperry 2001). Accumulating extracts gives the heart of many species of hardwood a darker colour than the sapwood (Plomion et al. 2001). On the oaks, the heartwood can be seen easily thanks to its dark coloration (Bary-Lenger and Nebout 1993, Vila-Lameiro and Diaz-Maroto 2005).

Bark is an important part in the tree growth. The bark protects the tree from the outside and against extreme weather elements (Schweingruber 2007). The phloem acts as the pipeline through which the food produced in the leaves is taken to the rest of the living tree. This section of cells lives for only a short time before it becomes part of the bark (Dinwoodie 2000).

Trees produce annual rings that reflect the changing environment. Rapid growth during the spring produces "earlywood" which is less dense and composed of large cells with thinner walls allowing for efficient water transport to support photosynthesis. This period is followed by slower growth, yielding "latewood", characterised by more densely packed, smaller cells, production of which stops for winter (Ramagea et al. 2017). In softwoods, the transition can be gradual, distinct, or a combination of each. In hardwoods, vessels may have a different size in early- and latewood (Vila-Lameiro and Diaz-Maroto 2005).

The potential vegetation that should cover most of Galicia would consist of a mixed hardwood forest with deciduous species dominance of *Quercus* species (Buide et al. 1998). Nowadays, the Galician oaks contain a great variety of ages and qualities, as a consequence of the diverse uses and conservation grade (Diaz-Maroto et al. 2005, 2006a). Oak forests occupy an area of 246,445 ha in Galicia, which corresponds to 18% of the forestry area (MAGRAMA 2011). The oak wood is little used by the forest industry, the existing small holdings in Galician land does not facilitate its industrial development. Oak forests management is virtually nonexistent and the oak use is very limited, mostly for firewood (Siry et al. 2005, Diaz-Maroto and Sylvain 2016).

On the other hand, today Galicia is a region with a high production of quality wines. It has five Origin Denominations (OD). Topography, climate, soils, and even the Galician landscape have contributed to the current quality of its wines. The wine producers are passionate about their land (Diaz-Maroto and Sylvain 2016). Wine producers with a greater tradition prefer not to make aging in French oak barrels and/or American because the aromas of wood are not Galician (Alanon et al. 2011a). In many cases, use the old French oak barrels in which the wood aromas have disappeared a long time, so the wine retains its authentic "aroma" of Galicia. Maybe, the best solution of this dilemma would be to use oak Galician barrels; the wine would keep its local origin and benefit of wood aromas. Therefore, using Galician oaks for manufacturing barrels would be a way to develop an important cooperage industry (Alanon et al. 2011b).

The aim was to carry out a whole description of the the bark, sapwood and heartwood of three species native oaks of Galicia (*Quercus robur*, *Q. petraea* and *Q. pyrenaica*), and its relationship with age for the manufacture of wine barrels, and later to properly estimate their physical properties for its use in cooperage.

MATERIAL AND METHODS

Study area, sampling and data measured

Galicia is located in NW Spain; it has an average altitude of 508 m and slopes bigger than 20% in more than half of its land (Fig. 1). There are a dominance of siliceous substrates, granite, schist, slates, quartzite; the climate is humid oceanic with Mediterranean influence in some areas. Annual precipitation varies between 600 to more than 3000 mm and the average temperature is next to 13ºC (Diaz-Maroto et al. 2006a). The study area was considered as a single unit, where zones for sampling were selected, taking care to include a representative number of oak stands with a minimum area ranged between 0.5 and 1 ha, which avoids edge effect problems. In total, 45 trees were selected in 15 different stands located of Galician provinces of Lugo and Ourense, from which we obtained 45 wood slices at 60 cm height on the tree trunk.

Fig. 1: Galicia location and its four provinces (A Coruña, Lugo, Ourense and Pontevedra) in north-western Spain in relation to its global context.

Given that 69% of the Galicia land is 600 m above sea level, many of the stands have steep slopes. This is a problem because a strong slope encourages the creation of tension wood with a heart outside centre, which is not suitable for the manufacture of barrels (Bary-Lenger and Nebout 1993, Lehringer et al. 2008). Tab. 1 shows the main characteristics of the trees felled (from normal tree to heart strongly off centre).

Tab. 1: Main characteristics of the trees felled and the wood slices.

Species	Normal tree	Heart a little off centre	Heart off centre	Heart strongly off centre	Total
Quercus robur	7	4	4	2	17
Quercus petraea	6	--	8	1	15
Quercus pyrenaica	5	2	3	3	13
Total	18	6	15	6	45

Software used in image analysis

Using a ruler with millimetre appreciation is possible to determine the length of the different parts of the tree trunk (Bakour 2003). However, we recommend working with some software, e.g. *ImageJ*, given its important advantages; it is a public domain image processing program. With ImageJ, it is possible estimate the area of each part of the tree trunk (bark, sapwood and heartwood) as well as their relative proportion. The method precision is greater, being possible to use a bigger number of samples considering the singularity of each one. *ImageJ* was designed with an open architecture and is multithreaded, so time-consuming operations such as image file reading can be done in parallel. It supports standard image processing functions, contrast manipulation, sharpening, smoothing, edge detection and median filtering. Spatial calibration is available to provide real world measurements in mm (Ferreira and Rasband 2012). Age measurement also was performed using *ImageJ* with a scanning resolution of 600 dpi. Finally, to study the possible linear relationship, two by two, between the different variables we used the Pearson correlation coefficient (Field et al. 2012).

Methodology description: Steps of the process

Photographs of wood slices were taken with a digital camera with macros, which uses a special structure that ensures best possible light and the distance between the camera and the wood samples is always the same. The accuracy of the image is optimal. Also, we have scanned all the samples in four resolutions: 300, 600, 900 and 1200 dpi, in the case where an analysis more precise will be necessary. Indeed, after the cutting of the samples, it is impossible to work on the surface of the wood slices because its quality is variable and depends of the plane we have used (Fig. 2).

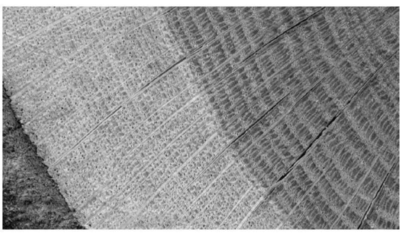


Fig. 2: Scanned image with a goodly quality of the surface of Q. pyrenacica sample.

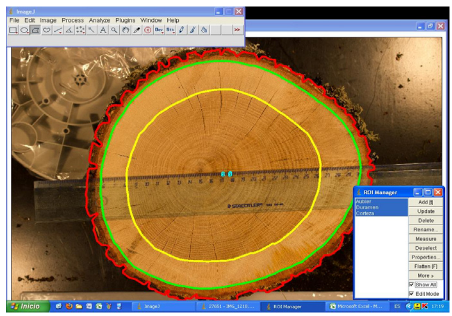


Fig. 3: Area of the heartwood (yellow line), sapwood (green line) and bark (red line).

The first step is to define the spatial scale of the image. In our case, the unit used has been the centimetre. Previously, reference must be selected as a known distance in a horizontal line. *ImageJ* automatically fills the distance in pixels depending on the selected longitude (Ferreira and Rasband 2012). Then we use the polygon tool to make selections irregularly defined by a segment series. To create a polygon we need to select line segments that correspond to the border of the different areas. *ImageJ* has automatic selection, Wand Tool. We can use the tolerance of this tool to define the selection border automatically, mainly for the bark, since its perimeter is irregular, and sometimes it's difficult to see clearly its limit. The sapwood area could be calculated by subtraction of the heartwood, and the bark area could be calculated by subtraction of the sapwood (Fig. 3).

Accuracy and precision

The measurement system accuracy is the degree of closeness of measurements of a quantity to that quantity's true value. The precision is the degree to which repeated measurements under unchanged conditions show the same results (CTI Reviews 2016). Although both words could be synonymous, there is an important contrast between them. A measurement system is designated valid if it is both accurate and precise (Taylor 1997, JCGM 2008).

RESULTS AND DISCUSSION

Measurement of the age of wood slices

The selection program of the samples follows the criteria following:

- Three species: *Quercus robur*, *Q. petraea*, and *Q. pyrenaica*
- Two wood types: sapwood and heartwood; additionally the bark (Fig. 3)
- Four tree types and wood slices: heart a little off centre, heart off centre, heart strongly off centre, and tree with a normal heart (Tab. 1)

The measurement of age was done with the *ImageJ* software with a scanning of a resolution of 600 dpi, since it offers a special pointer to assign a number to each ring of growth (Fig. 4). On the whole, the determination of the age was moderately easy. However, the poor state of samples of *Quercus petraea* (rotting) forced us to make assumptions about the final age. In addition, some samples have episodes of very low growth, a characteristic found in many hardwood trees in the study area. In these cases, the latewood is almost absent and it is very difficult to identify the separation of each growth ring (Meier 2015).

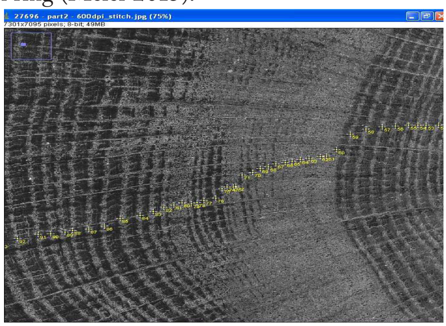


Fig. 4: Marking with yellow numbers corresponding to each ring of growth.

Variability of the bark, sapwood and heartwood in function of the age

Tab. 2 shows the area of the bark, sapwood and heartwood for each species. The average age of the trees and the age of the specific wood types are also shown to facilitate global interpretation.

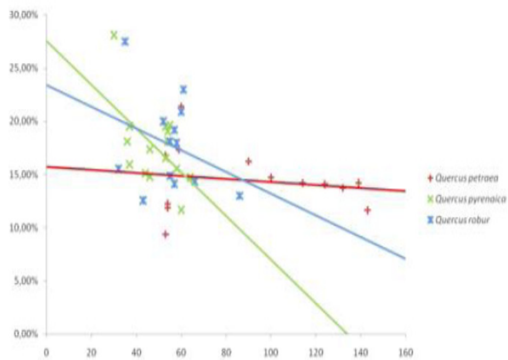
Tab. 2: Area of the bark, sapwood and heartwood for each species and average age in function of the species and the wood types.

Species	Number of trees	Type of wood	Average (%)	Standard deviation (%)	Coefficient of variation	Accuracy (%)	Minimum (%)	Maximum (%)	Average age	Age of sapwood	Age of heartwood	Annual growing (mm·year^{-1})
Quercus robur	17	B	22.9	14.3	62.7	1.4	12.0	30.3	55	20	35	5.1
		S	41.4	13.3	32.1	1.6	26.9	69.3	55	20	35	
		H	38.3	11.1	29.0	0.8	18.2	59.0	55	20	35	
Quercus petraea	15	B	16.7	7.1	42.2	1.2	8.8	22.0	90	23	68	3.3
		S	31.4	11.4	36.3	1.6	20.0	53.7	90	23	68	
		H	48.4	12.8	26.4	1.6	28.7	66.4	90	23	68	
Quercus pyrenaica	13	B	17.6	3.7	20.9	1.5	9.8	29.0	48	20	29	5.6
		S	44.7	13.6	30.5	0.7	12.9	60.6	48	20	29	
		H	37.7	14.6	38.8	1.3	11.7	70.1	48	20	29	

B: Bark, S: Sapwood, H: Heartwood

Quercus robur has a higher proportion of bark (22.9%) than *Quercus petraea* (16.7%) and *Quercus pyrenaica* (17.6%), but the high coefficient of variation (CV) associated with this value (62.7%) moderate this assertion (Tab. 2). Also, as expected, the accuracy of bark measurements is relatively low because of its extreme irregularities. Age does not seem, in this case, to have a significant influence in the bark proportion (Fig. 5); the values of the Pearson correlation coefficients are too small to conclude. On the contrary, the Fig. 6 shows a significant relationship between age and the proportion of the heartwood. The great Pearson correlation coefficients and the linear shape of the values check this assertion.

The relationship of the heartwood age, i.e. the number of rings in the heartwood area, and the proportion of heartwood makes it possible to obtain the best Pearson correlation coefficients for each of the studied species. The slope differences of the lines are due to different annual increase (Fig. 7). For example, the wood slices of *Quercus petraea* obtained of a mountain area with an altitude of 1200 m with steep slopes (Diaz-Maroto et al. 2006b), where growing conditions are difficult affect the width of the growth rings: with only 4 mm of average; this species has the lowest average width of growth ring (Diaz-Maroto and Sylvain 2016). A lower width in oak species results in a lower texture (Vila-Lameiro and Diaz-Maroto 2005); the proportion of earlywood is more important than the latewood (Pasztory et al. 2014).

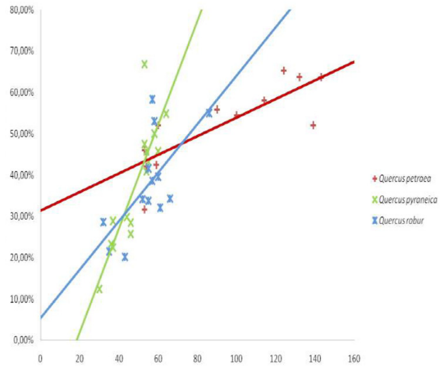


Quercus robur: y = -0.0010x + 0.2341; R2 = 0.1033
Quercus petraea: y = -0.0001x + 0.1574; R2 = 0.0300
Quercus pyrenaica: y = -0.0021x + 0.2754; R2 = 0.3122

Fig. 5: Evolution of the proportion of bark (%) (y-axis) versus age in years (x-axis).

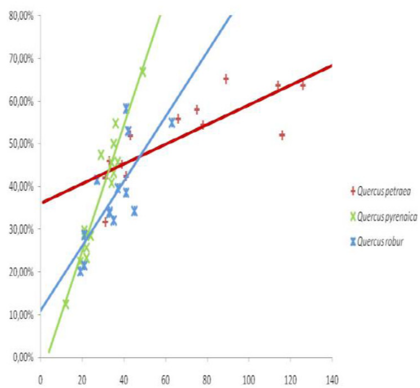
Ages does not seem, in this case, to have a significant influence on the sapwood proportion, the linear regression does not work very well and the values of the Pearson correlation coefficients are too small to conclude a correlation between the proportion of sapwood and age in any of the oak species studied.

This situation does not mean that there is not link between sapwood proportion and age (Lévy et al. 1992, Lebourgeois et al. 2004, Pasztory et al. 2014); the only conclusion is that these data are not able to reveal it. Probably, the variation of the values is too high: the coefficients of variation are superior to 30% for all tree species - *Quercus petraea* has a higher CV (36.3), followed by *Q. robur* (32.1), and *Q. pyrenaica* (30.5) - (Tab. 2), because our range of age is probably too small for *Quercus pyrenaica* (only 30 to 64 years) (Fernandez-Parajes et al. 2005).



Quercus robur: y = 0.0059x + 0.0533; R2 = 0.4555
Quercus petraea: y = 0.0023x + 0.3134; R2 = 0.6830
Quercus pyrenaica: y = 0.0126x – 0.2313; R2 = 0.7356

Fig. 6: Evolution of the heartwood proportion (%) (y-axis) versus age (years) (x-axis).



Quercus robur: y = 0.0076x + 0.1107; R2 = 0.5826
Quercus petraea: y = 0.0023x + 0.3612; R2 = 0.6446
Quercus pyrenaica: y = 0.0147x – 0.0450; R2 = 0.9197

Fig. 7: Evolution of the heartwood proportion (%) (y-axis) versus heartwood age (years) (x-axis).

Finally, we note that the accuracy of the heartwood area for *Quercus petraea* and *Q. pyrenaica* is less accurate than expected (> 1%) (Tab. 2). This situation can be explained by the presence of heartwood area without accurate limits. This is not the case on the wood slices of *Quercus robur* (Vivas 2000, Pot et al. 2013).

CONCLUSIONS

The assessment of the different proportions of bark, sapwood and heartwood of the Galician oaks and its relation with the age has allowed knowing the following aspects:

- The proportion of heartwood is positively proportional to the age of the tree.
- When a tree grows quickly, the proportion of heartwood is higher at the same age than in other trees.
- The rapid growth of *Quercus pyrenaica* causes the formation of large proportion of heartwood in a few years.
- In the samples studied, *Quercus petraea* has the largest proportion of heartwood, but the samples are older and their growth is slower than the other species.
- *Quercus robur* has an intermediate growth between *Quercus pyrenaica* and *Q. petraea*.
- In the Galician oaks, the heartwood proportion is an average between 21.8 and 62.8% (maximum and minimum values of the average ± standard deviation ± accuracy). However, the knowledge of the tree age and the heartwood age makes it possible to improve this range by using the obtained models.
- On the contrary, data from this study do not make it possible to respond to the existence of a relationship between age and proportion of bark and heartwood.

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Diaz-Maroto, I.J.*, Vila-Lameiro, P.
Universidad De Santiago De Compostela
Escuela Politécnica Superior
E-27002 Lugo
España.
Corresponding author: ignacio.diazmaroto@usc.es

Tahir, S.
École Superieure Du Bois
Rue Christian Pauc,
F-44036 Nantes Cedex 3
France