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CHEMICAL CHARACTERIZATION, HARDNESS AND TERMITE RESISTANCE OF *Quercus Cerris* HEARTWOOD FROM KOSOVO

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In memoriam of Dr. Thomas C. MANNES

ABSTRACT

Quercus cerris, the Turkey oak, is an oak species native to southern central and southeastern Europe, extending into southwestern Asia. It is present in a large extent in the forests of the Republic of Kosovo, and at present used mainly for bio-energy. The potential use of *Quercus cerris* wood for construction and higher value wood products has been investigated in the last years. However limited information is available on wood characteristics and performance, mainly regarding chemical composition and durability. The heartwood of *Quercus cerris* taken from 70-90 year-old trees grown in two sites in Kosovo was studied regarding resistance to termite attack, chemical composition and hardness. The heartwood contained only 6,7% extractives, with a small content of tannins. The wood density was on average 0,81 at 12% moisture content and Brinell hardness 36,2 MPa. It was classified as not durable against subterranean termites and therefore not suitable for external use in ground contact in termite areas unless adequately protected. Nevertheless, *Quercus cerris* wood showed adequate hardness and density for interior uses like flooring for domestic and commercial applications with moderate use.

Keywords: Brinell hardness, chemical analysis, density, *Reticulitermes grassei,* wood extractives.

INTRODUCTION

For centuries, European oaks have provided highly valued timber for carpentry, furniture making, construction and ship building. However, except for a few of the oak species e.g. *Quercus robur* and *Quercus petreae*, the knowledge on the technological properties of other species is still sparse. This is the case of *Quercus cerris*, the Turkey oak, a species native to southern central and southeastern Europe, extending into southwestern Asia (Danielewicz *et al.* 2014).

Quercus cerris is present in the forests of the Republic of Kosovo, which cover 42% of the country area and harbor a great potential in biomass for energy purposes and good quality timber for construction mainly from European beech (*Fagus sylvatica*) and oaks, with relevance to Turkey oak (*Quercus cerris*) (Bajraktari *et al.* 2014). *Quercus cerris* is one of the most used wood species in the country although mainly for bio-energy (Bouriaud *et al.* 2014).

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The possibility of using *Quercus cerris* wood for construction and in higher value wood products has been investigated in the last years (Bajraktari *et al.* 2014, Standfest *et al.* 2012a, Standfest *et al.* 2012b). The potential of *Quercus cerris* bark as a cork provider was also studied especially regarding one variety in Turkey, *Quercus cerris x cerris*, that has a thick bark with high cork content (Sen *et al.* 2011, Sen *et al.* 2016). Several authors have attempted wood modification processes (heat and steaming treatments) to improve the wood dimensional properties and to homogenize surface color (Todaro *et al.* 2012, Todaro *et al.* 2013). Other wood properties were also investigated e.g. density and moisture (Monaco *et al.* 2011), and bending strength (Karastergiou *et al.* 2005). However, there is still limited information on *Quercus cerris* wood characteristics and performance, mainly regarding chemical composition and durability.

The biological deterioration of applied timber is often viewed as a limiting factor for its improved used in building. The natural durability of a wood species, defined as its inherent resistance to wood destroying agents, can vary widely depending on tree age, geographical origin, and growing conditions. Decay resistance also varies within the stem e.g. it tends to increase radially from pith to the heartwood-sapwood boundary and longitudinally from crown to base (Stirling *et al.* 2015), and is typically connected to the wood chemical composition, in particular, with the extractives present (Daniels and Russell 2007, Gierlinger *et al.* 2003, Pâques and Charpentier 2015). Nevertheless, the reasons of resistance to decay may not be directly translated into resistance to insect attacks: for instance, Taylor *et al.* (2006) found that the total wood extractives amount was important but alone could not explain termite durability in *Thuja plicata* and *Chamaecyparis nootkatensis*, and Stirling *et al.* (2015) confirmed the findings for *Thuja plicata*. Density and hardness are also typically referred as influencing the natural resistance of wood to termite attack (Esenther 1997, Peralta *et al.* 2004, Arango 2006, França *et al.* 2016).

In the present work, the natural durability class for insect degradation, as defined by EN350 (2016) (Table 1), was determined for *Quercus cerris* heartwood from mature trees at harvest age grown in Kosovo. Subterranean termites from the species *Reticulitermes grassei* were used as models as they are accepted as the most dangerous insect species capable of degrading applied timber in Europe and elsewhere and their risk is expected to be higher in a changing climate scenario (Ewart *et al.* 2016). Tests were conducted according to EN 117 (2013) and the results obtained linked to the chemical analysis, density and Brinnel hardeness data obtained from paired test specimens.

Table 1. Wood durability classes to attack by subterranean termites according to EN350 (2016).

Durability Class	Description	Attack level
DC D	Durable	≥ 90 % "0 or 1" and max 10 % "2"; no "3" or "4"
DC M	Moderately durable	< 50 % "3, 4"
DC S	Not Durable	≥ 50 % "3, 4"

MATERIALS AND METHODS

Site characterization and sampling

Ten mature *Quercus cerris* L. trees were randomly selected and harvested from two naturally regenerated and unmanaged stands in the Republic of Kosovo: Blinaja (KB), located at 42°30'31''N – 42°30'38''N and 20°59'06''E – 20°59'15''E at 650-697 m of altitude, and Duboçak (KD) at 42°51'20''N - 42°51'23''N and 20°43'50''E - 20°44'02''E at 817-885 m of altitude. Both sites belong to a mixture of moderate continental and continental climates with 11,3ºC (KB) and 10,4ºC (KD) annual average air temperature, and 609,8 mm (KB) and 604,8 mm (KG) average annual precipitation.

The trees were randomly selected in each stand and characterized by measuring total height and diameter at 1,3 m above ground (d.b.h., as the mean of two crossed diameters). Tree age was approximately 70 and 90 years, respectively at KB and KD.

For stem quality characterization (Bajraktari *et al.* 2018) cross-sectional discs were taken at different stem height levels (base 1,3 m; 3,3 m; 5,3 m; 7,3 m and 9,3 m) and the logs between the first four discs were converted to boards. The boards were dried indoor under well ventilated conditions and the test specimens were taken from the heartwood. Unless otherwise described, the test specimens were obtained from the second log (1,3 m - 3,3 m).

Chemical analysis

Chemical summative analysis included determination of ash, soluble extractives in dichloromethane, ethanol and water, Klason and acid-soluble lignin, and the monomeric composition of polysaccharides. A total of 12 test specimens from the heartwood (6 from KB and 6 from KD) with 50 x 25 x 15 mm were individually analysed following procedures adapted from TAPPI standard methods (TAPPI 2004).

The test specimens were ground with a knife mill (Retsch SM200), sieved (Retsch ISO9001) and the 40-60 mesh fraction was kept for analysis. The ash content was determined by incinerating 1g of the sample at 525º overnight and weighing the residue (TAPPI 15 os-58).

The determination of extractives was adapted from TAPPI 204 cm-97, using a Soxhlet system with dichloromethane, ethanol and water during 6 h, 16 h and 16 h respectively. The extractives solubilized by each solvent were determined by mass difference of the solid residue after drying at 105ºC. The lignin content was determined in the extracted samples by acid hydrolysis with 72% sulphuric acid following TAPPI T 222 om-02. Klason lignin was determined as the mass of the solid residue after drying at 105ºC and the acid-soluble lignin was determined by the absorbance at 206 nm using a UV/VIS spectrophotometer (TAPPI Useful Method UM 250). The monosaccharides including neutral sugars and uronic acids as well as acetates were quantitatively determined in the hydrolysis liquor by High Performance Anion Exchange Chromatography. All determinations were made in duplicate samples.

Ethanol-water extracts

The ethanol-water extracts were prepared using 0,5g of the sample and 20 ml ethanol/water (50/50, v/v), for 30 min at 40 ºC in an ultrasonic bath. After filtration, the supernatant extract was used to determine the contents in total phenolics, condensed and hydrolysable tannins and flavonoids.

The total phenolics content was estimated according to the Folin-Ciocalteu method using gallic acid as a standard (Singleton and Rossi 1965, Miranda *et al.* 2016). Total flavonoids were quantified by an aluminium chloride colorimetric assay, and the results were expressed as mg of (+)-catechin equivalents on a dry extract base (Jia *et al.* 1999, Miranda *et al.* 2016). Tannin content was determined by the vanillin-H2SO4 method, and the results were expressed as mg of (+)-catechin equivalents on a dry extract base (Abdalla *et al.* 2014, Miranda *et al.* 2016).

Brinell hardness

Brinell hardness perpendicular to the grain was evaluated according to EN 1534 (2010) with test specimens with the following dimensions: 40 x 40 x 10 mm. Seven replicates from the second log of each tree felled and seven extra replicates from the first and third log of one tree per site in a total of 49 replicates for each location.

The tests were conducted at 20 ºC ± 2 ºC and 65 % ± 5 % relative humidity, using a universal machine AG 250KNIS-MO from Shimadzu, capable of measuring the applied load with an accuracy of 1 %. The test specimens is set to the machine table and a 10 mm steel ball indented into the surface of the wood at a steady and constant force in order to achieve 1 kN in 15 ± 3 s. The load was maintained for 25 ± 5 s and, after removal, the indentation was measured on images acquired immediately after testing with an Olympus SZX-ZB12 stereoscopic microscope and Olympus DP-Soft software. Crossed diameters (d1 and d2) were measured to evaluate the size of the deformation inflicted by the ball. The Brinell hardness values (HB) were determined according to Equation 1 in MPa,

$$HB = \frac{2F}{ND(D^2 - d^2)^{1/2}} \quad (1)$$

Where F is the force applied (N); d is the diameter of the indentation, in mm (average of two perpendicular diameters d1 and d2) and D is the diameter of the ball, in mm.

Determination of density

Density was calculated according to the Portuguese Standard NP616 (1973) on the same test specimens used for the determination of the Brinell hardness and termite resistance. All specimens were conditioned for one week at controlled temperature and humidity (20 ± 1°C; 65 ± 5%) and weighed. Dimensions of each specimen were then measured using a caliper and density calculated based on weight and volume and adjusted to 12% equivalent moisture content (EMC).

Termite resistance

The natural durability of the wood was evaluated according to the recommendations of EN350: 2016; therefore, the natural durability against the attack by subterranean termites was determined following the general procedure described in EN 117: 2012 with adaptations as described. Six replicates (50 mm x 25mm x15 mm) from the second log of each tree felled and six extra replicates from the first and third log of one tree per site in a total of 42 replicates for each location were tested.

The termites, *Reticulitermes grassei* (Clément), were collected from fallen logs from a forest area of *Pinus pinaster* Aiton, located 38º32.436' N 09º07.848' W, 18 m elevation. They were kept in Petri dishes with moistened filter paper inside a conditioned room (24 ± 2 ° C; 80 ± 5%) for a maximum of 10 days. Colonies of 250 workers (plus 1-3 soldiers and 3-5 nymphs) were established in 750 ml glass conical flasks with moisturized sand (Fontainebleau sand and water; 4:1 v/v) as substrate. The test specimens were placed over glass rings after installation of the termites in their respective containers, and the test run for eight weeks at 25 ± 2ºC and 80 ± 5 % relative humidity. Ten *P. pinaster* untreated test specimens with the same dimensions were also included as virulence controls. After the exposure period, the test specimens were removed and cleaned and the survival rate (expressed in %) was determined. A visual examination of the wood blocks was performed according to the criteria specified in the standard for the evaluation of the level of attack (0=no attack; 1=attempted attack; 2=slight attack; 3=moderate attack; 4=strong attack). The test is considered valid if all virulence control test specimens reach a final level of attack of "4" and have an average survival rate above 50%.

Statistical analysis

The descriptive statistic, correlation and regression analysis and analysis of variance (ANOVA or Student's t-test), where relevant, were performed using Microsoft Excel® (2010) and SigmaStat version 2.0 (Jandel Corporation).

RESULTS AND DISCUSSION

Chemical analysis

Table 2 displays the results regarding the chemical analysis of *Quercus cerris* heartwood. The analysis of variance showed no significant difference (P>0,05) between the two sampling sites for the mean values of total extractive content (P=0,415) and total lignin content (P=0,820).

Table 2. Chemical composition (% of the total dry mass) and monosaccharide composition (% of total monosaccharides) of the heartwood of *Quercus cerris* from KB and KD.

	KB	KD
Ash	0,88 (0,21)	0,97 (0,26)
Extractives		
Dichloromethane	1,02 (0,21)	1,01 (0,17)
Ethanol	2,12 (0,41)	2,65 (0,86)
Water	3,29 (1,06)	3,33 (0,86)
Total	6,43 (1,33)	6,99 (0,94)
Lignin		
Klason lignin	23,23 (1,36)	24,00 (1,41)
Soluble lignin	3,05 (0,30)	2,45 (0,24)
Total	26,27 (1,32)	26,45 (1,28)
Monosaccharides		
Ramnose	0,74 (0,08)	0,88 (0,12)
Arabinose	1,41 (0,12)	1,69 (0,08)
Galactose	1,98 (0,63)	2,18 (1,13)
Glucose	62,23 (2,41)	58,49 (2,46)
Xilose	30,41 (1,71)	33,06 (2,92)
Manose	2,07 (1,55)	3,04 (1,83)
Galacturonic acid	1,93 (0,14)	1,87 (0,13)
Acetic acid	0,23 (0,01)	0,21 (0,01)

Mean and standard deviation of 6 trees per site and 2 replicates per tree.

The heartwood of *Quercus cerris* presented the following average composition: ash 0,93% total extractable substances 6,7% and total lignin 26,4%. The extractives consisted mainly of polar compounds extracted by ethanol and water (5,7% of heartwood), corresponding to 85% of the total extractives.

The striking chemical feature of *Quercus cerris* heartwood is the low content in extractives (6,43% for KB and 6,99% for KD). In fact, oaks have in general a large amount of extractives. For instance, Sousa *et al.* (2009) reported values of total extractives between 18,8-19,3% and total lignin 22,6-23,7% for the heartwood of *Quercus faginea*. Carmona (2009) refers to total extractive contents between 14,8% and 15,7% (where the extractives in ethanol and water represent about 93% of the total extractives) in *Quercus robur*.

The chemical composition of the polysaccharides shows that glucose is the major sugar, corresponding to about 60,4% of the total monosaccharides present. The second most important sugar observed was xylose with a value of 31,7% which means that hemicelluloses in *Quercus cerris* heartwood are predominantly xylans with low contents of arabinose and acetyl groups.

The monomeric composition of polysaccharides is similar to that found for other oak woods in terms of predominance of glucose followed by xylose. Regarding *Quercus faginea*, Sousa *et al.* (2009) reported glucose and xylose values of 59,9% and 30,3% of the total monomers, respectively. A similar composition was reported for *Quercus laurina* and *Quercus crassifolia* woods with dominance of glucose (52,3–56,7%) and xylose as the second most abundant sugar (28,5–35,1%) (Ruiz-Aquino *et al.* 2015).

Composition of ethanol-water extracts

The results obtained for the ethanol-water extracts of *Quercus cerris* heartwood are presented in Table 3.

Table 3. Ethanol-water extracts of the heartwood of *Quercus cerris* from KB and KD: yield and content of total phenolics, condensed tannins and flavonoids. Mean and standard deviation of 6 trees per site.

	Blinaja	Duboçak
Extraction yield (%)	2,2 (1,3)	2,1 (1,0)
Total phenolics (mg GAE / g extract)	338,8 (152,8)	284,2 (97,9)
Condensed tannins (mg CE / g extract)	38,9 (26,1)	12,3 (6,0)
Flavonoids (mg CE / g extract)	67,4 (45,3)	61,2 (24,0)

There were no significant differences between the two locations, except for the condensed tannins that were higher in KB.

The total polyphenol content was on average 310,5 mg GA/g of extract, the average tannins and flavonoids 25,6 mg CE / g extract and 64,3 mg CE / g extract. No hydrolysable tannins were found. Lavisci and Scalbert (1991) have reported levels of polyphenols of 3-4 mg / g of *Quercus cerris* wood.

Quercus cerris heartwood shows comparatively low amount of phenolics and tannins in comparison with other oaks, normally used in cooperage, and where high values of hydrolysable tannins and the non-existence of condensed tannins are reported.

Brinell hardness and Density

The results obtained for the Brinell hardness are presented in Table 4 as well as the average density of each group of test specimens. Significant differences were found between sites for density ($p<0,001$) and Brinell hardness ($p<0,001$) that were lower in the KD samples.

Table 4. Brinell hardness and density of the heartwood of *Quercus cerris* from Blinaja and Duboçak. Mean and standard deviation of 7 replicates, maximum and minimum values measured.

		Blinaja	Duboçak
Brinell hardness (MPa)	Average (std dev)	38,48 (3,88)	33,90 (5,25)
	Minimum - Maximum	29,99 – 48,45	23,45 – 47,60
Density	Average (std dev)	0,83 (0,02)	0,78 (0,05)
	Minimum - Maximum	0,79 - 0,88	0,71 - 0,88

A positive correlation was found between density and Brinell hardness (Figure 1).

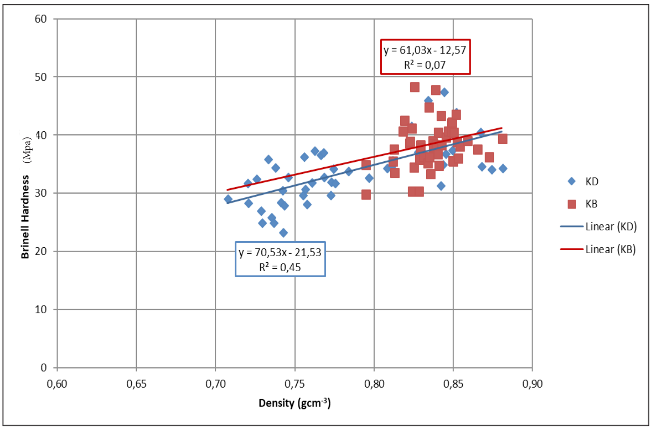

Figure 1. Relation between Brinell hardness and density of *Quercus cerris* heartwood.

The regression between density and hardness was significant for KD samples (R^2=0,45; P<0,0001), while it was not significant for KB samples (R^2=0,07; P=0,06).

The average density and Brinell hardness values from KB were close to the ones obtained on a previous study of wood from the same region (Standfest *et al.* 2012a, Standfest 2012b): 0,86 for density and 39,92 MPa for hardness. The average hardness values obtained for *Quercus cerris* wood are lower than values reported for *Quercus faginea* (50 MPa) or *Quercus suber* (56 MPa) but are nevertheless well within the required values for domestic flooring applications or commercial uses with moderate traffic (EN14354 2004).

Termite resistance

The results obtained for the rate of termite survival at the end of the test and grade of attack are presented in Table 5. The control test specimens of maritime pine (n=10) had an average density of 0,66±0,07 and at the end of the test showed an average level of attack of 4 and an average survival rate of 66,20% ± 15,37%.

Table 5. Wood density, survival and level of attack after 8 weeks of exposure to R, grassei of *Quercus cerris* heartwood from KB and KD. Mean and standard deviation of 6 replicates.

		KB			KD		
Tree	**Log**	**Density**	**Survival (%)**	**Level of attack**	**Density**	**Survival (%)**	**Level of attack**
I	2	0,81 (0,02)	54,40 (27,93)	3,33 (0,82)	0,82 (0,02)	58,20 (20,53)	3,33 (0,52)
II	2	0,79 (0,02)	67,27 (14,59)	3,67 (0,52)	0,79 (0,01)	33,53 (26,74)	3,33 (0,82)
III	1	0,80 (0,02)	59,27 (19,34)	3,50 (0,55)	0,79 (0,06)	45,53 (12,61)	3,33 (0,52)
III	2	0,77 (0,01)	53,47 (21,56)	3,67 (0,52)	0,87 (0,02)	45,93 (14,32)	3,17 (0,75)
III	3	0,83 (0,02)	55,93 (20,07)	3,83 (0,41)	0,76 (0,02)	29,40 (19,82)	3,67 (0,52)
IV	2	0,84 (0,06)	47,20 (27,00)	3,50 (0,55)	0,80 (0,03)	68,33 (8,23)	3,83 (0,41)
V	2	0,81 (0,01)	33,00 (12,54)	3,00 (0,55)	0,79 (0,02)	16,93 (16,67)	2,83 (0,75)
MEAN (n=42)		0,81 (0,03)	52,93 (21,94)	3,50 (0,55)	0,80 (0,04)	42,27 (23,26)	3,36 (0,66)

No significant differences were found between the *Quercus cerris* heartwood samples from both sites. On average, the survival rate was 47,60 % and the level of attack 3,43. No significant correlation was found between wood density and rate of survival. The percentage of test specimens graded "3 and 4" was determined and found to be 90,5% for KD and 97,8% for KB. Taking into account the criteria defined by EN350 (2016) to assign a subterranean termite durability class (Table 1) to a certain species of wood, the heartwood from *Quercus cerris* is classified as DC S (Not durable).

The fact that the species is susceptible to subterranean termites and has a heartwood classified only as "moderately durable" towards fungal decay (EN350 2016) leads to a recommendation of use preferably "out of ground contact" unless it is conveniently treated or otherwise protected, particularly in locations where termites are a risk.

Although other factors may influence the natural durability against termites, the susceptibility of *Quercus cerris* heartwood to termite attack is on line with the low extractives content found on the paired specimens (Table 2) and on the low content of tannins in the polar extracts (Table 3).

CONCLUSIONS

The heartwood of *Quercus cerris* has a low content of extractives namely of polar compounds of phenolic nature, namely of tannins.

The *Quercus cerris* wood is described as “not durable” against subterranean termites and its use in outdoor environments in ground contact should be avoided unless adequate protection is made.

Quercus cerris wood shows adequate hardness and density for interior uses like flooring for domestic and commercial applications, with moderate use.

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