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ORIGINAL

Cork influenced by a specific water regime—macro and microstructure characterization: the first approach

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Abstract

Cork is the most valuable non-wood product of the cork oak (*Quercus suber* L.). However, the cork oak sector may be at risk due to climatic and economic pressures on cork oak forests, affecting both the quantity and technological quality of products. At some sites, irrigation may present a solution for stimulating cork growth and thereby increasing production. This study presents an initial approach to characterizing cork grown in a forest stand associated with a specific water regime, by comparing cork growth on two plots—irrigated and a traditional rainfed—over an initial five-year period. Samples of cork tissue were analysed and several parameters were set: cell area, diameter, cell-wall thickness, number of cells, porosity, growth, and density. Irrigation plot samples showed on average: 25.83 ± 3.74 mm thickness, 5.17 ± 1.49 mm cork-ring width, 0.149 ± 0.041 g.cm^{-3} density, $13 \pm 3.4\%$ porosity coefficient in the tangential plane, 407.58 ± 268.22 µm^2 cell area in the tangential plane and 887.80 ± 449.14 µm^2 in the transverse plane, a total number of cells of 1232 ± 147 per mm^2, and 1.03 ± 0.30 µm cell-wall thickness; whereas traditional rainfed plot samples presented: 21.33 ± 5.48 mm thickness, 3.08 ± 1.44 mm cork-ring width, 0.167 ± 0.068 g.cm^{-3} density, $10 \pm 3.5\%$ porosity coefficient in the tangential plane, 304.31 ± 205.83 µm^2 cell area in the tangential plane and 752.45 ± 398.94 µm^2 in the transverse plane, a total number of cells of 1481 ± 153 per mm^2, and 1.204 ± 0.327 µm cell-wall thickness. As regards irrigation, two parameters, ring width and porosity coefficient, proved to be statistically significant, in contrast to density.

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Introduction

Portugal is regarded as the world's largest cork producer and exporter, accounting for 49.6% of annual global production and a revenue of around 815.6 M euros (APCOR, 2020). Despite this, according to the Inventário Florestal Nacional—National Forest Inventory, 11,300 ha of cork oak (including 3800 ha accounted for by regeneration of the species) were lost between 2005 and 2015 (ICNF 2015). Forest decline, jeopardizing cork production and affecting both quality and quantity, is due to changes in system management, mostly as a result of agropastoral intensification and/or mechanization (for example, soil disking), as well as pests and diseases and increasingly severe climatic conditions (Ribeiro et al. 2004, 2006, 2010; Pinheiro et al. 2008; Camilo Alves et al. 2013; 2017; Oliveira et al. 2016; Pinto Correia et al. 2018). Climate events, especially the annual distribution of precipitation, influence cork-ring growth, which is less marked during periods of drought (Schmidt et al. 2009; Vaz et al. 2011; Oliveira et al. 2016; Leite et al. 2019). Several authors have indicated a similar relationship between precipitation and cork-ring width (Oliveira et al. 1996a, 1996b; Caritat et al. 1996; Costa et al. 2002; Surový et al. 2009) and solar activity and cork-ring width (Surový et al. 2008), and presented evidence of further factors affecting cork quality: management practices, competition, debark pressure, and soil organic matter content (Montero et al. 1991, 1994, 1998; Montoya et al. 1984; Montoya 1985; Tinoco et al. 2009; Ribeiro et al. 2010; Ribeiro and Surový 2011).

The cork oak grows most intensely during two periods (Oliveira et al. 1996a; Vaz et al. 2011): (1) spring, when growth is greater due to the optimal conditions; and (2) autumn, when growth varies from low to moderate depending on temperature and rainfall. Oliveira et al. (1996a, b) mentioned the high dependency of annual growth dynamics on significant rainfall variation during spring, summer, and autumn. If the tree receives sufficient water, nutrients from twiglets migrate to evergreen leaves, initiating both new leaf production and elongation growth, followed by vascular cambium and phellogen activation—factors determining cork growth.

In the present study, we seek to determine the contribution of irrigation to cork production and the possible enhancement of production in terms of quantity and quality—the study´s hypothesis. However, management practices may exert an influence on cork characteristics (implemented silviculture model); thus, there is a need for providing an understanding of how these may modify cork's cellular structure and, consequently, its quality.

Cork tissue or phellem is defined as the group of cells belonging to the periderm—which derives from traumatic phellogen, as widely described in the literature (Natividade 1950; Graça and Pereira 2004; Pereira 2007, 2015). Its characteristics, as well as cork formation, influence the properties of final products, including three important quality parameters: growth, porosity, and density. Cork formation depends on genetic and environmental factors as well as the interaction between them. Pereira et al. (1987) described the structure of cork as homogeneous tissue with no intercellular spaces and thin-walled cells shaped like hexagonal prisms stacked transversely in columns. The different planes observed in cork structures are explained by Pereira (2015), who mentions a similar cell-wall thickness in three planes.

Porosity is a quality parameter defined by the volume occupied by lenticular channels that grow from pith to bark in both a radial and a transverse direction. It is affected by both genetic factors (lenticular channels and wood inclusions) and external factors (site-specific edaphic-climatic characteristics) (Pinto Correia et al. 2013). This may be observed in radial, transverse, and tangential sections: in the first two, pores have an elongated shape, perpendicular to cork rings, while in tangential sections pores are elliptically shaped (Fortes et al. 2004). Porosity is expressed by the porosity coefficient, whose values establish limits for the cork-stopper industry.

Density, one of cork's main structural characteristics (Fonseca et al. 1994), determines its suitability for different uses (Pereira et al. 1996) and may be influenced by a number of factors: geometry and cell dimension, autumn and spring cell dimension, the presence of lenticular channels, inclusions, discontinuities, cell-wall wrinkling, and the extent of porosity (Fortes et al. 2004; Pereira 2015; Anjos et al. 2008).

In industrial cork processing, the raw material is boiled, which causes radial expansion of about 15 and 6% transversely and tangentially, producing an increase in thickness. Porosity drops to 50% of gross values, and density also undergoes a small change of around 20% (Fortes et al. 2004). Although the samples used in this study were analysed raw, as the aim was to study the effect of the irrigation of cork oaks on the physiology of cork formation and its characteristics, references to boiled cork have been included throughout this paper due to their importance for the industrial process and the lack of raw cork references. However, this comparison may be made since the boiling process improves the characteristics of cork (leading to an increase in thickness and a decrease in porosity and density), and therefore, when dealing with raw cork, we have a good idea of what its characteristics will be following boiling.

The aim of the study

The aim of this study is to characterize the structure of cork from irrigated cork oaks from a forestry product perspective. Samples were compared with those from a traditional rainfed plot, both groups displaying a marked degree of variability. Cork growth was characterized in accordance with the same growth parameter: initial cork rings. The following parameters were set: cork density, thickness, ring width, porosity coefficient, the number of pores and pore areas, and cellular structure (area, diameter, cell-wall thickness, number of cells). The present study is part of an ongoing project and will provide the basis for the further characterization of cork from cork oaks under different water regimes.

Material and methods

Cork samples and study sites

Reproduction cork (*amadia*) raw samples from 24 trees at 130 cm height, collected during the 2017 harvest on two research plots maintained by University of Évora,

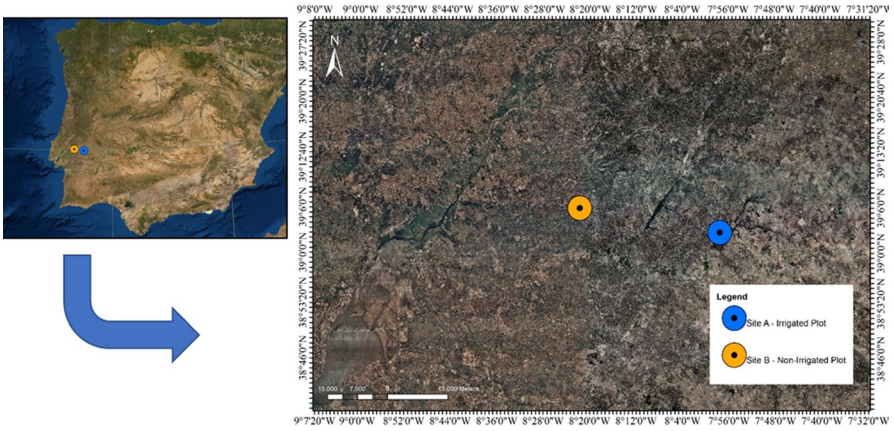

Fig. 1 Research sites: Site A—irrigated plot; Site B—non-irrigated plot; coordinate system: ETRS_1989_Portugal_TM06; Image: Direção geral do território, Portugal, 2018

Table 1 Mean ± Std. deviation for tree characteristics: total height, stem diameter at breast height, and stem height of harvesting

Trees		
Characteristics (average of 12 trees)	Site A—Irrigated plot	Site B—Traditional rainfed plot
Total height (m)	11.94 ± 1.40	10.88 ± 2.01
Stem diameter at breast height (cm)	198.55 ± 20.84	143.2 ± 34.19
Stem height of harvesting (m)	2.02 ± 0.53	1.68 ± 0.40

were taken as the subject of this study. Each plot has been subjected to a different treatment as regards water availability: at Site A irrigation was used, while Site B corresponds to a traditional rainfed system (Fig. 1). A total of 12 cork samples were collected at each site.

Samples were analysed in their raw state—before any industrial processing—in order to ascertain the real effect of irrigation on cork structure and physiological formation. Samples from site A were harvested following special authorization granted by the Instituto da Conservação da Natureza e das Florestas—Portuguese Institute for Nature Conservation and Forests.

Samples from Site A presented five complete rings over a six-year growth period. Though located on an 18-year-old plantation, with watering campaigns conducted by the producer from the beginning until 2017, following the same regime as the nearby olive grove, samples were harvested in 2017 from centenary trees (Table 1). Irrigation occurred once a week, from June to October, averaging 1928 m^3. ha^{-1} per year (Table 2). At the end of each round of irrigation, Inofert Plus 14:11:6 + 8 B (3.5 kg. ha^{-1}. $year^{-1}$) was supplied. Irrigation was drip-surface with one tube per

Table 2 Annual variation of precipitation and irrigation distribution at Site A

Annual precipitation (mm)			Irrigation distribution—Site A ($m^3.ha^{-1}$)
Complete year of cork growth	Site A—Irrigated plot	Site B—Traditional rainfed plot	
2008*		344.4	
2009*		307	
2010*		310.5	
2011	506.3	502.2	1400
2012	371.5	290.1	2500
2013	453.8	416.3	1800
2014	579.9	579.4	1350
2015	313.3	283	2600
2016	613.2	638	1250
2017	327.3	338.4	2600

*Years that are not part of cork growth for site A

plantation line and 2.1 L drip emitters spaced 0.75 m apart. The site presented soil characterized as a Low Satured Gleyic Luvisol. Cork samples from Site B (the control site) were also extracted from centenary adult trees (Table 1) located on a traditional rainfed plot (not forming part of a plantation) on Non-humic Litolic soil. Samples from Site B presented eight complete rings over a nine-year growth period, harvested in accordance with Portuguese law governing debarking. Trees at both sites had access to underground water.

Both sites presented a Mediterranean climate, with hot dry summers and mild winters (Fig. 2): mean annual rainfall and mean temperature: Site A 452.2 mm (Table 2) and 16.2 °C, respectively; Site B 400.9 mm (Table 2) and 15.8 °C, respectively.

Macrostructure analysis

Density and growth

For each sample, a cross section with 3 cm maximum length and 1.5 cm average thickness was taken using an elementary slicer machine (ABO with 220 mm incorporate blade and sharpener, Oggiona VA, Italy) and conserved under typical environmental conditions. The density and growth of polished samples (using P240 Rhynowood Indasa sandpaper) were analysed by means of X-ray technology using a QTRS-01X Tree Ring Analyser (Quintek Measurement Systems Inc., Knoxville, TN, USA). Measurements were performed automatically using QTRS-01X software (Quintek Measurement Systems Knoxville, Knoxville, TS, USA) (for image output, see Fig. 3). Calculations were made automatically using the following equation, relating X-ray attenuation and density:

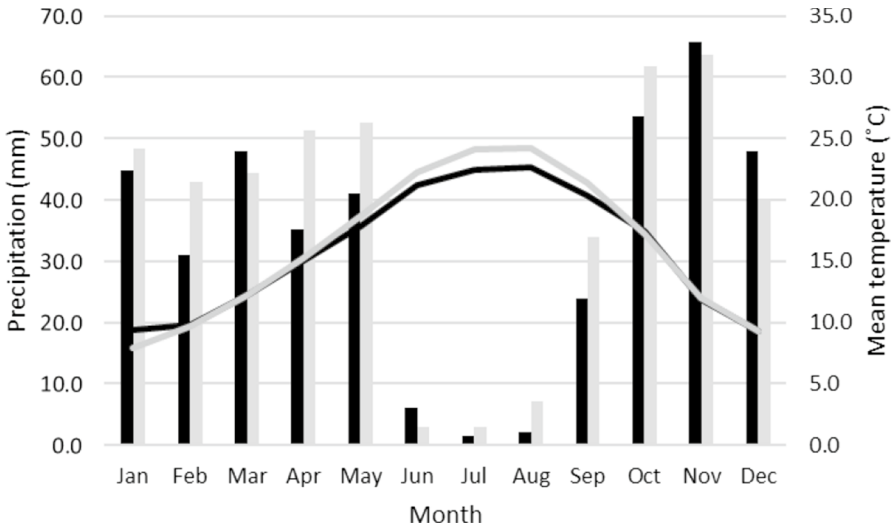

Fig. 2 Climate diagram showing temperature (lines) and precipitation (bars) at the study sites. Site A (irrigated plot) in grey and site B (traditional rainfed plot) in black, for the entire period of cork growth (2011–2017 and 2008–2017, respectively). Climatic data: IPMA—Instituto Português do Mar e da Atmosfera (Portuguese Institute for Sea and Atmosphere)

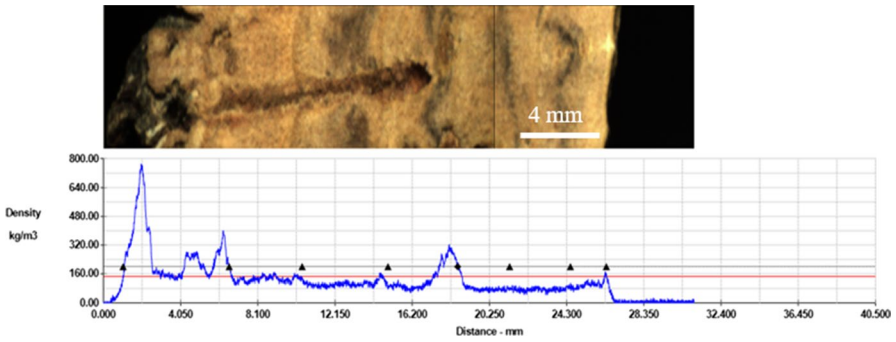

Fig. 3 Image output for one testing sample, using QTMR 01X software: density (kg.m^{-3}) expressed over the ring width (mm) along scan line from bark to pith

$\frac{\mathbf{I}}{\mathbf{I_o}} = e^{-\mu l t}$, where **I** is radiation beam intensity after passing through the sample, **Io** is radiation beam intensity not passing through the sample (from bark to pith), $\boldsymbol{\mu l}$ is the sample linear attenuation coefficient, and **t** is sample thickness.

Fixed parameters used included a 3.80 mass absorption coefficient, 200 threshold, and 50 dead band. Bark and belly half-rings were not accounted for in data analysis.

Porosity

Regarding porosity, samples were cut along the tangential and transverse planes (Fig. 4a, b) and polished prior to analysis. Digital images were obtained by means of a camera (AVT Marlin F-145C2, Stadtroda, Germany). Areas and numbers of pores along the two planes were measured using Image Pro-Plus 6.2 software (Media Cybernetics, Bethesda, USA), and porosity coefficients were calculated. Two regions of interest (ROI) were measured for each sample, with a total of 24 per plot.

Microstructure analysis

Biometric analysis

For biometric analysis, tangential and transverse samples were prepared (Fig. 5a, b). Tangential samples were taken using a movable blade microtome (Reichert with Jung blades), each 20 µm thick. For each sample, three images were obtained using a binocular magnifying glass (Nikon SMZ-10, Japan) with 40 × magnification and analysed using Image Pro-Plus 6.2 software (Media Cybernetics, Bethesda, USA). Four ROIs, randomly distributed per image, were measured (diameter, area and cell count).

Transverse samples were cut 1 mm thick. Using a polarized light microscope (Olympus BX50, Tokyo, Japan), nine images per sample were obtained. Images were distributed along the cork-ring width. Overall, 24 cells per image were analysed using Image Pro-Plus—6.2 software (diameter and area).

Cell-wall analysis by means of SEM

For scanning electron microscopic analysis of cork cell features, the transverse samples described in the section *Biometric analysis* were fixed on aluminium specimen holders using conductive double-sided adhesive carbon tabs coated with approximately 40 mm carbon, using an EMITECH K905 Carbon Coater (Emitech Ltd,

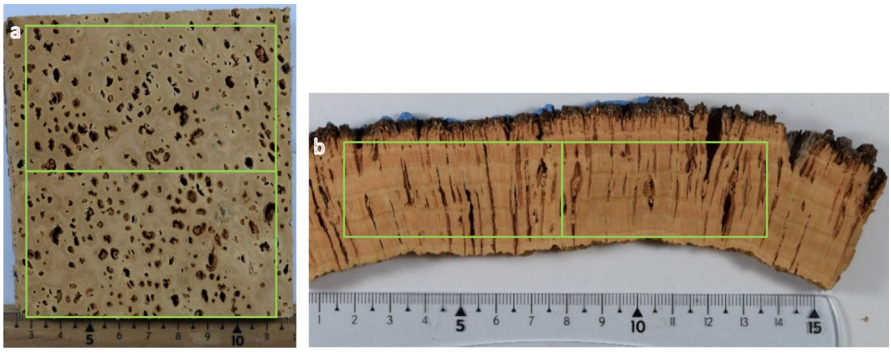

Fig. 4 Cork samples from the irrigated plot prepared for porosity analysis: a—tangential plane; b—transverse plane. Areas within the lines represent the regions of interest (ROI´s)

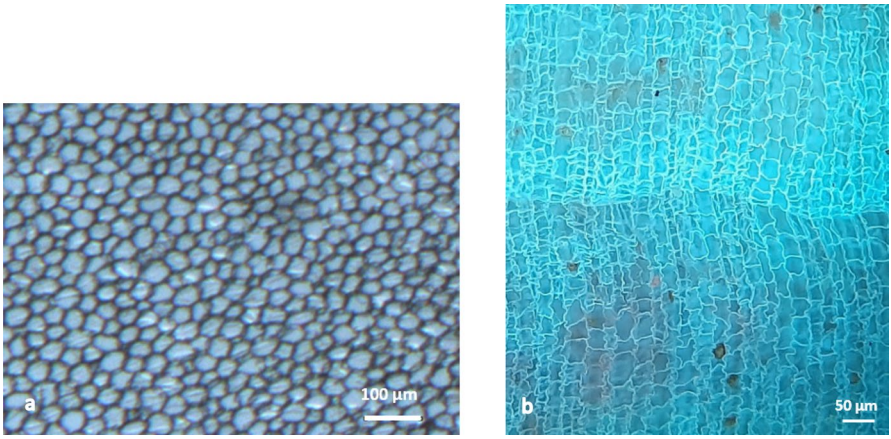

Fig. 5 Microphotographs of cork sample histological sections: a—tangential section (magnifying glass); b—transverse section (polarized light microscope)

Ashford, Kent, UK). To minimize charging effects, thus allowing for higher resolution, they were kept under high vacuum conditions in accordance with the description given by Crouvisier-Urion et al. (2019). For each sample (a total of 24), two images were obtained using MAPS software (MAPS version 2.1.38.1199, Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 10 kV beam energy and 2.5 spot size under high vacuum conditions with a scanning electron microscope (Quanta FEG 650, Thermo Fisher Scientific, Waltham, Massachusetts, USA). For each sample and image, 168 to 224 subframes were taken with high magnification and stitched together using MAPS software. The stitched SEM images (Fig. 6) were used for image analysis.

Cell-wall thickness was measured using the ImageJ 1.52a program (Wayne Rasband, National Institutes of Health, USA), and the general structure and features were observed by means of SEM images. Two images per sample were obtained

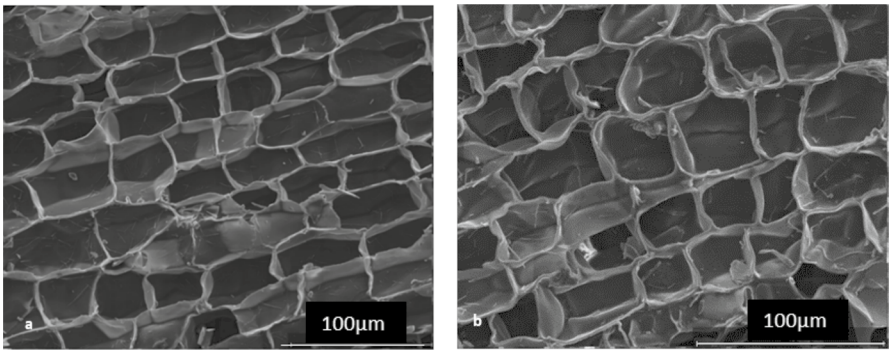

Fig. 6 Cork samples cellular structure from a transverse section obtained with scanning electron microscope: a—sample from the irrigated plot; b—sample from the traditional rainfed plot

and 200 measurements per image were taken, with a total of 400 cell-wall thickness measurements per cork sample.

Statistical analysis

Statistical analysis was conducted using JMP4.0.2 and SPSS v.25 software package (IBM Corp., Armonk, NY, USA). Analysis of variance (ANOVA) was performed to compare samples from the irrigated plot and the traditional rainfed plot, as well as comparing sample repetitions in each group due to the variability between trees.

Regions of interest for the different samples were compared and variability within trees analysed. A Gauss normal distribution fitted model (general linear models) was used to compare the main effects and evaluate the significant differences between means.

In the text, the following statistical explanation was used:

n.s.—not significant ($p > 0.05$); *—significant ($p < 0.05$); **—very significant ($p < 0.01$); ****—highly significant ($p < 0.001$).

Results and discussion

Macrostructure analysis

Growth and density

Cork thickness for irrigated and non-irrigated plots was 25.83 ± 3.74 mm (5 complete years of growth) and 21.33 ± 5.48 mm (8 complete years of growth), respectively, confirming the contribution of irrigation to cork growth (Table 3). The equivalent cork growth rings at the two sites (the first 5 rings) were compared. Samples from irrigated cork oaks revealed on average greater growth per ring (5.17 ± 1.49 mm) than those of non-irrigated cork oaks (3.08 ± 1.44 mm) (Table 3). Data from non-irrigated trees were in accordance with cork-ring width reported in the literature: Pereira (2007) recorded 3.50 mm cork-ring average growth (sampling across Portugal) and Chorana et al. (2019) an average of 2.43 mm cork annual growth in North-West Algeria. Both articles make reference to boiled cork, and, as

Table 3 Mean ± standard deviation for macroscopic characterization of cork thickness, ring width, total density and cork sample ring density by type of treatment (irrigated/non-irrigated). Cork thickness and total density considered the complete years of growth for samples from both plots. Values of cork-ring width and ring density were addressed to the same 5 initial rings of cork formation: Irrigated plot—2012 to 2016; Non-irrigated plot—2009 to 2013)

Treatment	Cork thickness (mm)	Cork-ring width (mm)	Total density ($g.cm^{-3}$)	Ring density ($g.cm^{-3}$)
Irrigated	25.83 ± 3.74	5.17 ± 1.49	0.149 ± 0.028	0.149 ± 0.041
Non-Irrigated	21.33 ± 5.48	3.08 ± 1.44	0.167 ± 0.068	0.167 ± 0.097

this paper deals with raw cork, it is expected that the average cork-ring width would be greater after boiling. Several studies cited environmental effects as the main factors affecting cork growth, particularly cork-ring growth decrease in response to episodes of drought (Costa et al. 2016; Oliveira et al. 1996a,b, 2016). Therefore, it can be stated that the irrigation silvicultural model implemented contributes to cork-ring width increase.

While irrigation resulted in an increase in cork growth, cork producers also find the growth-density relationship to be important. Cork density from irrigated cork oaks averaged 0.149 ± 0.028 g.cm^{-3} while cork density from non-irrigated cork averaged 0.167 ± 0.068 g.cm^{-3} (Table 3), which may be associated with the thinner cell walls of the cork from the irrigated plot. These characteristics affect the mechanical behaviour of cork (Knapic et al. 2016) and are responsible for the performance of cork products. For example, Oliveira et al. (2014) found a greater resistance to compression in samples with a high density and small ring width.

According to the literature, cork presents a high degree of variation as regards density values, depending on certain features, such as cell-wall thickness and lumen size. The findings of the present study (Table 3) were in accordance with those of Pereira (2007) in Knapic et al. (2016), who presented a density variation ranging from 0.120 to 0.170 g.cm^{-3}. Natividade (1934) found a density variation interval of 0.120–0.200 g.cm^{-3} for raw cork, similar to the results of the present study. Additionally, authors such as Silva et al. (2005) and Anjos et al. (2008) presented results (after boiling) of between 0.120 and 0.240 g.cm^{-3}. This wide range of values demonstrates the high degree of variability of cork. As already mentioned, the density of samples was determined in the present study for raw cork, which would be expected to produce slightly higher values than those reported in the literature (mostly regarding boiled cork) since the boiling procedure involves great decompression of cell walls and thus a decrease in density.

Analysis of variance (Table 4) showed a significant degree of influence by *Treatment* and *Ring* on ring width (p Value < 0.0001). Type of treatment was the main source of ring width variation, accounting for 46.59% of variation. In contrast, the source of *Ring* variation, which was highly significant, was only found to be 6.02%.

In contrast to growth, there was no significant difference in density associated with irrigation treatment. Variance, associated with the source *Trees within each*

Table 4 Analysis of variance in accordance with treatment (T) of different trees (Tr) and cork rings (R), for density and ring width

		Ring width			Density		
Source	DF	F	p Value	Var (%)	F	p Value	Var (%)
Treatment (T)	1	39.23***	< .0001	46.59	0.56 ns	0.0534	0
Tree/Treatment (Tr/T)	22	2.38**	0.0023	8.47	6.88***	< .0001	51.40
Ring (R)	4	5.70***	0.0004	6.02	2.91***	0.0258	3.49
R x T	4	4.18**	0.0038	8.14	1.38 ns	0.2469	1.39
Residual (R x Tr/T)	88		< .0001	30.77		< .0001	43.72

Table 5 Means ± standard deviation of macroscopic characterization as regards the coefficient of porosity, number of pores and individual pore area for cork samples, according to type of treatment

Treatment	Coefficient of porosity (%)		Number of pores		Individual pore area (mm^2)	
	Tangential	Transverse	Tangential	Transverse	Tangential	Transverse
Irrigated	13 ± 3.4	14 ± 4	385.50 ± 144.73	43.58 ± 9.18	1.448 ± 0.036	6.635 ± 0.311
Non-Irrigated	10 ± 3.5	9 ± 3	421.67 ± 110.85	30.71 ± 12.95	0.785 ± 0.033	4.284 ± 0.311

Table 6 Analysis of variance for treatment and trees, as regards the coefficient of porosity in the tangential and transverse planes

		Coefficient of porosity					
		Tangential			Transverse		
Source	DF	F	*p* Value	Var (%)	F	*p* Value	Var (%)
Treatment (T)	1	5.32*	< .0001	24.04	20.28***	< .0001	49.86
Tree/Treatment (Tr/T)	22	7.25***	< .0001	57.58	1.63 ns	0.1241	11.93
Residual	24		< .0001	18.38		0.005	38.21

treatment (Tr/T), presented a p value of < 0.0001 (Table 4), accounting for 51.40% of variation. In studies carried out by Silva (1996) and Marrafa (2016), similar results were found, as variation accounted for by the trees proved to be the main source of cork density variation, with a high degree of variability in terms of the genetic dependency of this characteristic. However, Marrafa (2016), Ribeiro et al. (2006) and Ribeiro and Surový (2011) stated that cork-ring width and density are affected by intraspecific competition (intense competition leading to smaller cork-ring width and higher cork density). Thus, these cork characteristics may be controlled using the irrigation silvicultural model, with a view to achieving forest-stand growth stock optimization over time and also individual tree annual cork-ring width and cork density objectives. In addition, Fonseca et al. (1994) reported a significantly negative relationship between ring width growth and density.

Porosity

Regarding the tangential coefficient of porosity, values of 13 ± 3.4% were recorded for the irrigated plot and 10 ± 3.5% for the non-irrigated plot (Table 5). As regards the transverse coefficient of porosity, the findings of the present study are in keeping with the coefficient reported by Pereira et al. (1996) ranging from below 2% to over 15%, while higher values were obtained from irrigated plot samples (14 ± 4%) than from non-irrigated plot samples (9 ± 3%). However, these values are expected to decrease after boiling, as indicated by Fortes et al. (2004).

A statistically significant relationship between the coefficient of porosity and type of treatment was found using a general linear model. Analysis of variance (Table 6)

demonstrated that *Treatment* was the principal source of variation of the transverse coefficient of porosity (49.86%), but this was not the case with the tangential plane, where *Trees within each treatment* was the main source of variation (57.58%). While porosity in the transverse plane corresponded to different years of cork growth, in the tangential plane it is accounted for by a single year of cork growth. This may be why in the tangential plane *Treatment* was not a significant source of variation as compared with the transverse plane. It would appear that porosity in the tangential plane is determined by genetic variability factors (seeing that the differences between individual trees were more important), whereas in the transverse plane environmental factors, such as irrigation, proved to be determining factors. Marrafa (2016) found that *Trees* was a highly significant source of variation as regards porosity in the tangential plane, while other factors associated with the environment (Year and Plot) were not, which is in accordance with the findings of the present study.

According to Silva (1996), greater soil water availability leads to larger pores. Additionally, Fortes et al. (2004) reported larger pores as a defect associated with fast-growing corks and recorded a pore length of 14 mm in the cross section and a diameter in the range 4–7 mm in the tangential section (these are higher values than those found in the present study). The same author also observed pore areas of less than 1 mm^2 for most pores in the tangential plane and this is more in keeping with the results of the present study (Table 5): a lower number of pores in the tangential plane for the irrigated plot (385.50 ± 144.73) and a higher coefficient of porosity (13 ± 3.4) and pore area (1.448 ± 0.036 mm^2) than the non-irrigated plot for which the recorded values were: 421.67 ± 110.85 pores, coefficient of porosity 10 ± 3.5, and pore area 0.785 ± 0.033 mm^2.

Regarding number of pores, analysis of variance (Table 7) demonstrated that *Treatment* was the main source of variation in the transverse plane (37.26%) but not in the tangential plane (0%), while *Trees within each treatment* was found to be the main source of variation in the tangential plane (86.46%).

The source of variation referred to as *Residual* (which represents repetitions for each sample) accounted for a significant variation in the number of pores in the transverse plane (42.94%) but only 13.54% in the tangential plane, thus constituting a source of great variability within the stem.

The area of individual pores of irrigated cork oaks presented values of 1.448 ± 0.036 mm^2 (Mean ± Std. deviation) in the tangential plane and

Table 7 Analysis of variance for treatment for trees, as regards number of pores in the tangential and transverse planes

		Number of pores					
		Tangential			Transverse		
Source	DF	F	*p* Value	Var (%)	F	*p* Value	Var (%)
Treatment (T)	1	0.49 ns	0.016	0.00	11.83***	< .0001	37.26
Tree/Treatment (Tr/T)	22	13.78***	< .0001	86.46	1.92 ns	0.0607	19.80
Residual	24		< .0001	13.54		0.0071	42.94

Table 8 Analysis of variance by treatment of tree samples, with regard to individual pore area in the tangential and transverse planes

	Individual pore area (mm^2)							
	Tangential				Transverse			
Source	DF	F	*P* Value	Var(%)	DF	F	*P* Value	Var(%)
Treatment (T)	1	13.135***	< .0001	1.73	1	8.340***	< .0001	0.19
Tree/Treatment (Tr/T)	22	16.921***	< .0001	1.61	22	2.823**	< .0001	0.21
Region/Tree/Treatment	24	0.846 ns	0.6789	0.000	24	0.911 ns	0.587	0.000
Residual	19,324		< .0001	96.65	1735		< .0001	99.60

Table 9 Mean ± standard deviation of biometric characterization of the tangential plane, measured in an area of 159,913 μm^2, by type of treatment (irrigated/non-irrigated)

Treatment	Number of cells per mm^2	Cell area (μm^2)	Max diameter (μm)	Min diameter (μm)
Irrigated	1232 ± 147	407.58 ± 268.22	27.19 ± 10.81	14.84 ± 6.24
Non-Irrigated	1481 ± 153	304.31 ± 205.83	22.92 ± 9.00	13.04 ± 5.46

6.635 ± 0.311 mm^2 in the transverse plane (Table 5), while cork from the non-irrigated plot presented lower values: 0.785 ± 0.033 mm^2 and 4.284 ± 0.401 mm^2 in the tangential and transverse planes, respectively (Table 5). Thus, cork from non-irrigated cork oaks presented smaller pores in both planes. It should be noted that in the tangential plane when irrigated cork oaks are compared to non-irrigated cork oaks a higher number of pores was found, which were smaller (Table 5).

Variation in individual pore area was mainly accounted for by repetitions (*Residual*) of each sample ($p < 0.0001$) by more than 96.7% and 99.6% in the tangential and transverse planes, respectively (Table 8). Despite the high level of significance in both planes ($p < 0.0001$) (Table 8), the sources of variation designated as *Treatment* and *Tree within each treatment* provided only a small contribution to variation in this case, below 2%.

Microstructure analysis

Biometric analysis

The cell area of cork samples from irrigated cork oaks was larger in both planes (tangential plane: 407.58 ± 268.22 μm^2; transverse plane: 887.80 ± 449.14 μm^2) than that of samples from the non-irrigated site (tangential plane: 304.31 ± 205.83 μm^2; transverse plane: 752.45 ± 398.94 μm^2) (Tables 9 and 10). With regard to the number of cells, the opposite was found: a lower number of cells were present in samples from the irrigated plot than those from the

Table 10 Mean ± standard deviation of biometric characterization in the transverse plane, measured in 24 cells, by type of treatment (irrigated/non-irrigated)

Treatment	Cell area (μm^2)	Max diameter (μm)	Min diameter (μm)
Irrigated	887.80 ± 449.14	44.06 ± 12.83	22.21 ± 6.76
Non-Irrigated	752.45 ± 398.94	38.66 ± 11.78	21.36 ± 6.66

traditional rainfed plot, the same pattern being found with regard to porosity in the tangential plane (a lower number of pores and a larger area) (Table 5).

Pereira (2007) reported cell diameter values in the range of 10–20 μm, while mean values recorded in the present study were in the upper range of values (Tables 9 and 10). Fortes et al. (2004) reported average cell areas ranging from 400 to 600 μm^2 for boiled cork. Tangential analysis findings for raw samples proved to be similar to the lower values recorded in the present study (Table 9). An increase in diameter and cell area is expected following boiling. However, in the present study, the findings were similar to those of Natividade (1934) as regards raw cork. An average cell height of 37.3 μm was recorded in the radial plane, in keeping with the 38.66 ± 11.78 μm found for maximum diameter on the non-irrigated plot in the transverse plane (Table 10). Natividade reported a 55–25 μm range in cork cell height.

A statistically significant relationship between cell area and type of treatment was established by means of a general linear model (Table 11). Following analysis of variance, it was concluded that in both planes each variation source was statistically significant ($p < 0.0001$), which means that *Treatment, Trees*, S*amples* and the *Regions of interest within each sample* all have an influence on cell area. However, *Treatment* presented a lower variation with regard to the transverse plane (0.245%), which means that irrigation may have a less marked influence on transverse cell area. In the tangential plane, *Sample* (45.54%) and *Region* (42.79%) sources—which represent the variation within tree—were those which accounted for most of the variation. *Region* was also an important source with regard to the transverse plane,

Table 11 Analysis of variance for treatment of tree, sample per tree, and region for each sample, as regards cell area in the tangential and transverse planes

	Cell Area							
	Tangential				Transverse			
Source	DF	F	*p* Value	Var (%)	DF	F	*p* Value	Var (%)
Treatment	1	44.282***	< .0001	2.765	1	5.868*	< .0001	0.245
Tree/Treatment	22	2.750**	< .0001	0.488	22	5.288***	< .0001	0.489
Sample/Tree/Treatment	48	5.258***	< .0001	45.543	48	1.171 ns	< .0001	3.356
Region/Sample/Tree/Treatment	216	5.081***	< .0001	42.785	144	4.541***	< .0001	78.600
Residual	62,191		< .0001	8.420	4968		< .0001	17.311

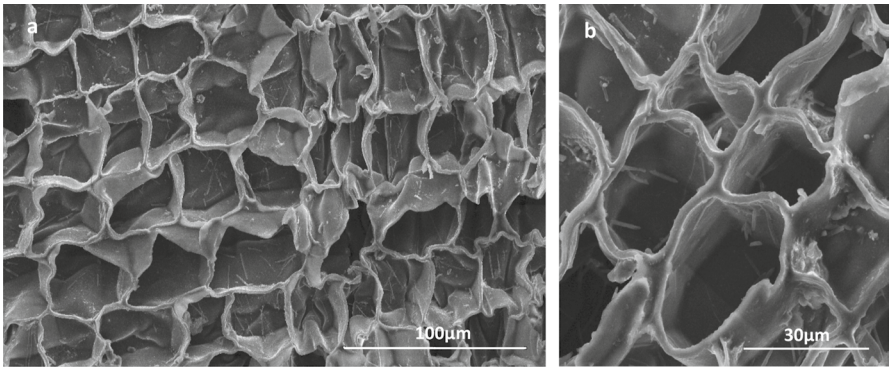

Fig. 7 Cellular structure of a cork sample from the irrigated plot obtained using SEM: a—observation of wrinkled and non-wrinkled cells; b—deposits on cell walls and artefacts in cell lumens

Table 12 Mean ± standard deviation of cork cell-wall thickness, by type of treatment

Treatment	Cell-wall thickness (µm)
Irrigated	1.031 ± 0.300
Non-Irrigated	1.204 ± 0.327

accounting for a 78.6% variation in cell area. The transverse plane indicated cork growth for the total number of years and this variability may be accounted for by differences in random cells measured in spring and autumn. It may also be accounted for by cell-wall wrinkling, once the analysis was made in raw cork (Fig. 7).

Cellular structure and cell walls

Cell walls provide the basis for cork properties. Through SEM it was possible to measure the cell-wall thickness and gain an enhanced view of the cork structure. Under both water regimes, it was possible to observe a number of columns composed of cells with a rectangular prism shape (Fig. 7), in accordance with the literature (Graça and Pereira 2004; Pereira 2007, 2015). Regarding cork from the irrigated site, some cell walls were wrinkled (Fig. 7a), which may be accounted for by cork which was not boiled, as cells thus did not have the opportunity to expand. In several samples from the two sites, cell lumens and walls showed some solid deposits with different shapes and sizes and soiled surfaces (Fig. 7b), similar to the deposits found by Xiaozhou et al. (2017) in *Quercus variabilis.*

Cork cell-wall thickness random measurements of irrigated cork oaks ranged from a minimum of 0.207 to a maximum of 2.834 µm, with a mean and standard deviation of 1.031 ± 0.300 µm (Table 12), whereas values for non-irrigated cork oaks ranged from a minimum of 0.362 µm to a maximum of 3.463 µm, with a mean and standard deviation of 1.204 ± 0.327 µm. These values provided confirmation of the lower cork density of sample from irrigated cork oaks, although the treatment effect on density is not statistically significant (Table 13).

Table 13 Analysis of variance by treatment for samples per treatment and ROI in each sample, as regards cell-wall thickness

	Cell-wall thickness			
Source	DF	F	*P* Value	VE (%)
Treatment	1	1007.423 ***	< .0001	11.6
Sample/Treatment	22	118.367 ***	< .0001	11.1
ROI/Sample/Treatment	24	47.858 ***	< .0001	14.7
Residual	9552			62.6

Natividade (1934) stated that values ranging from 1 to 2.25 μm are most frequently found in raw cork tissue, depending on the growth season, and the findings of the present study fell within this interval. In slow-growing cork oaks, such as those in Algeria and Morocco, thickness is usually greater (Natividade 1934), which causes slight elasticity.

All tested variation sources were highly significant ($p < 0.0001$, Table 13), demonstrating the important influence of irrigation on cork cell walls. The two regions of interest in each sample are a highly significant source affecting wall thickness variation, which reflects the great degree of variability within the tree ($p < 0.0001$) (Table 13). Individual cell variation (*Residue*) accounted for most wall thickness variation (62.6%), in accordance with the results presented by Silva (1996) and Chorana et al. (2019).

Conclusion

The comparison of cork samples from two different sites with distinct implemented silvicultural models was conducted. Site A was subject to a specific irrigation regime, while at Site B a traditional rainfed system was implemented.

Characteristics such as growth, density, and porosity were analysed, and tissues were examined (cell area, diameter, and cell-wall thickness).

The findings of this study demonstrate how greatly irrigation impacted most characteristics analysed. Meanwhile, the results of this study (mostly in accordance with published findings) are regarded as falling within the normal range for cork-stopper production, the most important use of cork.

Cork from the irrigated plot presented a greater thickness over a shorter formation period than that from the traditional rainfed plot over a regular formation period. The watering campaign, applied between June and October, contributed to cork-ring width increase, in accordance with the results of studies on the stimulation of greater growth (per ring) due to significant rainfall events (in spring, summer and autumn), carried out by Oliveira et al. (1996a, b), Caritat et al. (1996), Costa et al. (2002) and Surový et al. (2009).

In contrast to growth, no significant impact was found on density due to type treatment. However, some characteristics such as ring width, porosity, and cell-wall thickness may have contributed to observed density. Cork from the irrigated plot showed lower density values in keeping with biometric study results. In fact, cork

from Site A presented larger cells with greater transverse and tangential dimensions as well as thinner cell walls than cork from the traditional rainfed plot. Additionally, cork from the irrigated plot presented fewer cells per mm^2, which may affect certain properties of cork, such as its insulation capability, by decreasing the number of cells per mm^2, while the conductivity of heat and sound by cork is facilitated by a smaller number of cells. Cork from the irrigated plot showed higher porosity values in both planes than cork from the traditional rainfed plot.

In conclusion, irrigation proved to be a positive factor for cork growth (providing a contribution to growth monitoring), leading to increased cork production at a specific site and under specific conditions. Additionally, the silvicultural model implemented presented changes in cork features. Thus, the findings of this study indicate an opportunity for developing new irrigated cork oak silvicultural models aiming at the higher productivity of cork oak stands (under the same water regime conditions) and good cork technological quality, both factors being essential for achieving economic sustainability.

This study represents an initial approach to the characterization of cork in terms of a specific water regime and is part of ongoing research: the next steps will address different water regimes in order to ascertain optimal conditions for cork growth and thus achieve improved cork quality. Suggestions for future research are that the number of trees should be increased and a count of cell rows in cork rings should be conducted.

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Declarations

Conflicts of interest The authors declare no conflict of interest. All authors have read and agreed on the published version of the manuscript.

Data Availability The authors declare data transparency

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