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Climatic information in tree-ring width and vessel features of *Quercus ilex* L.

Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica da Professora Doutora Maria Cristina Amaral Penas Nabais dos Santos (Universidade de Coimbra) e do Doutor Filipe José Valente Campelo (Universidade de Coimbra).

José Manuel Ferrão Abrantes

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"O começo de todas as ciências é o espanto das coisas serem o que são."

Aristóteles

“Tal como as árvores registam nos seus anéis de crescimento as condições ambientais envolventes, nós, humanos, também formamos os nossos «anéis» ao longo dos anos, uns mais curtos e outros mais largos, os quais formam o todo que somos.”

Anónimo

Agradecimentos

Ao terminar esta etapa da minha formação académica, devo um agradecimento especial a algumas pessoas, as quais tornaram possível que esta fosse cumprida. Para alcançar esta solidez, elas tiveram um papel determinante. Umas ensinaram-me a construí-la, transmitindo-me, no dia-a-dia, os alicerces para a cimentar. Outras ajudaram-me a reconstruí-la, reforçando-a.

Não irei mencionar nenhum nome em específico, pois poderia ser injusto para alguém que, sem intenção, me poderia esquecer de referir. Para terminar, serei muito breve: apenas quero dizer a essas pessoas que as guardarei no meu coração.

Contents

Abstract

Holm oak (*Quercus ilex* L.), a diffuse to semi-ring porous tree, is a long-lived species widely distributed in the Mediterranean that has been recently used in dendroclimatological studies. In this work, we have compared the climatic signal of vessel features and tree-ring width (TRW) chronologies of *Q. ilex* from the Alqueva region (southeast Portugal), for the period 1942-2001. The features analyzed were maximum vessel lumen area (MAX), mean vessel lumen area (MVA) and vessel density (VDE). Each tree ring was divided into three equal parts, to guarantee that the vessels analyzed were formed within a similar time period.

Our results show that TRW was mainly correlated with late autumn/winter precipitation. MAX and MVA also showed a positive correlation with autumn precipitation, but only in the last portion of the ring. MAX and MVA were more sensitive to spring climatic conditions, with a positive correlation with precipitation and a negative one with temperature, a signal not detected in TRW. The climatic information of VDE was, to a great extent, the opposite of MAX and MVA, which can be related with the hydraulic design of tree-rings to balance conductivity and safety to embolism. MAX and MVA showed lag effects of climatic conditions, with climatic events occurring early in the growing season still affecting these variables in the last portion of the ring; while VDE seemed to be much more responsive to short-term climatic conditions, being correlated with climatic conditions along the growing season. The ecophysiological meaning of these results is discussed on the basis of tree physiology.

In conclusion, high-resolution time series of vessel features of *Q. ilex* can add new climatic information to TRW time series. This can be used to refine climate reconstructions and also to understand the wood anatomical plasticity, especially important in Mediterranean-type ecosystems that are likely to experience more frequent and intense droughts.

Key words: dendroclimatology, holm oak, Mediterranean climate, tree-ring width, vessel features

Resumo

A azinheira (*Quercus ilex* L.), uma árvore com poros difusos a semi-anelares, é uma espécie longeva largamente distribuída na região Mediterrânica que tem sido utilizada recentemente em estudos dendroclimatológicos. Neste trabalho, comparámos o sinal climático de cronologias das características dos vasos e do tamanho dos anéis de crescimento de *Q. ilex* na região do Alqueva (sudeste de Portugal), para o período entre 1942 e 2001. As características analisadas foram a área máxima do lúmen dos vasos, a área média do lúmen dos vasos e a densidade dos vasos. Cada anel de crescimento foi dividido em três partes iguais, de modo a garantir que os vasos analisados tivessem sido formados dentro de um período de tempo idêntico.

Os nossos resultados mostram que o tamanho dos anéis de crescimento esteve principalmente correlacionado com a precipitação do final do outono/inverno. As áreas máxima e média dos vasos também apresentaram uma correlação positiva com a precipitação de outono, mas apenas na última porção do anel. As áreas máxima e média dos vasos foram mais sensíveis às condições climáticas da primavera, com uma correlação positiva com a precipitação e uma negativa com a temperatura, um sinal não detetado no tamanho dos anéis de crescimento. A informação climática da densidade dos vasos foi, em grande medida, a oposta das áreas máxima e média dos vasos, o que pode estar relacionado com o desenho hidráulico dos anéis de crescimento para equilibrar a condutividade com a segurança ao embolismo. As áreas máxima e média dos vasos apresentaram efeitos retardados às condições climáticas, com eventos climáticos que ocorreram no início da estação de crescimento ainda a afectarem estas variáveis na última porção do anel; enquanto que a densidade dos vasos pareceu ser muito mais responsiva a condições climáticas a curto prazo, estando correlacionada com as condições climáticas ao longo da estação de crescimento. O significado ecofisiológico destes resultados é discutido com base na fisiologia das árvores.

Em conclusão, as séries temporais de elevada resolução das características dos vasos de *Q. ilex* podem adicionar nova informação climática às séries temporais do tamanho dos anéis de crescimento. Isto pode ser usado para refinar as reconstruções climáticas e também para perceber a plasticidade da anatomia da madeira, especialmente importante em ecossistemas Mediterrânicos que provavelmente irão experienciar secas mais frequentes e intensas.

Palavras-chave: dendroclimatologia, azinheira, clima Mediterrânico, tamanho dos anéis de crescimento, características dos vasos

1. Introduction

1.1. Vessel features time series: adding new climatic information to tree-ring width time series

Trees are long-lived organisms that can record climatic information in their annual rings. Therefore, tree rings can be used as proxies of past climate. Among the several climate proxies (e.g., ice cores, corals, pollen, lake and ocean sediments), tree ring records are generally the most accurate (IPCC 2007). The traditional tree-ring feature used in dendroclimatology is the tree-ring width (TRW), with an annual resolution (Fritts 2001). However, since early eighties, other wood anatomical variables, with a higher time resolution, such as vessel features, have also been used (Fonti *et al.* 2009b). Obtaining vessel time series is more time-consuming than obtaining ring-width series, but faster wood surface preparation techniques and semi-automated image analysis programs has reduced the workload needed for vessel measurements (Fonti *et al.* 2009a), which explains, in part, the increasing number of dendroclimatological studies that use vessel time series.

Several studies have demonstrated that vessel features can provide a different climatic signal compared to other tree-ring variables. García-González and Eckstein (2003) found a strong correlation between mean earlywood vessel area of *Quercus robur* L. and February to April precipitation, not present neither in earlywood width nor latewood width. Fonti and García-González (2004) showed that mean earlywood vessel area of *Castanea sativa* Miller contains climatic information (previous October and current February-March temperature) not recorded by TRW. Fonti and García-González (2008) found that mean earlywood vessel size of oak is controlled by spring precipitation in mesic sites, where ring-width variables have a weak climatic signal. Campelo *et al.* (2010) showed that in holm oak (*Quercus ilex* L.), maximum vessel area and mean vessel area of the largest 20-25 vessels from the first third of the ring have a better correlation with early spring and late spring precipitation, respectively, than TRW.

1.2. Influence of environmental conditions on vessel size

Vessel size can be affected by environmental conditions. Abundant water supply promotes the formation of large vessels, directly, by a high turgor pressure and a rapid vessel expansion (Tyree and Sperry 1989; Sass and Eckstein 1995) and, indirectly, by lowering the auxin concentration and, thus, slowing differentiation and allowing more vessel expansion (Kozlowski and Pallardy 1997; Aloni 2001).

Temperature may influence cambial sensitivity to auxin (Schrader *et al.* 2003), which in turn, may affect the process of differentiation of vessels (Aloni and Zimmermann 1983); it may also affect the storage of assimilates required for maintenance during the dormant period and the resumption of growth in the spring (Dickson 1991).

Vessel size is also a trade-off between water conducting efficiency and vulnerability to embolism, that is, although larger vessels have higher water transportation efficiency, they are more vulnerable to cavitation than smaller ones (Hacke and Sperry 2001; Sperry *et al.* 2006). On the other hand, vessels more resistant to cavitation are more energy-cost (Hacke and Sperry 2001).

1.3. *Quercus ilex* L.: a species with a great dendrochronological potential in the Mediterranean region

Holm oak, a semi-ring to diffuse porous tree (Schweingruber 1990), is a long-lived (Panaïotis *et al.* 1997; Patón *et al.* 2009) and drought-tolerant (Terradas and Savé 1992; Montserrat-Martí *et al.* 2009) species widely distributed in the Mediterranean Basin, being the dominant evergreen oak in the western part (Barbero *et al.* 1992; Terradas 1999; Blanco Castro *et al.* 2005) (Fig. 1).

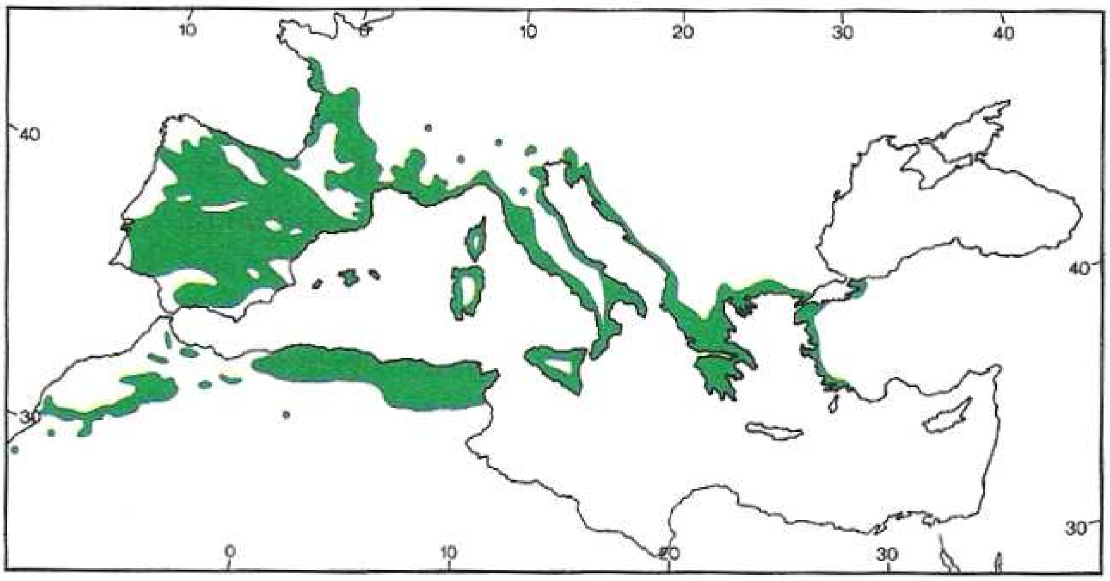


Fig.1. Distribution of *Quercus ilex* L. (adapted from Blanco Castro *et al.* 2005).

Its wide distribution, longevity and the possibility to accurately date old *Q. ilex* trees (Campelo *et al.* 2009; Gea-Izquierdo *et al.* 2009), despite the presence of double rings (Cherubini *et al.* 2003; Campelo *et al.* 2007), confers upon this species a great potential for dendrochronology. Although there are already some dendroclimatological studies that used tree-ring width chronologies of holm oak (Nabais *et al.* 1998-1999; Cherubini *et al.* 2003; Campelo *et al.* 2009; Gea-Izquierdo *et al.* 2009; Nijland *et al.* 2011), very few studies investigated the climatic signal of vessel features of this species (Corcuera *et al.* 2004; Campelo *et al.* 2010). Moreover, the longest vessel features chronology for this species spans only 20 years (Campelo *et al.* 2010) and, therefore, longer time series are needed to confirm the value of holm oak vessels for dendrochronological studies.

1.4. Objectives of the present work

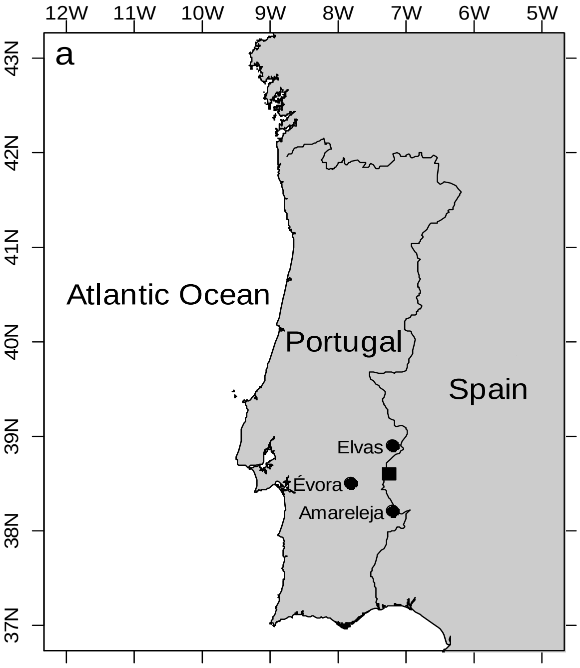
In this study, we investigated whether the variation in tree-ring width and vessel features (maximum and mean vessel areas and vessel density) of *Q. ilex* is driven by similar climatic factors. To test this hypothesis we have compared the intra- and inter-annual variation of the vessel features and the inter-annual variation of tree-ring width, with the intra-annual variation in the climate variables (monthly total precipitation and monthly mean temperature).

2. Materials and Methods

2.1. Study area and climate

The study area was located in the Alqueva region, near the Guadiana river (southeast Portugal; 38º36’N, 7º15’W; 140 m a.s.l.) (Fig. 2a). Before the construction of the Alqueva dam, which created the largest artificial lake in Europe, this area was characterized by a human managed forest of holm oak, named ‘Montado’. However, all the vegetation was cut down before the Alqueva reservoir started to fill in 2002.

The climate is Mediterranean, with a mean annual temperature of 16.1ºC and a total annual precipitation of 601 mm. Rainfall mainly occurs from October to April, with a dry period in summer. The climatic diagram was constructed by averaging climate data (monthly total precipitation and monthly mean temperature) from the three nearest meteorological stations: Amareleja (38º12’N, 7º12’W; 192 m a.s.l.), Elvas (38´54’N, 7º12’W; 208 m a.s.l.) and Évora (38º30’N, 7º48’W; 308 m a.s.l.) (Fig. 2b).



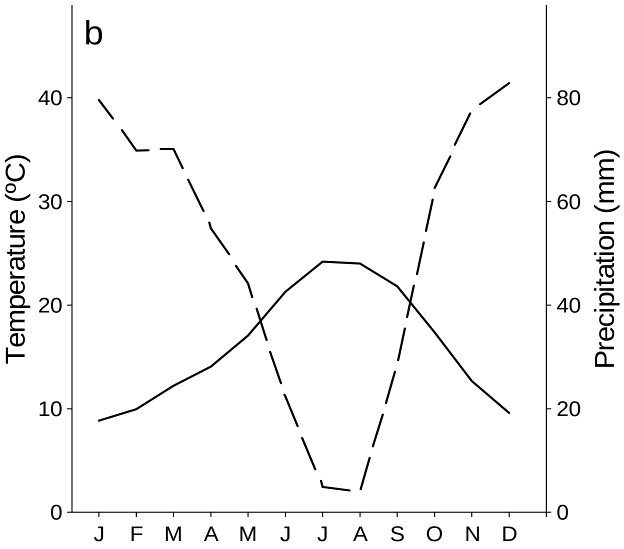


Fig. 2. (a) Study area (square) and the three closest meteorological stations (circles). **(b)** Climatic diagram using mean data of the three nearest metereological stations (Amareleja, Elvas and Évora) for the period 1941–2001. Data were obtained from Instituto de Metereologia of Portugal. Dashed and solid lines correspond to monthly total precipitation and monthly mean temperature, respectively.

2.2. Wood preparation and vessel measurements

In a previous work (Campelo *et al.* 2009), 30-cm-aboveground cross sections of twenty trees, cut down before the filling of the Alqueva dam, were used to study the climatic signal of the tree-ring width. In the present study, we have selected eleven out of these twenty trees, excluding trees with less than 60 years or with many tyloses, and analyzed one cross-section per tree.

Tree rings were visually crossdated, tree-ring width (TRW) was measured and the visual crossdating was statistically confirmed using the program COFECHA (Holmes 1983; Grissino-Mayer 2001). The samples were prepared for vessel measurements (Sass and Eckstein 1994). First, wood dust and tyloses were removed from vessel lumen with a high-pressure water blast. Afterwards, vessels were filled with white chalk, to increase the contrast between vessel lumen and the ground tissue. Images of the cross-section surface were captured on a radial path from the bark to the pith using a digital camera (Nikon DMX 1200F) attached to a stereomicroscope. The tangential width of these sections was larger than 7 mm.

ImageJ software (Abràmoff *et al.* 2004) was used to analyze the digital images. Tree-ring limits were identified by the differences in vessel lumen area and by the marginal parenchyma bands (Campelo *et al.* 2007). These limits and wood rays were used to establish, respectively, tangential and radial boundaries of sections analyzed for vessel measurements. For each tree ring, vessel lumen areas were measured. Size (1500–80000 μm^2) and shape (objects with a width/length ratio equal or lower than 0.60 were excluded) filters were defined to remove objects that were not vessels. Misrecognized vessels were manually corrected. Such corrections consisted of adding non-recognized vessels, deleting misrecognized vessels, splitting clustered vessels and correcting misrecognized vessel contours. After all manual corrections and before the measurement of the lumen area, vessel outlines were smoothed to an elliptical shape (Fig. 3). In order to determine the relative radial position of each vessel within the tree ring, the coordinates of its centroid were recorded.

2.3. Data analysis

The period for the analysis was 1942–2001 (60 years). In order to separate vessels formed during different periods, each tree ring was radially divided into three equal parts (Fig. 3). Maximum vessel lumen area (MAX), mean vessel lumen area (MVA) and vessel density (VDE) were determined for each one.

Site chronologies of TRW and vessel variables (MAX, MVA and VDE) were obtained from individual time series. Non-climatic growth trends, associated with low-frequency variability, were estimated, by fitting a cubic smoothing spline with a 50% frequency cut-off of 32 years, and removed, by dividing the original data by the fitted curve (Cook and Peters 1981). The growth indices obtained were averaged to build the chronologies.

The statistical quality of chronologies was evaluated by four parameters commonly used in dendrochronology: mean correlation between trees (R_{bt}), expressed population signal (EPS), mean sensitivity (MS) and first-order autocorrelation (AR_1) (Wigley *et al.* 1984; Briffa and Jones 1990; Fritts 2001). The EPS value measures how well a chronology based on a finite number of trees represents the hypothetical population chronology, so that, the higher the R_{bt} and the sample size, the higher the EPS. First-order autocorrelation is a measure of the degree to which growth in a given year is similar to growth in the preceding year. Mean sensitivity measures the relative difference between tree-ring variables of consecutive rings (Fritts 2001).

We performed a principal component analysis (PCA) to evaluate differences among tree-ring variables. Growth/climate relationships were analyzed by Pearson’s correlations calculated between tree-ring variables and monthly climatic data (total precipitation and mean temperature) from October of the previous year (t-1) to November of the current year (t). The following monthly grouped periods were also correlated with tree-ring variables: November$_{(t-1)}$–March$_{(t)}$ and April$_{(t)}$–May$_{(t)}$.

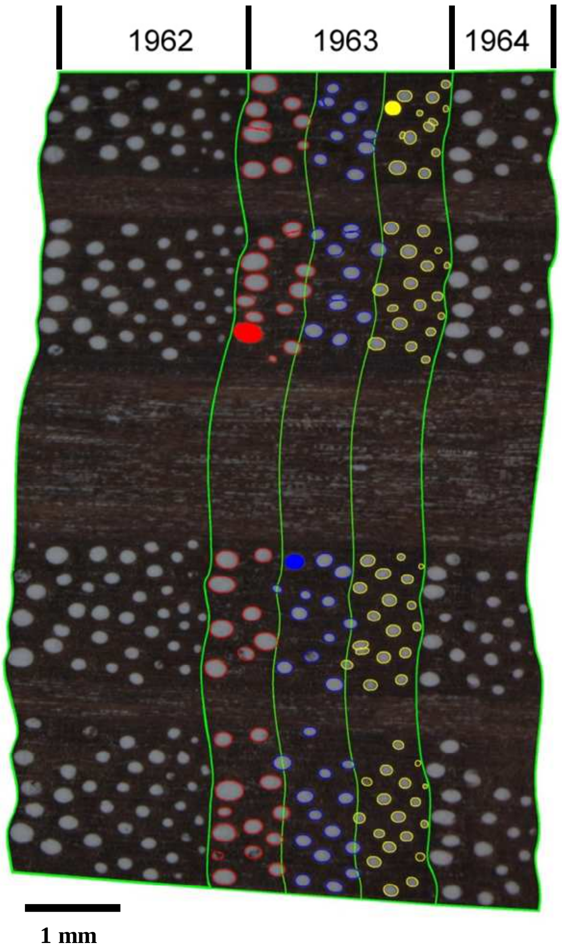


Fig. 3. Example of an image of *Quercus ilex* wood surface used to determine the lumen area of vessels and their position within the ring. Green lines are used to delimit tree rings (1962–1964) and also to radially divide the ring 1963 into three equal parts. Vessels of the first, second and third parts are bounded by red, blue and yellow contours, respectively. The largest vessel of each third is filled.

3. Results

3.1. Vessel distribution and time series of tree-ring variables

The lumen area of all measured vessels (*n* = 54,926) had a mean value of 10,510 μm^2 and ranged from 1,291 μm^2 to 77,547 μm^2. Vessel size decreased gradually across the ring, mainly towards its end (Figs. 3, 4). Small vessels occurred throughout the ring, but were more frequent at the final part, while large vessels were more frequent at the beginning (Figs. 3, 4). Their frequency distribution was skewed to the left, with few large vessels and many small vessels (Fig. 5).

Vessels were larger in the first third of the ring (higher MAX and MVA), but more frequent in the last portion (higher VDE) (Fig. 6). In the detrended series, where only high-frequency variation was retained, the growth index of TRW showed a higher year-to-year variation as compared to the growth indices of vessel variables (Fig. 6).

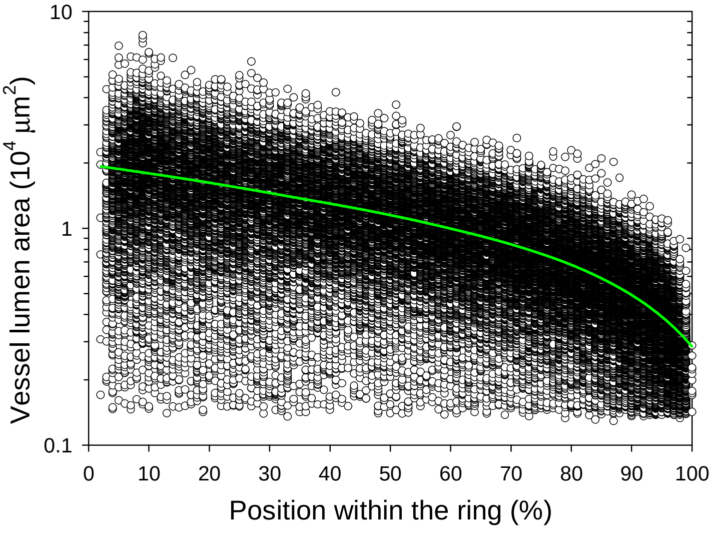


Fig. 4. Distribution of all measured vessel lumen areas across the ring; the green line represents the smoothing curve with a span width of 0.70.

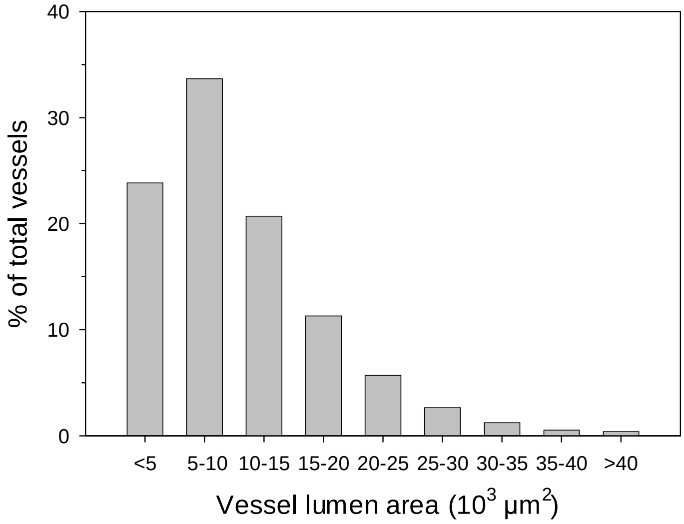


Fig. 5. Frequency distribution of lumen area of *Quercus ilex* vessels.

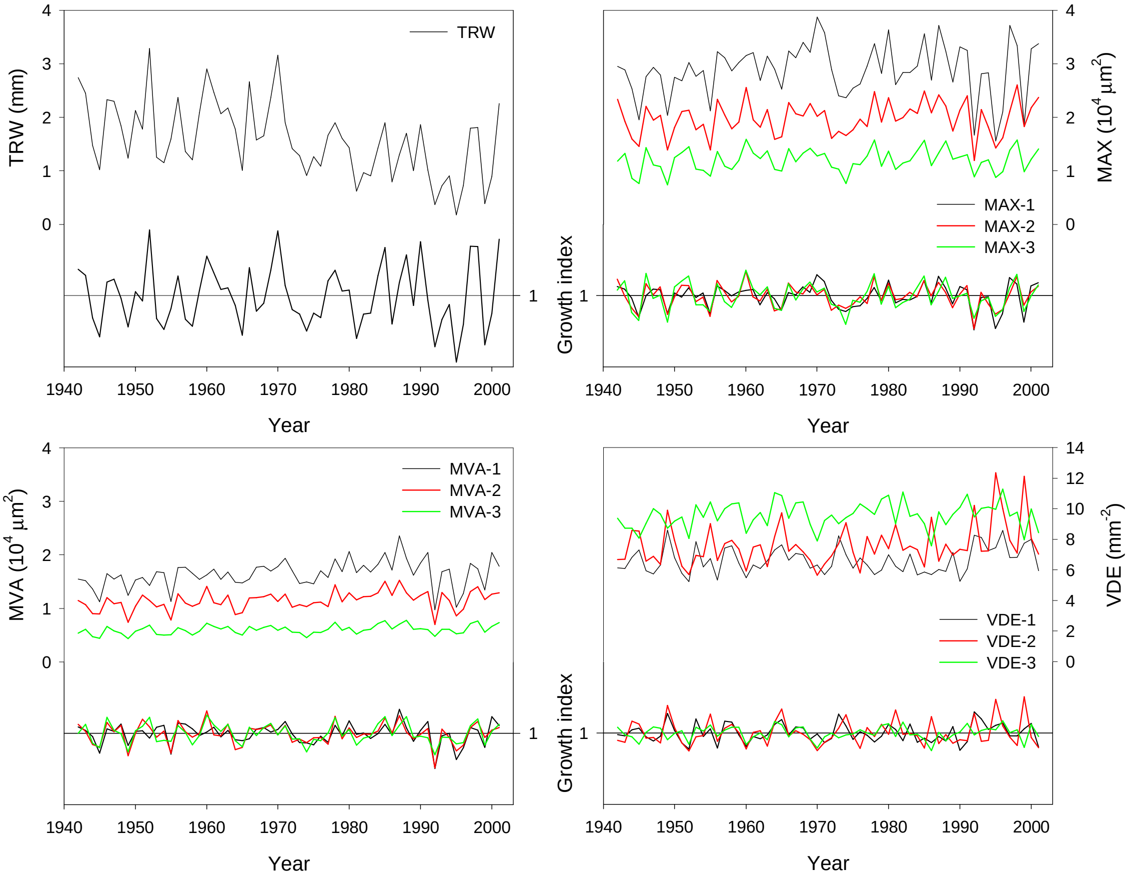


Fig. 6. Raw and standardized time series of tree-ring width (TRW), and of maximum vessel area (MAX-1, MAX-2, MAX-3), mean vessel area (MVA-1, MVA-2, MVA-3) and vessel density (VDE-1, VDE-2, VDE-3) of each third of the ring, for the period 1942–2001.

3.2. Quality of chronologies

TRW had a very high common signal, while all vessel variables yielded EPS values lower than the critical value of 0.85 proposed by Wigley *et al.* (1984) (Table I). EPS values of vessel lumen area variables (MAX and MVA) were higher than those of VDE. All variables were not significantly affected by previous growth (very low AR_1 values). TRW had a higher year-to-year variation (higher MS values) than vessel variables (Table I).

Table I. Correlation between trees (R_{bt}), expressed population signal (EPS), mean sensitivity (MS) and first-order autocorrelation (AR_1) for tree-ring width (TRW), and for maximum vessel area (MAX-1, MAX-2, MAX-3), mean vessel area (MVA-1, MVA-2, MVA-3) and vessel density (VDE-1, VDE-2, VDE-3) of each third of the ring, for the interval 1942–2001.

	TRW	**MAX**			**MVA**			**VDE**		
		1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
R_{bt}	0.556	0.266	0.255	0.320	0.207	0.316	0.291	0.158	0.197	0.101
EPS	0.926	0.784	0.774	0.824	0.723	0.822	0.804	0.653	0.710	0.529
MS	0.451	0.171	0.178	0.200	0.145	0.153	0.138	0.123	0.183	0.100
AR_1	0.100	0.042	-0.089	0.022	-0.029	-0.067	0.102	0.045	-0.111	-0.021

3.3. Comparison among variables

The differences in the information contained in the tree-ring variables are summarized in a principal component analysis (PCA). The two first principal components were responsible for 79.9% of the total variance (67.4% and

12.5%). The first axis showed a separation between VDE variables and all the other variables (Fig. 7). In the second axis, there were clear differences between VDE-3 and all the other tree-ring features, whereas MAX-1, MVA-1 and VDE-1 expressed small differences in relation to TRW (Fig. 7).

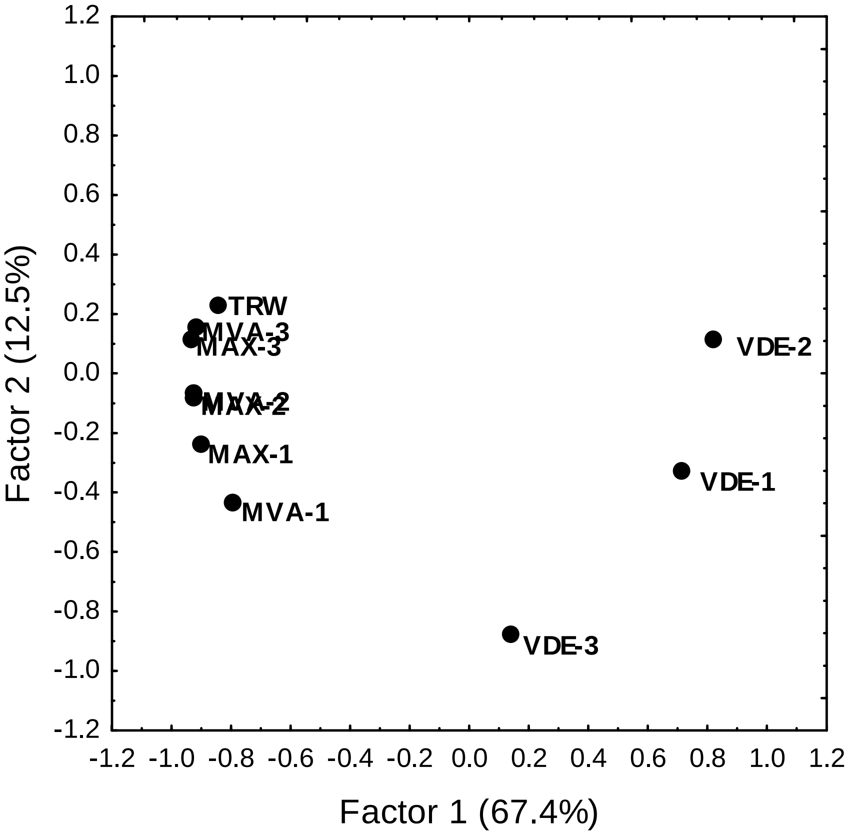


Fig. 7. Principal component analysis of all tree-ring variables analyzed.

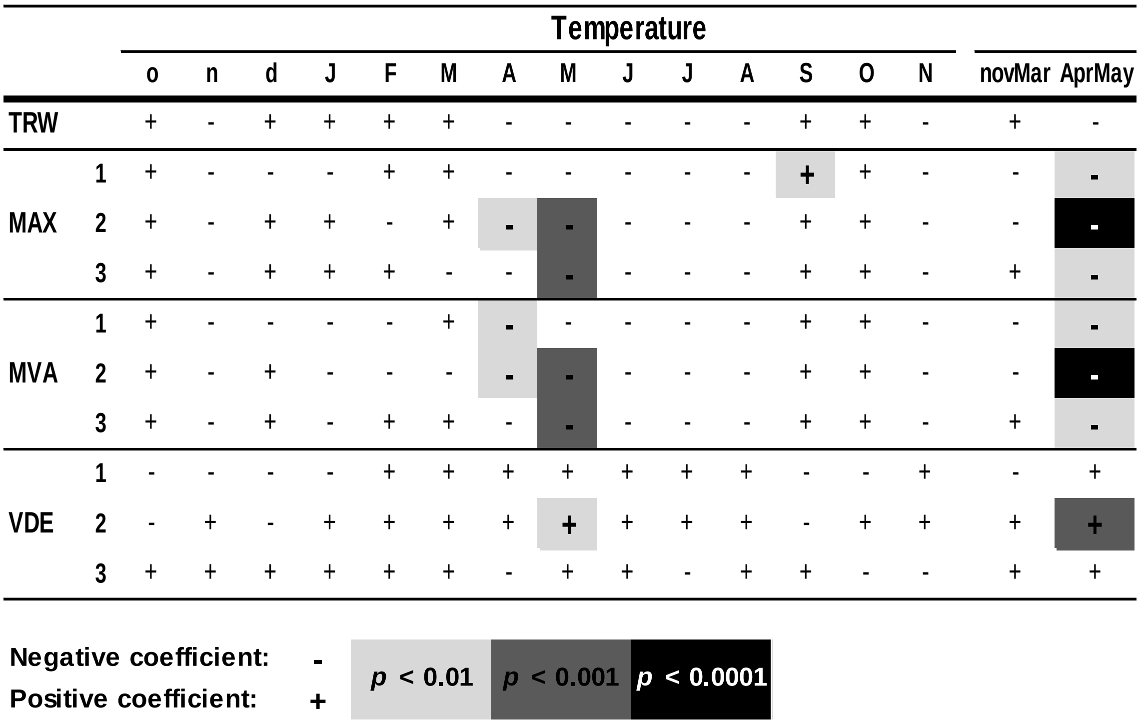
3.4. Tree-ring growth – climate relationships

Regarding climate-growth relationships, TRW, MAX and MVA were positively correlated with late autumn/winter precipitation. MAX and MVA were also positively and negatively correlated with spring precipitation and temperature, respectively (Table II). VDE presented negative correlations with late autumn/winter, spring and early summer precipitation and positive correlations with spring temperature (Table II).

The analysis of vessel features at different radial positions showed that the climatic signal varied across the tree ring. For MAX and MVA, the second third of the ring had a stronger correlation with spring temperature than the adjacent parts, as well as the third part with late autumn/winter precipitation (Table II). In the case of VDE, the first third of the ring maximized the correlation with previous autumn/winter precipitation, while precipitation and temperature in spring and precipitation in early summer were only correlated with the second and third parts of the ring, respectively (Table II).

Table II. Pearson's correlations between the standardized tree-ring variables (TRW, MAX-1, MAX-2, MAX-3, MVA-1, MVA-2, MVA-3, VDE-1, VDE-2, VDE-3) and the climatic variables (monthly mean air temperature and monthly total precipitation), for the period 1942–2001. The last two columns are groups of months (novMar: November$_{(t-1)}$–March$_{(t)}$; AprMay: April$_{(t)}$–May$_{(t)}$).

		Precipitation															
		o	n	d	J	F	M	A	M	J	J	A	S	O	N	novMar	AprMay
TRW		+	**+**	**+**	**+**	+	+	+	+	+	+	+	-	+	+	**+**	+
	1	+	+	+	+	+	+	+	+	+	+	+	-	+	-	**+**	+
MAX	2	+	+	+	+	+	+	**+**	+	-	-	+	+	+	+	+	**+**
	3	+	**+**	+	+	+	+	+	**+**	+	+	+	-	+	+	**+**	**+**
	1	+	+	+	+	+	+	**+**	+	-	+	+	-	+	-	+	+
MVA	2	+	+	+	+	+	+	**+**	+	-	-	+	-	+	+	+	**+**
	3	+	**+**	+	+	+	+	+	**+**	+	+	+	-	+	+	**+**	**+**
	1	-	**-**	-	-	-	-	-	-	-	-	-	+	-	+	**-**	-
VDE	2	-	-	-	-	-	-	-	-	-	+	-	+	-	-	**-**	**-**
	3	+	-	+	-	+	+	-	-	**-**	+	-	-	-	+	+	-



		Temperature															
		o	n	d	J	F	M	A	M	J	J	A	S	O	N	novMar	AprMay
TRW		+	-	+	+	+	+	-	-	-	-	-	+	+	-	+	-
MAX	1	+	-	-	-	+	+	-	-	-	-	-	**+**	+	-	-	**-**
	2	+	-	+	+	-	+	**-**	**-**	-	-	-	+	+	-	-	**-**
	3	+	-	+	+	+	-	-	**-**	-	-	-	+	+	-	+	**-**
MVA	1	+	-	-	-	-	+	**-**	-	-	-	-	+	+	-	-	**-**
	2	+	-	+	-	-	-	**-**	**-**	-	-	-	+	+	-	-	**-**
	3	+	-	+	-	+	+	-	**-**	-	-	-	+	+	-	+	**-**
VDE	1	-	-	-	-	+	+	+	+	+	+	+	-	-	+	-	+
	2	-	+	-	+	+	+	+	**+**	+	+	+	-	+	+	+	**+**
	3	+	+	+	+	+	+	-	+	+	-	+	+	-	-	+	+

Negative coefficient: -

Positive coefficient: +

$p < 0.01$ $p < 0.001$ $p < 0.0001$

4. Discussion

4.1. Comparisons of tree-ring variables chronologies

The study of climatic signals in high-resolution time series of wood anatomical variables can add new information to the classical dendrochronological methods of ring width measurements. Moreover, vessels at different radial positions are formed at different periods during the growing season and can incorporate different information about climate (García-González and Fonti 2006). Therefore, to maximize their climatic signal it is important to select and compare vessels based on their relative radial position.

Analyzing the chronology quality of the tree-ring features measured, TRW presents a higher common signal (higher Rbt and EPS values) and year-to-year variation (higher MS values) than vessel variables. These results are consistent with previous works (García-González and Eckstein 2003; Tardif and Conciatori 2006; Fonti and García-González 2008; Campelo et al. 2010). Although the EPS assesses the statistical quality of the chronologies, it does not show their climatic information. Therefore, to know their climatic signal it is necessary not only to compare tree-ring variables with climate, but also among them because they may not have an independent response to the environment (Wimmer and Grabner 2000; Fonti and García-González 2004). Thus, a principal component analysis (PCA) was performed. The PCA aggregated TRW, MAX and MVA, while VDE was separated from those variables. So, TRW, MAX and MVA store similar information, suggesting that these variables are controlled to a great extent by similar climatic parameters. Additionally, the PCA showed that VDE-3 was separated from VDE-1 and VDE-2, indicating that they may be controlled by different climatic parameters.

4.2. Climatic signal and its ecophysiological meaning

In the study area, vessel features and tree-ring width showed highly significant correlations with monthly climate data, which confirms the strong influence of climate on holm oak growth.

The main climatic signal revealed by the TRW of *Q. ilex* was the positive correlation with late autumn/winter precipitation. Thus, the refill of soil water reserves during winter, when rainfall reaches its maximum, is a major determinant of tree growth in the following growing season. This was also observed in previous studies with *Q. ilex* using TRW time series (Zhang and Romane 1991; Nabais *et al.* 1998-1999; Cherubini 2003; Corcuera *et al.* 2004; Campelo *et al.* 2009).

MAX and MVA also showed a significant positive correlation with late autumn precipitation, but only in the third part of the ring. Trees produce large vessels at the beginning of the growing season, when water availability is high, increasing the hydraulic conductivity according to the Hagen-Poisseuille's law (Tyree *et al.* 1994). Although *Q. ilex* has a diffuse to semi-ring porous wood, MAX and MVA were higher in the first portion of the tree-ring. As the season progresses, water availability decreases and vessels with a smaller lumen area are produced in response to low turgor pressure (Domec and Gartner 2002; Abe *et al.* 2003). These vessels are more resistant to drought-induced embolism than wider vessels (Sperry and Tyree 1988; Lo Gullo and Salleo 1993; Sperry *et al.* 1996). Thus, the last portion of the tree-ring is much more sensitive to the availability of water, and maybe the reasoning behind why MAX and MVA only showed a significant correlation with late autumn precipitation in this part of the tree-ring.

VDE showed a strong negative correlation with late autumn/winter precipitation, but only in the first portion of the tree-ring, when MAX and MVA showed the higher values. It was also observed that VDE was higher in the last portion of the tree-ring. This can represent a strategy to increase the safety of vessels to embolism (vessels with a lower lumen area), but at the same time keeping some conductive efficiency (more vessels per area) when water availability is lower. So, on the contrary, in the beginning of the growing season, when there is a higher availability of water, it is more important to have fewer but larger vessels to increase hydraulic conductivity.

Vessel features showed climatic signals not revealed by TRW, namely with precipitation and temperature in spring. MAX and MVA showed a positive correlation with spring precipitation (April-May) and a negative one with spring temperature (April-May), with MVA showing this correlation along the three parts of the tree-ring. VDE showed a negative correlation with early summer precipitation (June), only in the last portion of the tree-ring, and a positive correlation with spring temperature in the second part of the tree-ring. High temperatures during spring can potentially increase evapotranspiration and water stress and to reduce vulnerability to embolism, vessel lumen area is smaller and/or vessel density is higher. High temperatures also increase the cambial sensitivity to auxin, resulting in smaller vessels due to a faster process of differentiation and less vessel expansion before secondary wall deposition (Aloni and Zimmermann 1983; Schrader *et al.* 2003).

In general, VDE showed the opposite correlations with climate when compared with MAX and MVA, suggesting a combined strategy to balance the safety to embolism and conductive efficiency (Hacke and Sperry 2001; Sperry *et al.* 2006). Thus, in years with low availability of water in winter and spring, vessels have a lower lumen area, but their density tends to be higher, possibly to compensate the hydraulic efficiency.

4.3. Final remarks

TRW, MAX and MVA have some common climatic information, namely a positive correlation with previous autumn and winter precipitation. MAX and MVA also showed a positive and negative correlation with spring precipitation and temperature, respectively, a climatic signal not found in TRW. The climatic signal of VDE was to a great extent the opposite of the one found in MAX and MVA, revealing that VDE somehow compensates MAX and MVA in the trade-off between vulnerability to embolism and hydraulic conductivity.

MAX and MVA showed lag effects of climatic conditions, meaning that climatic conditions occurring early in the growing season can still affect vessel size later in the growing season. VDE seems much more responsive to short-term climatic conditions, with the first portion correlated with late autumn/winter precipitation, the second portion with spring temperature, and the last portion with early summer precipitation.

The study of wood anatomical variables of Mediterranean trees can add new climatic information to the tree-ring width time series. Recent climate projections for the Mediterranean area predict a decline in precipitation during spring and summer (Gao and Giorgi 2008; Somot *et al.* 2008). From our results, spring precipitation affects MAX and MVA and can have a major impact on the hydraulic conductivity of *Q. ilex*. Thus, more studies on xylem hydraulic and wood anatomical plasticity are important to understand the consequences of a long-term increased drought.

5. References

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