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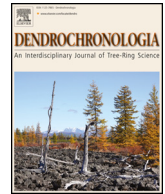
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Original Article

Towards a new approach for dendroprovenancing pines in the Mediterranean Iberian Peninsula



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ABSTRACT

Dendroprovenancing studies frequently use site chronologies to identify the origin of archaeological and historical timber. However, radial growth (tree-ring width, TRW) of tree species is influenced by both local and regional climate scales. Here we investigate how the use of annually-resolved Blue Intensity (BI) measurements can enhance dendroprovenancing precision of black pine (*Pinus nigra* Arn.) and Scots pine (*P. sylvestris* L.) on the Iberian Peninsula. Principal Component Gradient Analyses (PCGA) was used to assess geographical patterns of annual variation in different TRW and BI proxies of pine trees from two mountain ranges in the Central System and Andalusia in Spain. Local climate-growth relationships were quantified to identify underlying causes of identified groups with diverse growth patterns. Two distinct elevational groups were observed when performing PCGA on latewood BI time series with the response to summer drought as the main factor causing the differences. Both *P. nigra* and *P. sylvestris* BI time series were found to be more related to summer drought at low-elevation sites showing an increase in sensitivity at lower latitudes. PCGA of TRW time series allowed to discriminate between trees from Andalusia and Central System within the elevation groups. February and October temperatures were found to be the main climatic factors causing the differences in TRW time series among the high-elevation sites, whereas for low-elevation trees it was the average winter temperature influencing TRW. A subsequent leave-one-out analyses confirmed that including latewood BI time series improves the precision of dendroprovenancing of pine wood in the Iberian Peninsula.

1. Introduction

Identifying the geographical origin of timber using dendroprovenancing methods has provided insights into the use of local and foreign wood resources in a specific time and place, allowing inferences about the organisation of timber trade networks during past centuries (Bridge, 2012). Dendroprovenancing of wood from a given species is traditionally done by comparing tree-ring width (TRW) time series derived from (pre)historical wood with a set of available TRW reference chronologies (Eckstein and Wrobel, 2006). The source area is then detected by a maximum match (e.g. using Student's *t*-value or/and correlation coefficient), but this method has strong pitfalls (Bridge, 2012), particularly when strong teleconnections are found between forests located far away from each other (e.g. Domínguez-Delmás et al.,

2013). Moreover, reference site chronologies from living trees frequently do not reach back in time for successful dating of timber, and therefore often regional tree-ring width chronologies without exact known origin are used. Seeking to improve the accuracy of dendroprovenancing, other methods based on more site-dependent variables have shown promising results, such as the use of vessel-size oak chronologies in Spain (Akhmetzyanov et al., 2019), and stable carbon isotopes of pine species in SW USA (Kagawa and Leavitt, 2010). Wood anatomical structure of historical timber can also be used for species identification, and thus, based on species distribution, potential sources area of the timber can be identified (Romagnoli et al., 2007). Moreover, Esteban et al. (2012) have found more pronounced variation in wood biometry between provenances of black pine populations rather than within population, indicating strong potential of wood anatomy for use

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in dendroprovenancing studies. In addition, qualitative wood anatomical features were also found to play an important role in identification of timber origin, e.g. abnormal small earlywood vessels of a specific year in Baltic oaks (Fletcher, 1975), or ray frequency and ray parenchyma cell frequency in pines (Martin et al., 2010). Tree-ring based variables can also be combined with other timber tracing techniques based on pyrolysis-gas chromatography mass spectrometry (Vinciguerra et al., 2011) or on mass spectrometry to assess the composition of species-specific and site-specific organic (Traore et al., 2018) and inorganic wood substances (Hajj et al., 2017; Paredes-Villanueva et al., 2018), or on DNA analyses (Paredes-Villanueva et al., 2019; Vlam et al., 2018) to link a piece of timber to a specific tree population.

It is noteworthy that maximum latewood density of conifers (MXD) has been proven to be a more robust variable than TRW for temperature reconstructions in cool temperate climates (Grudd, 2008; McCarroll et al., 2013). MXD series are found to have a stronger correlation with the temperature during the growing season, at least in northern mid- and high latitudes (Anchukaitis et al., 2013; Wilson et al., 2016), lower autocorrelation and lag effects (Briffa et al., 2002), and are also less disturbed by other ecological signals than climate (Rydval et al., 2015). These characteristics make MXD series a very promising tool for provenancing historical timbers of conifer species. However, the costs for generating MXD data are high, both time-wise and financially (Campbell et al., 2007), which results in a restricted number of studies that use this method for provenancing purposes.

However, the improvements of digital image analyses have evolved towards an easier and cheaper alternative to MXD: the blue intensity (BI), i.e., the intensity of the blue channel of light reflected from the wood surface (c.f. McCarroll et al., 2002). Minimum latewood blue intensity (LWBI), which can easily be measured from scans of tree cores, shows high similarities to MXD (Björklund et al., 2014; Rydval et al., 2014) because it reflects similar wood characteristics as MXD, such as combined lignin, cellulose and hemicellulose content in the cell walls of latewood tracheids (Björklund et al., 2014; Wilson et al., 2014; Wilson et al., 2017). McCarroll et al. (2002) linked blue reflectance to the amount of lignin in the wood, as lignin absorbs most of the energy of short-wavelength light (blue light) (Schubert, 1965), whereas with the increase of the wavelength, e.g. red or green light, the absorption decreases, thus reducing sensitivity to lignin content, and consequently, to the wood density. The BI method has been used largely to extract climatic signals from tree rings and to build multi-century temperature reconstructions in cool temperate environments (Fuentes et al., 2018), and recently was also applied in tropical forests (Buckley et al., 2018). Pioneer work exploring the use of LWBI data to provenance wood of historic buildings in Scotland showed great potential (Wilson et al., 2017), whereas the possibilities of using maximum earlywood blue intensity (EWBI) for historical studies have yet to be tested. However, we still lack a comprehensive assessment of LWBI-suitability for dendroprovenancing in water-limited environments like in the Iberian Peninsula, where trees endure a double climatic stress of cold winters and summer droughts (Mitrakos, 1980). Thus, it would be of interest to assess how BI can help dendroprovenancing techniques in an area with drought limitations, with a historically strategic position in trade routes, and high abundance of cultural heritage suitable for dendroarchaeological research.

Classical TRW-based dendrochronology has successfully been applied in Spain for dating and provenancing timbers of Scots and black pine (respectively *Pinus sylvestris* and *P. nigra*) of different historic buildings (Domínguez-Delmás et al., 2017; Domínguez-Delmás et al., 2018; Richter and Eckstein, 1986; Richter and Rodríguez Trobajo, 1986). However, provenancing of black pine in this territory could be restricted by the fact that TRW site chronologies show stronger correlations with chronologies of similar-elevation sites located hundreds of kilometres away, rather than with nearby chronologies at different elevations (Domínguez-Delmás et al., 2013). In Bavaria (Germany), Norway spruce (*Picea abies*) chronologies originating from sites located

in close vicinity but at different elevations also showed a low mutual correlation with each other (Wilson and Hopfmueller, 2001). Elevation was also found to be the main driver of variation in TRW for three pine species in the Eastern Mediterranean region (Touchan et al., 2016). This large-scale elevation-based similarity in growth patterns has advantages for dendrochronological dating, as it allows for the creation of so-called “regional chronologies” covering large areas at similar elevations. Yet, the additional small-scale network of chronologies using a combination of wood features (e.g. ring-width based method and wood anatomical properties) may improve timber provenancing studies (Akhmetzyanov et al., 2019).

Here, we assess whether variables obtained through BI-based measurements can improve the dendroprovenancing accuracy of Scots pine (*Pinus sylvestris* L., PISY) and black pine (*Pinus nigra* subsp. *salzmannii* Arn., PINI) timber on the Iberian Peninsula. Our objectives are (a) to evaluate whether BI can improve the precision of previous TRW-only dendroprovenances at different elevations and (b) to test whether BI is a suitable proxy under dry Mediterranean conditions to more precisely identify the geographical origin of these pine species in the Iberian Peninsula. We hypothesize that BI-based time series contain a different and stronger climatic signal than TRW, which will allow for pinpointing the elevation and geographic origin of timber with higher precision, thereby overcoming the above mentioned limitations of TRW series.

2. Materials and methods

2.1. Study areas and species

The study was carried out along an elevational gradient located on the north-facing slopes of the Spanish Central System (CS) (Sierra de Guadarrama National Park) and Cazorla, Segura and Las Villas Natural Park in Andalusia, southern Spain (AND), two mountain woodlands dominated the former by Scots pine (*Pinus sylvestris* L.), and the latter by black pine (*P. nigra* subsp. *salzmannii* Arn.) above 1000 m. a.s.l. Historical evidence and dendrochronological studies show that these forests were intensively managed since ancient times up to the mid-20th century for the exploitation of construction timber (Trobajo, 2008). As a result, wood from these forests can nowadays be found in historic buildings of different periods.

Both areas have a prevalent Mediterranean climate with marked continental influence, characterized by dry summers and cool winters (Sánchez-Salguero et al., 2015). The total annual rainfall is 1266 mm for CS and 1100 for AND, and the mean annual temperature is 6.5 °C and 11.7 °C for CS and AND, respectively. The bedrock is mainly formed by granite and gneiss in CS and by limestone and dolomites in AND.

2.2. Sample collection and data acquisition

Between 2014 and 2016, as part of the ForSeaDiscovery project (<http://forseadiscovery.eu/>), field samplings were carried out in three pine stands for each region considering high-elevation > 1500 m a.s.l and low-elevation < 1500 m a.s.l sites. Two PISY stands in CS were sampled at high-elevation (ARF and FFR) and one mixed stand with PISY and PINI individuals at low elevation (JRS/JRN; Table 1). In the case of Andalusia (AND) two sampled PINI stands were located at high elevation (PMB and NAV) and one PINI stand at low elevation (LIN; Table 1).

The largest and apparently oldest pine trees with no visible damage to crowns or stems were selected for sampling, and two to four 5-mm tree-ring cores were taken from each tree at breast height. Sample sizes varied between study sites due to the different number of suitable trees at each stand (see Figure S1 for the detailed information on sampling area).

Due to varying quality of collected samples, cores were divided into two groups: a) for tree-ring width (TRW) measurements, and b) for the blue intensity (BI), TRW, earlywood width (EW) and latewood width

Table 1
Characteristics of the study site and sampling strategies.

Mountain range	Forest	Code	Coord.	Species	Bed rock	Elevation (m a.s.l)	#trees TRW	#trees BI	Dbh (cm)	Mean #rings
Sierra de Guadarrama	Arroyo Frío	ARF	40°47' N 3°59'W	PISY	MG	1917	83	13	58	146 ± 42
	Fuenfria	FFR	40°47' N 4°2'W	PISY	MG	1900	30	11	93	357 ± 90
	La Jarosa	JRS	40°39' N 4°9'W	PISY	MG	1421	14	11	61	169 ± 49
	La Jarosa	JRN	40°39' N 4°9'W	PINI	MG	1421	27	11	58	386 ± 100
Sierra de Cazorla	Linarejos	LIN	37°55' N 2°54'W	PINI	SL	1191	64	14	49	160 ± 32
	Navanoguera	NAV	37°55' N 2°48'W	PINI	SL	1585	63	12	59	150 ± 88
	Poyos de la Mesa	PMB	37°53' N 2°54'W	PINI	SL	1596	17	10	86	331 ± 121

Code - abbreviation used for the study forest;

Coord. - coordinates of the study site;

PISY and PINI - *Pinus sylvestris* and *Pinus nigra*, respectively;

MG and SL - magmatic granite and sedimentary limestone, respectively;

#trees TRW - the number of trees used for ring width, earlywood width, and latewood width measurements;

#trees BI - the number of trees used for blue intensity measurements;

Dbh - average diameter at breast height;

#rings - mean number of rings per tree.

(LW) measurements. For the latter, increment cores with no obvious cracks or wood discolorations were selected. Samples selected for the TRW analyses were air dried, glued onto wooden holders, and cut using a sliding microtome (Gärtner and Nievergelt, 2010). TRW was measured using a Velmex measuring table (Velmex Inc., Bloomfield NY, USA) coupled to a stereo- microscope (Olympus SZ60). Crossdating was first performed on a visual basis, i.e. comparing the annual fluctuations of ring- width among samples and eventually checked statistically using COFECHA (Grissino-Mayer, 2001). Identified missing rings were inserted with width value of 0.01 mm.

Prior to the BI measurements, the specifically selected tree cores underwent a treatment to remove organic extractives, i.e. resins, which may cause a bias in the measurements (Buckley et al., 2018). Samples were first surfaced with a sliding microtome and then immersed in acetone. After 24 h, acetone was renewed to prevent saturation by extractives, as some cores, particularly those of *P. nigra*, were very resinous. Samples were kept immersed in acetone at room temperature for 72 h in total, as this time interval was found to be sufficient to remove all the extractives (Rydval et al., 2014). Afterward, the cores were scanned at a resolution of 2400 dpi with an Epson Expression 10000XL scanner. To ensure comparability of the generated data among different laboratories, an IT8 Calibration Target IT8.7/2 was used to calibrate the scanner using the SilverFast Auto8 calibration procedure integrated into the SilverFast Ai v.6.6 professional scan software. In order to minimize any influence of ambient light, the scanning surface was isolated from its surroundings by placing a box, fitted to the shape of the scanner.

BI measurements were carried out using CooRecorder 9.0.1 (Larsson, 2018). Two different BI variables were measured, namely minimum latewood blue intensity (LWBI), and maximum earlywood blue intensity (EWBI). The raw BI data are essentially inversely related to density (Campbell et al., 2007); therefore, in order to facilitate the interpretation, LWBI data were inverted first by multiplying each value by -1, and then adding the constant 2.56 (related to light intensity scale 0–255) to remove any negative values. Since colour differences between sap- and heartwood of conifer trees can cause a bias in the BI measurements, ΔBI was also calculated as a difference between EWBI and raw LWBI (Björklund et al., 2014). CooRecorder measures BI values by placing a window around each tree-ring point, and the mean BI value of the window is then calculated. The window parameters are adjustable, and were selected as follows: width – 100 pixels, offset – 3 pixels, depth – 200 pixels. For details of BI measurements and window parameters we refer to the Cybis website (Cybus, 2018). The option “Not available” was selected for the BI values of missing rings.

Before further processing, ring-width measurements were averaged per tree and then, in order to remove age-related trends, averaged ring-width series were detrended using a smoothing cubic spline (Cook

et al., 1992) with a frequency response of 50% at a wavelength of 32 years to remove low-frequency variations and emphasize high-frequency variations; for this we used the *dplR* package (Bunn, 2010) in R 3.4 (R Development Core Team, 2017). Statistics to assess the quality and characteristics of tree-ring series were calculated for the period 1900–2014 (Table 2), notably first-order autocorrelation of raw width data (AC), mean sensitivity (MS) of indexed TRW or BI values, mean correlation between trees (r_{bt}) and variance accounted for by the first principal component (PC1).

2.3. Spatial grouping of time series

Assessment of geographical patterns of annual variation in the measured/calculated variables was done using Principal Component Gradient Analysis (PCGA) (Buras et al., 2016). PCGA allows for identifying clusters of shared growth patterns at individual tree scale and visualizing whether these clusters are related to available (categorical) explanatory variables such as site or elevation. PCGA was performed on all measured variables (TRW, EW, LW, EWBI, LWBI, and ΔBI) over a common interval, including the maximum number of trees and years, to investigate whether differences in trees origin, i.e., CS or AND, were reflected in the identified clusters of the respective selected variables. In order to test whether elevation is a prevailing factor causing differences in the measured variables and climate-growth responses, trees were also classified into high and low elevation groups for each species. PCGA loadings were coloured according to the corresponding categorical explanatory variable (i.e. either CS vs. AND or high vs. low elevation). In this way, the observation of clustered colours indicates that the chosen proxy clearly differs between the chosen category (see e.g. Buras et al., 2018; Rehschuh et al., 2017).

2.4. Gradient analyses

Individual tree ranks obtained from the PCGA were used to identify the main climatic factors driving the grouping of the measured variables. That is, we computed single tree-climate correlations (Spearman's rank correlation to account for non-normal distribution of data) with available climate data and tested for a systematic variation of correlation scores along the PCGA rank using Spearman's rank correlation. Gridded CRU TS 4.01 climate data were downloaded over the period 1901–2015 (Harris et al., 2014) from the KNMI climate explorer (<https://climexp.knmi.nl/start.cgi>) with a 0.5° grid cell. In addition, Standardized Potential Evapotranspiration Index (SPEI) integrated over 1–24 months was calculated for each study site (Vicente-Serrano et al., 2010), and used for climate correlation analysis. The SPEI is useful to identify time-dependent contrasting geographical and elevational growth responses to drought (see Pasho et al., 2011a). Afterwards, we

Table 2

Spearman correlations calculated between tree-ring variables (TRW tree ring width, EW earlywood width; LW latewood width; Δ BI, delta blue intensity, EWBI, earlywood blue intensity, LWBI, latewood blue intensity) for each site. The correlation was computed over the 1900-2014 period. Asterisks indicate significant correlation (** $p < 0.01$, * $p < 0.05$).

Central System							Andalusia									
Site	TRW	EW	LW	Δ BI	EWBI	LWBI	Site	TRW	EW	LW	Δ BI	EWBI	LWBI			
ARF	TRW	–	0.95**	0.81**	0.77**	–0.01	0.69**	PMB	TRW	–	0.94**	0.88**	0.81**	0.13	0.79**	
	EW	–	–	0.61**	0.68**	–0.16	0.55**		EW	–	–	0.71**	0.72**	0.01	0.64**	
	LW	–	–	–	0.74**	0.30**	0.80**		LW	–	–	–	0.80**	0.27**	0.86**	
	Δ BI	–	–	–	–	0.03	0.89**		Δ BI	–	–	–	–	0.04	0.89**	
	EWBI	–	–	–	–	–	0.38**		EWBI	–	–	–	–	–	0.36**	
	LWBI	–	–	–	–	–	–		LWBI	–	–	–	–	–	–	
FFR	TRW	–	0.94**	0.80**	0.80**	0.12	0.75**	NAV	TRW	–	0.94**	0.89**	0.75**	–0.19*	0.68**	
	EW	–	–	0.59**	0.66**	–0.03	0.57**		EW	–	–	0.75**	0.65**	–0.24**	0.54**	
	LW	–	–	–	0.85**	0.42**	0.91**		LW	–	–	–	0.75**	–0.06	0.78**	
	Δ BI	–	–	–	–	0.11	0.88**		Δ BI	–	–	–	–	–0.21*	0.87**	
	EWBI	–	–	–	–	–	0.48**		EWBI	–	–	–	–	–	0.10	
	LWBI	–	–	–	–	–	–		LWBI	–	–	–	–	–	–	
JRS	TRW	–	0.95**	0.71**	0.63**	0.10	0.62**	LIN	TRW	–	0.94**	0.89**	0.72**	–0.01	0.67**	
	EW	–	–	0.51**	0.48**	0.00	0.46**		EW	–	–	0.72**	0.59**	–0.17	0.50**	
	LW	–	–	–	0.81**	0.31	0.84**		LW	–	–	–	0.77**	0.19*	0.81**	
	Δ BI	–	–	–	–	0.13	0.94**		Δ BI	–	–	–	–	–0.03	0.92**	
	EWBI	–	–	–	–	–	0.33**		EWBI	–	–	–	–	–	0.25**	
	LWBI	–	–	–	–	–	–		LWBI	–	–	–	–	–	–	
JRN	TRW	–	0.97**	0.85**	0.85**	–0.27**	0.76**	JRN	TRW	–	0.97**	0.85**	0.85**	–0.27**	0.76**	
	EW	–	–	0.73**	0.77**	–0.33**	0.66**		EW	–	–	–	0.73**	0.77**	–0.33**	0.66**
	LW	–	–	–	0.90**	–0.04	0.90**		LW	–	–	–	–	0.90**	–0.04	0.90**
	Δ BI	–	–	–	–	–0.14	0.92**		Δ BI	–	–	–	–	–0.14	0.92**	
	EWBI	–	–	–	–	–	0.15		EWBI	–	–	–	–	–	0.15	
	LWBI	–	–	–	–	–	–		LWBI	–	–	–	–	–	–	

investigated whether these correlations varied significantly along the determined PCGA gradient using Spearman's rank correlation. In this analysis a strong correlation would indicate a directional variation of climate correlations along the PCGA gradient.

To test for significance in differences of the polar coordinates of the different categories derived from the PCGA, the Wilcoxon rank sum test was performed. A non-parametric test was chosen due to the non-normal data distribution. Finally, we computed regional chronologies, i.e. the average of all individual time series representative of each geographic and elevational category. These regional chronologies were again correlated with monthly temperature and precipitation using a 15-month window, from August in the prior growing season to October in the current growing season, and with 1–24 months SPEI. Such a 15-month time window ending in October of the current season was selected based on studies by Camarero et al. (2010) and Poljanšek et al. (2019). According to Camarero et al. (2010) latewood formation of *P. sylvestris* both in low (340 m a.s.l.) and high (1200 m a.s.l.) elevations under Mediterranean climates has ended in mid-October. In Bosnia and Herzegovina, Poljanšek et al. (2019) found that xylem formation of *P. nigra* at low elevation (390 m a.s.l.) ended in the middle of August in 2012, and in the middle of September in 2011. The correlation analysis with climatic factors was done to assess whether the climatic effects behind the gradients' analyses were also the main factors limiting/enhancing tree growth in the study areas.

2.5. Leave-one-out analyses

To test if incorporation of BI time series improves the performance of wood provenances, a leave-one-out analysis (LOOA) was performed following the steps described by Akhmetzyanov et al. (2019) based on the Euclidian distance between the left-out tree with trees from the same geographical origin or elevation. LOOA was first performed on all trees using only BI time series to see the 'correct' wood elevation identification, and then on TRW time series of subgroups derived from the BI PCGA. The latter was performed to understand if including more variables would lead to a more accurate provenancing of the collected material.

3. Results

3.1. Characteristics of chronologies

The average length of derived TRW time series varied both between and within areas (Table 1). Among the sites from CS, the longest series were derived from trees at JRN and, whereas JRS and ARF series were considerably shorter. Trees of comparable age to the latter were found at LIN and NAV, whilst trees from PMB had an age close to trees from JRN and FFR (Table 1).

The mean TRW values varied across sites as well, with the largest values in ARF, JRS and NAV and smallest in JRN (Fig. 1a). A similar pattern was observed for the EW, but not for the LW, where the largest mean value was found in LIN (Table S1). As for the BI variables, the largest mean LWBI values were found in JRN and LIN, while ARF trees had the smallest average LWBI (Fig. 1b). All sites showed similar mean Δ BI, but at LIN the value was considerably higher, probably due to the low EWBI value (Table S1).

The correlation between trees (r_{bt}) within each stand varied from 0.69 (NAV from AND) to 0.57 (FFR from CS) (Table S2), confirming a high growth coherency among trees from the same provenance. Some tree-ring variables were highly correlated for all sites, i.e. TRW and EW/LW (Table 2). BI variables were also correlated among each other and with ring-width variables, but EWBI showed considerably lower and largely insignificant correlations with other variables.

3.2. Spatial grouping of time series

3.2.1. PCGA of geographical provenances

The common interval for the PCGA performed for all 196 TRW time series comprised the common period from 1863 to 2014. The analyses failed to group the TRW series according to their geographical origin, as a set of trees from the CS (from low elevation) grouped together with AND trees (Fig. 2a). The cluster of incorrectly assigned trees composed of both *P. nigra* and *P. sylvestris* from JRS/JRN (CS low-elevation group). PCGA of LW and LWBI time-series per study region did not reveal any clear grouping according to geographical provenance

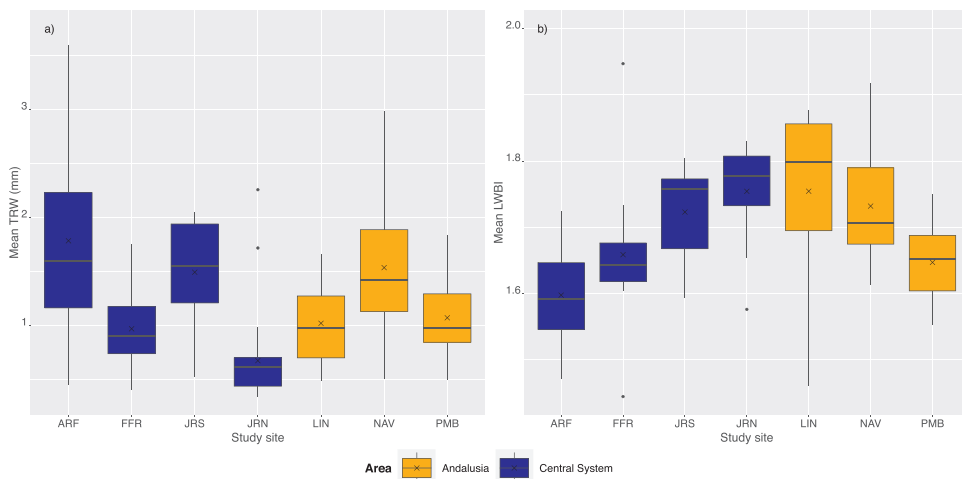


Fig. 1. Mean TRW (a) and LWBI (b) of series from the Central System (blue) and Andalusia (orange). Black crosses indicate average time series length per study site (abbreviations for study sites as in Table 1). Lower and upper box boundaries 25th and 75th percentiles, respectively, line inside box median, lower and upper error lines 10th and 90th percentiles, respectively, filled circles data falling outside 10th and 90th percentiles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

(Fig. 2b-c). At the same time, the PCGA of LW time series (common interval 1902–2014, 78 series) yielded separation between trees from high and low elevations, but also featured nine inconsistently assigned series (Fig. 2e). The PCGA of LWBI (common interval 1902–2014, 61 series) however, showed a clear grouping of trees according to their elevation origin with only one incorrectly assigned series (Fig. 2e)

($p < 0.01$, Wilcoxon test). PCGA of EW showed similar results to TRW analyses (Figure S2-a), while PCGA of ΔBI revealed comparable results to LW BI (Figure S2-c), but having more outliers. PCGA of EW BI did not result in any meaningful grouping of time series (Figures S2b). No meaningful grouping of time series per study species was found (Figure S3).

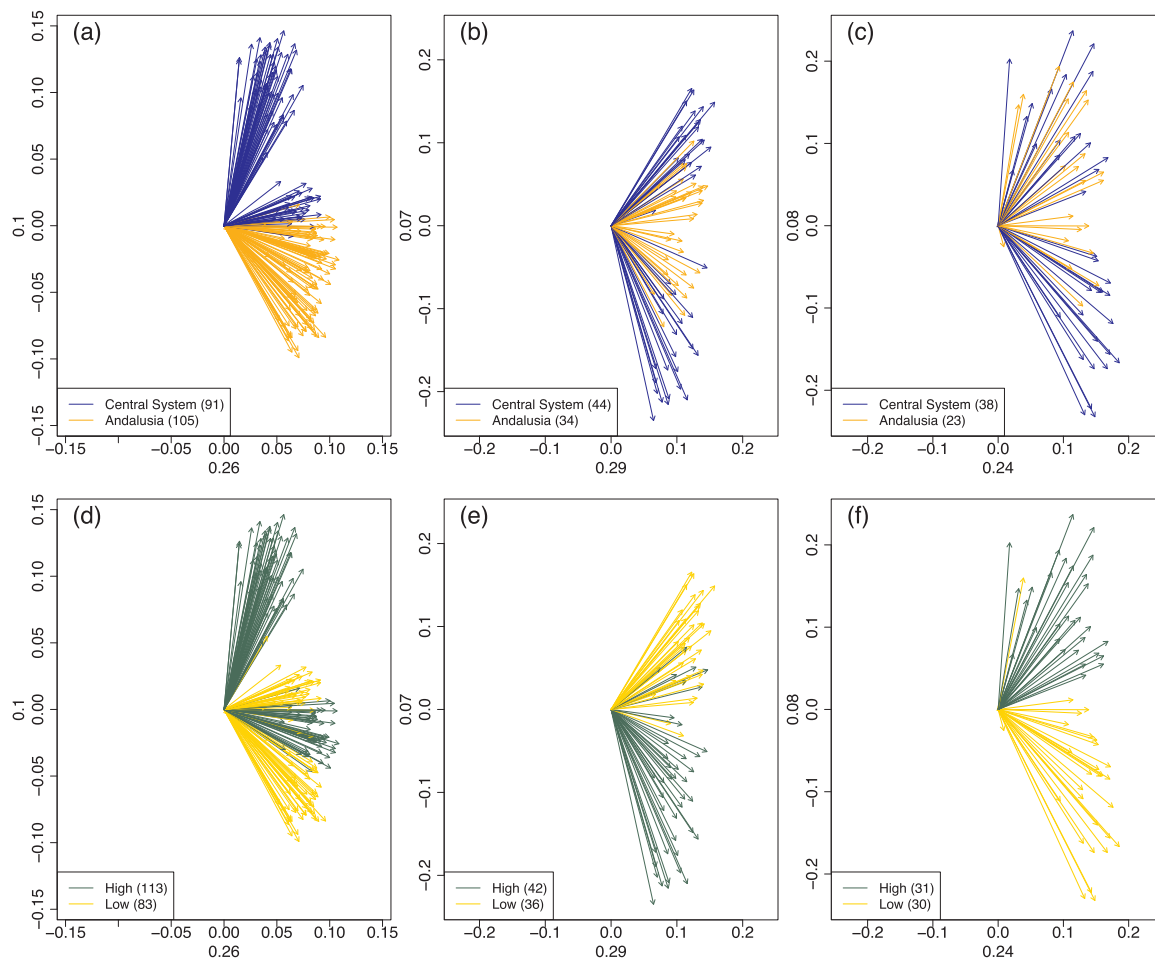


Fig. 2. Loadings plots for PCGA for study regions (upper panels) and for elevation subgroups (lower panels), for time series of tree-ring width index (a and d), latewood width (b and e), and latewood blue intensity (c and f). Low-elevation sites included LIN, JRS and JRN; high- elevation sites are PMB, NAV, ARF and FFR. Each arrow represents the loadings on the first two principal components from one tree. Values in parenthesis represent number of trees in the respective category. Values on the x- and y-axes represent the amount of explained variance by the respective component. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

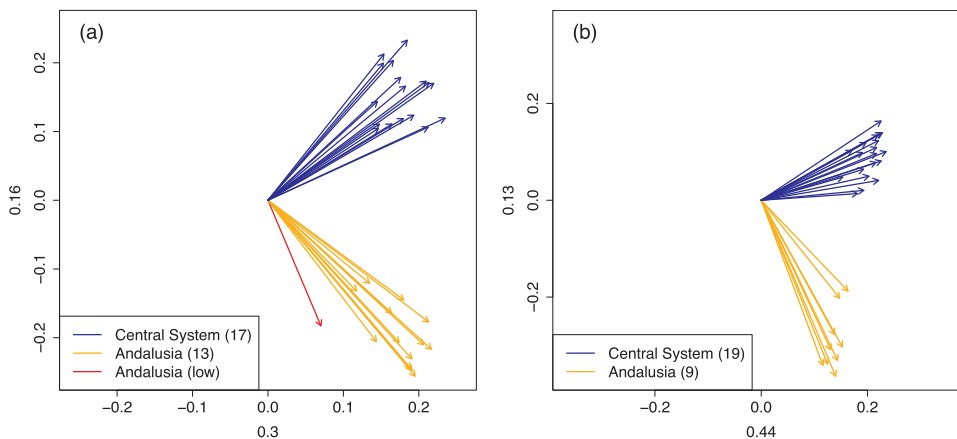


Fig. 3. Loadings plot for the PCGA based on tree-ring width index of a) trees from high elevations (ARF/FFR from CS, and NAV/PMB from AND), one “Andalusia (low)” red arrow represents the outlier from the LWBI PCGA; b) trees from low elevations (JRS/JRN from CS, and LIN from AND). Each arrow represents the loadings on the first two principal components from one tree. Values in parenthesis represent number of trees in the respective category. Values on the x- and y-axes represent the amount of explained variance by the respective component. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

3.2.2. PCGA of elevation groups

PCGA based on TRW of elevation groups as identified by the PCGA using LWBI showed a clear distinction between CS and AND trees both within high and low elevational subgroups (Fig. 3a and 3b, respectively) ($p < 0.01$, Wilcoxon test). The common interval of PCGA of trees coming from lower elevations was 1894–2014 (121 years) and involved 28 TRW time-series, whereas for the trees from higher elevations it was 1888–2014 (127 years) and involved 31 trees. The CS low-elevation group comprised both *P. nigra* (9 individuals) and *P. sylvestris* (10 individuals). However, difference between the species was not reflected in PCGA of the respective elevational group (Figure S4). PCGA of other selected variables also showed a similar grouping of trees according to their geographical origin within the elevational groups, especially EW and LW time-series (Figure S5).

3.3. LWBI and TRW climate-growth relationships

3.3.1. Drought sensitivity of the selected variables

The correlation analysis between TRW and SPEI showed a significant positive association between growth and drought index, but these associations were found to be increasing if using LWBI chronologies (Fig. 4). A positive and significant relationship between summer SPEI was recorded at 3–4 months long scales and low-elevations trees’ LWBI chronologies, whilst correlations with TRW were considerably weaker. High-elevation trees did not show any significant relationship with drought, neither in TRW nor in LWBI chronologies.

3.3.2. TRW and BI responses to climate within elevational groups

Assessment of climate growth-relationships between regional TRW and LWBI chronologies within the elevation subgroups and climatic factors revealed a strong negative effect of temperature of the previous autumn and positive effect of current winter temperature on high-elevation trees from Andalusia (AND), whereas such strong effects were not observed for trees from the Central System (CS) of the same elevation group (Fig. 5). On the other hand, the trees from CS at high-elevation were negatively affected by average October temperature, while this signal was very weak in Andalusian trees. A strong positive effect of summer precipitation was found in LWBI for low-elevation sites, with a stronger correlation at CS (Fig. 5). Furthermore, the average winter temperature was found to be the main factor driving the growth of trees in Andalusia for both elevations, while only February temperature showed significant correlations with the Central System chronology (Fig. 5).

3.4. Variations of climate-growth relationships along the PCGA gradient

Climate correlations varied significantly along the PCGA-gradient for several parameters and months as indicated by Spearman’s rank

correlation (Fig. 6). The strongest correlations were found with the summer SPEI-3. The gradient derived from the PCGA based on LWBI time series (Fig. 2c) reflects the strong correlation between LWBI time series of individual trees with summer temperature and precipitation, but the strongest correlations were found with the summer SPEI-3 (Fig. 6).

Also, the ranking of the PCGA using TRW on elevational subgroups revealed significant variation of climate correlations along the corresponding gradient. That is, high elevation gradients revealed a significant variation in response to temperature and precipitation before the start of the growing season (Fig. 7a), whereas low elevation gradients revealed a significant variation of correlations with summer/early autumn and previous autumn growing conditions (Fig. 7b).

3.5. Leave-one-out analyses

The leave-one-out analyses demonstrated a highly correct assignment of trees to the elevation groups based on LWBI (100 % and 93 % to high and low elevations, accordingly), whereas for TRW and ΔBI the percentages were lower (Table 3). Performing a similar analysis within the elevation groups as defined by PCGA on LWBI, TRW performed best, i.e. 100 % of series were correctly assigned for both elevation subgroups. The same analyses for LW BI and ΔBI showed lower percentages (Table 3).

4. Discussion

In this study, the analysis of dendroprovenancing in Mediterranean climates using the Blue Intensity technique improved the precision separating the sites (in comparison to the results obtained by traditional TRW series), and the understanding of climate effects at different elevations. Our findings prove the potential of LWBI to identify the geographical origin of the studied pine species, which opens the door to implement this method to provenance historic pine timbers in the Iberian Peninsula.

4.1. The added value of latewood blue intensity for pine timber provenancing

Application of multivariate techniques on individual time series of pine trees from the Mediterranean area revealed that a combination of ring-width and blue intensity enhances the precision of dendroprovenancing (Table 3). By combining time series of LWBI with the commonly used TRW in a two-step procedure we were able to overcome the strong teleconnections observed over large distances for *P. nigra* growing below 1500 m a.s.l. (e.g. Domínguez-Delmás et al., 2013) (Figs. 2 and 3). In the first step, LWBI allows assigning time series to their elevation category (Fig. 2f), while further analyses of TRW series

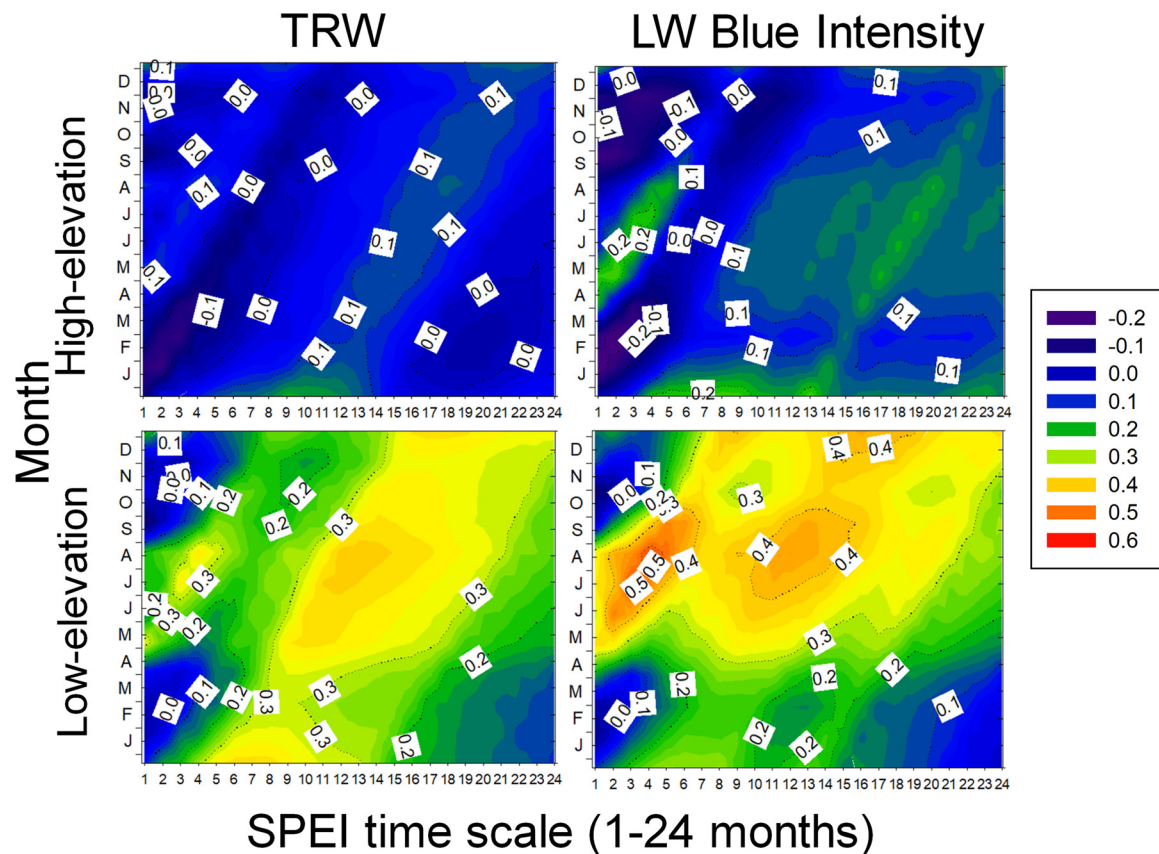


Fig. 4. Drought associations of the regional chronologies by elevations assessed by relating ring-width indices (TRW) and latewood blue intensity (LWBI) to the Standardized Precipitation–Evapotranspiration Index (SPEI) considering up to 24-month long scales (x- axes). Correlations were calculated for the common and best-replicated period 1900–2014 and considering the months of the current growth year (y-axes). Correlation values above +0.20 and below –0.20 are significant at $p < 0.05$ level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

within these elevation categories allowed the identification of trees' geographic origin with high precision (Table 3). This was possible due to the observed differences in the climate signal captured by the two selected variables, i.e. a summer drought signal in the LWBI and temperature and precipitation before and at the end of the growing season for TRW series (Figs. 4 and 5). The difference in summer drought signal strength among elevation groups is triggered by the difference in average summer temperature between low and high elevation sites, i.e. sites from higher elevations experience less drought compared to lower elevations sites (e.g. Sánchez-Salguero et al., 2015). BI values were also found to express stronger relationships with climate than TRW in areas with temperature as the main growth limiting factor i.e. at high latitudes (Campbell et al., 2007; Fuentes et al., 2018), as well as in regions with no dominant climate signal in TRW series, e.g. in Caucasus (Dolgová, 2016), or Pyrenees (Büntgen et al., 2008; Wilson et al., 2017). This is explained by the fact that TRW chronologies contain aggregated information on conditions throughout the whole growing season, which also includes factors not related to climate (Cook, 1985), while LWBI, as a surrogate of maximum latewood density (MXD), specifically reflects summer conditions hence contains a stronger climatic signal (Björklund et al., 2014; Wilson et al., 2014). These factors likely explain why the combined LWBI and TRW approach can be used to identify the geographical origin of conifer wood in Mediterranean areas with higher precision than with TRW alone.

High provenancing precision can also be achieved by creating a dense reference network of chronologies (Domínguez-Delmás et al., 2015). However, creating long TRW chronologies from lower elevations is sometimes hampered by intensive logging in the past (Domínguez-Delmás et al., 2013). In those areas, developing chronologies using proxies with a complementary climate signal, such as BI, may serve to

overcome the lack of long-lived trees at low-elevation. Additional studies involving historic timber are needed to test this hypothesis.

4.2. TRW and BI climate-growth relationships

To our knowledge, this is the first study examining the potential of BI together with TRW for dendroprovenancing in dry areas, where trees experience a severe lack of soil water. Most of the studies involving BI were done at higher latitudes where trees' secondary growth as well as BI is positively affected by summer temperatures (e.g. Björklund et al., 2014; Wilson et al., 2014). But, due to the strong similarity between the BI and MXD variables (Campbell et al., 2007), BI-climate associations can be interpreted as MXD-climate one. In this sense, at several sites MXD was found to be strongly and directly dependent on conditions prevailing during late summer and early autumn, i.e. when the latewood is formed (Briffa et al., 1998). This strong association between latewood density and climate conditions at the end of the growing season are related to cell-wall lignification of the latest formed tracheids, which may take place long after the cells division (Gričar et al., 2005).

Under drought stress, conifer trees were found to produce smaller tracheids with thicker cell walls (Bouriaud et al., 2005; Park and Spiecker, 2005), leading to the production of denser wood thus allowing tracheids to endure high xylem tension resulting from low water potential (Hacke et al., 2001). In our study, however, we observed a positive association between BI chronologies from low elevation trees and drought index (Fig. 4). Similar positive correlations between summer SPEI and MXD were found for *Pinus uncinata* trees from the Pyrenees, which has been explained by the enhancement of carbohydrate production and tracheid lignification during wet summers

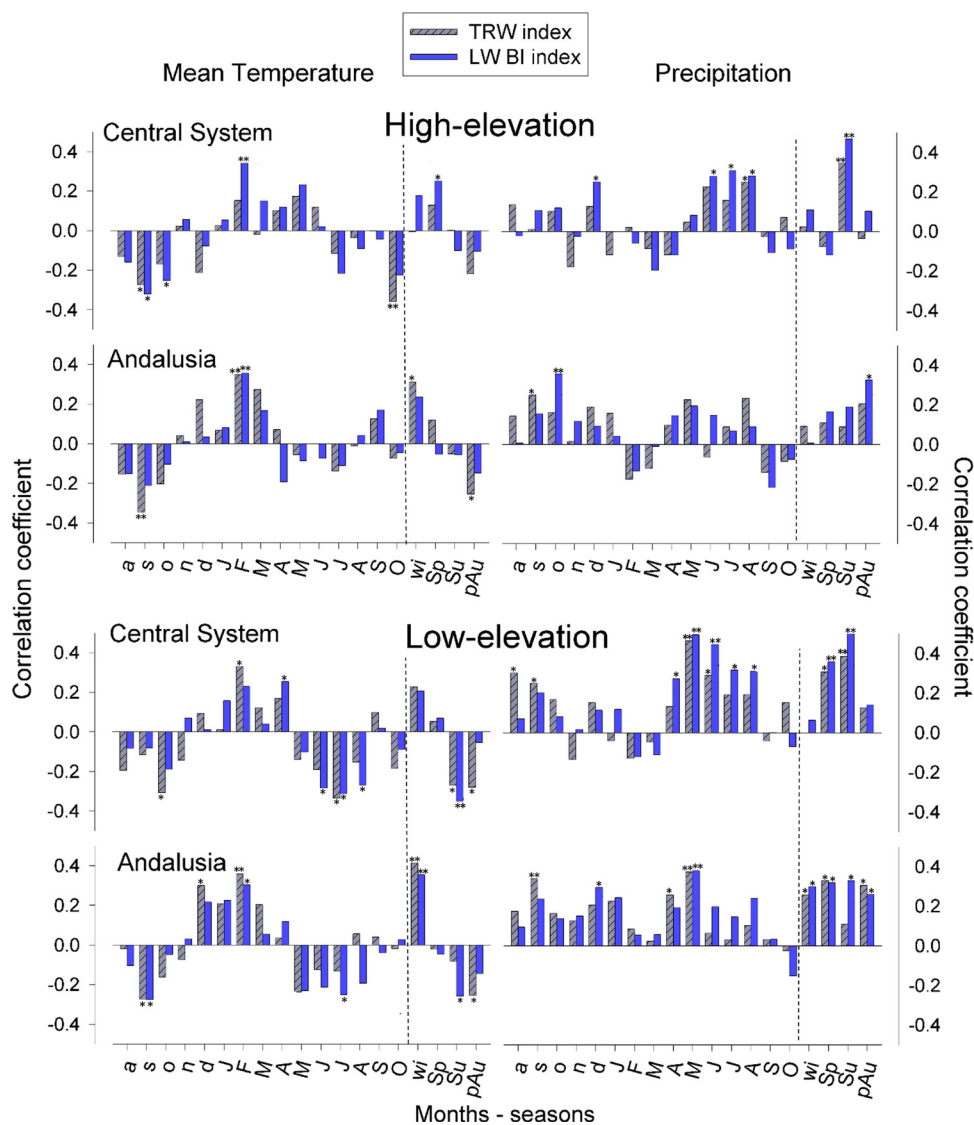


Fig. 5. Bootstrapped correlation coefficients between tree growth variables-climate associations for the studied sites assessed by relating ring-width indices (TRW) and latewood blue intensity (LWBI) to seasonal and monthly climatic variables (mean temperature and precipitation). The temporal window of analyses includes from previous August (previous-year months and seasons are abbreviated by uppercase letters) up to current October (current-year months and seasons (i.e., An-annual; wi- winter; sp-spring; su-summer; pAu-previous autumn) are abbreviated by lowercase letters). Dashed lines separate between monthly and seasonal climatic variables, asterisks indicate significant correlation (** $p < 0.01$, * $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

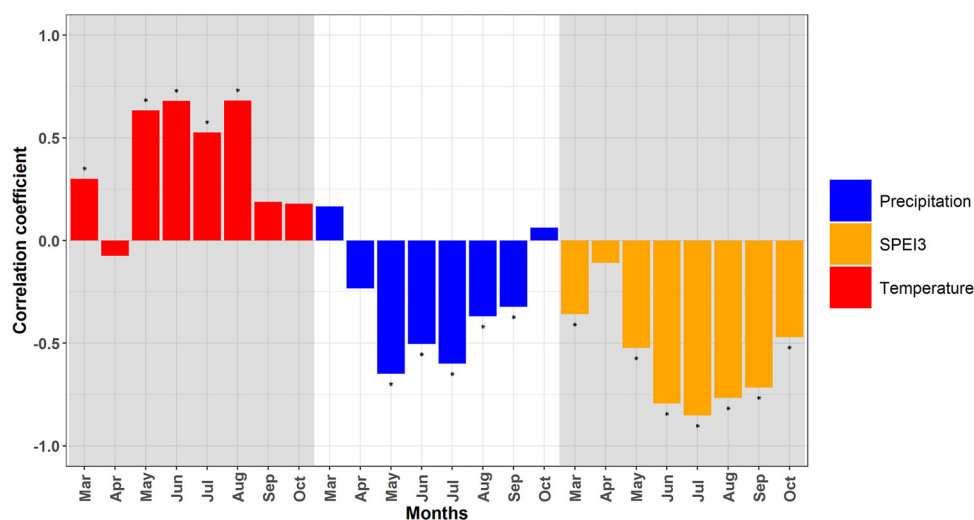


Fig. 6. Correlation between ranks of all trees derived from LWBI PCGA, with the correlation between individual LW BI time series and average temperature (red), precipitation (blue) and 3-months-long scale Standardized Precipitation Evapotranspiration Index - SPEI (orange). Asterisks indicate significant correlations ($p \text{ adj.} < 0.01$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

(Galván et al., 2015). Presumably, similar processes may lead to the observed correlations with LWBI in our study. The positive correlation with 12-month scale SPEI (Fig. 6) reflects that wood density in our study trees depends on the amount of available water during the entire

year preceding the current growing season (c.f. Camarero et al., 2013). Such a long-term influence of previous-season conditions on latewood production/MXD was also found for *P. halepensis* in eastern Spain (Pasho et al., 2011b), *P. pinaster* (Caminero et al., 2018); *P. nigra*

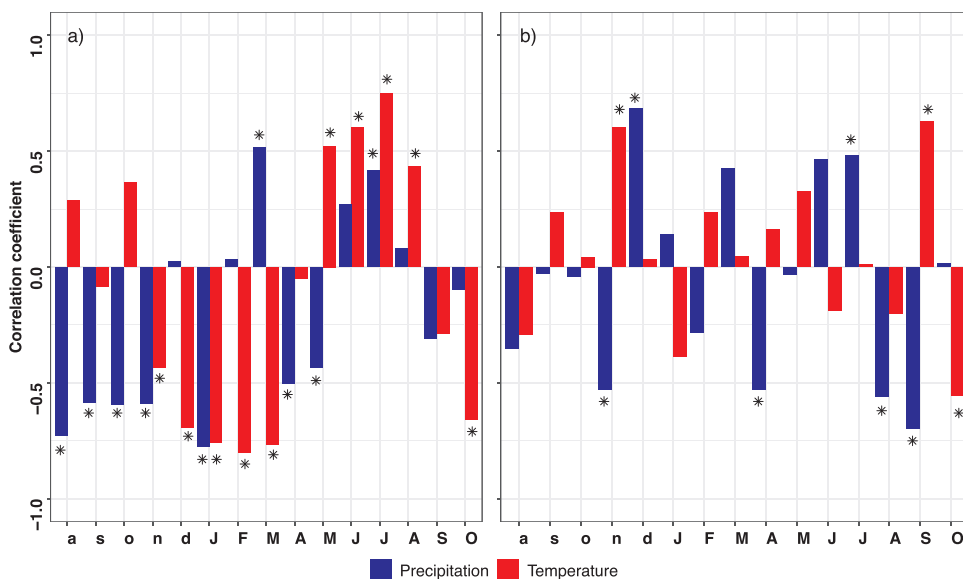


Fig. 7. Correlation between individual tree PCGA ranks with the correlation between individual tree-ring width time series of a) high elevation trees and climatic factors and b) low elevation trees and climatic factors, Tav_g – average monthly temperature, Prec – monthly precipitation. The temporal window of analyses includes from previous August (previous-year months) are abbreviated by uppercase letters) up to current October (current-year months); asterisks indicate significant correlation (p adj. < 0.01).

Table 3

Rates of success for the leave-one-out approach. High, Low - success rates of assigning trees to sites, from high and low elevations, respectively. Central System (CS) and Andalusia (AND) indicate success rates of assigning trees to each site. For the latter, trees from predefined by the PCGA of latewood blue intensity time series from each elevational group, i.e., high elevation and low elevation, were analysed separately.

	High	Low	High elevation		Low elevation	
			CS	AND	CS	AND
LW_BI	100 %	93 %	100 %	88 %	100 %	66 %
TRW	52 %	96 %	100 %	100 %	100 %	100 %
ΔBI	76 %	96 %	100 %	100 %	88 %	100 %

(Camarero et al., 2013), and for *P. uncinata* from the Pyrenees, but only for the second half of the twentieth century (Galván et al., 2015). This signal can be explained by benefits the tree has from carbohydrate synthesis and storage under favourable conditions in the previous year (Galván et al., 2015).

The observed negative effect of previous autumn temperature on TRW chronologies from Andalusia (Fig. 5) together with positive precipitation was also found for *P. nigra* chronologies growing in the same region (Domínguez-Delmás et al., 2013), for *P. nigra*, *P. sylvestris* and *P. uncinata* chronologies from other regions of Spain (Andreu et al., 2007). Positive previous autumn precipitation on TRW was also found for *P. canariensis* from the Canary Islands (Rozas et al., 2013). These results can be explained by the extension of the growing season due to the warmer autumn temperatures and hence consumption of carbohydrates and lower reserves formation for the next growing season, which eventually leads to the formation of narrower tree rings (Andreu et al., 2007). These results were also confirmed by Camarero et al. (1998). A strong positive effect of winter temperature on Andalusian TRW chronologies may also be related to the change of the vegetation period: colder winters may postpone growth resumption in the following springs and consequently depress ring formation (Domínguez-Delmás et al., 2013) and, vice versa, warmer winters might induce an earlier start of physiological activity in the tree and thus extend the length of the growing season (Lebourgeois, 2000). TRW chronologies from the Central System did not exhibit such a strong correlation with winter temperature. Instead, low elevation TRW chronologies were negatively/positively affected by summer temperature/precipitation, whereas high elevation chronologies were found to be negatively associated with late autumn temperature and positively with summer

precipitation (Fig. 5). The strong positive summer precipitation effect found in our study is in line with other studies on pines in the Mediterranean area (Sánchez-Salguero et al., 2015), as well as in dry areas in central Europe (Eilmann et al., 2010; Eilmann et al., 2011; Lebourgeois, 2000), where pine trees suffer from a lack of water which results in the reduction of secondary growth. The negative influence of October temperature found for high-elevation TRW chronology was also observed in mid- and low-elevation trees from the Central System (Sánchez-Salguero et al., 2015), where trees still have high photosynthetic rates while high temperatures inhibit secondary growth.

It is important to stress that our study is representative for two coniferous species on the Iberian Peninsula. For this dataset, the presented approach succeeded in dendroprovenancing the wood from two different areas and elevations. Whether this approach is applicable to other species and/or Mediterranean areas remains to be tested in future studies. Moreover, this approach must be extensively tested on archaeological timber, as degradation processes and their associated changes of cellulose and lignin content in the wood might lead to misleading results in the blue intensity measurements. In addition, possible changes in blue intensity results caused by the use of different resin extraction techniques on degraded timber must also be assessed in order to establish a protocol that allows for the standardisation and comparison of results.

5. Conclusion

Our study suggests that a stepwise, multivariate (PCGA) approach combining latewood blue intensity (LWBI) with tree-ring width enhances the precision of dendroprovenancing. Therefore, our study provides a meaningful contribution to increasing the accuracy when identifying the origin of historical timber in the Iberian Peninsula, and opens the door to extend the research area to the entire Mediterranean basin.

Applying this approach through larger spatiotemporal scales and including additional tree-species may help identifying ancient trade networks currently unknown, contributing to deepen our knowledge about the socio-economic development of Mediterranean cultures in different periods.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

influence the work reported in this paper.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.dendro.2020.125688>.

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