Forestry *An International Journal of Forest Research*

Institute of
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Climatic signals of tree-ring width and intra-annual density fluctuations in *Pinus pinaster* and *Pinus pinea* along a latitudinal gradient in Portugal

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Tree-ring width integrates the prevailing environmental conditions during the whole growing season (annual resolution). Other wood anatomical traits, like intra-annual density fluctuations (IADFs), imprint environmental conditions within the growing season (sub-annual resolution). IADFs are anatomically characterized by latewood-like cells within earlywood or earlywood-like cells within latewood. Under climate change scenarios, it is increasingly important to understand which traits are more relevant in the adjustment of long-lived species. We established a latitudinal transect along Portugal and compared tree-ring width and the frequency of IADFs in *Pinus pinaster* growing under temperate and Mediterranean climate, and of *P. pinaster* and *P. pinea* growing under Mediterranean climate. *Pinus pinaster* growing under temperate and Mediterranean climate showed similar correlations between climate and tree-ring width, whereas *P. pinaster* and *P. pinea* under Mediterranean climate presented a different pattern of climate–growth correlations. We therefore suggest that the climate–growth response is species dependent. However, the climate–IADFs correlation was driven by site conditions and less related to the species. We propose that the correlations between climate and growth are conserved within the distribution range of a species, whereas IADFs function as a finer morphological adjustment to the environmental conditions during the growing season.

Introduction

Phenotypic plasticity plays an important role in plant fitness (Chuine, 2010) and is especially important in long-lived trees (Lupi *et al.*, 2010; de la Mata and Zas, 2012). Long-lived trees have to cope with climatic fluctuations on a long-term basis, witnessing and recording those fluctuations in variable tree-ring width, a fingerprint of the tree's adjustment to climate conditions (Fritts, 2001). The variability of wood anatomical traits, like tree-ring width, is dependent on the vascular cambium activity that is ruled by both endogenous (genetic) and exogenous (environmental) conditions (Dufour and Morin, 2010; Hölttä *et al.*, 2010; Rossi *et al.*, 2012). Xylem cells present plastic traits like the lumen area and cell wall width that can be linked to functional traits like hydraulic conductivity (Tyree and Ewers, 1991; Anfodillo *et al.*, 2012), and physical traits like wood density (Rathgeber *et al.*, 2006). Wood density changes from earlywood (less dense) to latewood (denser) but also at finer spatial scales, named intra-annual density fluctuations (IADFs), with latewood-like cells within earlywood or earlywood-like cells within latewood (Fritts, 2001). IADFs are induced by environmental conditions during the growing season that modify cambial activity and therefore can give climatic information with intra-annual resolution (Campelo *et al.*, 2007, 2013; de Micco *et al.*, 2007; Rozas *et al.*, 2011; Novak *et al.*, 2013).

If these anatomical traits change across the geographic and climatic range of a species, then they can be seen as important morphological adjustments to climate change conditions (Mäkinen *et al.*, 2002; Fonti *et al.*, 2010; Huang *et al.*, 2010). Previous studies showed that the frequency of IADFs is dependent on genetics, tree-age, tree-ring width, length of the growing season and environmental conditions (Rigling *et al.*, 2001; Wimmer 2002; Campelo *et al.*, 2007, 2013; Novak *et al.*, 2013), making difficult the comparison among different sites and species. Nonetheless a broader geographical network of IADFs would give valuable information on the variability of climatic conditions within the growing season and the relative contribution of each factor for the formation of IADFs.

Current climatic scenarios predict that by the end of this century, the Mediterranean basin will suffer a decrease of 10–20 per cent in annual precipitation, together with an increase of 3–3.5°C in the mean annual temperature, associated with more frequent and severe drought periods (IPCC, 2007). Within this framework, it is essential to understand how wood anatomical traits change along climatic gradients and/or among different species (Villar-Salvador *et al.*, 1997; Miyamoto *et al.*, 2010).

To understand how climate and/or tree species affect wood traits, we have compared tree-ring width and IADF formation in *Pinus pinaster* under temperate and Mediterranean climate, and

in *P. pinaster* and *P. pinea* under Mediterranean climate. *Pinus pinaster* and *P. pinea* grow under drought-prone conditions of the Mediterranean basin and can be found on sandy soils, with low water-holding capacity. *Pinus pinaster* has a small geographical range, in the western Mediterranean basin, and it grows under contrasting water availabilities, from oceanic to arid conditions (Correia *et al.*, 2008; Corcuera *et al.*, 2010; de la Mata *et al.*, 2012) being used as a model species to study the molecular response to drought stress (Perdiguero *et al.*, 2013). It is considered a drought-avoiding species showing sensitive stomata and fast osmotic adjustment in response to water stress (Nguyen-Queyrens *et al.*, 2002). *Pinus pinea* shows a wider distribution, all around the Mediterranean basin, and although it presents a low level of genetic diversity (Vendramin *et al.*, 2008), it has a high level of plasticity (Mutke *et al.*, 2005). It is a more thermophilic and xerophytic species and is usually found on poorer soils (Barbéro *et al.*, 1998).

The specific objectives of the study are to identify and compare the climatic variables that most strongly correlate with (1) tree-ring width and IADFs of *P. pinaster* growing under temperate and Mediterranean climate and (2) with *P. pinaster* and *P. pinea* growing under Mediterranean climate and (3) to determine whether the climatic signal of tree-ring width and IADFs are species-specific and/or related to the climatic conditions.

Materials and methods

Study areas

The study areas are located in Portugal along a latitudinal gradient ranging from Caldas do Gerês (42°N 8°W), Leiria (40°N 8°W), closer to the Atlantic coast of Portugal, and Mértola (38°N 7°W), in the inner part of Portugal (Figure 1). The altitude in Caldas do Gerês is ~1500 m, in Leiria is ~200 m and in Mértola is ~400 m a.s.l.

The monthly average mean air temperature and total precipitation from 1940 to 2006 were obtained from the nearest $0.5° \times 0.5°$ grid point at http://climexp.knmi.nl/. The climate is temperate-like in Caldas do Gerês, with a mean annual temperature of 11°C and 1235 mm of annual precipitation, and Mediterranean-like in Leiria and Mértola, with a mean annual temperature of 15 and 17°C, and 912 and 592 mm of annual precipitation, respectively (Figure 1). The length and intensity of the dry period increases with decreasing latitude, as observed in the climatic diagrams (Figure 1). The different climatic conditions were reflected in the type of vegetation, Mediterranean-like in the southern latitude (40 and 38°N), dominated by evergreen trees, and temperate-like vegetation in the northern latitude (42°N), dominated by deciduous trees.
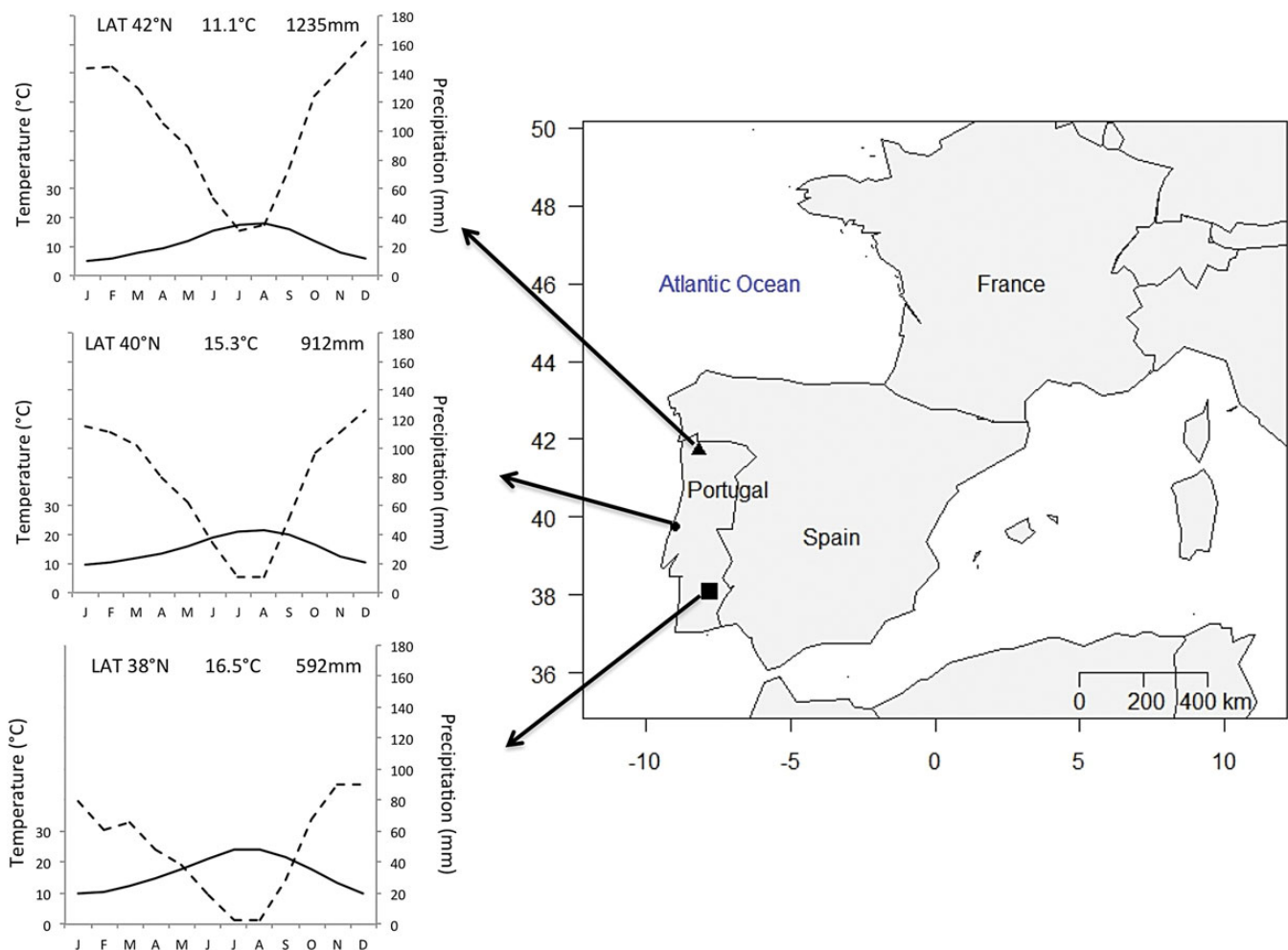
Figure 1 Location of the three sampling sites and the correspondent climatic diagram.

Sampling and chronology construction

The areas at 42 and 40°N were pure stands of *P. pinaster* under different macroclimatic conditions, temperate and Mediterranean, respectively. Although we have screened for populations of *P. pinaster* at 38°N, they were too young (<40 years old), making the comparison with northern populations unsuitable. The area at 38°N was a pure stand of *P. pinea*.

In Caldas do Gerês (42°N) and Leiria (40°N), 18 and 22 trees of *P. pinaster* were cored in 2007 and 2006, respectively, and in Mértola (38°N), 17 trees of *P. pinea* were cored in 2002. Two cores were taken from each tree, at breast height, with an increment borer (0.5 cm in diameter), in the north–south direction. Afterwards, the cores were air-dried, mounted on a wooden support and sanded with progressive finer sand paper to highlight tree-ring patterns. Tree-rings were visually cross-dated using standard dendro-chronological techniques (Stokes and Smiley, 1996). Tree-ring width was measured to the nearest 0.01 mm using a linear table, LINTAB (FrankRinn S.A., Heidelberg, Germany) and the program TSAP Win (Rinn, 2003). The cross-dating accuracy was then checked using the program COFECHA (Holmes, 1983). None of the trees showed correlations with the master chronology <0.3, thus were all used for further analysis. To remove age-related growth trends and competition, a two-step detrending was applied to each individual series, using the packages 'dplR' (Bunn, 2008) and 'detrendeR' (Campelo *et al.*, 2012) of the R freeware program (http://www.cran.r-project.org). In the first step, a negative exponential or a straight line with slope ≤ 0 was fitted to each individual ring-width series. In the second step, a smoothing cubic spline curve with a 50 per cent frequency cut-off and response period of 60 years was fitted to the dimensionless series. To remove the previous year effect, an autoregressive model was fitted to the standardized indices. Finally, to reduce the influence of isolated outlier values, a biweight robust estimate of the mean was applied and a residual chronology obtained. The descriptive statistics to compare the residual chronologies along the latitudinal gradient are shown in Table 1. The mean sensitivity is a measure of the year-to-year variability in width of consecutive tree-rings, the first-order autocorrelation is a measure of the year-to-year growth similarity, the expressed population signal (EPS) is a measure of the statistical quality of the mean residual chronology as compared with an infinitely replicated chronology and the mean between-trees correlation (rbt) is a measure of the similarity in growth among trees.

Intra-annual density fluctuations

Intra-annual density fluctuations in *P. pinaster* and *P. pinea* are easily distinguishable from annual tree-ring boundaries by the gradual transition in cell size and wall thickness at the outer borders of these anatomical structures (Campelo *et al.*, 2007). The IADFs were classified based on the radial position within the tree-ring: Type E with latewood-like cells within the earlywood and Type L with earlywood-like cells within the latewood (Campelo *et al.*, 2007). The visual identification of IADFs was made on correctly dated cores, using a stereomicroscope (magnification up to 25×). Because of the variability of IADF occurrence throughout the stem, an IADF in a given ring was only considered if present in both cores. The relative frequency of IADFs was calculated as $F = n_i/N$, where n_i is the number of trees showing an IADF on both cores in the year i and N is the number of trees in that year. To correct the bias generated by the changing sample depth over time, a stabilized IADF frequency was calculated as $F_{stab} = F \times n^{0.5}$ (Osborn *et al.*, 1997).

Tree-ring width, intra-annual density fluctuations and climate

The reliable period of the chronologies was considered when EPS values were >0.85, a threshold widely used in dendrochronological analysis (Wigley *et al.*, 1984). Thus, the reliable common period to compare all populations was from 1940 to 2002. The climate–growth relationships were estimated for that period, using monthly mean temperatures and total precipitation values as climatic predictors, and the residual index chronologies, or stabilized IADF frequencies, as the dependent variables. Spearman's correlation coefficients between tree-ring width, or the chronologies of the stabilized IADF frequencies, and the climate parameters (temperature and precipitation) were analysed from October of the previous year to November of the current year.

Results

Chronologies and general statistics

The length of the chronologies varied from 101 to 131 years (Table 1). Comparing the three chronologies, values of mean sensitivity were higher for *P. pinaster* at 40°N, although the standard

Table 1 Descriptive statistics of *Pinus pinaster* and *P. pinea* chronologies along a latitudinal gradient from 42 to 38°N in Portugal

Species	*Pinus pinaster*		*P. pinea*
Latitude	42°N	40°N	38°N
Chronology length (years)	1907–2007 (101)	1876–2006 (131)	1896–2003 (107)
Number of trees (cores)	18 (36)	22 (44)	17 (34)
Mean ring width (mm)	2.60	2.17	3.36
Median ring width (mm)	2.39	1.81	2.83
Mean sensitivity	0.27	0.30	0.27
Standard deviation (mm)	1.22	1.54	1.97
First-order autocorrelation			
Raw series	0.70	0.76	0.72
Residual series	0.03	0.02	0.03
Mean LW/EW	0.57	0.57	0.41
Common interval analysis for the period 1940–2002			
Number of trees (cores)	18 (36)	22 (44)	13 (23)
EPS	0.91	0.94	0.88
rbt	0.29	0.31	0.32

LW, latewood; EW, earlywood; EPS, expressed population signal; rbt, mean between-trees correlation.
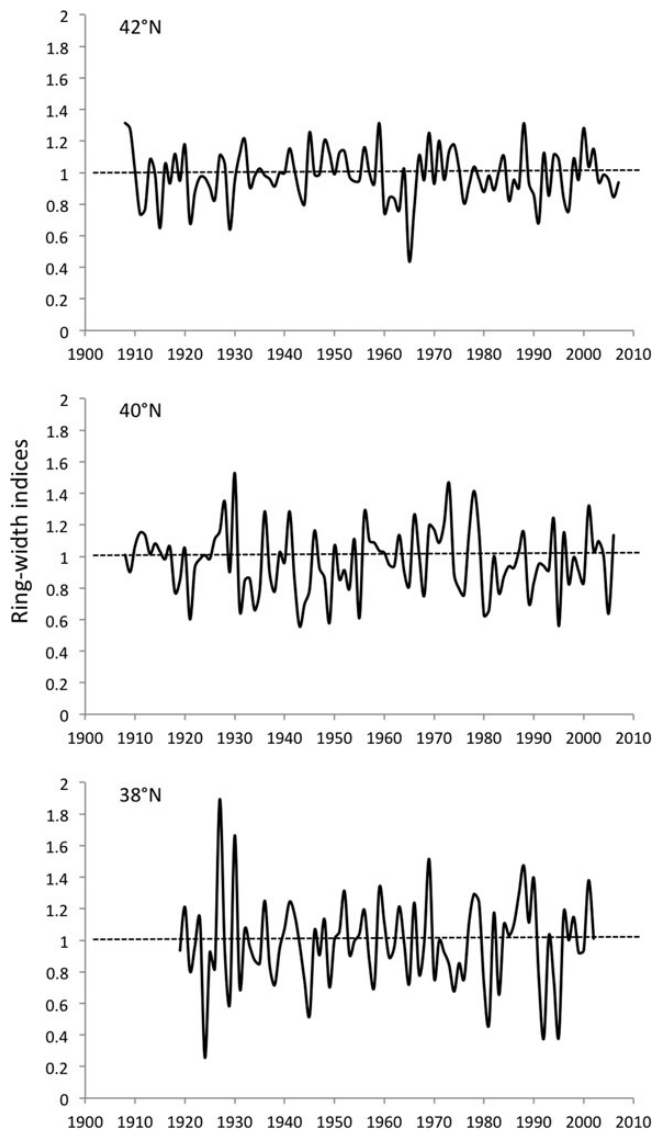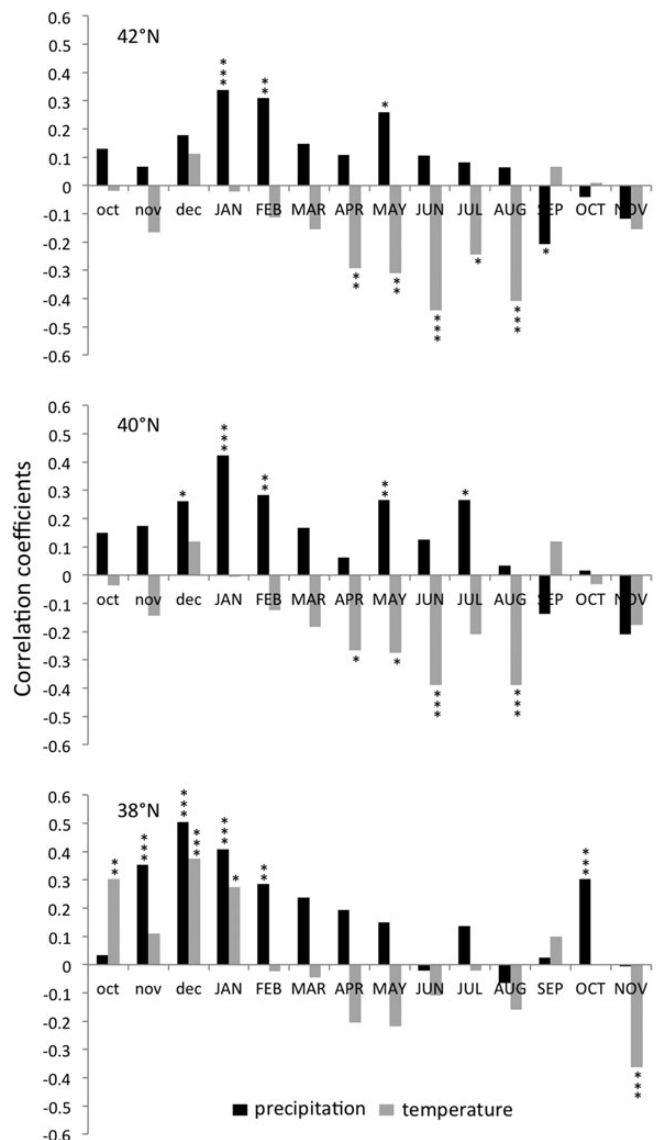
Figure 2 Ring-width residual chronologies of *Pinus pinaster* at 42 and 40°N and *P. pinea* at 38°N for the period 1908–2007.

Figure 3 Correlation between tree-ring width residual chronologies and monthly climatic data along the latitudinal gradient. One, two or three asterisks indicate that correlations are significant at the 95, 99 and 99.9 % levels, respectively.

deviation was higher for *P. pinea* at 38°N (Table 1). The first-order autocorrelation of the raw series was higher for *P. pinaster* at 40°N, and the ratio of latewood/earlywood was higher for *P. pinaster* at 42 and 40°N (Table 1). The EPS value for the common interval of analysis to compare the three chronologies was higher than the critical value of 0.85 proposed by Wigley *et al*. (1984), suggesting a strong common climate signal in the chronologies. The rbt value was higher for *P. pinea* at 38°N (Table 1). The annual variability in ring-width indices of the residual chronologies increased with decreasing latitude (Figure 2).

Tree-ring width and climate

Along the latitudinal gradient, tree-ring width of *P. pinaster* and *P. pinea* showed a common positive correlation with January and February precipitation (Figure 3). Concerning only the species *P. pinaster*, tree-ring width and climate correlations were quite

similar at 42 and 40°N (Figure 3). Growth was positively correlated with winter (January and February) and spring (May) precipitation and negatively correlated with spring (April and May) and summer (June and August) temperatures. At 38°N, tree-ring width of *P. pinea* was positively correlated with autumn/winter precipitation (previous November and December, January and February) and temperature (previous October, December and January) (Figure 3). It also showed a positive correlation with the precipitation of October and a negative one with the temperature of November.

Intra-annual density fluctuations and climate

Most of the IADFs found in *P. pinaster* and *P. pinea* were located in the latewood, characterized by earlywood-like cells within

Table 2 Stabilized latewood IADF frequency of *Pinus pinaster* and *P. pinea* along a latitudinal gradient from 42 to 38°N in Portugal

Species	*P. pinaster*				*P. pinea*	
Latitude	42°N		40°N		38°N	
	Tree	Both cores	Tree	Both cores	Tree	Both cores
Latewood IADFs (%)	50.4	34.2	27.8	16.9	37.5	28.9

latewood. IADFs were more frequent at latitude 42°N followed by 38°N and 40°N (Table 2).

IADFs of *P. pinaster* at 42°N showed a positive correlation with the temperature of June and August (Figure 4). IADFs of *P. pinaster* at 40°N showed a negative correlation with the precipitation of January and March, and a positive correlation with precipitation in September and October and with the temperature of November and December (Figure 4). IADFs of *P. pinea* at 38°N showed a strong positive correlation with precipitation in September and October (Figure 4).

Discussion

Our results showed that the climate–growth correlation patterns are species-specific and conserved within a range of climatic conditions. On the contrary, the climate–IADFs correlation patterns are less species-specific and more dependent on the growing conditions. IADFs constitute a plastic response and reveal an anatomical adjustment of tracheids to the prevalent environmental conditions at shorter time-scales. The plasticity of IADFs can be important in the adjustment of trees to climate change conditions in the Mediterranean. Our data contribute to elucidate the main climatic drivers of tree growth for *P. pinaster* and *P. pinea* and the factors determining the formation of IADFs.

Pinus pinaster *under temperate and Mediterranean climate*

The climate–growth correlations of *P. pinaster* showed a similar pattern along the latitudinal gradient, with a positive correlation with precipitation in January, February and May, and a negative correlation with June and August temperature. Precipitation in winter is important to recharge the soils and aquifers before the onset of the growing season, reinforced afterwards by spring precipitation, during the peak of the growing season. The strong negative correlations with June and August temperatures might be indirectly connected with the closure of stomata to reduce excessive transpiration and the consequent reduction in photosynthetic capacity and growth. This reinforces the idea that *P. pinaster* is a drought-avoiding species (Nguyen-Queyrens *et al.*, 2002).

The similar climate–growth correlation pattern under different macroclimatic conditions (temperate and Mediterranean) suggests a species-specific response to climate. Rigling *et al.* (2001) also observed similar climate–growth correlation patterns of *P. sylvestris* under different climatic zones and soil substrates. This is consistent with large-scale-spatial studies of tree species within their natural distribution (Cook and Cole, 1991; Frank and Esper, 2005). Nonetheless, the climatic differences between the temperate and Mediterranean sites might not be big enough to induce a distinct climate–growth response in *P. pinaster* (Alía
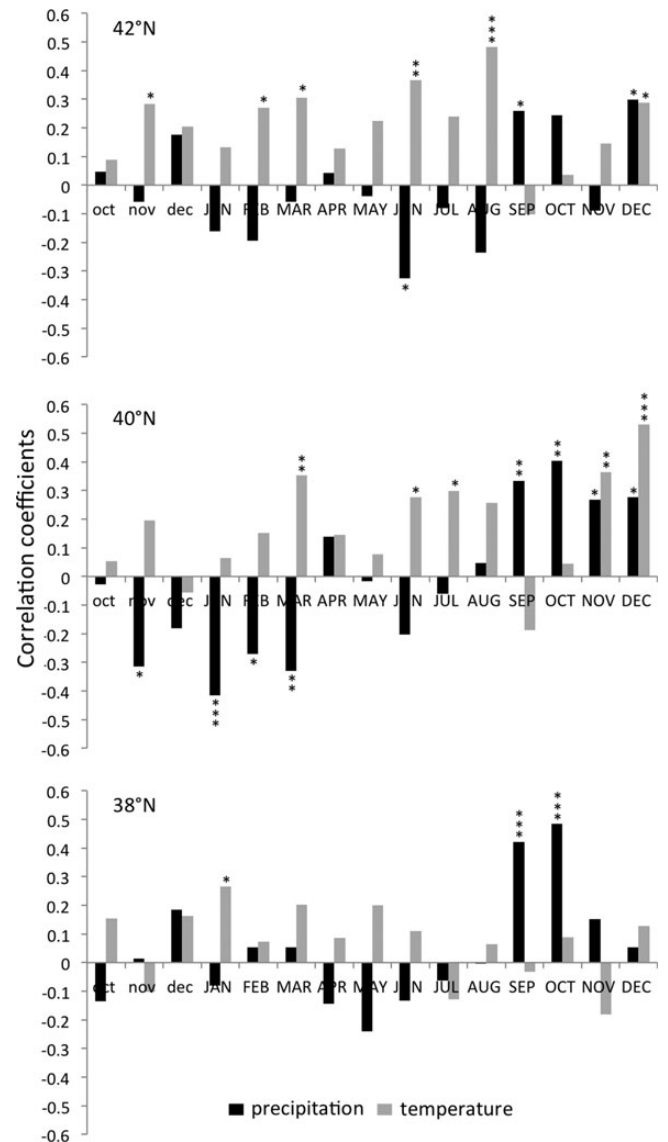
Figure 4 Spearman rank-order correlation between the master chronology of standardized frequency of IADFs and monthly climatic data along the latitudinal gradient. One, two or three asterisks indicate that correlations are significant at the 95, 99 and 99.9 % levels, respectively.

et al., 1997; Correia *et al.*, 2008; Corcuera *et al.*, 2010). Thus, a broader geographical database including more populations of *P. pinaster* under contrasting climatic conditions is needed to reveal if the growth responses to climate is species-specific.

Most of the IADFs found in *P. pinaster* were located in latewood. IADFs are anatomically characterized by changes in the wall/lumen ratio of the tracheids and are the result of changes in environmental conditions (Wimmer *et al.*, 2000; Rigling *et al.*, 2002). Differences in the morphology of the xylem cells are ultimately dependent on cambial activity, which in turn is highly dependent on the tree water status during the growing season (Larson, 1994; Rozenberg *et al.*, 2002). The shift from early- to latewood is closely related to the availability of soil moisture and triggered by drought stress (Domec and Gartner, 2002). Latewood formation involves a limited radial expansion and an increase in wall thickening of the cambial derivatives (Uggla *et al.*, 2001). An increase in soil moisture later in the season promotes radial expansion of tracheids and a decrease in cell wall deposition resulting in the formation of earlywood-like cells within latewood (Denne, 1974; Uggla *et al.*, 2001).

The correlation pattern of IADFs and climate was different for *P. pinaster* growing under temperate or Mediterranean climate. Latewood IADFs of *P. pinaster* growing under temperate climate were positively correlated with summer temperature, whereas under Mediterranean climate, latewood IADFs were positively correlated with autumn precipitation and autumn/winter temperature. Experiments performed on several conifers to test the effect of temperature on tracheid development showed that tracheid wall thickness decreased and tracheid lumen area increased with increasing temperature (Denne, 1971). However, if high temperatures are associated with low soil moisture, latewood-like cells are produced (Domec and Gartner, 2002). This could explain the different triggering climatic factors in the formation of latewood IADFs of *P. pinaster* under temperate and Mediterranean climate. Under temperate climate, there is no prominent dry season and thus the high summer temperatures have the effect of decreasing tracheid cell wall thickness and increasing tracheid lumen area, as described in the literature (e.g. Vaganov *et al.*, 2006). However, under Mediterranean climate, latewood IADFs are only produced if autumn precipitation is enough to alleviate water deficit after the summer drought (de Luis *et al.*, 2011; Novak *et al.*, 2013), reinforced afterwards by the effect of above average autumn/winter temperatures on tracheid development.

Pinus pinaster *and* P. pinea *under Mediterranean climate*

Pinus pinaster and *P. pinea* presented a different pattern of climate–growth correlations under similar macroclimatic conditions suggesting that the climate–growth response is influenced by the species. A similar result was obtained by de Luis *et al.*, (2009) who studied *P. halepensis* and *P. pinea* growing under Mediterranean climate. However, we must take into account that in our study, *P. pinaster* and *P. pinea* were not growing exactly under the same climatic conditions, which limits the interpretation on the relative contribution of genetics and environment on tree-growth response.

Tree-ring growth of *P. pinea* and *P. pinaster* showed a common positive correlation with winter precipitation, and *P. pinaster* growth was also correlated with spring precipitation. *Pinus pinea* showed a strong positive correlation with winter temperature and we hypothesize that *P. pinea* in the southernmost latitude starts the growing season earlier. A study with poplar showed that cumulative high temperatures from late winter to early spring induced an early start of cambial cell division, xylem differentiation and an extended growth period (Begum, 2008).

Tree-ring width of *P. pinaster* under Mediterranean climate showed a negative correlation with summer temperatures whereas there were no correlations for *P. pinea*. These results suggest that *P. pinaster* can grow during summer whereas *P. pinea* enters a quiescent stage. Probably, in the southernmost latitude, the summer is too dry to maintain cambial activity and xylem cells differentiation (Campelo *et al.*, 2007, 2009).

Tree-ring width of *P. pinea* showed a positive correlation with precipitation in October suggesting a second growth period after the summer pause (Campelo *et al.*, 2007), a signal not present in *P. pinaster*. *Pinus halepensis* growing in Spain under Mediterranean climate showed two major growth phases, one in spring and another in autumn, with low or no cambial activity during summer (de Luis *et al.*, 2007; Camarero *et al.*, 2009; Novak *et al.*, 2013). This was also observed in other Mediterranean species like *Arbutus unedo* (Battipaglia *et al.*, 2010) and *Erica arborea* (Battipaglia *et al.*, 2014). Precipitation after the summer drought can resume cambial activity with the formation of new cells and/or cambial derivatives that did not complete their differentiation before the summer can now become more turgid, increasing the tree-ring width (Vieira *et al.*, 2013).

The strong negative correlation of tree-ring width of *P. pinea* and temperature in November is not so straightforward to explain. High temperatures increase plant respiration, and if this is associated with a lower photosynthetic capacity due to a reduction of the day-length in late autumn, the available carbohydrates for growth diminish (Denne, 1974; Dodd and Fox, 1990) reducing tree-ring width.

Latewood IADFs of both *P. pinea* and *P. pinaster* growing under Mediterranean climate were positively correlated with autumn precipitation. High precipitation in autumn can increase the turgor pressure of tracheids that are still under differentiation, increasing their radial expansion and producing earlywood-like cells (Campelo *et al.*, 2007; Vieira *et al.*, 2010, 2013).

Under Mediterranean climate, there was a negative correlation between *P. pinaster* latewood IADFs and the precipitation from previous winter and current spring, a signal neither present in *P. pinaster* under temperate climate nor in *P. pinea* under Mediterranean climate. Low precipitation in winter and spring can affect photosynthesis and the production of new cells by the vascular cambium, ultimately translated in a reduction of tree growth (Körner, 2003). Additionally, cell wall thickening of latewood cells is an expensive and long-lasting process of carbon deposition (Deslauriers *et al.*, 2003). However, it is a lower priority carbon sink, in relation to respiration, root growth and storage (Oribe *et al.*, 2003; Polak *et al.*, 2006; Deslauriers *et al.*, 2009). We suggest that if low precipitation in winter and spring can significantly reduce carbon acquisition, cell wall deposition in latewood tracheids might not be completed, resembling earlywood-like cells.

Species vs. climate: some final remarks

Our results indicate that the tree-ring growth responses to climate are species-specific. Thus, within the geographical distribution range of a species, which encompasses a certain range of climatic conditions, the correlations between climate and growth are conserved. On the contrary, the climatic parameters that control the formation of IADFs are site-dependent, and not so much dependent on the species. In this sense, IADFs represent an important plastic trait of the adjustment of trees to the variability of environmental conditions within the growing season. In our

sites, most of the IADFs were located in the latewood for both species, indicating good environmental conditions after the summer drought (Rigling *et al.*, 2001; Campelo *et al.*, 2007). Earlywood-IADFs indicate stressful conditions during the spring growth (Battipaglia *et al.*, 2010) but were quite rare in our samples. In regions with a long growing season, like the Mediterranean, a broader geographical network of IADFs chronologies would give valuable information to reconstruct the variability of climatic conditions within the growing season.

Under the scope of climate change, it is foreseen an increase in temperature and frequency of extreme events for Portugal (Ramos *et al.*, 2011). According to our results, a higher temperature in winter might induce an earlier start of the growing season benefiting *P. pinea*. Higher temperatures in spring and summer can increase evapotranspiration and thus water stress with a negative effect in *P. pinaster*. IADFs can be in the forefront of the adjustment of trees to climatic changes. If spring becomes more stressful in the Mediterranean, then an increase in the frequency of earlywood-IADFs would be expected. More studies are necessary to quantify the adaptive value of IADFs, namely their effect on the hydraulic adjustment of trees to the availability of water (De Micco *et al.*, 2007, 2012; Battipaglia *et al.*, 2014).

Funding

Conflict of interest statement

None declared.

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