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ORIGINAL PAPER

Climatic control of intra-annual wood density fluctuations of *Pinus pinaster* in NW Spain

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Abstract Tree rings of *Pinus pinaster* often contain intra-annual density fluctuations (IADFs), which have been attributed to the succession of dry and rainy periods typical of Mediterranean climate, but their formation has not been studied yet under Atlantic climate. We analyzed the occurrence and climatic significance of replicated IADFs in ten monospecific stands in NW Spain. The frequency of IADFs was higher than previously reported for this species under Mediterranean conditions and consistently decreased with increasing elevation. The formation of bands of latewood-like tracheids within the earlywood was favored by dry previous August, cold previous winter and dry April. Bands of earlywood-like tracheids within the early latewood were also favored by low winter temperatures. However, their occurrence was geographically heterogeneous, with two groups of stands being defined by their distances to the shoreline. In coastal stands, cold May–August triggered IADFs formation, while in inland stands their formation was favored by dry May–July. Regional winter temperatures and April water balance were strongly related to the East Atlantic (EA) pattern, which greatly conditioned the occurrence of IADFs in the earlywood and the early latewood. By contrast, the presence of bands of earlywood-like tracheids in the late latewood was independent of the EA pattern, being strongly related to warm conditions in spring and especially to a wet October. The link between regional climate and the EA pattern strongly controlled the physiological processes that determine intra-annual growth dynamics and short-term cell enlargement of *P. pinaster* in NW Spain.

Keywords Dendrochronology · False tree-rings · *Pinus pinaster* · Atlantic climate · Water balance · East Atlantic pattern

Introduction

The integration of the effects of several environmental factors operating at multiple time scales on tree-ring growth makes difficult to understand the underlying mechanisms responsible for triggering intra-annual cambial activity and growth dynamics (Fritts 2001). In fact, cambial activity in conifers may vary at a shorter time scale within the growing season, leading to the formation of intra-annual bands of tracheids with distinctive appearance, differing from those typical of the early- or latewood parts of the ring (Vaganov et al. 2009). Like the most widely used ring-width records, small variations in wood density within a tree ring are morphologically preserved in the wood structure and can be easily differentiated and analyzed any time.

The intra-annual density fluctuations (IADFs) in conifers are areas of the tree ring where wood density changes as a response to a particular combination of environmental conditions that modify the rates of cambial activity

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(De Micco et al. 2007). The term IADF includes the so-called false rings, intra-annual bands, light latewood rings and double or multiple rings, which can be anatomically characterized by latewood-like tracheids within the earlywood, or earlywood-like tracheids within the latewood (Wimmer et al. 2000; Girardin et al. 2001; Rigling et al. 2001). The visual characterization of IADFs is given by changes in the wall/lumen ratio of the tracheids, which corresponds with intra-ring variations in the $^{13}C/^{12}C$ isotopic composition of cellulose, suggesting that IADFs are associated to plastic responses to changes in physiological stress (Park et al. 2006; De Micco et al. 2007).

Several external disturbance events such as insect outbreaks, and different climatic triggers such as droughts, flooding, snowfall, or frosts have been identified as causes of IADF formation (Rigling et al. 2002; Hoffer and Tardif 2009; Edmondson 2010). In addition, individual features such as canopy position, growth rate and tree age can also influence their formation (Copenheaver et al. 2006; Bogino and Bravo 2009; Vieira et al. 2009). Usually, the presence of IADFs in the earlywood is related to dry springs followed by moist conditions, while moist summers can cause IADFs in the latewood (Wimmer et al. 2000; Bouriaud et al. 2005). A wide variety of woody species under Mediterranean climate characterized by summer drought and a high inter-annual variability in precipitation and temperature commonly form IADFs (Cherubini et al. 2003). Among these species, IADFs in the Mediterranean pines *P. halepensis*, *Pinus pinaster* and *P. pinea* have been attributed to an irregular rainfall regime during the growing season (Campelo et al. 2006; De Luis et al. 2007; De Micco et al. 2007; Bogino and Bravo 2009).

Pinus pinaster is a forest species of the western Mediterranean basin, inhabiting a wide range of environmental and physiographic conditions. Its main populations are located in the Iberian Peninsula, growing in both natural and planted woodlands under climatic conditions ranging from the extremely dry and warm summers of the Mediterranean areas in central and southern Spain to the mild and humid climate of the Atlantic coast at its western range boundary in the Iberian Peninsula (Alía et al. 1997). Despite the great productive and conservational importance of *P. pinaster* under Atlantic conditions, its intra-annual growth dynamics and sensitivity to limiting climatic factors have not been studied in this region yet. Under Mediterranean climate, *P. pinaster* presents IADFs in both the early- and latewood due to the succession of dry and rainy periods during the growing season (Bogino and Bravo 2009; Vieira et al. 2009). The pronounced genetic and physiological differences between the Atlantic and Mediterranean populations (Bucci et al. 2007; Correia et al. 2008), and the contrasting climatic regimes of both regions, suggest that other environmental factors different from the alternation between dry and wet periods may be critical for the intra-annual growth dynamics of *P. pinaster* under Atlantic climate.

Regional climate in Spain is controlled by large-scale circulation patterns, such as the North Atlantic Oscillation and El Niño-Southern Oscillation, which significantly influence rainfall regimes (Rodó et al. 1997; Trigo et al. 2004). Therefore, large-scale climatic patterns may also influence intra-annual growth of *P. pinaster* through determining the variability of regional climate conditions in NW Spain. This study is aimed at (1) characterizing the occurrence patterns of intra-annual wood density fluctuations in *P. pinaster* over a tree-ring network at its northwestern range boundary in NW Spain, and (2) identifying the regional and large-scale climatic factors that drive the formation of different IADF types under Atlantic climate.

Materials and methods

Study area

The study area comprises the region of Galicia, NW Spain, where *P. pinaster* grows at its northwestern range boundary and covers over 47% of the forested area in pure or mixed stands, being one of the most important commercial woods. Since the past century, this species has been extensively planted in Galicia (Fig. 1a), and the resulting even-aged stands are typically managed through short rotations of 40–50 years. Regional climate is temperate and humid, with a mean annual temperature of 12.7°C for the period 1967–2005, ranging between 10.1 and 14.5°C. The mean annual precipitation is 1,300 mm, ranging from 870 in the southern inland area to 1,800 mm near the coast under more oceanic influence.

Climate data

We used monthly gridded data from the datasets of the Climate Research Unit, University of East Anglia (CRU TS 3, $0.5° \times 0.5°$), taken from the Web site of the Royal Netherlands Meteorological Institute (http://climexp.knmi.nl/), for mean temperature (T) and total precipitation (P) for the period 1967–2006. Since maximum temperature is coupled to a minimum of rainfall during summer time, precipitation data were just used to calculate water balance (WB) as $WB = P - PET$, where PET is the potential evapotranspiration estimated as a function of monthly mean temperatures and geographical latitude (Thornthwaite 1948). Mean monthly WB ranged between −73 mm in July and 145 mm in December–January, showing a tendency to water deficit from June to August. To characterize the climatic determinants of IADFs formation in

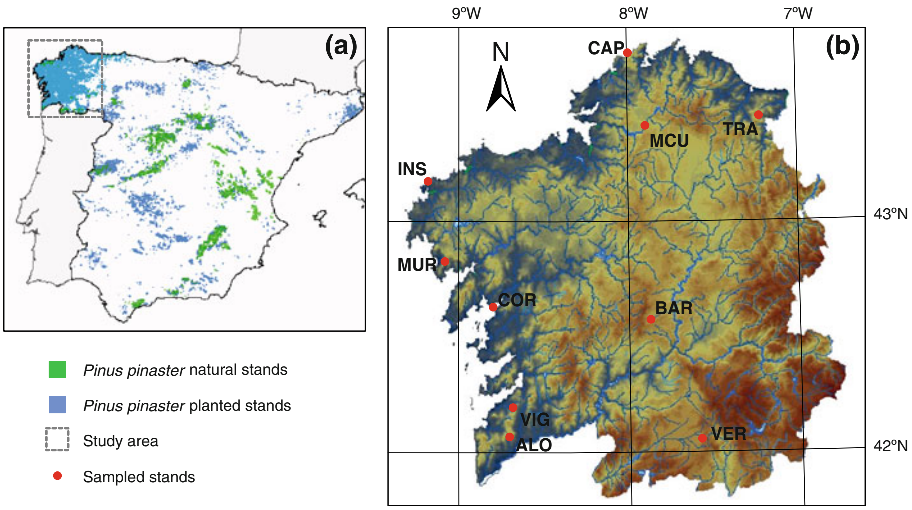

Fig. 1 **a** Location of the study area, and distribution of natural and planted *Pinus pinaster* in Spain (http://iniagis.inia.es/Pinus%20pinaster/). **b** Location of the sampled stands in Galicia, NW Spain. Stand codes are shown in Table 1

P. pinaster at a regional scale, mean values from the 0.5° × 0.5° grid boxes comprised within the region (7.0–9.5°W, 42.0–44.0°N) were calculated.

Monthly indices of a series of teleconnective patterns (North Atlantic Oscillation, East Atlantic pattern, East Atlantic/West Russia pattern, Scandinavia pattern, Tropical/Northern Hemisphere pattern, Polar/Eurasia pattern) were obtained from the Web site of the NOAA/National Weather Service, Climate Prediction Center, Maryland, USA (ftp://ftp.cpc.ncep.noaa.gov/wd52dg/data/indices/tele_index.nh). Among them, only the East Atlantic (EA) pattern showed significant relationships with IADFs records of *P. pinaster*. EA pattern is a prominent mode of low-frequency variability structurally similar to the NAO, which consists of a north–south dipole of anomaly centers spanning the North Atlantic from east to west, whose positive phase is associated with above-average surface temperatures in Europe (Barnston and Livezey 1987).

Monthly climatic variables were seasonalized to values for winter (December–February), spring (March–May), summer (June–August) and autumn (September–November). The considered window for exploring the relations between climate and tree growth were from previous June (Jun(–1)) to current November (Nov) for monthly values, while for seasonal values they spanned from previous summer (Sum(–1)) to current autumn (Aut). In addition, annual values were calculated by averaging monthly values from previous October (Oct (–1)) to current September (Sep).

Sampling, tree-ring dating and tree age estimation

We sampled ten monospecific stands located both at the coastal and inland areas along the full elevation range of *P. pinaster* in Galicia, from sea level to 855 m (Fig. 1b, Table 1). We measured DBH (bole diameter at 1.30 m), and took at least two increment cores per tree from 15 to 24 dominant trees per stand. The cores were air-dried, glued onto wooden mounts, and polished with successively finer grades of sandpaper, until the xylem cellular structure was visible in the transverse plane. The tree-ring series were absolutely dated by assigning calendar years to the rings following standard procedures (Fritts 2001). Total ring widths were measured under magnification to the nearest 0.001 mm with a sliding-stage micrometer (Velmex Inc., Bloomfield, NY, USA) interfaced with a computer. The software COFECHA (Grissino-Mayer 2001) was used to quantitatively check for crossdating errors in the ring width series. All series with potential dating errors or weakly correlated with the master site chronology were corrected when possible or discarded.

In cores showing the pith, tree age at coring height was determined by the number of crossdated rings. In partial cores showing the arcs of the inner rings, the pith was located using a graphical method based on the convergence of xylem rays at the pith (Rozas 2003), and the number of missing rings toward the pith was estimated by extrapolating the mean growth rates from the innermost five rings in the cores. Tree age was estimated based on the oldest core per tree. No corrections for the number of missing rings due to coring height were performed.

Intra-annual wood density fluctuations

The adequately dated cores were visually examined under magnification for identification of IADFs, which were distinguished from actual tree rings due to their non-sharp transitions in opposite to the marked boundaries between annual rings (Wimmer et al. 2000; Park et al. 2006). Based on the previous definitions for *P. pinaster*, IADFs were classified into three types according to their appearance and intra-ring position (Vieira et al. 2009). IADFs type E were

Table 1 Site and tree characteristics of the *P. pinaster* stands

Stand name	Code	Latitude (N)	Longitude (W)	Elevation (m)	Number of sampled trees	DBH ± SD (cm)	Age ± SD (years)
Monte Aloia	ALO	42°05′	8°41′	530	21	46.2 ± 9.3	33 ± 6
A Barrela	BAR	42°32′	7°51′	620	24	66.7 ± 15.4	45 ± 4
A Capelada	CAP	43°40′	7°59′	340	23	45.0 ± 9.0	42 ± 3
Illa de Cortegada	COR	42°37′	8°47′	20	22	54.4 ± 9.5	52 ± 6
Monte Insua	INS	43°08′	9°09′	10	15	52.8 ± 11.8	34 ± 9
Marco da Curra	MCU	43°22′	7°53′	590	20	29.6 ± 3.7	44 ± 1
Muros	MUR	42°48′	9°04′	155	22	56.6 ± 5.9	54 ± 3
Trabada	TRA	43°25′	7°13′	640	23	45.7 ± 6.8	50 ± 5
Verín	VER	42°04′	7°33′	855	23	41.6 ± 3.5	43 ± 4
Vigo	VIG	42°12′	8°39′	365	19	59.6 ± 8.3	55 ± 7

DBH diameter at 1.30 m above ground

bands of latewood-like tracheids within the earlywood (Fig. 2a). By contrast, IADFs types L and L+ were bands of earlywood-like tracheids within the latewood (Fig. 2b), and near the transition between the latewood and the earlywood of the following ring (Fig. 2c), respectively.

An IADF in a given ring was considered when present in at least one core per tree, and was tabulated. The percent frequencies of cores and rings per stand showing IADFs of types E, L, and L+ were computed as descriptive statistics of the IADF distributions. The relative frequency of IADFs was calculated as $F = n_x/N$, where n_x is the number of trees showing IADFs in the year x, and N is the number of trees in that year. Moreover, the variance bias induced by the varying number of tree rings per year was corrected by calculating the stabilized IADF frequency as $F_{\text{stab}} = F \times N^{0.5}$ (Rigling et al. 2001). F_{stab} series were calculated separately for the types E, L and L+ at each stand. In order to estimate the dependence of IADFs frequency on ring age and ring width, Spearman's correlations between ring age, mean ring width, and F_{stab} of each IADF type, were calculated.

We tested if the occurrence of IADFs in *P. pinaster* was independent from both inter-site distances and differences between site elevations using the standardized Mantel statistic (R_M). The Mantel test compares two similarity or distance matrices computed for the same objects, and behaves like a correlation coefficient, being bounded between −1 and 1 (Legendre and Legendre 1998). To calculate R_M, we used the matrices of similarity between F_{stab} chronologies and descriptive statistics of the IADF distributions for the period 1967–2005, compared to the matrices of geographical distances and elevation

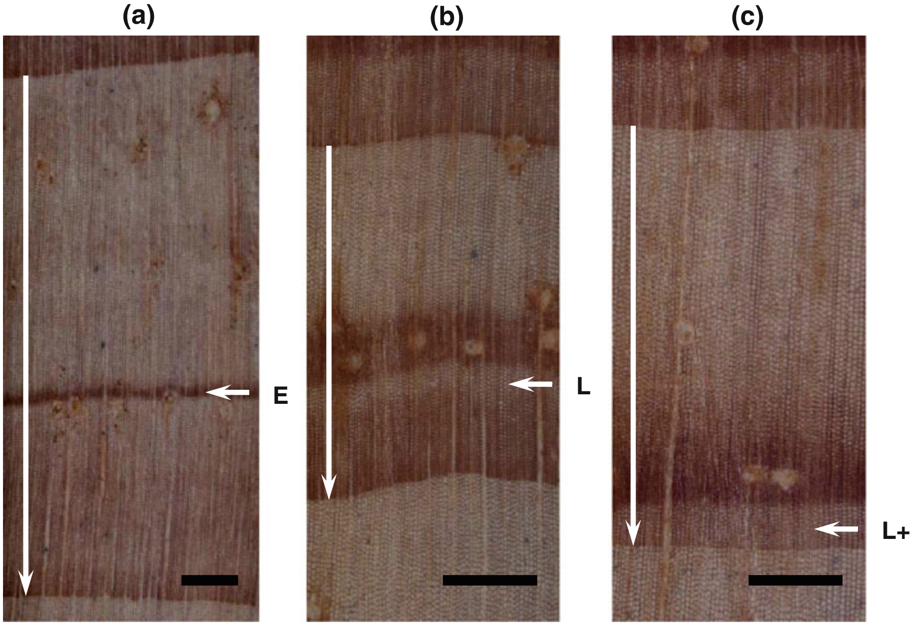

Fig. 2 Aspect of the intra-annual wood density fluctuations of the types E (**a**), L (**b**) and L+ (**c**) in *P. pinaster* based on their anatomical appearance and intra-ring position. Vertical arrows show the extension of the complete annual ring. *E* Latewood-like tracheids within the earlywood. *L* Earlywood-like tracheids within the latewood. *L+* Earlywood-like tracheids near the transition between the latewood and the earlywood of the next ring. *Scale bars* 1 mm

differences among stands. We used Spearman's correlation as a measure of similarity between F_{stab} chronologies, and $1 - D_{\mathrm{norm}}$ as similarity between descriptive statistics, being D_{norm} the normalized difference ranging between 0 and 1 (Legendre and Legendre 1998). Since we compared similitude matrices with distance matrices, positive and negative R_{M} values would indicate more and less similar patterns, respectively, at increasing distances or elevation differences. The statistical significance of R_{M} was tested by means of 9,999 random permutations of one of the distance matrices, to obtain the expected distribution of the statistic under the null hypothesis of independence. R_{M} was calculated with the Mantel function from the PopTools v3.1.0 add-in for Microsoft Excel (http://poptools.org/).

Relationships between IADFs and climate variability

To analyze the relationships between climate and IADFs frequency at a regional scale, we excluded from the analyses those years showing IADF frequencies that did not show any significant replication within a stand. We used the definition given by Schweingruber et al. (1990) of "pointer year" to refer to a group of trees in which most of them display an event—in our case a density fluctuation– in the same year. The most conspicuous IADFs replicated amongst a significant proportion of trees at each stand were considered using a significance test that identifies frequencies higher than a given background value (Edmondson 2010). This background value represents the approximate frequency of an entirely random inter-annual occurrence, and was calculated as the ratio between all IADFs and the total number of dated rings at a given stand. The frequency F was considered significant when the value $F - (F\,(1 - F)/N)^{0.5}$ exceeded the random background value (Edmondson 2010). Pointer years showing significant IADFs were identified separately for the types E, L and L+ in each stand, and a regional distribution was calculated for each type as the year-by-year sum of significant events at all stands (Appendix S1).

The analyses for relating climate and IADFs frequency were performed along 1967–2005, the common period for all IADFs records. Due to the abundant zero values in the regional chronology of IADFs type E, we calculated the anomalies of all climatic variables for pointer years in comparison to the expected means in the remainder years without IADFs for the period of analysis (Masiokas and Villalba 2004). Monthly, seasonal and annual climatic variables were normalized, and the deviations of means from years showing IADFs type E, with respect to years not showing IADFs, were calculated. Standard deviations above 0.5 or below −0.5 were considered as significant positive or negative anomalies, respectively. Logistic regression analysis was also used to identify those independent explanatory variables with a significant effect on the probability of occurrence of IADFs type E at a regional level. The model was depicted by $P = 1/(1 + e^{a+\Sigma bi Xi})$, where P is the probability of an IADF occurrence ($P = 0$ indicates non-pointer year, $P = 1$ indicates pointer year), a is the constant of the model and bi are the coefficients for the independent variables Xi. Logistic regression was performed by a forward stepwise procedure and the best adjustment was obtained by maximum likelihood (Sokal and Rohlf 1995).

For IADFs types L and L+, the reduced number of null values allowed the use of correlation analysis to identify the main climatic variables that determined the regional IADF chronologies. To account for non-normality of regional IADF pointer year chronologies, we calculated Spearman's rank correlations between the regional chronologies of types L and L+ and the monthly, seasonal and annual climatic variables. In addition, to evaluate the diversity of climatic triggers at a local scale, Spearman's correlations between local F_{stab} distributions and monthly gridded T and WB were calculated. Finally, the relationships between regional climate variability and EA pattern were explored by means of Pearsons's correlation. The statistical analyses were performed with the SPSS 15.0 for Windows package (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of trees and IADF distributions

The mean DBHs of dominant trees at the sampled stands varied between 29.6–66.7 cm, and the mean estimated ages between 33–55 years (Table 1). The reduced standard deviations of tree ages suggested an even-aged origin for the majority of the sampled stands. Almost 100% of the 498 cores analyzed showed IADFs (Table 2). The proportion of cores with IADFs type E was quite variable, ranging between 2.2 and 88.2%, while the proportion of cores with IADFs in the latewood was consistently high. For IADFs type L, the proportions ranged between 58.3–100%, and 87.5–100% for the type L+. A total of 19,513 tree rings were analyzed, with the proportion of rings with IADFs varying between 15.9 and 89.5% depending on the site. The proportion of rings showing IADFs type E ranged between 0.0 and 9.0%, while the types L and L+ were comparatively more abundant, ranging between 3.7–38.8% for type L, and 7.9–66.9% for type L+.

Patterns of IADFs occurrence

No inter-site common trends in the relationships between the frequency of IADFs and tree-ring age or ring widths were found. For the IADFs type E, there were no

Table 2 Descriptive statistics of the IADF distributions

Code	Period	Cores					Rings				
		N	% IADFs	% E	% L	% L+	*N*	% IADFs	% E	% L	% L+
ALO	1967–2006	36	100.0	33.3	97.2	100.0	1,081	54.0	2.3	26.3	35.8
BAR	1961–2006	50	100.0	14.0	94.0	100.0	1,951	69.7	0.7	11.9	61.9
CAP	1966–2006	45	97.8	20.0	97.8	97.8	1,693	30.7	0.6	14.7	17.2
COR	1953–2005	34	100.0	88.2	100.0	100.0	1,541	89.5	9.0	38.8	66.9
INS	1967–2007	26	100.0	61.5	100.0	92.3	822	45.7	2.8	13.7	34.4
MCU	1961–2006	46	100.0	2.2	97.8	100.0	1,914	36.1	0.0	13.6	26.8
MUR	1951–2006	42	100.0	30.9	100.0	95.2	2,172	56.2	0.8	15.7	47.5
TRA	1951–2006	48	100.0	16.6	95.8	89.6	2,427	15.9	0.4	8.4	7.9
VER	1964–2006	48	95.8	27.1	58.3	87.5	1,894	18.3	0.7	3.7	13.9
VIG	1951–2007	33	100.0	63.6	100.0	100.0	1,563	53.0	3.0	28.3	31.3

N total number of cores or rings analyzed, *% IADFs* percentage of cores or rings showing intra-annual density fluctuations, *% E*, *% L* and *% L+* percentage of cores or rings showing density fluctuations of the types E, L and L+, respectively

The period includes at least five trees

significant relationships of F_{stab} with age and ring width. For types L and L+ some correlations were statistically significant, both positive and negative, but no general pattern was observed (Appendices S1, S2).

The similitude between F_{stab} chronologies was neither related to inter-site distances nor to the difference among site elevations, as shown by the Mantel test (Table 3). The only exception was the IADFs type L, which showed a negative relationship with inter-site distances, suggesting less similar patterns of IADFs frequency when increasing distance. The statistics for IADFs distribution were independent of the geographic distance, but were in general significantly and negatively related to elevation differences. In addition, negative correlations with elevation were found for the proportion of cores showing IADFs types E ($R = -0.71$, $P = 0.021$) and L ($R = -0.65$, $P = 0.041$). The only exception was the proportion of cores showing IADFs type L+, which did not show a great inter-site variation. These results suggest more frequent IADFs occurring at lower than at higher sites, irrespective of their geographical position.

Climatic significance of IADFs

Composite regional distributions of pointer years for significant IADFs were developed for each IADF type, according to the random background frequencies for pointer year recognition (Appendix S1). For IADFs type E, the 16 pointer years within the period 1967–2005 showed below-average temperature in December and previous winter, below-average water balance in previous August and April, and below-average EA indices in previous December and April (Fig. 3a). According to the logistic model, the independent explanatory variables with a positive effect on the probability of occurrence of IADFs type E were lower EA indices in April (Wald's test $\chi^2 = 4.94$, $P = 0.026$, coefficient $b = -0.009$), and reduced water balance in previous August ($\chi^2 = 4.27$, $P = 0.039$, $b = -0.038$).

The regional record of pointer years showing IADFs of type L was significantly correlated with both T and EA variability, but independent from any variation in WB (Fig. 3b). Pointer years for IADFs type L were negatively correlated with winter and annual T, and also with EA variation in previous December, previous winter, and its annual variation. The local distributions of F_{stab} for IADFs type L were negatively correlated with T in January–February and May–August at the coastal sites (CAP, COR, INS, MUR, VIG), but in the period May–August positive correlations with T were found at inland sites (ALO, BAR, MCU, VER) (Fig. 4a). Moreover, F_{stab} for IADFs type L in these inland sites showed also negative correlations with WB in May–July. All these results suggest that temperature and water availability in the growing season are site-dependent triggers for IADFs type L formation, exerting contrasting local effects on intra-annual cambial activity.

By contrast, the regional record of pointer years for IADFs type L+ is significantly correlated with both T and WB variability, but independent from EA pattern (Fig. 3c). Positive correlations with T in April, August, spring, and the complete year were found. For WB, positive correlations were found in October and autumn, and negative correlations in January and the complete year, were found. The local analysis showed that, in more than 50% of the cases and without any distinction between the coastal and inland stands, IADFs type L+ responded positively to T in March–May and August, and also positively to WB in October (Fig. 4b). Moreover, high WB in May impacted negatively on the formation of IADFs type L+.

Table 3 Mantel tests for the comparison of inter-site distances and differences between site elevations, with the similarities between F_{stab} site chronologies and chronology statistics for the different types of IADFs

	Variables	Inter-site distances		Site elevations	
		R_M	P value	R_M	P value
IADF chronologies	F_{stab} E	0.08	ns	0.13	ns
	F_{stab} L	−0.30	0.021	−0.14	ns
	F_{stab} L+	−0.15	ns	0.16	ns
IADF chronology statistics	% cores E	0.01	ns	−0.73	<0.001
	% cores L	0.25	ns	−0.64	<0.001
	% cores L+	0.11	ns	−0.24	ns
	% rings IADFs	0.05	ns	−0.57	<0.001
	% rings E	−0.04	ns	−0.61	<0.001
	% rings L	0.07	ns	−0.57	<0.001
	% rings L+	0.04	ns	−0.49	0.001

ns non-significant test

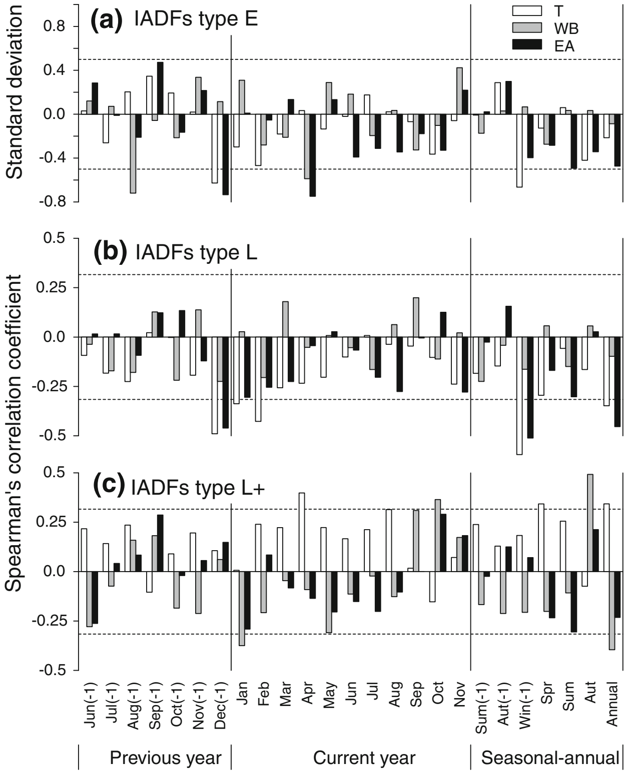

Fig. 3 Standard deviations from mean temperature (*T*), water balance (*WB*) and East Atlantic pattern (*EA*) in those years with significant IADFs type E (**a**), and Spearman's correlation coefficients between the significant IADFs of types L (**b**) and L+ (**c**) and mean monthly, seasonal and annual T, WB and EA in the period 1967–2005. Horizontal lines indicate the standard deviations −0.5 and 0.5, or the lowest significant ($P < 0.05$) correlation coefficient, in absolute value

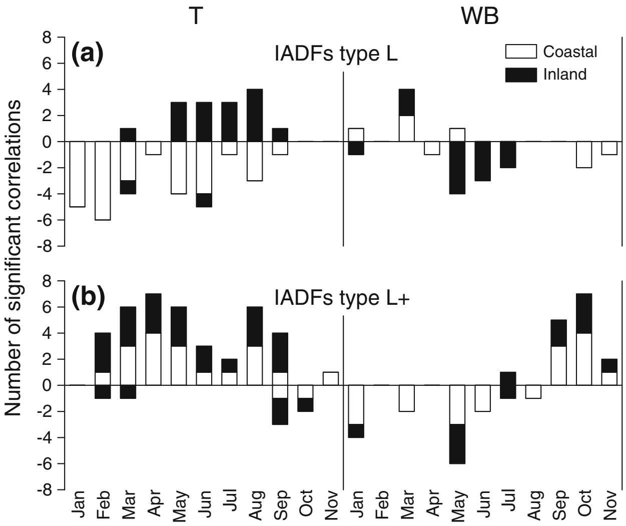

Fig. 4 Number of significant Spearman's correlation coefficients between the F_{stab} chronologies for IADFs of types L (**a**) and L+ (**b**) and local gridded monthly temperature (*T*) and water balance (*WB*). Coastal (CAP, COR, INS, MUR, VIG) and inland (ALO, BAR, MCU, TRA, VER) stands are differentiated. Negative numbers refer to negative correlations

According to the obtained correlations, the main regional climate driver for the formation IADFs type L was previous winter T, which showed a decreasing number of pointer years strongly related to the ascending trend of winter temperature (Fig. 5a). For the type L+, the main climatic driver at a regional scale was autumn WB, with more frequent IADFs in those years with enough water availability in autumn (Fig. 5b).

Several climatic factors revealed as major determinants of IADFs formation in *P. pinaster* showed to be directly dependent on EA pattern variability. For instance, mean temperature of December and winter of the previous year were highly positively correlated with EA in December (Fig. 6a) and winter (Fig. 6b), respectively. In addition, annual temperature was positively correlated with annual EA ($R = 0.49$, $P = 0.001$). Moreover, water balance in April showed a positive correlation with April EA (Fig. 6c).

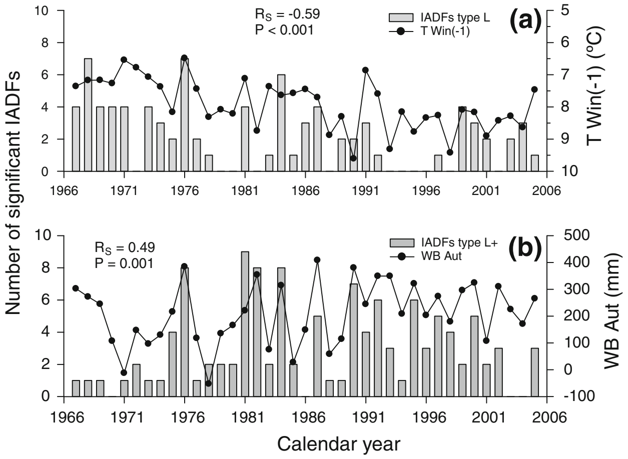

Fig. 5 **a** Comparison of the observed number of significant IADFs type L and mean temperature (T) in previous winter. Note the reverse scale of T axis. **b** Comparison of the observed number of significant IADFs type L+ and water balance (*WB*) in autumn. The Spearman's correlation coefficients (R_S) and their significance values (P) are shown

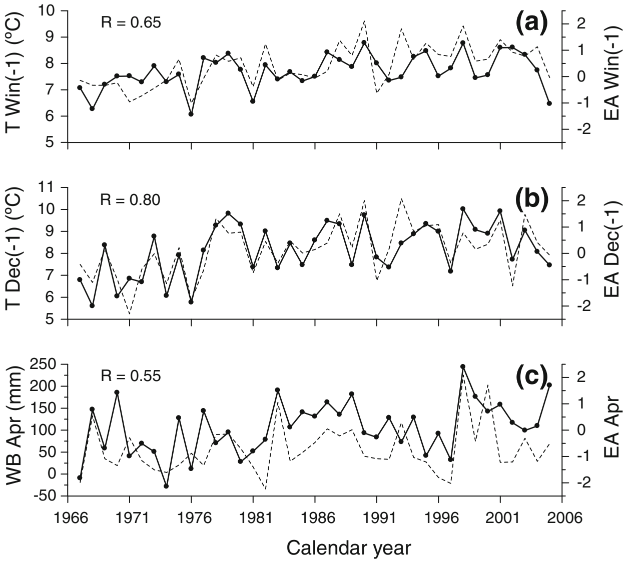

Fig. 6 Comparison of regional climatic variables for T and WB (*dashed lines*) and the EA pattern (*solid lines and dots*) for previous winter T (**a**), previous December T (**b**), and April WB (**c**). Pearson's correlations (R) between EA and the regional climate series in 1967–2005, all of them significant at the 0.001 level, are shown

Discussion

Patterns of IADF occurrence

The frequencies of IADFs previously described for different pine species under a wide variety of climatic conditions were considerably lower than those reported in this paper. Under boreal or temperate climate, IADFs were observed in 9% of the tree rings at maximum (Wimmer et al. 2000; Rigling et al. 2001; Copenheaver et al. 2006), and under Mediterranean climate, they were observed in up to 15–32% of the rings (Campelo et al. 2006; Bogino and Bravo 2009; Vieira et al. 2009). In our study, eight out of ten stands showed IADFs in more than 30% of the rings, six stands in more than 50%, and a maximum frequency of 89.5% of the rings with IADFs was found. Despite IADFs are assumed to be special features or anomalies in normal tree-ring growth, in the light of the observed frequencies, IADFs are more the rule than the exception in *P. pinaster* under mild Atlantic climate.

Studies on several pines, including *P. pinaster*, showed that the frequency of IADFs was negatively related to tree age and positively to growth rates, being more abundant in the juvenile period and during or immediately after periods with wider rings (e.g., Copenheaver et al. 2006; Bogino and Bravo 2009; Hoffer and Tardif 2009; Vieira et al. 2009). Our findings, however, showed no dependence among IADFs frequency, cambial age, and ring width, which can be due to the reduced age of our trees in comparison with other works describing age-related effects. However, age-related effects can be important in determining the high frequency of IADFs observed, since the sampled trees are not much older than 55 years, and IADFs frequency is usually much higher for younger trees (Vieira et al. 2009).

The frequency of IADFs was strongly dependent on elevation, with more abundant IADFs at lower than higher elevations. These evidences suggest that the stressful effects of specific triggering factors causing short-term reduction/reactivation of cambial activity and cell enlargement decrease with increasing elevation. A longer span of the growing season at a lower elevation has probably also contributed to higher frequencies of IADFs.

As previous publications on pine species (Rigling et al. 2001, 2002; Campelo et al. 2006; Vieira et al. 2009), we found a higher frequency of IADFs in the latewood than in the earlywood of *P. pinaster*. Reductions of cambial activity at the early growing season are less likely than a later growth reactivation, probably due to the different duration of growth periods for early- and latewood, and to more regular weather conditions during early in the active season. In *P. halepensis* under warmer and xeric conditions, earlywood formation lasts for only 2 months (March to April–May), while latewood growth is more time-consuming, extending up to 6–8 months from May–June to November–December (De Luis et al. 2007; Camarero et al. 2010). Accordingly, our results suggest that latewood growth of *P. pinaster* in NW Spain could last for at least 6 months, from May to October–November.

Climatic determinants of wood density fluctuations

We interpret the dependency of IADFs on climate on the basis of particular preconditioning and triggering climatic factors, whose combined effects produced the observed fluctuations in wood density.

Climatic drivers for the occurrence of IADFs in the earlywood of *P. pinaster* showed to be relatively heterogeneous throughout the study area, with very few IADFs per stand, while a clear combination of climatic drivers for their formation was identified at a regional scale. Below-average water availability in previous late summer (August), or below-average temperature in previous winter (December), are preconditioning factors for the formation of bands of latewood-like tracheids within the earlywood. Both factors are related to the amount of carbohydrates stored in the previous season and used for growth in spring (Hansen and Beck 1994; Lacointe 2000). Water availability in summer can modulate the amount of carbohydrates at the end of the growing season, considering that summer drought is not as restrictive as under Mediterranean climate, while photosynthetic activity of *P. pinaster* can be inhibited by low temperatures during winter (Medlyn et al. 2002). Below-average water balance in April appears to be the actual triggering factor for IADFs type E, in combination to low carbohydrate reserves at the beginning of the active season. Such relationships to water availability in the early growing season were found for other conifers in different regions. For instance, the frequency of IADFs in the earlywood of *P. nigra* in the northern Alps was negatively correlated with May precipitation (Wimmer et al. 2000), earlywood density of *Picea abies* in France responded strongly to fluctuations in soil water reserves in late spring-early summer (Bouriaud et al. 2005), and drought in early summer induced the formation of IADFs in the earlywood of the Mediterranean pines *P. pinea* and *P. halepensis* (Campelo et al. 2006; De Luis et al. 2007). Moreover, carbon gain in winter can be allocated to growth of fine roots, increasing their absorptive capacity at the onset of following growing season (Lacointe 2000), which can contribute to proper cell enlargement during the earlywood formation, and thus reduce the frequency of IADFs type E. These findings highlight the importance of water deficit for the short-term inhibition of cell enlargement at the beginning of the growing period, which combined with a low carbon availability can induce false-rings formation in the earlywood of *P. pinaster* under Atlantic climate.

The formation of IADFs type L is trigged by climatic conditions in late spring and summer, but winter temperature regulating photosynthesis and the amount of carbohydrates available for the following season seemed to be also a major preconditioning. Radial growth depends on tree water status as a controlling factor for the metabolism of the entire tree, but it also depends on the carbon balance as a source of energy for metabolic activity and of compounds for the cambial activity (Zweifel et al. 2006). In evergreen conifers, the carbon supply for needle formation and cambial activity is provided by mobilization of stored reserves, complemented with new photosynthates assimilated even during the cold season (Hansen and Beck 1994). Relatively high rates of winter photosynthesis are stimulated by elevated temperatures in the previous winter (Schaberg et al. 1998; Medlyn et al. 2002), and bands of earlywood-like tracheids in the latewood seemed to occur less frequently when high temperatures occurred in previous winter. Therefore, a negative carry-over effect due to less stored carbohydrates seems to be detrimental for growth, probably causing an inhibition of cambial activity and the earlier beginning of latewood formation due to limited carbon reserves. If latewood is formed earlier, the probability of cambium reactivation due to the occurrence of any favorable condition increases.

The climatic triggers for IADFs type L had a geographically heterogeneous distribution within the study area, with two different groups of stands. In coastal stands, IADFs type L were favored by low temperatures in May–August, when periods of low water availability are common, though less pronounced than under a Mediterranean climate (Martínez Cortizas et al. 1994). The high water use efficiency and growth potential of *P. pinaster* even under moderate water stress (Correia et al. 2008), can explain the greater frequency of IADFs type L if low temperatures in late spring and summer alleviate drought stress and promote cambium reactivation. By contrast, a low water availability coupled with high temperatures in late spring-summer increased the probability of latewood IADFs occurrence in inland stands. Cambium reactivation may be promoted by positive water balance at shorter time scales than the monthly periods adopted in this work, as probably occurs in our inland study stands. These climatic determinants for IADFs formation are not consistent with those previously described in the literature, in which the combined effects of cool springs-drought summers (Hoffer and Tardif 2009), or the succession of cool and warm conditions in the active period (Rigling et al. 2002) facilitate the formation of IADFs. But these previous works were performed in boreal pines under other limiting climatic conditions, and they did not distinguish different types of IADFs according to their intra-ring position, then these previous studies could not be comparable with our results.

Density fluctuations of type L+ were preconditioned by warm/dry conditions during most of the growing season, particularly by a warm period from March to May in dry years, and triggered by a wet October. This dependency on climatic factors is consistent with those described for other Mediterranean pines. In *P. pinea* and *P. halepensis*, the occurrence of a spring-summer drought combined with early autumn precipitation favored the formation of IADFs in the latewood (Campelo et al. 2006; De Luis et al. 2007). Moreover, Vieira et al. (2009) showed that IADFs in the late latewood of *P. pinaster* under Mediterranean conditions are strongly linked to wet autumns, mainly in October. This response was uniformly observed throughout our study area, suggesting that the formation of IADFs type L+ responds to very specific climatic factors, and has a wide geographical and even inter-specific validity among Mediterranean pine species.

A relevant finding of this paper is that IADFs in the earlywood and the early latewood of *P. pinaster* in NW Spain were strongly linked to the EA pattern, but not those in the late latewood. This significant connection with EA was due to a direct link between EA variation and the regional climatic drivers for the formation of IADFs at the beginning and the middle of the growing season. In particular, regional climate is strongly related to EA pattern for temperature in previous winter, and also for water balance in April, which are major preconditioning and triggering factors, respectively, for the formation of IADFs types E and L. Although some connection between tree-ring growth and other large-scale patterns, like the North Atlantic Oscillation, have been previously observed in southern Europe (Piovesan and Schirone 2000; Rozas et al. 2009), this work demonstrates for the first time a strong link between IADFs formation and the EA pattern, mediated by a robust large-scale modulation of regional climate.

IADFs and growth plasticity in *P. pinaster*

As other Mediterranean pines, *P. pinaster* evolved during the Pliocene under tropical-like climate, before the onset of the Mediterranean climate, as a component of the pre-Mediterranean Arcto-Tertiary flora (Verdú et al. 2003; Petit et al. 2005). This species survived to a past gradual increase of aridity during the transition to Mediterranean conditions, which may have led to its characteristic growth plasticity (Chambel et al. 2007). The expected growth behavior should be partially related to the intra-annual reduction/reactivation of photosynthetic and cambial activities typical of Mediterranean and subtropical environments showing seasonal aridity (Cherubini et al. 2003). Also the vegetative cycle of Mediterranean pines is usually markedly bicyclic or polycyclic, often showing two or more growth units in each annual shoot (Heuret et al. 2006; Barthélémy and Caraglio 2007). Polycyclism is a characteristic expressing adaptation to intermittent favorable conditions for vegetative growth that is more frequently expressed under Atlantic humid conditions (Alía et al. 1997). The hormone indol-3-acetic acid is produced in the younger shoots and exported basipetally to induce the production of xylem and regulate the developmental processes during xylogenesis (Aloni 2001), so that we can hypothesize that shoot growth and cambial activity should be related. Further investigations on intra-annual tree-ring growth based on the high-resolution dendrometer measurements and xylogenesis (e.g., De Luis et al. 2007; Camarero et al. 2010), and their relationships with shoot enlargement, should inform us about the physiological and ecological significance of IADFs as adaptive traits.

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