Climate factors promoting intra-annual density fluctuations in Aleppo pine (Pinus halepensis) from semiarid sites

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Research was carried out on the wood formation process and the intra-annual density fluctuations (IADFs) in Mediterranean Aleppo pine (Pinus halepensis Mill.) trees from the coastal site of Guardamar, and inland Maigmo in south-eastern Spain. Samples taken at breast height of the trees were inspected to define the frequency of tree rings with latewood-like cells in earlywood (E-ring) and with earlywood-like cells in latewood (L-ring). L-rings were generally more frequent, especially on the warmer, dryer, coastal site of Guardamar. Dendrochronological techniques using tree-ring features vs. climate showed that L-rings were formed in Guardamar when August and/or September precipitation was higher than normal and in Maigmo when August precipitation was higher than normal. The formation of E-rings was promoted when winter and early spring were warmer than long term average. By studying intra-annual tree-ring formation at a cellular level, we found that at Maigmo in 2004 (MAI2004) all monitored trees presented a normal ring with normal earlywood and latewood and gradual transition between both. At Guardamar in 2005 (GUA2005) a typical L-ring was formed in the majority of monitored trees. In GUA2005 the wood formation started before February and the transition from early- to latewood occurred in late spring. In summer, the cambial cell production occurred at a very low rate, but an increased production of xylem cells took place in September when the amount of precipitation was twice as high as the long term average. During this period, a band of earlywood-like cells was formed, followed by the production of latewood-like cells that continued until the end of December. The normal ring in MAI2004 was formed because climatic factors triggering IADF did not occur (no precipitation in August 2004). After a modest cell production of cambium in September, only few latewood-like cells were produced. According to the strong agreement of results obtained from studying long-term dendrochronological series and intra-annual information on wood formation, such combined study indicates a high potential for use in explaining the environmental signals registered by a tree during different phases of wood formation.

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In the Mediterranean climate, characterized by mild and rainy winters and hot dry summers, cambial activity is not always associated with regular dormancy in the cold season, as it is in temperate or boreal climatic zones (Cherubini et al., 2003). Moreover, due to high variability in climate conditions in the Mediterranean, cambial activity can also vary from year to year in line with the climatic conditions. Due to this the trees can be subjected to one or two interruptions in cambial activity; one during the winter caused by low temperatures and one during the summer triggered by high temperatures and lack of precipitation (Liphschitz and Lev-Yadun, 1986; Lev-Yadun, 2000; Nicault et al., 2001; De Luis et al., 2007) or even the radial growth might be continuous through the year.

In Mediterranean pines, anatomical features of wood often differ from normal patterns, with typical earlywood and latewood and a fairly gradual transition between them (Schweingruber, 1988). Intra-annual density fluctuations (IADFs), in the literature also referred to as false or double rings (Schulman, 1938; Fritts, 1976; Kramer and Kozlowski, 1979) can often be observed (Battipaglia et al., 2010). They can be mainly observed as latewood-like cells within earlywood or earlywood-like cells within the latewood that are formed depending on the current year’s temperature and amount and distribution of precipitation (Campelo et al., 2006; De Luis et al., 2007).

The relationship between IADF formation and climate has been studied in various tree species (Wimmer et al., 2000; Rigling et al., 2001; Masiokas and Villalba, 2004; Campelo et al., 2006, 2007). However, it often seems to be almost impossible to determine precisely when the controlling event had occurred if only the relative position of the IADF in the tree-ring is known (Rigling et al., 2002).

Combined studies using long dendrochronological series and more detailed information on wood formation on an intra-annual scale proved to be useful for explaining the environmental signals registered by a tree during different phases of wood formation (Čufar et al., 2008). On one hand, information on the influence of environmental factors on tree growth provided by dendrochronology may be useful for drawing up the climate factors triggering IADF formation. On the other hand, detailed studies on wood formation during the growth season in particular years may represent valuable information valid for verification of information derived from dendrochronological results.

The aims of this study were: (i) to quantify the occurrence of different types of IADFs in the wood of Aleppo pine (Pinus halepensis Mill.) growing at one coastal and one inland site, Guardamar and Maigmo in south-eastern Spain, (ii) to use dendrochronological techniques to define which climatic factors promote the formation of IADFs, and (iii) to present the development of tree-rings at cellular level and to verify the influence of identified climatic factors on the formation of normal rings or IADF.

2. Materials and methods

2.1. Study sites

The study was carried out in two forest areas located on the south east of Spain in the province of Alicante. In both sites, the study forests represent even-aged Aleppo pine (P. halepensis) stands planted at the beginning of the 20th century. Guardamar del Segura (GUA) is a sand dune ecosystem located on the coast (38° 6’N, 0° 40’W; 5 m a.s.l.) where the climate is semi-arid Mediterranean (mean annual temperature 17.4 °C, and annual sum of precipitation 268 mm). The other site, Maigmo (MAI) is located not far away, but inland and at a higher altitude (38° 3’N, 0° 38’W; 844 m a.s.l.) where climate conditions are generally wetter and colder (13.5 °C and 359 mm). The average pattern of temperature and rainfall distribution throughout the year is shown in Fig. 1. The two locations have similar seasonal rainfall distribution, with two maxima, one in spring and one in autumn, separated by a summer drought characterized by high temperatures and low precipitation that typically persist for approximately three months in Maigmo and up to eight months in Guardamar (De Luis et al., 2009a).

2.2. Tree-ring data and intra-annual density fluctuations (IADFs)

In 2001 we selected at each of the sites 15 dominant trees. They were approximately 80 years old with diameters at breast height (DBH) of 38.2 ± 6.2 cm in Guardamar and 42.5 ± 6.8 cm in Maigmo. The selected trees had no visible damage. We took two to four cores at breast height from each – additional details on Guardamar chronology can be found in De Luis et al. (2009b). The cores were mounted on wooden supports and sanded with progressively finer sandpaper to obtain a smooth cross-section. For cross-dating, we took digital pictures of each core, identified signature rings along two radii, and dated the tree-rings. Next, the widths of tree-rings were measured to the nearest 0.01 mm using the TSAP Win programme. Possible dating and measurement errors were checked with the COFECHA computer programme (Holmes, 1994). An expressed population signal (EPS) of 0.85 was used to determine the length of the chronology (Wigley et al., 1984). The analyses were made for the common period 1919–2000.

Cross-dated tree-rings were examined for IADFs with a stereomicroscope magnifying up to 25-fold. Finally, a total of 6821 tree-rings where examined (4529 from Guardamar and 2292 from Maigmo). The rings with typical earlywood and latewood and a gradual transition between these were defined as normal rings. IADFs were identified when such gradual transition was interrupted. They were classified according to latewood-like cells in earlywood (E-ring) and earlywood-like cells in latewood (L-ring), as proposed by Campelo et al. (2006) (Fig. 2).
The frequency of IADFs per year, $F$, was calculated as the ratio:

$$F = \frac{N}{n},$$

where $N$ is the number of tree-rings that present the same type of IADF in a given year and $n$ is the number of observed tree-rings (the missing tree-rings that were frequent in Guardamar site were not considered).

The changing sample depth ($n$) over time generates a bias for the frequency variance. To address this problem, the adjustment proposed by Osborn et al. (1997) was applied to improve the stability of variance:

$$f = F_n^{0.5},$$

where $f$ is the stabilized IADF frequency.

On both sites, the relation between the stabilized IADF frequency and meteorological data was explored using the Spearman's rank order correlation test (Campelo et al., 2006). For this, we used the $f$ series as dependent variables, while the independent variables were the monthly mean temperatures and the monthly sums of precipitation from January to December of the current year from the nearby meteorological stations (De Luis et al., 2009a).

2.3. Anatomical observations of xylem development

The wood formation process was followed in Maigmo during 2004 (MAI2004) and in Guardamar during 2005 (GUA2005). On both sites, the trees of characteristics similar to those of the trees used for dendrochronological analyses were selected. The trees were mature, isolated and healthy *P. halepensis* aged approximately 80 years, with diameters from 17 cm to 46 cm. In MAI2004, sampling was made on six trees at monthly intervals from March until December. In GUA2005 sampling was also made on six trees, but twice a month, from February to December.

The samples (25 mm × 10 mm × 10 mm) were taken with a chisel and knife at a breast height of each tree. They contained intact tissues of phloem, cambium and outer xylem. Immediately after removal from a tree, they were fixed in formalin–ethanol–acetic acid solution (FEA). Sample blocks were then dehydrated in a graded series of ethanol (30%, 50%, and 70%).

With MAI2004, transverse sections (25 μm thick) were prepared using a Leica SM 2000R sliding microtome. With GUA2005, the sample blocks were reduced to dimensions of 2 mm × 2 mm × 3 mm and embedded in paraffin using a Leica TP 1020-1 tissue processor for dehydration in a graded series of ethanol (70%, 90%, 95% and 100%) and bio-clear (d-limonene) for paraffin infiltration (Rossi et al., 2006). Cross-sections of 12 μm thickness were prepared on a Leica RM 2245 rotary microtome, using disposable Feather N35H blades. For better adhesion of the sections, glass slides were pre-treated with albumin. Sections were dried at 70 °C for half an hour and cleaned of residual paraffin by immersing the slides in bio-clear and ethanol.

Finally, all sections were double-stained, first with Safranin (0.5% in 95% ethanol) and then with Astra blue (0.5% in 95% ethanol), and embedded in Euparal resin. The equipment used for observations, semi-automatic counting, and measuring of cells and tissues at various stages of their development consisted of: (a) a Nikon Eclipse 800 light microscope (bright field and polarized light), (b) digital camera DS-Fi1, and (c) NIS elements BR3 image analysis system.

We followed the seasonal dynamics of xylem formation by distinguishing among differentiating tracheids [postcambial (extension) growth, secondary cell wall formation, and lignification] and mature tracheids as in De Luis et al. (2007). The presence of tracheids in postcambial or extension growth (ET) was used to estimate whether the cambium was producing new cells or not. Date of transition from earlywood to latewood formation was also recorded.

3. Results

3.1. Intra-annual density fluctuations and climate

In the colder inland site Maigmo, E-rings were only detected in 0.25% of analyzed tree-rings and in 1% of analyzed years. This was different than in the coastal site Guardamar, where E-rings were identified in 1.25% of analyzed tree rings and in 14% of analyzed years (Fig. 3a).

The frequency of L-rings was considerably higher than the frequency of E-rings on both sites. L-rings were more frequent in Guardamar than in Maigmo. Thus, in Guardamar, L-rings were identified in 9.9% and in Maigmo in only 3.8% of analyzed tree-rings. Similarly L-rings were detected in 58% of analyzed years in Guardamar and only in 37% in Maigmo (Fig. 3b).

The Spearman's test obtained between stabilized IADF frequency and climate in Guardamar suggests that the formation of E-ring is promoted when winter and spring are warmer than normal (positive relation) (Fig. 4a).
Climatic factors promoting the formation of L-ring slightly differ between the two sites. Thus, the results suggest that L-rings are formed in Guardamar when August and/or September precipitation is higher than normal (Fig. 4b). By contrast, in Maigmo, L-rings are formed when August precipitation is higher than normal (Fig. 4c).

3.2. Intra-annual dynamic of wood formation

3.2.1. Formation of a normal tree ring in MAI2004

Intra-annual observations revealed that, in all investigated trees, wood formation started before mid-March 2004 when the first samples were taken (Fig. 5a). Production of earlywood cells took place from March to July. In July the first latewood cells with narrower radial dimensions were detected. Production of the xylem cells continued until August, when a decrease in cambial activity was observed and almost no expanding latewood cells were noticed. In September, after the first autumn rains (Fig. 5b), the rate of cambial cell divisions increased again and about 20% of the xylem growth ring was produced in the period between September and October. These newly formed cells had the characteristics of latewood and no IADFs were detected in any of the analyzed trees. Development of latewood tracheids continued until December (Fig. 5b and c).

3.2.2. Development of L-ring in GUA2005

In Guardamar in 2005, wood formation started before 18 February, before our first sampling (Fig. 5a). The rate of cell production increased until mid-April and then decreased until August, when almost no activity was noticed. By the end of June the formation of earlywood was completed and the first latewood cells with narrower radial dimensions were observed. During summer, only a few new cells were formed and their characteristics corresponded to latewood. In September, after the first autumn rains (Fig. 5c) an increase of cell divisions occurred, which reached a second maximum of cell production in mid-October. The cells produced in September had enlarged radial dimensions and therefore resembled earlywood, whereas cells produced during October and November had small lumina and thick cell walls, such as is typical of latewood. On the last sampling, on 26 December, new formed cells were not detected adjacent to the cambium but we still observed some cells undergoing maturation process (Fig. 5b).

In contrast to MAI2004 (where a normal ring was formed in all trees), in GUA2005 a typical L-ring was formed in most of the trees. The differences in structure can be explained by differences in dynamics of wood formation. In GUA2005, the period of earlywood production was longer than in MAI2004. In both cases, cambial activity did not stop in summer but the rate of cells division strongly decreased. After summer, in MAI2004, a slight increase in cell production occurred but the cells differentiated into latewood. Contrarily, in Guardamar, a strong increase in cell divisions occurred after the first autumn rains and the new formed cells had earlywood characteristics. As a consequence, a normal tree ring
Fig. 5. (a) Number of differentiating tracheids (ET) including postcambial cells, cells undergoing secondary cell wall formation and lignification and number of mature tracheids (MTs) in the currently formed growth ring. The line above shows the period of earlywood-like (EW) (bold line) and latewood-like (LW) cells (dotted line) formation. (b) Seasonal formation of the tree-ring. Tree-ring in Maigmo 2004 gradual transition from early- to latewood, in Guardamar 2005 with L-type IADF having earlywood like cells in the latewood. Scale bars 100 μm. (c) Weather conditions during the year 2005.

was formed in Maigmo while an L-ring was formed in Guardamar (Fig. 5b).

4. Discussion

Our results indicate that the duration of the growing season might be extended up to 11 months in P. halepensis growing in warm semi-arid conditions of Spain (GUA). Contrarily, in inland forest stand (MAI) where cold conditions in winter may act as an additional limiting factor, beginning of the growing season was delayed and the length of the growing season was shortened to 9–10 months.

Results of our current and previous study revealed that the dynamics of cambial activity in P. halepensis is characterized by two major growth phases, one in spring and another in autumn. They are interrupted in summer period when the cambium might remain active but cell divisions occur at a very low rate (De Luis et al., 2007).

This indicates high seasonal plasticity which is in agreement with results of other studies in Aleppo pine growing in different environmental conditions (Gindel, 1967; Serre, 1976a,b; Attolini et al., 1990; Nicault et al., 2001; Camarero et al., 2010) and suggests that the cambium is able to remain active throughout the whole year if climate conditions are favourable.

Our results confirmed that IADFs are frequent in P. halepensis at the Maigmo and Guardamar sites. It was also shown that, despite limitations, the climatic signal obtained through analysis of dendrochronological series of intra-annual features can serve as indirect evidence of climate influence on wood formation. Thus, dendrochronology in combination with research into wood formation can be used to provide new insights into the role of climatic parameters on the intra-annual wood dynamics, as also shown by Čufar et al. (2008) in European beech (Fagus sylvatica).

Our dendrochronological results in P. halepensis from Mediterranean forests suggest that intra-annual density fluctuations in tree-rings are more frequent on drier sites where the availability of water is limited. Fritts (1976) has already suggested that trees
growing in extreme conditions respond strongly to climatic variations and accordingly have more frequent growth anomalies. Our results are also in agreement with studies by Campelo et al. (2006) and Vieira et al. (2009) in southern Portugal, where the climatic response of Pinus pinea and Pinus pinaster proved to be higher in areas with more severe summer drought. Similarly, our results indicate that most of the IADFs were observed in latewood (L-rings) and that their presence was correlated with fluctuations in climate parameters during the growing season.

However, our results only partially agree with those of Campelo et al. (2006) and Vieira et al. (2009) concerning identification of the climate factors triggering IADFs. Since in south Portugal the L-type IADFs were associated with above-average precipitation in early autumn (mainly October) our study in SE Spain suggests that L-rings are related to above-average precipitation in August (Maigmo), or in September at the warmer site (Guardamar). Completely different results are observed for the formation of E-rings. While Campelo et al. (2006) stated that E-rings are linked to above-average precipitation in early summer, we found that E-rings were only formed on the drier and warmer site (Guardamar) and that they were linked to warmer conditions during winter and dry conditions in March.

The nature of these differences is difficult to interpret. The fact that the studies in Portugal and Spain were done on sites with different climate conditions (the amount of precipitation in the study areas of Portugal was nearly double compared to our study sites) and on different pine species may be among the reasons for this. Recently it was also shown that there are significant differences among different pine species in regard to their sensitivity to climate (De Luis et al., 2009b).

Thus, particularly in P. halepensis growing in semiarid environments, E-rings are formed when abnormally warm conditions occur at the beginning of the growing season. If early spring is abnormally warm, latewood-like cells may be formed within earlywood. If spring conditions later change to more normal ones, the cambium starts to produce earlywood cells and their production continues until the beginning of summer. According to this, E-rings are less likely to occur in Maigmo, where winter is usually notably colder than in Guardamar.

On the other hand, L-rings are generally frequent and likely to occur on both sites. They are related to unusual precipitation events in August (Maigmo) or in August and/or September (Guardamar). In this regard, our results suggest that L-rings are formed (or are not formed) in dependence of the rate of increase in cambial activity in late summer/early autumn. Thus, if conditions in late summer/early autumn remain dry, the cambium does not increase production of new cells or if the amount of precipitation is very low the cells formed during autumn differentiate into latewood cells. However, if favourable conditions occur (higher precipitation than normal), the rate of cell divisions strongly increases and a new band of earlywood-like cells is formed during late spring and summer period (L-ring formation). According to this, we can hypothesize that the increased cambial activity and L-ring formation occurs in August in colder sites (Maigmo), but may be delayed to September in warmer ones (Guardamar). It must be noted here that mean annual temperature in Maigmo in August (22.2 °C) is even lower than mean annual temperature in Guardamar in September (23.1 °C). Differences in climate factors triggering L-ring formation on both sites may also explain different temporal patterns in frequency of L-rings observed on two sites.

The indirect evidence on factors promoting IADF formation during 1916–2000 has been partially verified with results on wood formation in MAI2004 and GUA2005. Thus, according to dendrochronologically derived hypotheses, a normal ring in MAI2004 was expected to be formed because climatic factors triggering IADF did not occur (no precipitation in August 2004). Therefore, latewood cells started to be formed with the onset of drought (June–July) and latewood cells were also formed during autumn.

Also according to dendrochronologically derived hypotheses, an L-ring was expected in GUA2005 where precipitation in September was notably above average (56 mm in 2005 vs. 28.7 mm average 1916–2000). Under these conditions, we observed that the increased cambial activity after summer drought was more intense and a band of earlywood-like cells was formed early in autumn. Wood formation samples confirmed that fairly pronounced L-rings were observed in the trees during 2005.

According to this, the increase in temperatures and decrease in precipitation predicted for Mediterranean areas in the context of climate change (Christensen et al., 2007) may cause important modification in the growth phenology of trees (Peñuelas et al., 2002). According to our results, in P. halepensis growing in semiarid conditions, an extension of the growing season (earlier onset of activity and later halt) can be expected, but this extension may not necessarily translate into higher growth rates, because a more intense and longer summer break can also be expected.

However, modifications in seasonal climate regimes may also induce changes in the latewood/earlywood ratio that will result in modifications to the hydraulic and mechanical properties of wood (Froux et al., 2002; De Micco et al., 2008; Martinez-Meier et al., 2008).

The different types of wood formed during the early and late growing seasons have very different hydraulic properties. Earlywood in conifers is specialized for conducting water, whereas latewood provides mechanical stability and a large amount of stored water. The corresponding hypothesis is that an increase in the quantity of latewood may increase the resistance to cavitation, or in other words, that a higher proportion of latewood tends to increase resistance to drought (Domec and Gartner, 2002; Martinez-Meier et al., 2008).

Within the global warming perspective, the survival of trees is certainly related to their ability to preserve conductivity in their hydraulic system. Observed site-to-site and year-to-year variability in climatic signals and wood formation seems to be in line with phenological plasticity of P. halepensis (De Luis et al., 2007, 2009b) as traditionally described. Similarly, ability of species to produce different type of cells (earlywood or latewood cells) in different periods may be also interpreted as an important adaptation of trees to maintain equilibrium among capacity to conduct water, mechanical stability, and resistance to cavitation. All this could play an important role in acclimatization or/and adaptation to new, changed climate conditions.

However, as the climate strongly differs from year to year in the Mediterranean area, it is necessary to continue studies on cambial activity for several years in order to learn more on the influence of climate on wood formation and on physiological processes behind climate–growth relationships. Combined and complementary information obtained from long term tree-ring chronologies and intra-annual wood formation will certainly contribute to this scientific task.

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