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Age-dependent variations of radial growth, intra-annual density fluctuations and pointer years in *Pinus pinaster* under Mediterranean climate

Joana Margarida Soares Vieira

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica da Professora Doutora Cristina Nabais (Universidade de Coimbra).

Joana Margarida Soares Vieira

2008

Aos meus pais

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Abstract

Dendrochronology generally assumes that climate-growth relationships are age independent once the biological growth trend has been removed. However, tree physiology changes with age, namely photosynthetic capacity and hydraulic conductivity. Additionally, in a scenario involving climate change it is important to verify if the relationship between tree growth and climate is also changing. We tested whether the radial growth response to climate and the frequency of intra-annual density fluctuations (IADFs) and pointer years (PY) of *Pinus pinaster* Ait. varied with age. It was also investigated whether the climatic factors triggering IADFs formation were stable over time. Trees were sampled in Pinhal de Leiria (Portugal), and were divided in two age-classes: young (<65 years-old) and old (>115 year-old).

Earlywood and tree-ring width of young *P. pinaster* trees were more sensitive to climate influence while the response of latewood width to climate was stronger in old trees. Young trees start the growing season earlier, thus a time window delay occurs between young and old trees during which wood cells of young trees integrate environmental signals. Intra-annual density fluctuations (IADFs) in tree-rings are produced as a response to variations in climatic conditions during the growing season, particularly water availability. Young trees usually have a longer growing season and respond faster to climate conditions, thus young *P. pinaster* trees presented a higher frequency of IADFs compared with old trees. Most of the IADFs were located in latewood and were positively correlated to autumn precipitation. The radial-growth response of *P. pinaster* to climate and the IADFs frequency were age dependent. The use of trees with different age to create a tree ring chronology for climate studies can increase the resolution of climatic signals. Age-dependent responses to climate can also give important clues to predict how young and old trees react to climate change.

If IADFs frequency was higher in young *P. pinaster*, how was the frequency of IADFs when old trees were young? The frequency of IADFs in old *P. pinaster* trees was analyzed from 1900 to 2006. The high frequency of latewood IADFs during the beginning of the 20th century was probably age related, since IADFs are more prone to occur in younger and wider tree-rings. From the 40s to the 70s IADFs frequency decreased in parallel with a general increase of the tree-ring width. From 1970 onward, it was observed an increase of IADFs frequency and a decrease of tree-ring width. The

increase of IADFs frequency in narrower rings suggests that climate conditions favoring IADFs formation were becoming more frequent. Latewood IADFs were positively correlated with September to December precipitation and with November and December temperature, but this correlation changed over time. In the last 30 years, the precipitation in October and the temperatures of November and December increased, partly explaining the higher frequency of IADFs since the 70s. Our results suggest that the frequency of IADFs and the change of their frequency over time can help us understand how trees are being affected by climatic changes.

The frequency of extreme climatic events is increasing in the last 50 years. These climatic extremes are recorded in tree-rings width, with the formation of wide and narrow tree-rings, named pointer years (PY). In *P. pinaster* narrow PY were formed in exceptionally dry years and wide PY in years of abundant precipitation. Narrow PY were more frequent than the wide ones and young trees presented more PY than old trees, showing that young trees are more sensitive to drought conditions. Additionally, most of the narrow PY occurred after the 70s, confirming that the frequency of extreme climatic events is increasing in the last decades. The climatic signal extracted from the PY chronologies was stronger for specific months suggesting that these chronologies are suited for climatic reconstructions.

In conclusion, there are age-dependent responses to climate in *P. pinaster* trees growing in the Mediterranean region. It is also important to notice that climate changes in the Mediterranean are already being recorded in tree-rings. The study of trees with different age, IADFs and PY frequencies can bring additional climatic information to tree-ring chronologies, important to study past climate conditions.

Resumo

Um dos pressupostos da dendrocronologia diz que a resposta climática é independente da idade da árvore, desde que a espessura dos anéis seja transformada num índice onde a tendência de anéis mais jovens serem mais largos é removida. Contudo, a fisiologia da árvore, nomeadamente a capacidade fotossintética e a condutividade hidráulica, alteram-se com a idade. Adicionalmente, face às alterações climáticas actuais, é importante verificar se a relação entre o crescimento radial das árvores e o clima também se alterou. Para tal, verificámos se a resposta do tamanho do anel de crescimento ao clima, a frequência das flutuações intra anuais de densidade da madeira (IADF – do inglês intra-annual density fluctuation) e de “anos extremos” (PY – do inglês “pointer year”) em *Pinus pinaster* Ait. variou em função da idade da árvore. Investigou-se também se os factores climáticos que induzem a formação de IADFs foram estáveis ao longo do tempo. As árvores estudadas foram amostradas no Pinhal de Leiria (Portugal) e divididas em duas classes de idade: jovens (< 65 anos) e velhas (> 115 anos).

O tamanho do lenho primaveril e do anel revelou uma maior sensibilidade ao clima nas árvores jovens enquanto o tamanho do lenho tardio apresentou melhor sinal climático nas árvores velhas. As árvores jovens iniciam o crescimento mais cedo, criando-se assim um período em que apenas as árvores jovens podem registar informação climática. Os IADFs são estruturas anatómicas produzidas em resposta a variações nas condições ambientais durante o período de crescimento, nomeadamente disponibilidade de água. As árvores jovens apresentaram uma maior frequência de IADFs do que as árvores velhas, pois além de geralmente possuírem uma época de crescimento maior, também respondem com maior rapidez às alterações climáticas. A maioria dos IADFs foi encontrada no lenho tardio apresentando uma correlação positiva com a precipitação de Outono. O registo climático presente no tamanho do anel e na frequência de IADFs revelou ser dependente da idade da árvore. A utilização de árvores de diferentes idades na criação de cronologias para estudos climáticos pode aumentar a resolução do sinal climático. A diferente resposta de árvores velhas e jovens pode ainda ser útil na previsão do impacto das alterações climáticas no crescimento das árvores.

Como a frequência de IADFs foi superior nas árvores jovens de *P. pinaster*, qual seria a frequência de IADFs nas árvores velhas quando estas eram jovens? Para

responder a esta questão, foi estudada a frequência de IADFs presente no lenho tardio de árvores velhas, de 1900 a 2006. No início do século XX as árvores apresentaram uma frequência elevada de IADFs, provavelmente por corresponder ao período em que as árvores eram jovens. No período seguinte, de 1940 a 1970, apesar de se formarem anéis mais largos, a frequência de IADFs diminuiu. De 1970 até à actualidade a frequência de IADFs voltou a aumentar apesar dos anéis formados serem mais estreitos. O aumento da frequência de IADFs em anéis mais estreitos sugere que as condições climáticas que desencadeiam a sua formação estão a aumentar de frequência. Os IADFs do lenho tardio apresentaram uma correlação positiva com a precipitação de Setembro a Dezembro e com as temperaturas de Novembro e Dezembro. Nos últimos 30 anos, a precipitação de Outubro e as temperaturas de Novembro e Dezembro aumentaram, explicando parcialmente o aumento da frequência de IADFs registado desde os anos 70. Os nossos resultados sugerem que as diferenças encontradas na frequência de IADFs ao longo do tempo podem ser úteis para compreender o impacto das alterações climáticas no crescimento das árvores.

A frequência de eventos climáticos extremos tem vindo a aumentar nos últimos 50 anos. Estes eventos ficam registados nos anéis de crescimento de árvores através da formação de anéis largos ou estreitos, denominados “anos extremos” (PY). Em *P. pinaster*, PY estreitos foram formados em anos excepcionalmente secos enquanto PY largos foram formados em anos com precipitação elevada. A frequência de PY estreitos foi maior do que a de PY largos e as árvores jovens apresentaram maior frequência de PY estreitos, revelando que estas são mais sensíveis à seca. Adicionalmente, a maioria dos PY foi registada depois de 1970, confirmando que a ocorrência de eventos climáticos extremos tem vindo a aumentar nas últimas décadas. O sinal climático obtido nas cronologias de PY foi mais forte para meses específicos, demonstrando o potencial deste tipo de cronologias para efectuar reconstruções climáticas.

Em conclusão, o sinal climático de árvores de *P. pinaster* varia com a idade. É ainda importante referir que as alterações climáticas sentidas no Mediterrâneo têm vindo a ser registadas nos anéis de crescimento de árvores. O estudo de árvores de idades diferentes, da frequência de IADFs e de PY pode fornecer informação climática adicional à obtida a partir de cronologias de tamanho do anel para estudar as condições climáticas do passado.

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CHAPTER I

General Introduction

1.1. Tree Rings: A Storyteller of Climate

Dendrochronology is the science that studies and dates tree-rings. Tree-ring series can be synchronized or "crossdated" because trees in the same region share the same climatic signal (Fritts, 2001). "Crossdating" is the technique by which the characteristics of rings from two or more trees are compared and a calendar year is assign to each of them (Yamaguchi, 1991; Fritts, 2001). For example, in a semi-arid climate where water is the main factor limiting tree growth, a dry year is characterized by the formation of a narrow ring, whereas in a wet year, a wider ring is formed. Thus, the pattern imprinted in the rings will allow the crossdating process.

Dendrochronology is a wide spread science, used in various fields such as climatology, hydrology, fire ecology, archaeology and others. For example, it has been successfully used in dating historical buildings (e.g. Park et al., 2007), volcanic eruptions (e.g. Yamaguchi, 1993), droughts (e.g. Stahle et al., 2007), climate reconstruction in different geographic regions (e.g. Briffa et al., 1992; Cook et al., 2002; Frank and Esper, 2005), and more recently to study how trees are responding to climate change (Martinelli, 2004; Ammann and Wahl, 2007).

Although the climatic reconstruction using tree rings is a powerful toll, lately the so called 'divergence problem' related to climate change has been posed (for a review see D'Arrigo et al., 2008). This 'problem' has been reported in alpine or subalpine environments and is reflected in the reduction of forest growth and temperature sensitivity in response to temperature increase (Briffa et al., 1998; Wilmking et al., 2004; Esper et al., 2005; Frank et al., 2005). Various authors have reported a 'divergence' in the response of trees to climate, mainly since 1970, when trees were responding positively to temperature, now they are responding to precipitation.

The ’divergence problem’ is questioning the accuracy of climate reconstructions based on tree-rings, since they were build under the principle of uniformitarism, which states that the climatic processes operating in the past are the same as those that can be observed operating in the present (Fritts, 2001). But, currently, what we are assisting is a change in the trees response to climate due to a temperature increase trend. This trend in the calibration period does not represent the range of conditions during the past, questioning the reliability of the climatic reconstructions (Ammann and Wahl, 2007).

1.2. Intra-Ring Features: Looking Inside the Growing Season

Dendrochronology typically uses tree-ring width in its investigations but other wood ring features can also be used, such as wood density, false rings or intra-annual density fluctuations (IADFs), and lumen area of wood cells (vessels or tracheids). The inclusion of intra-ring features in dendroclimatology confers a higher resolution to the analyses and allows a better understanding of the relation between tree-growth and climate. The first attempt to look inside the growing season was made through the separate analysis of earlywood and latewood (e.g. Lebourgeois, 2000). This type of studies can infer the start of the earlywood and latewood formation and what climatic conditions limited its growth. Sanchez-Vargas et al. (2007) used microdensity profiles of *Pinus pinaster* Ait. to describe temporal weather changes throughout the growing season.

Another intra-ring feature commonly used in dendroclimatology is the study of IADFs. IADFs are special anatomical features formed in response to environmental changes during the growing season (Villalba and Veblen, 1994; Rigling et al., 2001) and can occur within the earlywood and latewood. Earlywood IADFs are characterized by latewood-like cells within earlywood and latewood IADFs are characterized by the presence of earlywood-like cells within latewood (Fritts, 2001). IADFs allow a more detailed screening of the climatic conditions within the growing season, especially when used for the reconstruction of the final part of the growing season, since these structures are more frequent in the latewood (Villalba and Veblen, 1994; Rigling et al., 2002). Campelo et al. (2007) compared the frequency and climatic signal of IADFs in *Pinus pinea* L. growing in the inland and coastal area of the South of Portugal. He was able to correlate IADFs frequency with monthly precipitation, and using a linear regression model proved that, for the inland area, the inclusion of IADFs as a ‘dummy’ variable increased 20% of the variance predicted by latewood alone for October precipitation (Campelo, 2007).

1.3. Xylogenesis: the ultimate link between wood rings and climate

The vascular or lateral cambium is a secondary meristem, formed by a few layers of cells with the ability to divide indefinitely. During the growing season the cambium cells divide periclinally producing secondary phloem (outside) and secondary xylem (inside). The process of wood formation or xylogenesis is divided in stages separated in time and space: first the division of the cambium cells; then the enlargement and finally cell wall thickening and autolysis of its contents (Vaganov et al., 2006). The process of wood formation was described by Wilson et al. (1966) and is illustrated in Figure 1.

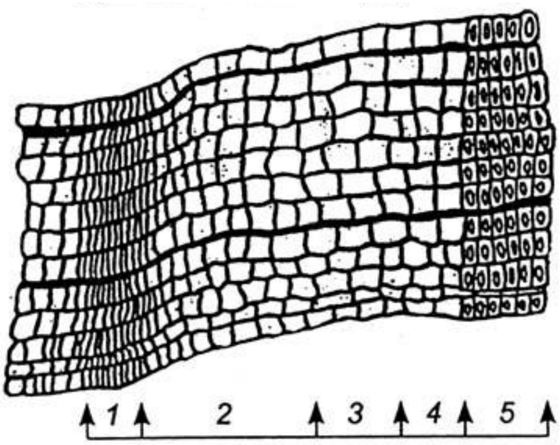

Figure 1 - Tree-ring formation, after Wilson et al. (1966): *1* cambial zone; *2* enlarging zone; *3* secondary wall thickening zone; *4, 5* mature tracheids of the current and previous year, respectively. (Adapted from Vaganov et al., 2006).

Xylogenesis is a dynamic process controlled by both internal (e.g. genetic, age, hormones) and external factors (e.g. temperature, moisture) that determine the rate and the number of cells formed at each stage.

The weather conditions during the growing season determine the beginning and end of the cambium activity. Photoperiod and temperature have an important role in the break of dormancy in temperate environments. Resumption of the vascular cambium is triggered by the above factors and the hormone auxin, produced by the developing shoots (Raven et al., 1999).

Tree rings are formed by two types of wood: earlywood and latewood. Earlywood is formed in the beginning of the growing season. The tracheids have wide lumen area and thin cell walls and their main function is water transportation. Latewood is formed in response to photoperiod and water stress in the end of the growing season and is characterized by smaller tracheids with thicker cell walls. Thus, precipitation also plays an important role in xylogenesis, especially in a semi-arid environment as the Mediterranean where water stress can determine an early end of the growing season

(Fritts, 2001) and/or the onset of latewood formation (Vaganov et al., 2006). The seasonal dynamics of climate are thus reflected in the wood so each ring contains an image of the time at which it was formed, projected onto the ring size and ultimately on the wood anatomy.

1.4. Objectives

The main objective of the present dissertation is to analyse the climatic signal of tree-ring width and IADFs of *Pinus pinaster* and to determine whether the trees response to climate is age-dependent and time stable within the present climate change conditions.

1.5. Thesis Layout

The present thesis is composed of three chapters which emphasize different aspects of *P. pinaster* response to climate.

In **Chapter II** we investigate if *P. pinaster* tree-ring width response to climate and IADFs frequency is age-dependent. For that we study two age-classes of *P. pinaster*: old (>115 years) and young trees (< 65 years). To determine the climate-growth relationships, response function and Spearman’s correlation analyses are performed. IADFs frequency and their correlation with climate are also investigated for both age classes.

In **Chapter III** we investigate whether the relation between climate and *P. pinaster* latewood width and IADFs frequency are stable over time. For that we analyse the old trees IADFs frequency from 1900 to 2006, perform a moving correlation analyses for the IADFs frequency and a moving response function for the latewood width, using a time window of 40 years.

In **Chapter IV** we investigate the distribution of “pointer years” in old and young trees to verify if the growth extremes are environmental determined and/or age-dependent.

The last section presents the final conclusions.

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CHAPTER II

Age-dependent responses of tree-ring growth and intra-annual density fluctuations of *Pinus pinaster* to Mediterranean climate

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2.1. Introduction

The number of tracheids produced by the cambium, their lumen area, and the thickness of cell walls are controlled by both physiological processes and environmental conditions (Jardon et al., 1994; Vaganov et al., 1996; Nicault et al., 2001; De Micco et al., 2007; Rossi et al., 2008). High temperatures in spring promote the synthesis of cell wall components, but reduce the time of tracheid development in the secondary wall thickening zone and as result, the early tracheids have thinner cell walls (Nicault et al., 2001). The formation of latewood occurs in the summer and is influenced by water stress and photoperiod (Vaganov et al., 2006). The increased time length of cell wall material deposition results in the formation of tracheids with thick walls (De Micco et al., 2007; Uggla et al., 2001).

Besides the normal transition between earlywood and latewood in tree rings, intra-annual density fluctuations (IADFs) can occur. IADFs are characterized by latewoodlike cells within the earlywood and earlywoodlike cells within the latewood (Fritts, 2001). These structures are formed in response to changing climatic conditions during the growing season (Masiokas and Villalba, 2004) and their radial position within the ring is determined by the time the triggering factor occurred (Campelo et al., 2007). In *Pinus pinea* L., growing under a Mediterranean climate, earlywood like cells within latewood were positively correlated to above-average precipitation in September or October (Campelo et al., 2007). *Pinus nigra* Arn. growing in the Austrian Alps showed earlywood IADFs positively correlated with a wet April, dry May and wet June (Wimmer et al., 2000).

The inclusion of IADFs in dendrochronological studies allows a more detailed analysis of climatic events within the growing season (Wimmer et al., 2000). However, in the literature it is suggested that younger trees have higher IADF frequency than older trees (Copenheaver et al., 2006; Masiokas and Villalba, 2004; Rigling et al., 2002; Villalba and Veblen, 1994), since younger trees have a longer growing season (Rossi et al., 2008) and respond faster to changing environmental conditions (Villalba and Veblen, 1994). Few studies have investigated whether climate-growth response is consistent across different age classes. Some studies showed that the climatic signal was maximized in older trees (Szeicz and Macdonald, 1994; Carrer and Urbinati, 2004; Rossi et al., 2008; Yu et al., 2008). And others (Linderholm and Linderholm, 2004) that older trees were more sensitive to summer temperature, while younger trees were more responsive to temperatures in the peak of the growing season.

Most of the age-dependent tree-ring growth response studies were held under subarctic climate (Szeicz and Macdonald, 1994) or high-altitude environments (Rossi et al., 2008; Yu et al., 2008), where the growing season is short (2-3 months) and tree growth is mainly limited by temperature. However, the response of tree growth to climate according to tree age has not yet been investigated under a Mediterranean climate, where water stress is the main limiting factor and the growing season is longer (6-8 months). The aims of the present study were to analyse whether the response of earlywood, latewood and tree-ring width to climate and the frequency of different types of IADFs in *Pinus pinaster*, growing under a Mediterranean climate, were age-dependent.

2.2. Materials and Methods

2.2.1. Study area

Our study site is located in Mata Nacional de Leiria (Figure 2), in the northwest coast of Portugal, a managed forest of *Pinus pinaster* Ait. and *Pinus pinea* L.. *Pinus pinaster* covers 98% of the afforested area (10 618,83 ha), making Pinhal de Leiria the largest continuous forest of this species in Portugal (Amaral, 1980). It was established by D. Afonso III in the XII century to prevent the progression of sand dunes, but it was with D. Dinis (1279-1325) that the afforestation was intensified and reached the present dimension.

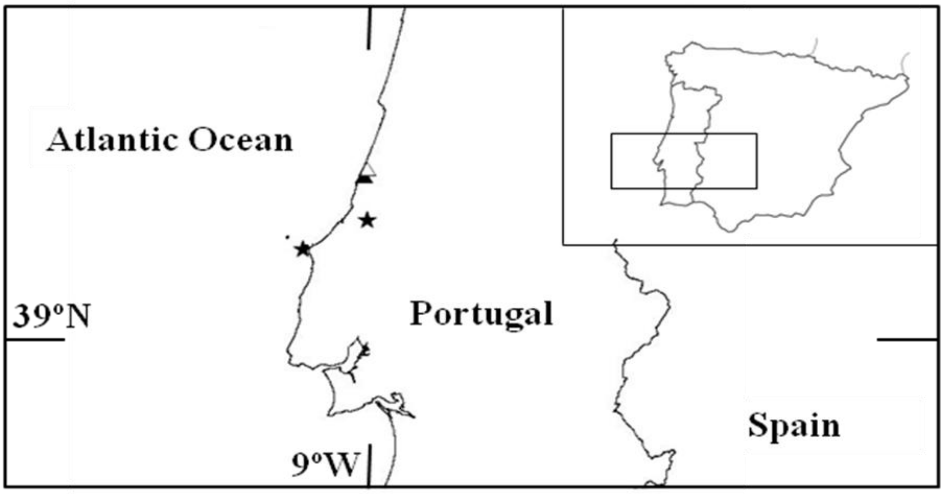

Figure 2 - Location of the study areas and meteorological stations (★). The full and open triangles represent the sampling areas of old (>115 years) and young trees (< 65 years) respectively.

The forest is divided in even-aged plots (600 to 1100 ha) and managed since 1882 (Amaral, 1980). The main purpose of the management plan was the production of construction wood, thus the stands were maintained in a way that ensured a regular harvest of wood. The average age of cut is 70 years but some stands, in recreational areas, are maintained for longer periods of time.

The climate is typically Mediterranean with maximum rain fall in autumn and winter and a pronounced summer drought. The total annual precipitation is 700mm and the average annual temperature is 15ºC. The data was obtained from the nearest meteorological stations, Cabo Carvoeiro and Alcobaça (Figures 2 and 3).

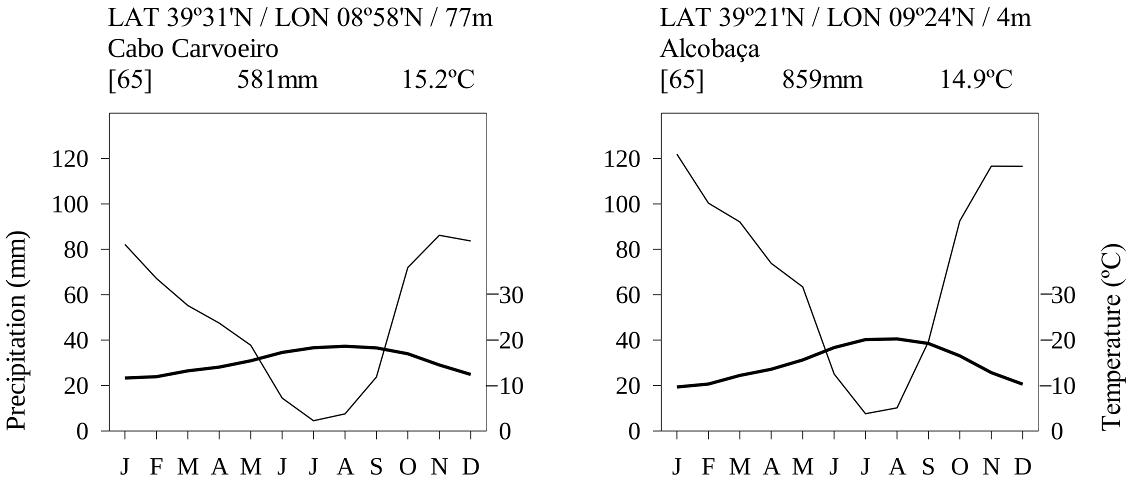

Figure 3 - Climate diagram, for the period 1941-2006. The diagrams consist of monthly mean precipitation (mm, thin line) and temperature (ºC, thick line). Data from Instituto Nacional de Meteorologia, Portugal.

2.2.2. Pinus pinaster – Phenology and Distribution

The species chosen for the present dissertation was *Pinus* pinaster Ait.. The distribution area of *P. pinaster* covers the Mediterranean basin and North Africa (Pereira, 2002). In Portugal, *P. pinaster* is a wide distributed species with a strong representation in the Portuguese forest (Godinho-Ferreira et al., 2005) (Figure 4).

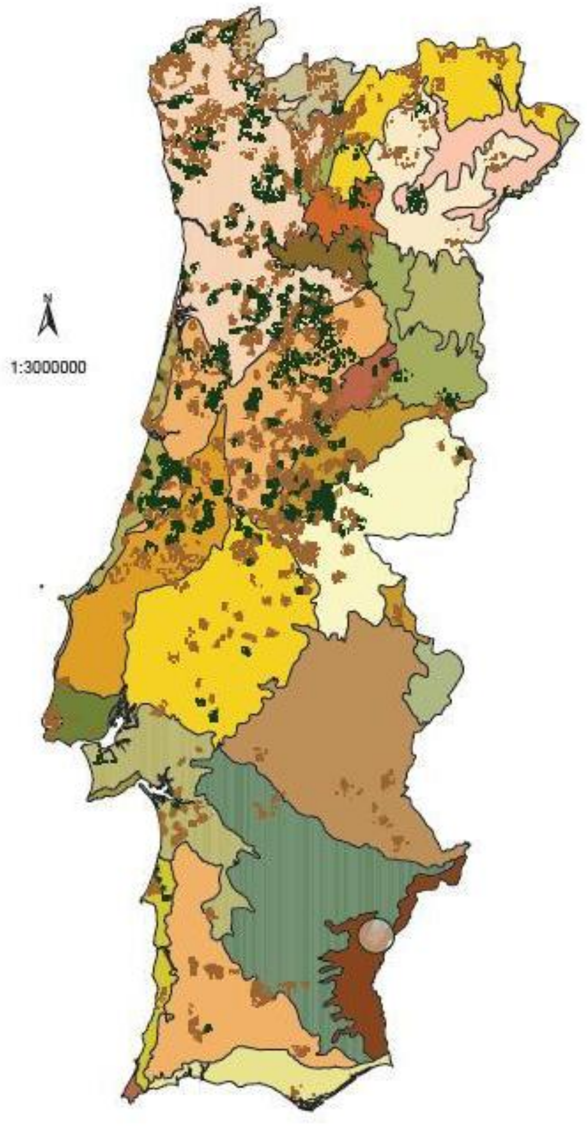

Figure 4 – *Pinus pinaster* distribution in continental Portugal. The brown spots represent closed and low forest and the green spots open and tall forests. The background colors represent biogeographical units. (Adapted from Godinho-Ferreira et al., 2005).

Pinus pinaster is a species that tolerates different climatic conditions, generally occurring in warm temperate regions with oceanic influence. It has been cultivated to stabilize sand dunes and coastal areas because of its deep root system. It is also an important source of resin and wood.

This species has a fast growth rate until 60 years old, time at which the growth rate reduces (Pereira, 2002). They can reach 20 – 40m in height and have a dense and irregular crown. The cone production starts when the tree reaches 6 years old and becomes regular in 10 – 15 years. The cones appear between late winter and mid spring; full seeds crop in 3-5 years (Pereira, 2002).

The genus *Pinus* has been successfully used in dendroclimatological studies in different climatic ecotypes (Fritts 2001; Rigling et al. 2002; Cherubini 2003; Campelo et al. 2007). In the Mediterranean, *P. pinaster* was used by De Micco et al. (2007) in a dendroecological study focused on the isotopic determination in IADFs. Studies of the adaptive response of wood formation to weather events were also developed in *P. pinaster* (Sanchez-Vargas et al. 2007; Bogino and Bravo 2008). These studies exemplify the potential of this species in dendroclimatology, justifying its choice for the present dissertation.

2.2.3. Tree Ring Data

Two cores were taken per tree, at breast height, with an increment borer, 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 dendrochronological techniques (Stokes and Smiley, 1996). Earlywood, latewood and tree-ring width were measured to the nearest 0.01 mm using a linear table, LINTAB (Frank Rinn S.A., Heidelberg, Germany) and the program TSAP-Win (Rinn, 2003). The crossdating accuracy was then checked using the program COFECHA (Holmes, 1994). Trees exhibiting correlation values with the master chronology below 0.4 were excluded. In the end 20 trees remained in the young category and 22 in the old one.

To remove age-related growth trends and competition a two-step detrending was applied to each individual series, using the program ARSTAN (Cook, 1985; Briffa and Cook, 1990). 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% 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. Several descriptive statistics were used to compare the age-class residual chronologies (Table I).

2.2.4. Response function analyses

The relation between climate and earlywood, latewood and tree-ring widths, for the period 1942-2006, was investigated by bootstrapped response function analyses using the program PRECON (Briffa and Cook, 1990; Serre-Bachet F., 1990; Fritts, 1999). This procedure is a particular multiple regression model where the independent variables (mean monthly temperature and total monthly precipitation) are first transformed in principal components, to remove the possible interactions between them. The dependent and the independent data sets were drawn by 999 random samplings with replacement from the original data (Guiot, 1990, 1991). For each iteration the dependent data was used to construct a model to predict the relation between tree-ring width and climatic variables (calibration) and the independent data was used to verify the accuracy of the model obtained (verification). The result is a measure of the strength of the climate-forcing signal relation (Briffa and Cook, 1990). The significance of each regression coefficient was provided by the ratio between the average value estimated from the results of 999 simulations and its standard deviation (Briffa and Cook, 1990). When the ratio is ≥ 2, ≥ 2.58 or ≥ 3.3, the significance of the corresponding regression attains 95, 99, or 99.9% of probability, respectively. Finally, the overall significance of the model reaches 95% of probability when the averages of the multiple correlation values are at least twice its standard deviation, as in the case of the partial regression coefficients (Guiot, 1991).

2.2.5. Intra-Annual Density Fluctuations

The correctly dated cores were visually examined for IADFs using a stereomicroscope magnifying up to 25-fold. IADFs were easily distinguished from annual tree rings, as illustrated in Figure 5, since IADFs showed a non sharp transition in opposite to the annual rings boundary (Fritts, 2001). The IADFs were classified based on the radial position within the ring: Type E with latewoodlike cells within the earlywood; Type L with earlywoodlike cells within the latewood and type L^+ with earlywoodlike cells between latewood and earlywood of the next tree ring (Campelo et al., 2007). Because of the variability of IADFs tangentially and vertically within tree rings along the stem the IADFs were only considered when present in both cores, in the same tree ring (Kuo and McGinnes, 1973).

The frequency of IADFs per year, F, was calculated as the ratio:

$$F = N/n$$

where N is the number of trees that showed an IADF type in a given year and n is the total number of observed trees. The changing of the sample depth, n, in time created a bias which was addressed by the adjustment proposed by Osborn et al. (1997) by calculating an adjusted IADFs frequency:

$$f = F\, n^{0.5}$$

where *f* is the stabilized IADF frequency.

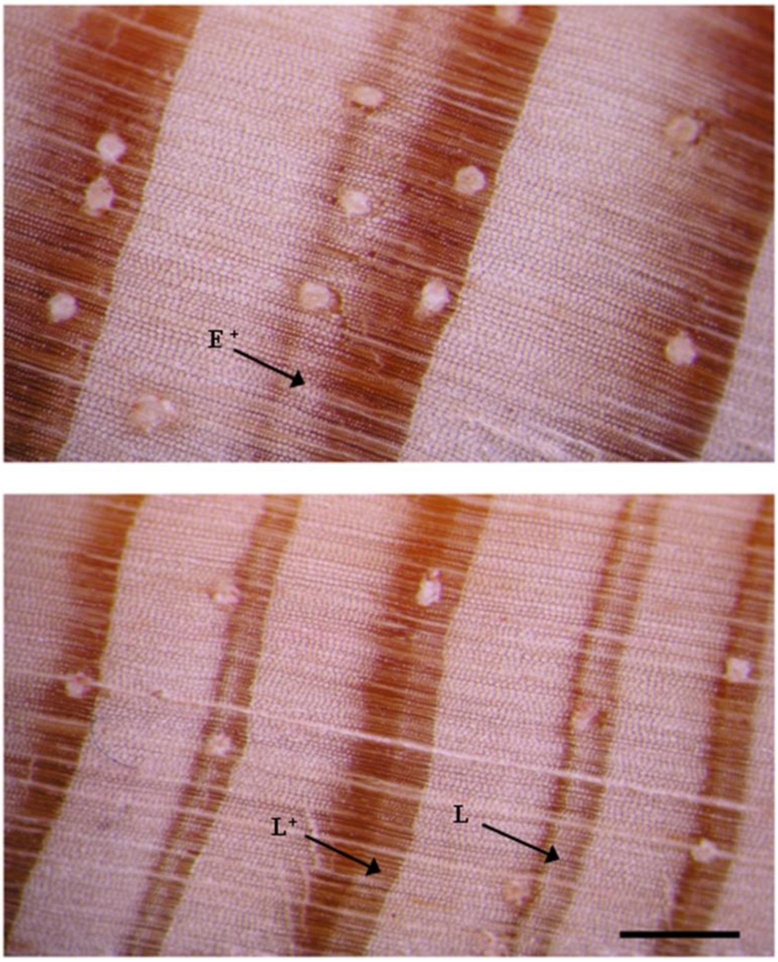

Figure 5. Anatomical structure and relative position within a tree ring of the different types of intra-annual density fluctuations (IADFs) in *Pinus pinaster* (magnification 16x), the bar represents 1mm.

2.3. Results

2.3.1. Tree-Ring Chronologies

The residual chronologies are present in Figure 6, the total time span of the residual chronologies extend from 1818 to 2006 for the old trees and from 1933 to 2006 for the young ones.

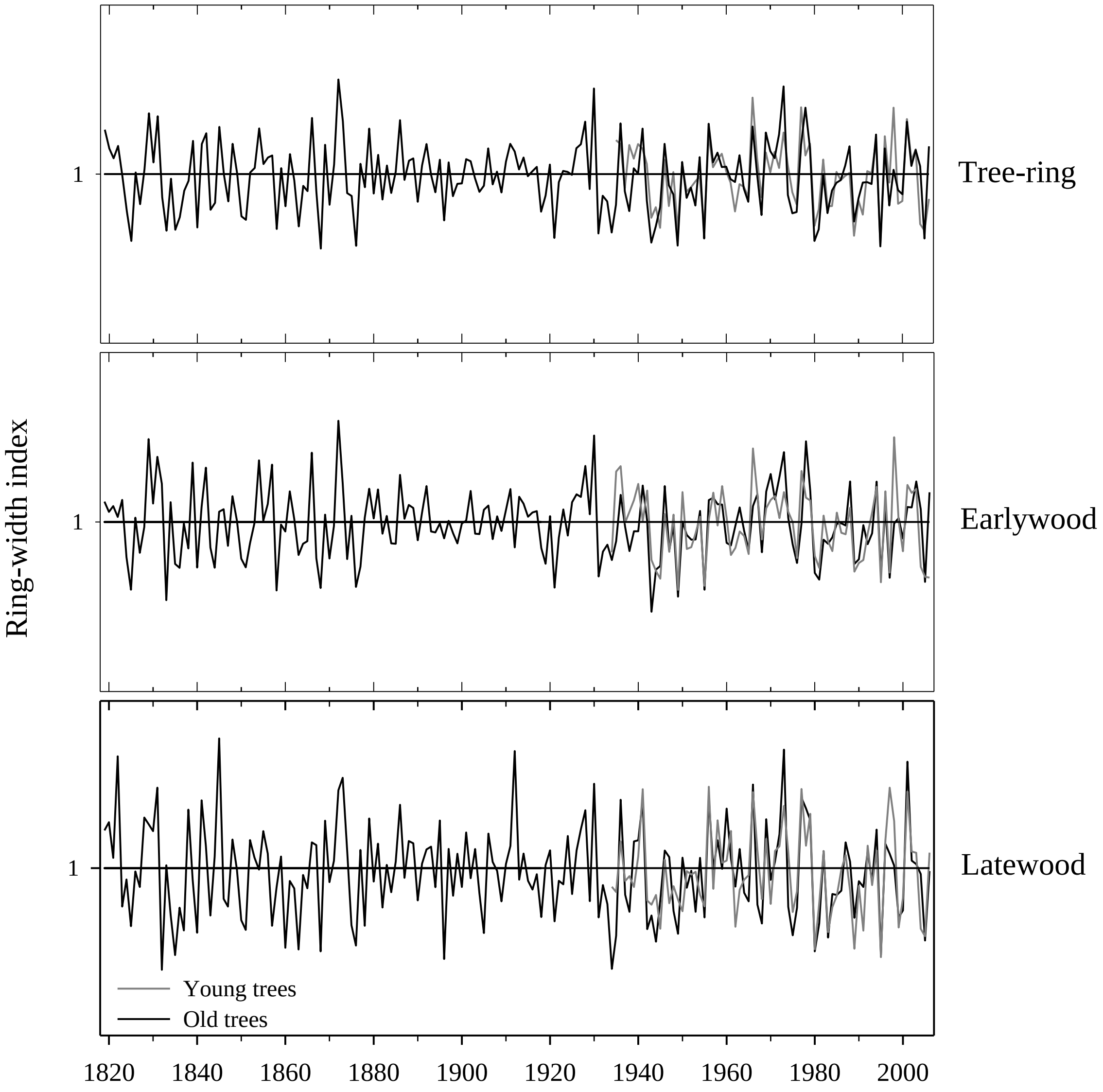

Figure 6. Residual ring width indices of *Pinus pinaster* for the period of 1900 – 2006. The gray line represents young trees and black line the old ones.

The parameters calculated with ARSTAN to characterize the chronologies are presented in Table I. Mean sensitive was higher for latewood. The expressed population signal (EPS) was above the critical value of 0.85 proposed by Wigley et al. (1984) for both age-categories, indicating a strong common signal. The EPS value measures the degree to which a particular chronology drawn from a finite number of trees portrays the theoretical chronology based on an infinite population (Wigley et al., 1984).

Table I. Descriptive statistics of earlywood, latewood and tree-ring width residual chronologies for the total time span and for the common interval analysis

	Old			**Young**		
	Ring Width	Earlywood	Latewood	Ring Width	Earlywood	Latewood
Start	1818	1818	1818	1933	1933	1933
End	2006	2006	2006	2006	2006	2006
Lengh (years)	189	189	189	74	74	74
Raw ring width (1/100 mm)	220	136	84	264	180	84
Residual chronologies:						
Mean sensitive	0.23	0.22	0.33	0.21	0.22	0.29
Standart Deviation	0.20	0.20	0.27	0.19	0.20	0.25
Skewness	0.10	0.16	0.33	0.10	0.15	0.36
Kurtosis	0.01	0.33	0.10	-0.12	-0.35	-0.09
First order autocorrelation	0.02	0.05	-0.03	0.00	0.03	0.03
Common interval analysis (1942-2006):						
No. of trees (no. of cores)	21 (41)	21 (41)	21 (41)	12 (16)	12 (16)	12 (16)
Rbt	0.29	0.29	0.30	0.39	0.34	0.37
EPS	0.91	0.90	0.90	0.89	0.86	0.88
Variation of First Eigenvector (%)	35	32	34	44	39	42

2.3.2. Climate-Growth response

The bootstrapped response functions, calculated for the period 1942-2006, are presented in Figure 7 and describe the relationship between residual chronologies and climate. Response function analysis showed that climate accounted for a high amount of variance in ring-width chronologies, ranging from 56 to 65% (Table II) and it was higher in young trees for earlywood and tree-ring width and in old trees for latewood width.

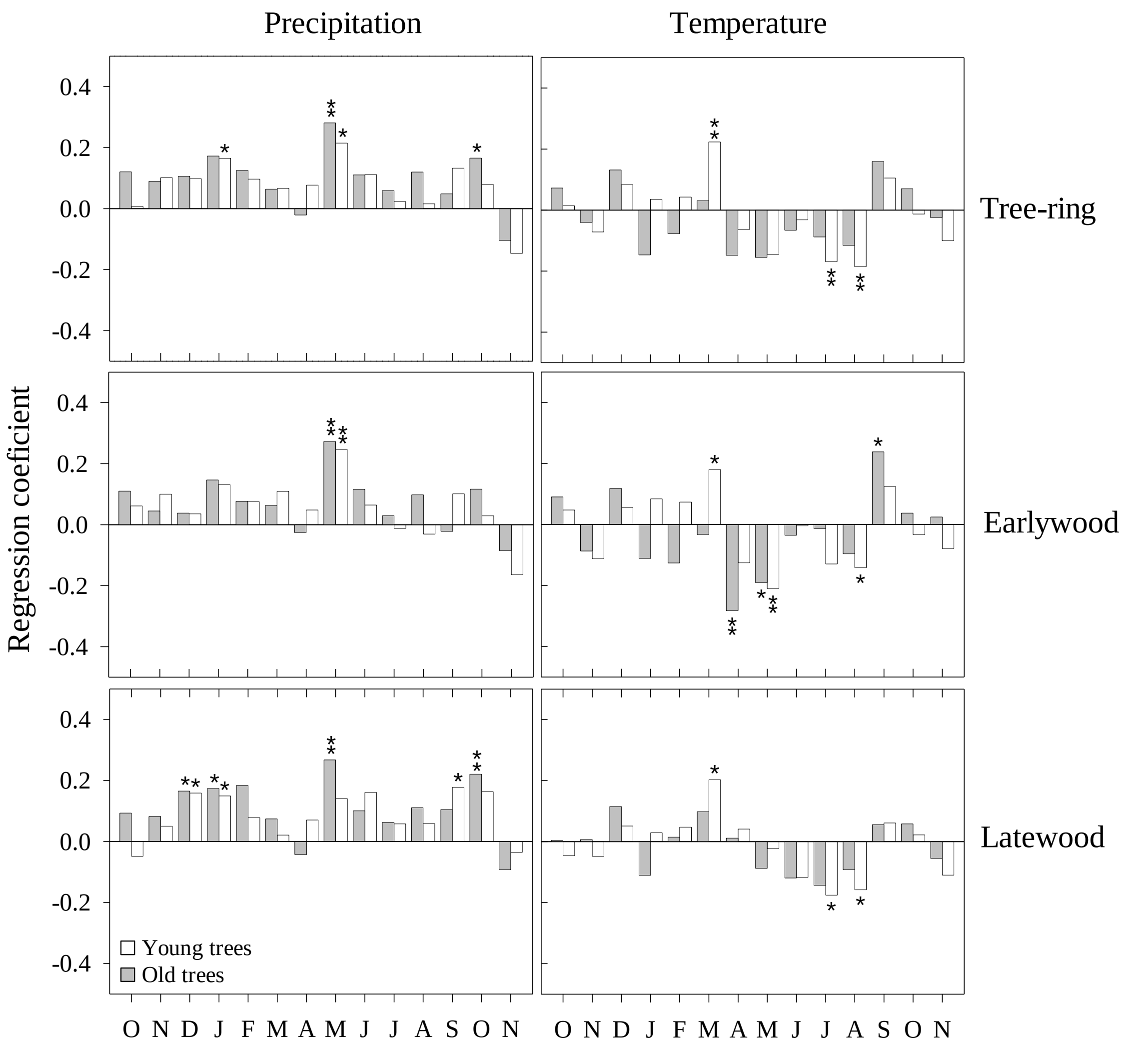

Figure 7. Response function analyses of earlywood, latewood and tree- ring width residual chronologies for *Pinus pinaster* young and old trees and monthly climatic data from October $_{(t-1)}$ to November $_{(t)}$ in the period from 1942 – 2006 (open bars – young trees; closed bars – old trees), data from Instituto Nacional de Meteorologia, Portugal. Significance classes: * p < 0.05; ** p < 0.01.

Tree-ring width of young trees responded positively to precipitation in January and May and to temperature in March but negatively to July and August temperatures (Figure 7). The old trees showed a positive response towards May and October precipitation.

Earlywood width, in both age-categories, showed a positive response to May precipitation and a negative one to May temperature (Figure 7). In young trees, earlywood width also showed a positive response to March temperature and a negative one to August temperature. Earlywood width of old trees showed a negative response to April temperature and a positive one to September temperature.

Latewood width in young trees was enhanced by precipitation in previous December, January and September (Figure 7). Regarding the temperature effect, latewood width of young trees exhibited a positive response to March temperature and a negative one to July and August temperature. In old trees, latewood width showed a positive response to precipitation in previous December, January, May and October.

Table II. Calibration and Verification regression coefficients of the response function analyses and percentage of variance explained by climate.

	Correlation coefficient of calibration	Correlation coefficient of verification	% of variance explained by climate	Overall significance of the model
Tree-ring				
Young	0.859 ± 0.035	0.514 ± 0.141	65	$p < 0.001$
Old	0.818 ± 0.050	0.414 ± 0.161	58	$p < 0.05$
Earlywood				
Young	0.851 ± 0.037	0.478 ± 0.150	63	$p < 0.01$
Old	0.812 ± 0.047	0.361 ± 0.161	57	$p < 0.05$
Latewood				
Young	0.816 ± 0.050	0.354 ± 0.163	56	$p < 0.05$
Old	0.828 ± 0.049	0.435 ± 0.157	59	$p < 0.01$

2.3.4. Intra-annual density fluctuations

Young trees showed the highest stabilized frequency of IADFs, being the type L^+ the most frequent (Table III). IADFs located within the earlywood were less frequent. Occasionally, more than one type of IADF was observed in the same tree ring.

Table III. Descriptive statistics of the IADFs distribution.

	Old	**Young**
Number of trees (cores)	22 (44)	20 (40)
Trees with IADFs	22	18
Number of rings analysed	1064	1250
Number of missing rings	8	90
Rings with IADFs (%)	12.31	19.84
Rings with IADFs type E (%)	0.19	1.92
Rings with IADFs type L (%)	5.17	4.24
Rings with IADFs type L^+ (%)	6.95	13.76

The distribution of IADFs in relation to calendar years is shown in Figure 8. The distribution of IADFs type E is not shown since few trees exhibit this type of IADF. The stabilized frequency of IADF type L ranged from 0 to 2 in the young trees and from 0 to 2.32 in the old ones. For the IADF type L^+ the frequency distribution ranged from 0 to 2.23 in young trees and from 0 to 1 in the old ones.

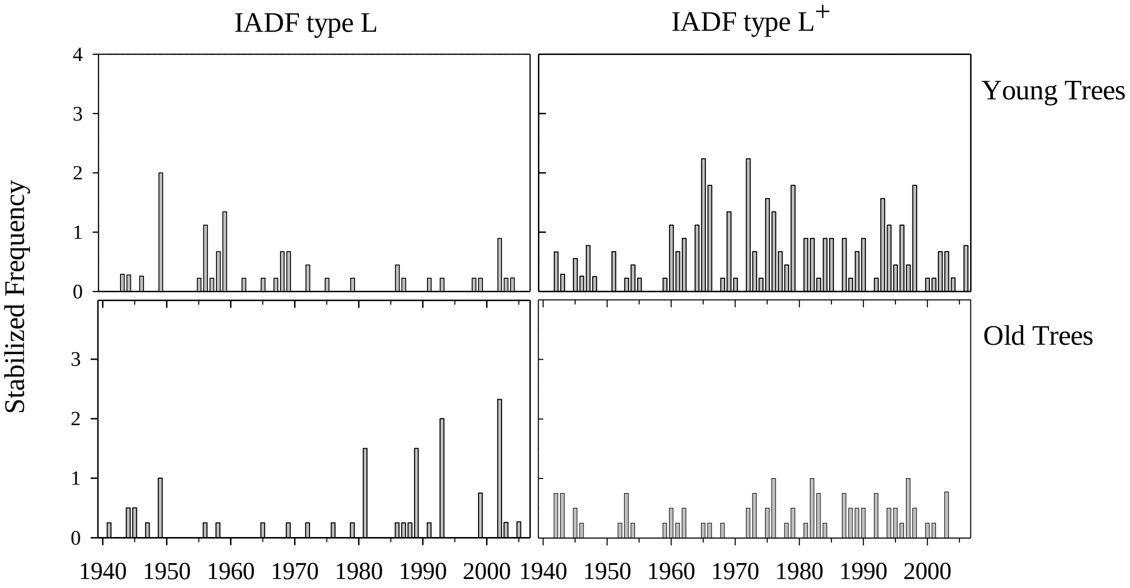

Figure 8. Stabilized IADF frequency in relation to calendar year.

Spearman's correlation coefficients between the chronologies of stabilized IADFs frequencies and total monthly precipitation and mean temperature were analysed from January to December of the current year (Figure 9). In young trees, IADF type L was positively correlated with March and September precipitation and negatively correlated with March and November temperatures. In old trees, IADF type L was negatively correlated with May precipitation and positively with October precipitation. IADF type L^+ in young trees was positively correlated with precipitation in February, September and October, whereas in old trees IADF type L^+ was positively correlated to precipitation in October and December and with November temperature.

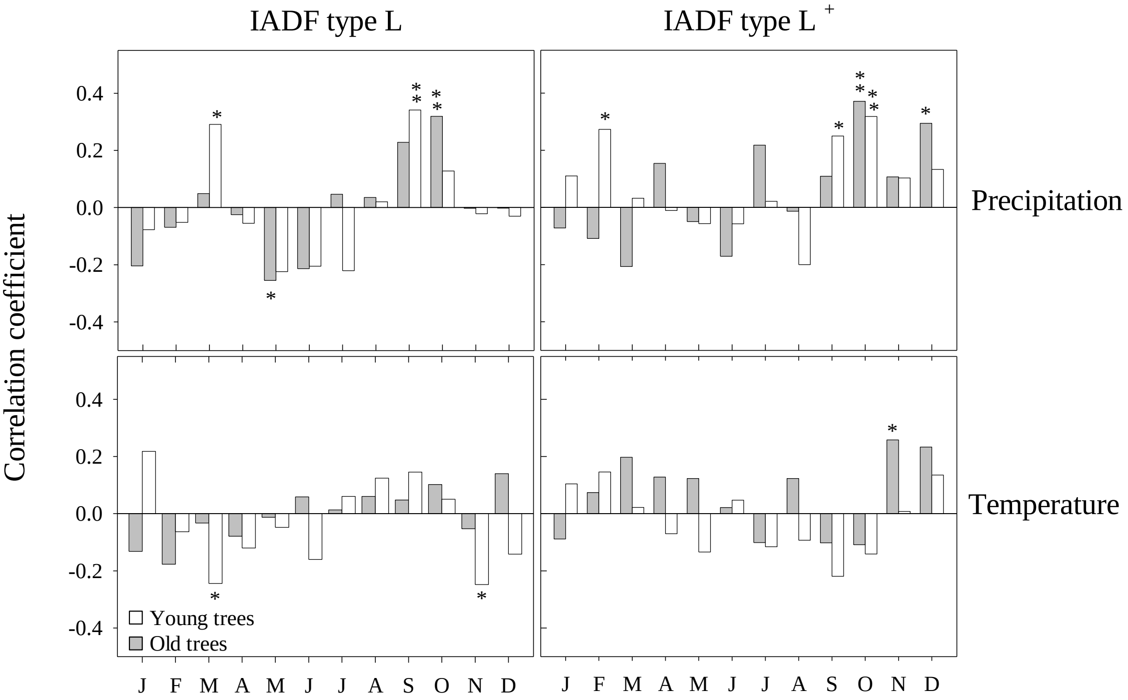

Figure 9. Spearman's correlation coefficient between IADFs stabilized frequency of young and old trees and monthly climatic data from 1941 – 2006 (open bars – young trees; closed bars – old trees). Significance classes: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

2.4. Discussion

Response function analyses showed that earlywood and tree-ring width of young *P. pinaster* trees were more sensitive to climate influence while the response of latewood width to climate was stronger in old trees. Rossi et al. (2008) showed that old conifer trees started their growing season later, compared to young trees. If this is also true for *P. pinaster*, it is expected that younger trees start responding to climatic conditions earlier. The response of young *P. pinaster* trees to March temperature could be related to an earlier start of the cambial activity, with a longer period for earlywood formation. Latewood is initiated by photoperiod and water stress (Vaganov et al., 2006). The efficiency of water translocation through a tree declines with increasing age and/or height, due to a non-optimal network of xylem conduits with a tapered structure (Anfodillo et al., 2006; Ryan et al., 2006; West et al., 1999). As a result, water deficits may become more pronounced with age and this is reflected in the higher response of latewood width to climate, as observed in older trees of *P. pinaster*.

In both age classes high precipitation in May induced the formation of wider earlywood bands and consequently, wider tree rings. Although in the Mediterranean climate the majority of the precipitation does not typically fall in May (Touchan et al., 2007), it provides the necessary moisture and optimum temperatures for photosynthesis, with earlywood being formed mainly during this month (Lebourgeois, 2000). This was also reported for *P. pinaster* in central Spain (Bogino and Bravo, 2008), for *Pinus nigra* in south-eastern Spain (Martín-Benito et al., 2008) and for *P. pinea* in the south of Portugal (Campelo et al., 2007).

Temperatures in May had a negative effect on earlywood width of both age classes, and April temperature also produced a negative effect on old trees (Figure 7).

High temperatures in spring can induce water stress, which in turn will limit the photosynthetic capacity of plants by inducing stomatal closure to avoid water loss (Baquedano and Castillo, 2007). Tree-ring and latewood width of young trees were sensitive to high temperatures in July and August. High temperatures in summer, associated with low precipitation, reduce cambial activity and radial cell expansion (Antonova and Stasova, 1997; Deslauriers and Morin, 2005).

In dendrochronology, the incorporation of special ring features such as IADFs can improve the resolution of the climate signal within the growing season (Wimmer, 2002). IADFs were shown to be a useful tree-ring feature to reconstruct monthly precipitation (Wimmer et al., 2000). Several studies with the genus *Pinus* showed a good correlation between IADF formation and climate (Campelo et al., 2007; De Micco et al., 2007; Rigling et al., 2001; Wimmer et al., 2000). However, Ringling et al. (2002) were not able to correlate climate and IADFs formation in *Pinus sylvestris* L. growing in Siberia. Due to the short term growing season in Siberia (2-3 months), the mean monthly climatic data was probably not sufficiently precise to describe IADF formation.

Total IADF frequency was higher in young than in old *P. pinaster* trees (Table III). Several studies have also shown that IADFs were more frequent in wider and younger tree-rings (Copenheaver et al., 2006; Rigling et al., 2001; Villalba and Veblen, 1994). This could be due to a faster response of young trees to changing factors (Villalba and Veblen, 1994) and/or to a longer growing season of young trees (Rossi et al., 2008). In *P. pinaster* of both age classes, IADFs were mainly correlated with precipitation in autumn (Figure 9). A wet autumn associated with favourable temperatures, resumes cambial activity resulting in the formation of an IADF (Wimmer et al., 2000). The increase of water availability will increase the turgor of xylem cells under differentiation, blocking the thickening of cell walls, producing earlywood like cells within latewood (Wimmer et al., 2000). This indicates that lignification can switch on and off in response to environmental conditions (De Micco et al., 2007). Most of the IADFs were of type L^+. The accumulation of substances in cell walls close to the end of the growing season, when climatic conditions are less favorable, can be affected and tracheids do not reach full maturity, looking like earlywood cells.

When comparing the latewood width response function analyses with the IADFs correlations we can observe that the climatic signal is slightly different (Figures 7 and 9). In young trees, latewood width and IADF type L responded to September precipitation, and IADF type L^+ responded to both September and October precipitation. In old trees, latewood width responded to October precipitation while IADF type L was correlated to October precipitation and IADF type L^+ to October and December precipitation and November temperature. Therefore, IADFs can detail and reinforce the climatic signal of latewood width chronologies in the Mediterranean climate.

To our knowledge this is the first study to show an age-dependent tree-ring growth response in a water limited ecosystem. Earlywood and tree-ring width of young *P. pinaster* trees were more sensitive to climate, while latewood width response to climate was stronger in old trees. This is probably related to the fact that young trees start the growing season earlier, thus a time window delay occurs between young and old trees during which only young trees integrate environmental signals. Age-dependent responses to climate can give important clues to predict how differently young and old trees react to climate change. Young *P. pinaster* trees seem to be more sensitive to summer temperatures than old trees. Therefore, the predicted increase in summer temperatures in the Mediterranean region can alter the functioning and structure of *P. pinaster* forests. Further studies including other species and areas of the

Mediterranean Basin are needed to fully understand how trees of different ages respond to climate change and how this can affect Mediterranean forest ecology.

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CHAPTER III

Long term changes in the relationship between climate and Intra-annual density fluctuations of *Pinus pinaster* in the Mediterranean

Submitted as:

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3.1. Introduction

Dendroclimatology generally assumes that the relationship between tree-ring growth and climate is stable over time. Nevertheless, within a scenario of climate change it is important to verify if this assumption holds or if this relationship is also changing. In the last decade several studies have reported a changing climate-growth relationship over time in tree-ring series of temperature-limited ecotones (Wilmking et al., 2004; Bunn et al., 2005; Driscoll et al., 2005; Buntgen et al., 2006; Oberhuber et al., 2008; Wilmking and Myers-Smith, 2008). In alpine regions the radial growth of different tree species increased after the middle of the 20th century, which can be related to climate change (Bunn et al., 2005; Oberhuber et al., 2008). Additionally, tree line populations are showing a weakened response to temperature and an increased response to precipitation in recent decades, which has been referred as the ‘divergence problem’ (for a review, see D'Arrigo et al., 2008).

Since climate changes are affecting tree growth in the alpine regions, what is happening with trees growing in the Mediterranean region? According to the IPCC report (IPCC, 2008), it is expected that the Mediterranean region will become warmer and drier, with a predicted increase of maximum summer temperature and a decrease of total precipitation, intensifying the risk of drought. In the Mediterranean climate, summer induces a “drought-imposed rest” and after this quiescent period, if water availability and favorable temperatures return, a second growth period can occur (Cherubini et al., 2003). This bimodal growth pattern was already observed for other species in the Mediterranean area, such as *Quercus ilex* L. (Campelo et al., 2007a) and *Pinus halepensis* Mill. (Nicault et al., 2001; De Luis et al., 2007). The impact of climate in trees is reflected in several wood-ring characteristics, namely tree-ring width and ultimately in wood cell features. Intra-annual density fluctuations (IADFs) are special anatomical features formed in response to climate conditions along the growing season (Fritts, 2001; Masiokas and Villalba, 2004). Latewood IADFs, characterized by earlywood-like cells within latewood, are produced after the summer drought during the second growth peak (Campelo et al., 2007b; De Luis et al., 2007; De Micco et al., 2007). In the Mediterranean area, these structures were positively correlated with precipitation in September or October (Campelo et al., 2007b; De Luis et al., 2007), whereas in subarctic or alpine environments IADFs were correlated with summer temperature (Wimmer et al., 2000; Rigling et al., 2002; Masiokas and Villalba, 2004). Sanchez-Vargas et al. (2007) related within-ring density of *P. pinaster* to weather variation within a growing season in France and showed that the cambium was controlled by water availability. This plasticity in the tracheids anatomy reflects the importance of water on cambial activity and wood formation.

The intra-annual resolution of IADFs can reveal if the response of trees to climate is changing in recent decades. The aim of this research paper is to find whether the climatic factors triggering IADF formation in *Pinus pinaster* Ait. growing under a Mediterranean climate are stable over time.

3.2. Material and methods

3.2.1. Study area and tree-ring data

The study area and the procedure for sampling, cross-dating and standardization are described in chapter II, sections 2.2.1 and 2.2.3 (pp 17 and 20, respectively). In this chapter, however, only the old trees were studied.

3.2.2. Intra-Annual Density Fluctuations

IADFs were identified from 1900 to 2006, as described in chapter II, section 2.2.5 (p 22). The frequencies and stabilized frequencies were obtained as previously described (p 22) however in this chapter, due to the smaller number of trees, IADFs from both cores were used.

3.3.3. Climate-growth relationships

Due to the fact that most IADFs were located in the latewood, the relation between climate and latewood width was evaluated with a bootstrapped response function analyses, using the program PRECON (Briffa and Cook, 1990; Fritts, 1999). The response function procedure is explained in chapter II, section 2.2.4 (p 21).

As climate and tree growth relationships may change over time, statistical calibration between the latewood width residual chronology and monthly climate was tested for temporal changes using a moving response function analyses. A moving response function employs a fixed number of years that progressively slide across time to compute the response coefficients (i.e. first calculation 1942–1982, second calculation 1943–1983, etc.) (Biondi, 1997).

The moving response function analyses was performed using the program PRECON (Fritts, 1999). Correlations were computed between the residual chronology of latewood width and climate parameters from previous October to current November, using a total of 28 predictors. To maintain enough degrees of freedom a moving interval of 40 years was used (Biondi, 1997).

3.3.4. IADFs and climate

The climatic data was transformed by means of Z score transformation in dimensionless indices:

$Z = (x - \overline{X}) / SD$

where x is the monthly climatic value for a given year, $\overline{X}$ the average value from 1941 to 2006 and SD its standard deviation.

The relationship between the stabilized IADFs frequency and the monthly climatic indices were computed using Spearman's correlation coefficients for a moving time window of 40 years (i.e. first period 1941–1980, second period 1942–1981, etc.), to identify the climatic factors that produced the highest correlation scores.

3.3. Results

In the beginning of the 20th century, when trees were approximately 30 years old, the IADFs frequency was high, then showed a decrease from the 40s to the 70s, and then increased until the present (Figure 10). The IADF type L was positively correlated with September and October precipitation while IADF type L^+ was positively correlated with October, November and December precipitation and with November and December temperature (Figure 11).

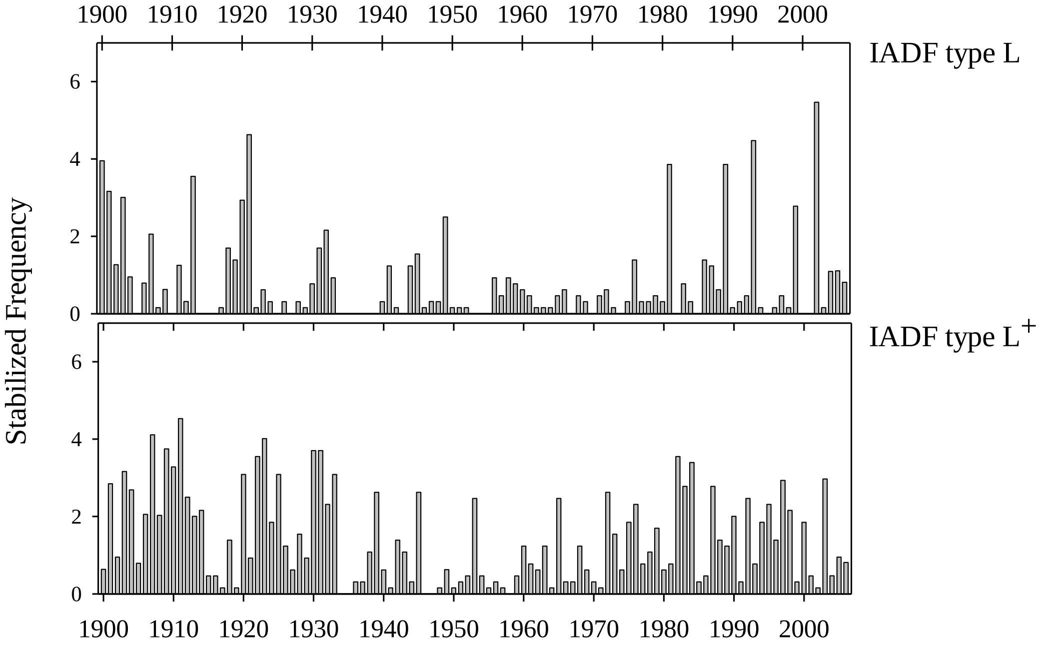

Figure 10. IADF frequency of *Pinus pinaster* in relation to calendar year.

To check if the climatic factors triggering IADF formation changed over time, a moving correlation coefficient was calculated for the months with significant correlation coefficients. The relation between IADF type L and September precipitation was significant for the periods 1941–1980 and 1946–1985 and decreased in the last periods (Figure 12). The correlation between October precipitation and IADF type L also decreased and from 1959 onward lost significance. Although IADF type L did not present a significant correlation with temperature for the period 1941–2006 (Figure 11), the relation between this IADF and November temperature became significant after 1949 (Figure 12).

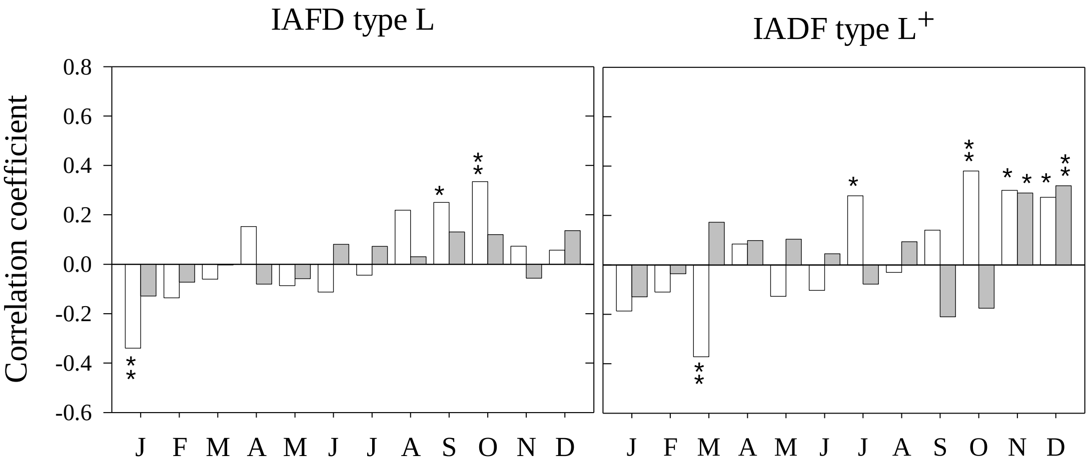

Figure 11. Spearman's correlation coefficient between the master chronology of IADFs frequency of *Pinus pinaster* and monthly climatic data (open bars – total precipitation; closed bars – mean temperature) from 1941 to 2006. (*$p < 0.05$; **$p < 0.01$).

The moving correlations between IADF type L^+ and October precipitation remained significant throughout 1941–2006 (Figure 13). Regarding the correlations between IADF type L^+ and November precipitation although it was significant for the overall period, 1941–2006 (Figure 11), the moving correlation analyses did not present any significant periods (Figure 13). The correlations between December precipitation and IADF type L^+ were not significant in the first periods, 1941–1980, afterwards the correlations gained significance, until 1992, time at which it decreased and lost significance. The analyses of the moving correlation between IADF type L^+ and temperature revealed that November temperature was not significantly correlated with IADF type L^+ for the entire period (Figure 14). The correlations with December temperature had an increasing tendency and from 1959 until 2003 were significant (Figure14).

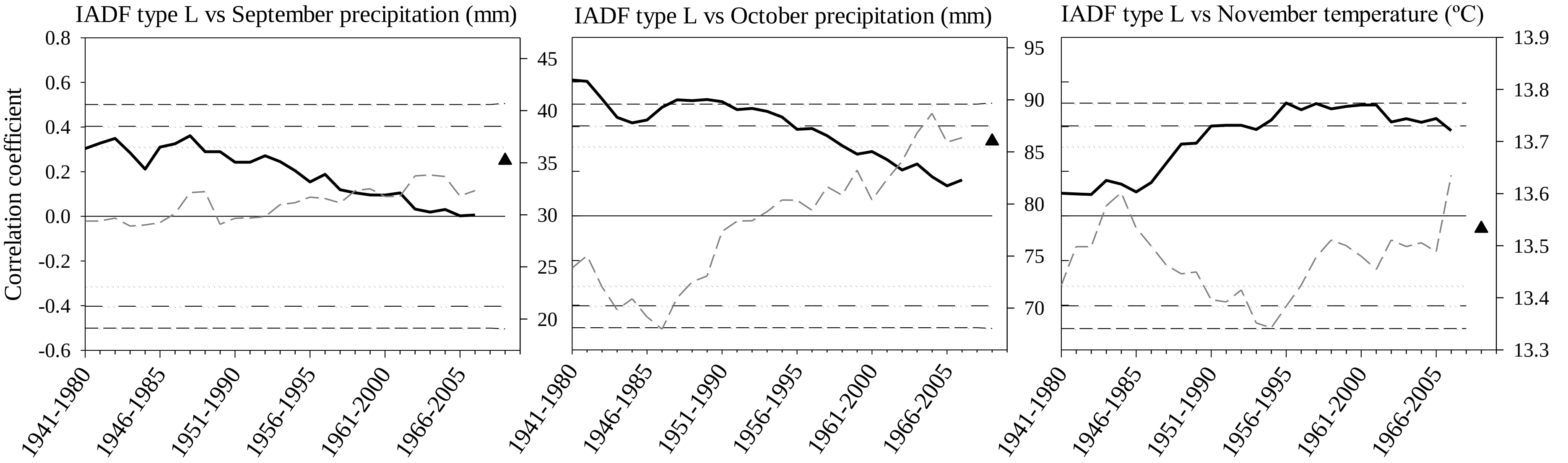

Figure 12. Moving correlation analyses between the IADF type L stabilized frequency and the monthly precipitation in September and October and temperature in November, for the period 1941–2006, using a 40-year window. Full line, moving correlation coefficient value; dashed grey line precipitation/temperature moving average; triangle correlation coefficient between IADF stabilized frequency and monthly precipitation/temperature for the period 1941–2006. Horizontal lines represent significance intervals (dot line $p < 0.05$; dash-dot line $p < 0.01$; dash line $p < 0.001$).

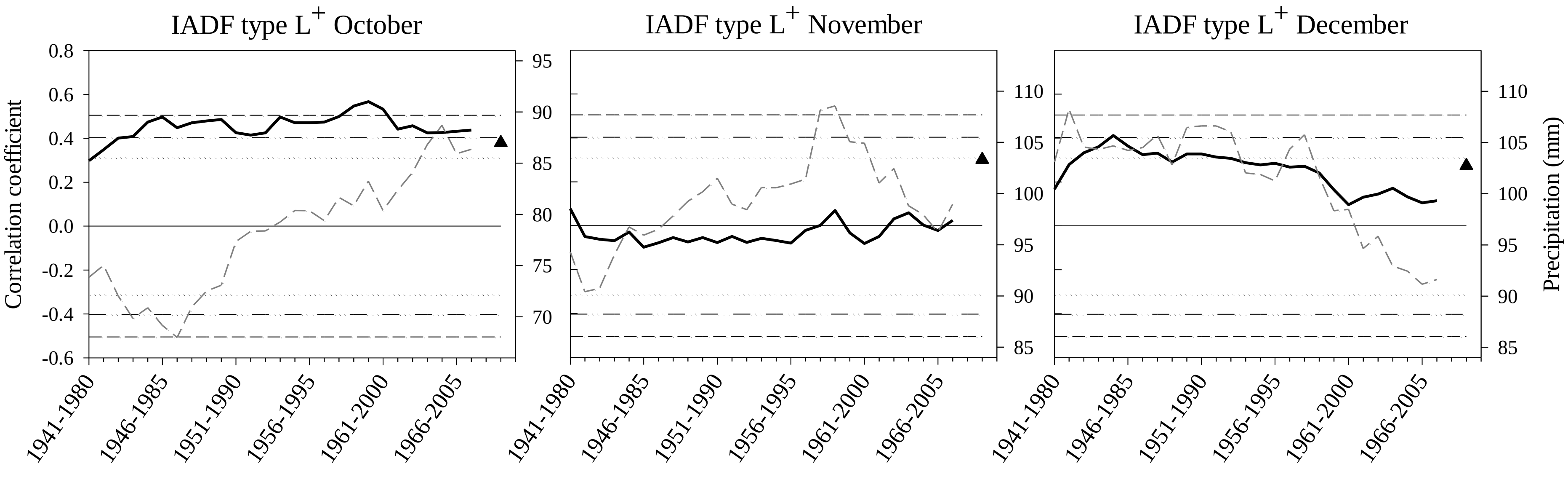

Figure 13. Moving correlation analyses between the IADF type L^+ stabilized frequency and the monthly precipitation in October, November and December for the period 1941–2006, using a moving window of 40-years. Full line, moving correlation coefficient value; dashed grey line precipitation moving average; triangle correlation coefficient between IADF stabilized frequency and monthly precipitation for the period 1941–2006. Horizontal lines represent significance intervals (dot line $p < 0.05$; dash-dot line $p < 0.01$; dash line $p < 0.001$).

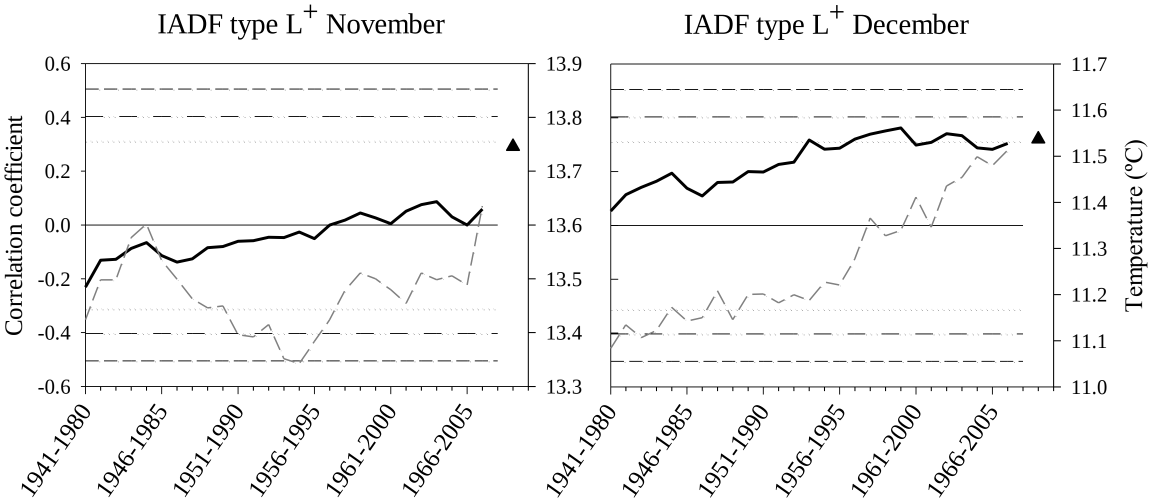

Figure 14. Moving correlation analyses between the IADF type L^+ stabilized frequency and the monthly temperature of November and December, for the period 1941-2006, using a 40-year moving window. Full line, moving correlation coefficient value; dashed grey line, temperature moving average; triangle, correlation coefficient between IADF stabilized frequency and monthly temperature for the period 1941–2006. Horizontal lines represent significance intervals (dot line $p < 0.05$; dash-dot line $p < 0.01$; dash line $p < 0.001$).

According with the available meteorological records, precipitation and temperature in September did not change significantly from 1941 to 2006. However, for the same period, October precipitation increased 20mm and the temperature dropped 0.3ºC, November precipitation dropped 5mm and temperature increased 0.3ºC and December precipitation decreased 10mm and temperature increased 0.4ºC.

Since most of the IADFs were located in the latewood we analysed the latewood response function for the period 1941–2006 (Figure 15). Latewood width showed a positive correlation with precipitation in January, May and October and a negative one with July temperature. Because the response of IADFs to climate changed in the last 65 years, we also investigated if the latewood response changed, using a moving response function analysis (Figure 16). The latewood moving response function showed a high significant positive correlation with May precipitation from 1955 onwards. The correlation between latewood and October precipitation became significant after 1959 for the following periods: 1959–1998, 1962–2001, 1964–2003 and 1966–2006.

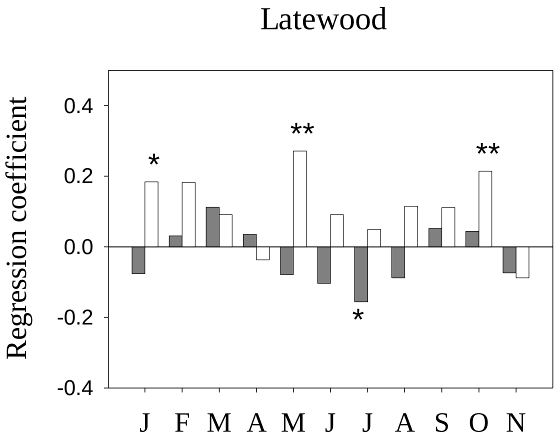

Figure 15. Response function analyses of latewood width residual chronologies for *Pinus pinaster* and monthly climatic data (closed bars – mean temperature; open bars – mean precipitation) from January to November in the period from 1941-2006. (* $p < 0.05$; ** $p < 0.01$).

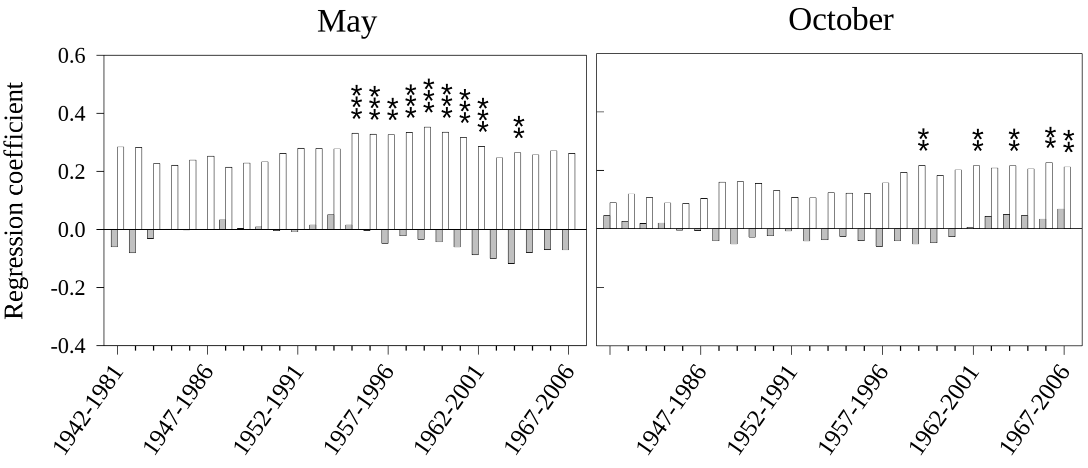

Figure 16. Moving response function analyses of latewood residual chronology of *Pinus pinaster* and monthly precipitation of May and October for the period 1941-2006, using a moving window of 40 years. (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

3.4. Discussion

From the beginning of the 20th century until the 40s the frequency of latewood IADFs of *P. pinaster* was high (Figure 10). This could be age related since IADFs are more prone to occur in younger and wider tree-rings (Villalba and Veblen, 1994; Rigling et al., 2001; Copenheaver et al., 2006). From the 40s to the 70s, although wider rings were formed (Figure 17), the IADFs frequencies decreased, except in particular years such as 1949 (for IADF type L) and 1953 (for IADF type L^+). The IADFs frequencies increased after the 70s, which was not related to the occurrence of wider rings, since tree-ring width decreased in this period. What is then determining the observed pattern of IADFs frequency after the 40s?

An increase in October precipitation has been observed in the last 50 years, and associated with an increase of the temperature in November and December probably created the optimum conditions for a second growth peak, explaining the increased IADFs frequency since the 70s. Latewood width moving response function also showed a significant response to October precipitation since 1960, reinforcing the idea that the cambium is still dividing and that new xylem cells are added to latewood, increasing its width (Figure 16).

A shift in the climatic parameters controlling IADFs formation was observed in the period from 1941 to 2006. From the 60s onwards, the formation of IADFs type L was less controlled by September and October precipitation, whereas November temperature became more relevant (Figure 12). October precipitation increased 20mm after the 60s and is probably no longer a limiting factor for the formation of IADFs type L. November temperature also increased 0.3ºC, and is becoming a controlling factor for IADFs type L formation. The formation of IADFs type L^+ was dependent on

October precipitation throughout the entire period of study (1941–2006) (Figure 13). However, it seems that since the 60s there is an increasing correlation with the temperatures of November and December (Figure 14). Warmer conditions in November and December probably affect the enlargement and secondary cell wall deposition stages, forming cells resembling earlywood.

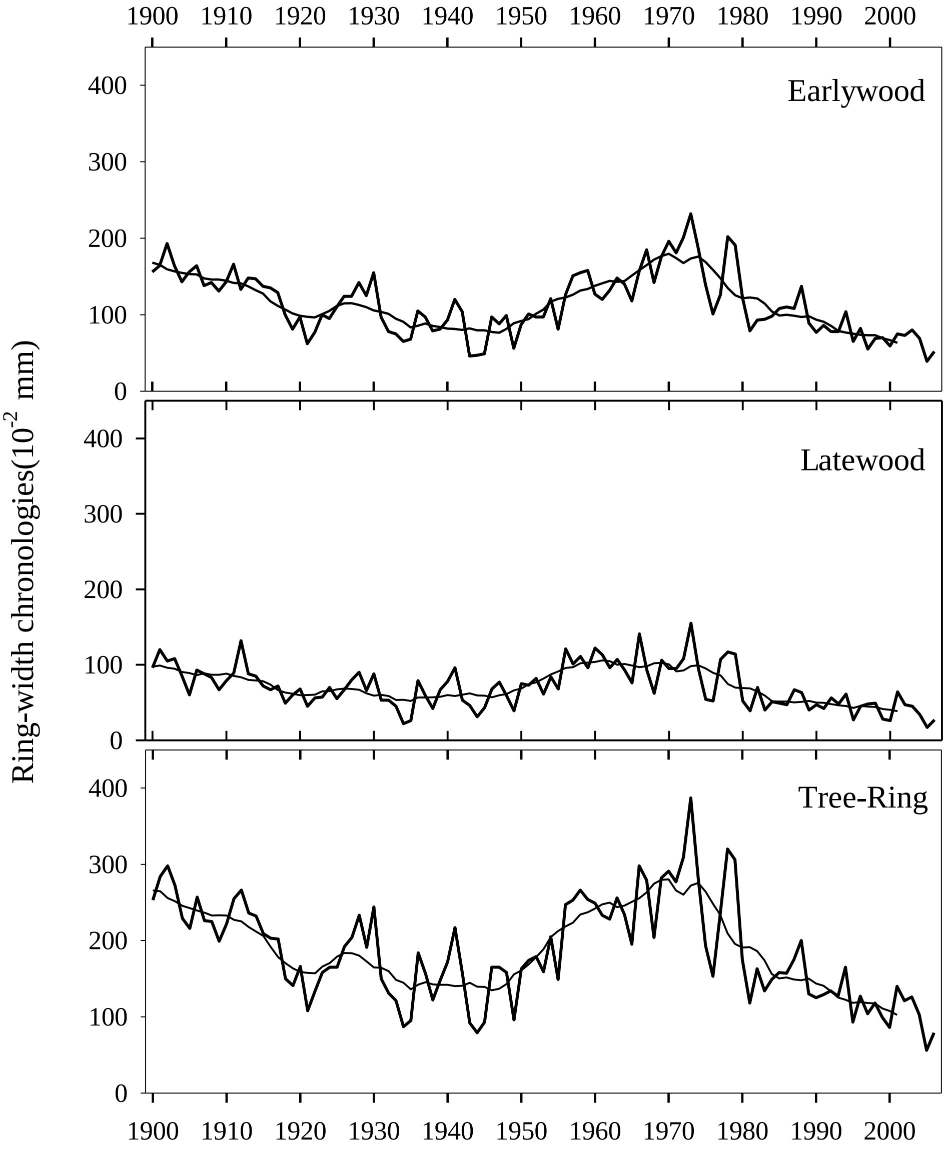

Figure 17. Raw ring-width chronologies of *Pinus pinaster* (thick line) with a 10 years moving average (thin line).

The Mediterranean climate is changing and there are evidences that the summer temperature increased 0.4ºC over the last 50 years (Xoplaki et al., 2006). Andreu et al. (2007a) working with *Pinus nigra* Arnold, *Pinus sylvestris* L. and *Pinus uncinata* Ramond ex. DC, in Spain, reported a change in the trees response to climate in the second half of the 20th century. They detected a higher occurrence of narrower rings linked to an increase in the water-stress period from mid to late summer. The growth synchrony among the pine forests was also stronger, indicating that climate is becoming more limiting to growth. Bogino and Bravo (2008) also detected a change in the climate-growth relation of *P. pinaster* from Spain since 1980, some climatic variables (May temperature and April and May precipitation) had changed from a non-significant to a significant relation with tree-growth. In the eastern Mediterranean, Sarris et al. (2007) found a decrease in the tree-ring width of *Pinus brutia* Ten. due to a reduction in precipitation, which allowed them to predict that tree-growth will become more dependent on deep soil water.

In conclusion the IADFs correlation with climate in *P. pinaster* changed over time, as detected by the moving correlation analyses, making this type of approach a better tool to understand the ‘behavior’ of trees facing climate change. Apparently, the climatic conditions for tree growth after the summer drought improved after the 60s, with trees taking more advantage of a second growth peak with the formation of latewood IADFs. However, the increasing risk of drought predicted for the Mediterranean (IPCC, 2008) will also increase the risk of exhaustion of deep soil water reserves, important for the growth of trees.

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CHAPTER IV

Age-dependent response of *Pinus pinaster* tree-rings to climatic extremes in Mediterranean climate

4.1. Introduction

The temporal distribution and frequency of climatic extremes is important to characterize the trends of climate and to understand their impacts on forests and tree growth (Frank et al., 2005). According to these authors, the climate and tree-ring width extremes “can be defined as events occurring towards the tail ends of – their – statistical distributions”.

Dendrochronological investigation is traditionally made through the study of tree-ring width, which represents an “average climate-growth relation” (Schweingruber et al., 1990). However, the study of single year events, pointer years (PY) characterized by an abrupt change in growth, are used to date past climatic extreme events (Yamaguchi, 1991; Stokes and Smiley, 1996). The term PY is related to cross-dated rings from different trees, whereas the term event year is related to observations and measurements of single tree-ring samples (Schweingruber et al., 1990).

Pointer years are normally used in a present/absence analyses but they can also be used to develop chronologies. Tardif and Conciatori (2006) compared a tree-ring width chronology to an event-year chronology and found identical results which emphasized the utility of event-year chronologies in tree-ring studies. Recently several studies have developed chronologies based on dichotomous variables (e.g. presence/absence of intra-annual density fluctuations) and used them to obtain climatic signals that were not present in the traditional ring-width chronologies (Wimmer et al., 2000; Campelo et al., 2007; Chapter II).

Pointer year formation is related to episodes of growth release and/or reduction, thus the analyses of its frequency has been successfully used in the identification of extreme climatic events such as drought (MacDonald and Tingstad, 2007), winter frosts

(Lebourgeois, 2007) and atmospheric circulation (Knapp et al., 2002). In a temperate environment (France), Lebourgeois (2007) compared the PY formation in two species, *Abies alba* Mill. and *Picea abies* Krast and concluded that narrow PY were formed in years with winter frosts and/or summer drought.

In the Mediterranean narrow PY are mostly formed in response to drought (Ogle et al., 2000; Dobbertin, 2005; Bigler et al., 2006; Pichler and Oberhuber, 2007) thus PY can be used as proxies for precipitation. The study of their past frequency and the response of trees after abrupt growth reductions can help us to predict how forests will react to a scenario of increasing frequency of drought episodes (IPCC, 2008). It is also important to understand how trees of different age react to extreme climatic events. Bigler et al. (2006) studied drought as a possible factor causing *Pinus sylvestris* L. mortality in the Swiss Alps. They concluded that single-year droughts caused growth reduction but trees recovered in the following years however, multi-year droughts reduced tree growth for several years, decreasing the trees vigor and ultimately leading to its death.

The aims of the present study are (i) to identify PY in *Pinus pinaster* Ait. trees of different age: young and old; (ii) to determine whether PY formation is age-dependent; (iii) to determine the climatic extremes that lead to its formation and (iv) to determine the advantages of the study of PY in relation to the traditionally tree-ring width studies.

4.2. Materials and Methods

4.2.1. Study area and Tree-ring data

The study area and the procedure for sampling, cross-dating and standardization are described in chapter II, sections 2.2.1 and 2.2.3 (pp 17 and 20, respectively).

4.2.2. Pointer Year

A VBA routine for Excel was created to determine the PY based on the ratio growth change. This routine compared the tree-ring width of a given year with the mean of the n previous years, in this study $n = 3$. This short “window” could result in the discrimination against dry years embedded in extended droughts (Knapp et al., 2002), however our aim was to identify single-year drought events. A tree-ring was considered a pointer year when at least 80% of all cores were 20% larger or narrower than the mean of the previous three years. Finally, an average of the growth ratio change of all cores was calculated, in order to determine the strength of the PY. To better identify positive and negative PYs the mean values were subtracted by 1, so in the graphic the narrow PY appear negative and the wide ones positive.

4.2.3. Climatic Relationships

To evaluate the relationship between PY and climate and to compare the climatic signal of PY and tree-ring width chronologies a bootstrap response function analyses was performed using the program PRECON (Fritts, 1999). The response function analyses were computed between the residual ring-width and PY chronologies and monthly climatic data from previous October to current November, as described in chapter II, section 2.2.4 (p 21).

4.3. Results

The PY analysis revealed 11 years showing a sudden increase or decrease of growth (Figures 18 and 19), with narrow PY more frequent than wide PY. Most of the PY were present in both age classes: 1945, 1976, 1981, 1994 and 2005. From the period 1940-2006, a total of 7 PY were identified for the old trees, 2 wide and 5 narrow. For the young trees 9 PY were identified, 1 wide and 8 narrow. All of the narrow PY identified for old trees were also present in young trees. The wider PY for old trees was 1946 as for young trees it was 1966 (Figure 19). The narrower PYs were 1981 and 2005 for old and young trees, respectively. In both age classes, for the period under study, the frequency of PY was higher since the 70s (Figure s 18 and 19).

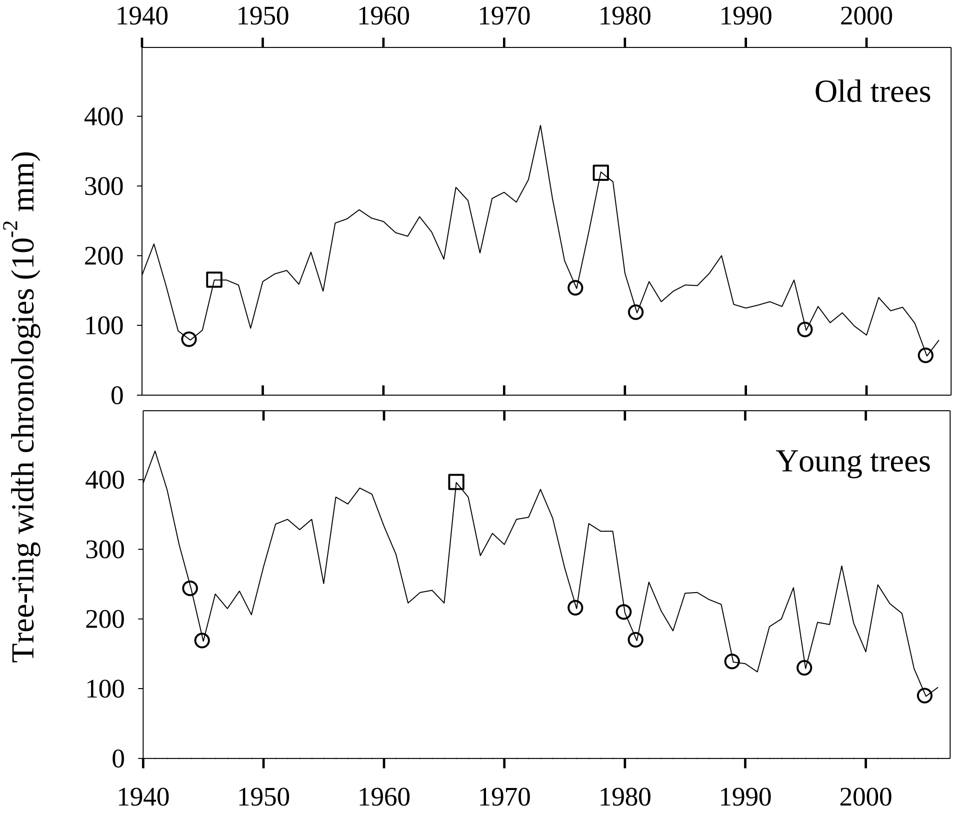

Figure 18. Raw tree-ring width chronologies of *Pinus pinaster* with the PY identified, (circles, narrow PY; squares, wide PY).

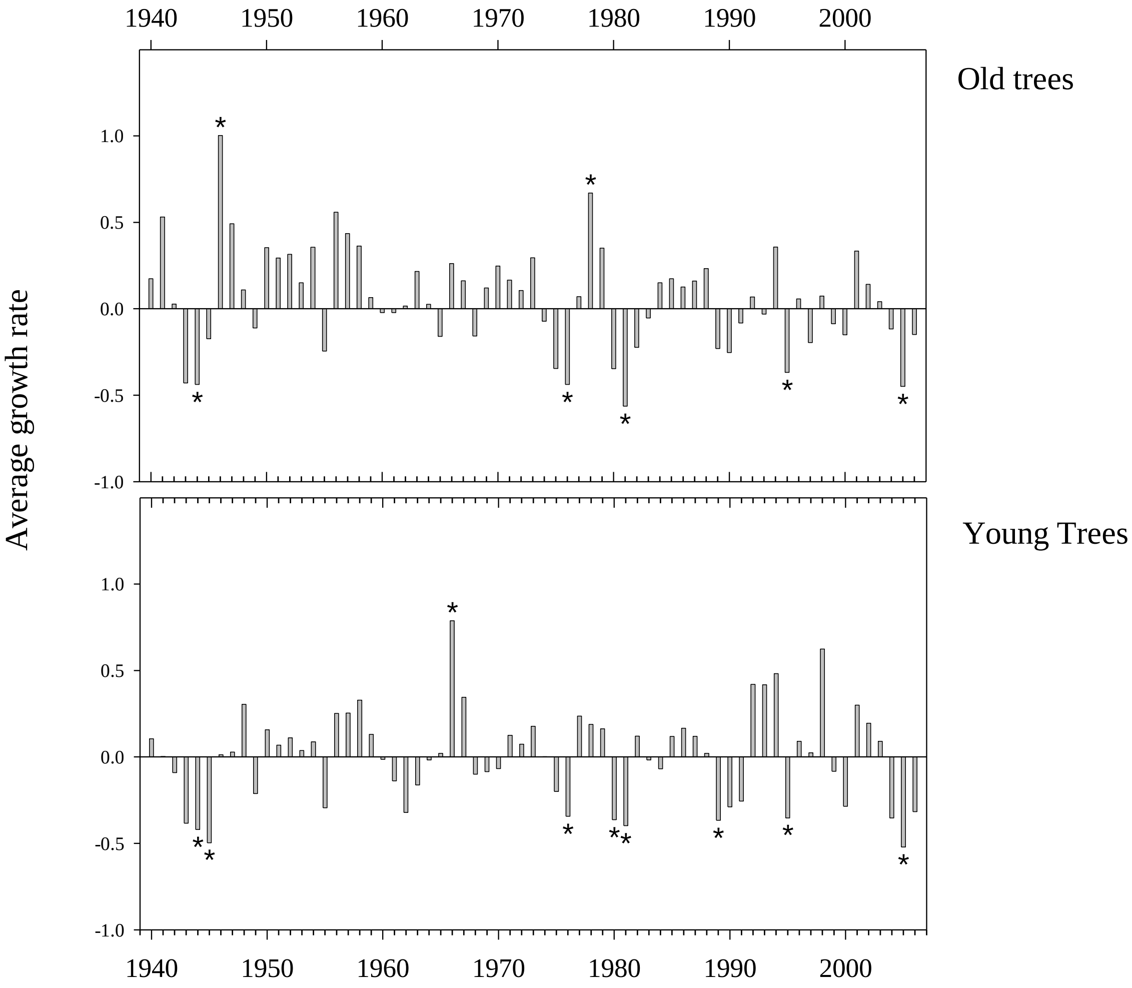

Figure 19. Average growth rate chronologies, PY are identified with (*), the wide PY are positive and the narrow PY negative.

The narrower PY in both age classes (1981 and 2005) corresponded to two extreme drought years with total annual precipitation below half (350mm) the annual mean precipitation for the period 1941-2006 (700mm) (Figures 3 (p 18) and 20).

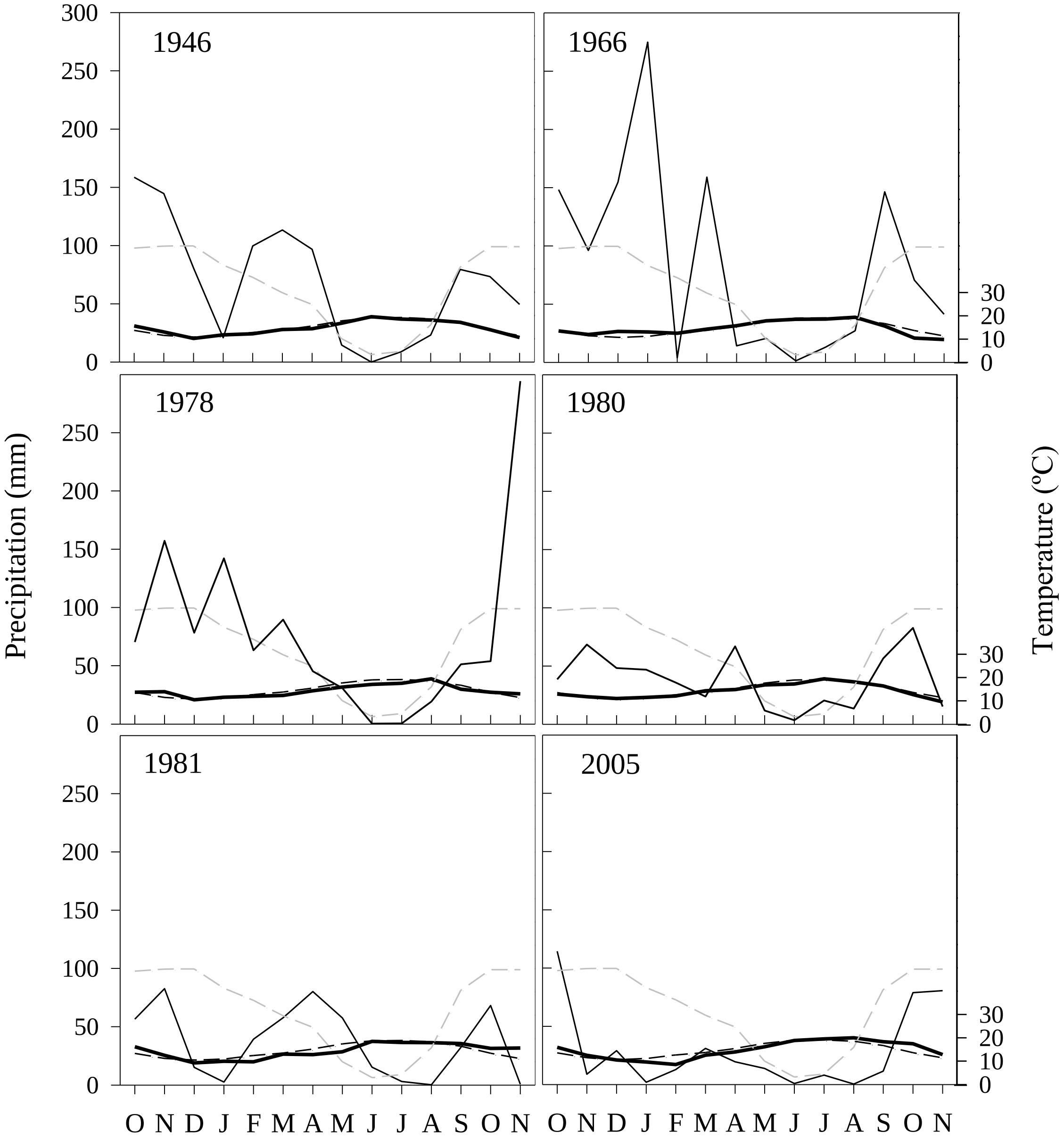

Figure 20. Climatic diagrams for the extreme years: 1946 and 1966 wide PY; 1981 and 2005 narrow PY; 1978 a wide PY just for old trees and 1980, a narrow PY just for young trees. Thick black line temperature; dashed black line average temperature 1941-2006; thin black line precipitation; dashed grey line average precipitation1941-2006.

There were years with different responses for each age classes, such as the year 1946 and 1978, wide PY for old trees, 1966 a wide PY for young trees and 1980 a narrow PY for young trees (Figures 18 and 19). The year 1946 was characterized by high precipitation in the previous winter and spring and a warmer winter previous to growth but colder spring and autumn (Figure 20). The year 1966 was characterized by high precipitation, except in March and May, and high temperatures in January and February (Figure 20). In the year 1978 precipitation was above average in the previous December and February and the temperatures were below average in April, May and June (Figure 20). The year 1980 was a dry year, with below average precipitation during winter and spring (Figure 20).

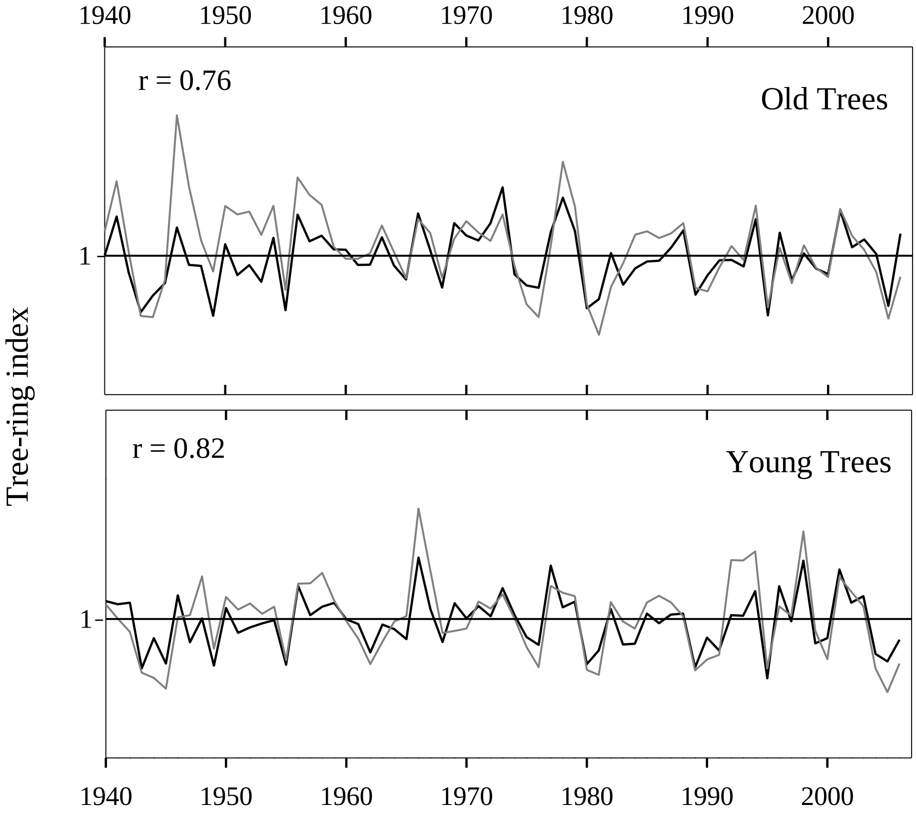

Figure 21. Overlapping between point year (PY) and tree-ring width (TRW) chronologies, for old and young trees (black line TRW and grey line PY chronology). In the left superior corner is present the correlation coefficient between the chronologies.

PY and tree-ring width (TRW) chronologies were very similar with a high correlation coefficient between them (Figure 21). In order to compare the climatic signal of both chronologies, a response function analysis was performed (Figure 22). Comparing both chronologies, the young trees TRW response function presented a positive significant response to January precipitation and a negative one to August temperature that was not detected in the PY response function. Regarding old trees, the TRW response function detected a positive response to October precipitation that was not present in the PY response function. The PY response function showed a negative response to January temperature, while the TRW response function did not and the response to May precipitation was stronger (Figure 22). Concerning the strength of the model, the TRW was stronger than the PY (Table IV).

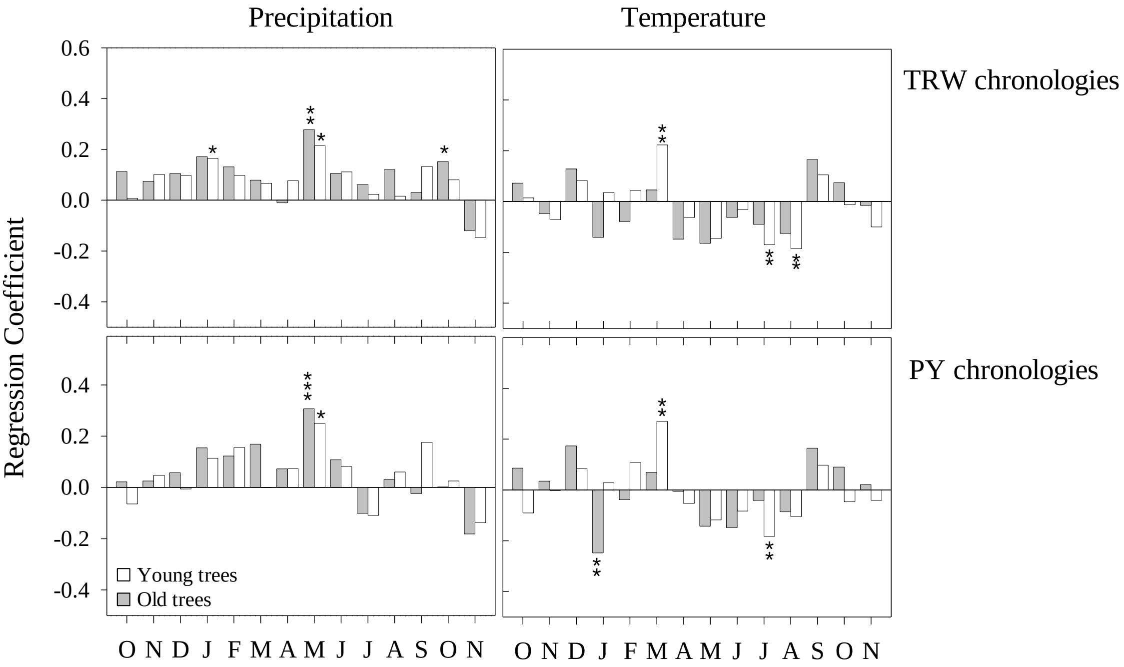

Figure 22. Response function analyses between TRW residuals and PY chronologies of *Pinus pinaster* and monthly climatic data from October$_{(t-1)}$ to November for the period from 1941-2006 (open bars – young trees; closed bars – old trees; *p < 0.05; **p < 0.01).

Table IV. Correlation coefficients of calibration and verification of the response function.

		Correlation coefficient of calibration	Correlation coefficient of verification	% of variance explained by climate	Overall significance of the model
Tree-ring width	Young	0.859 ± 0.035	0.514 ± 0.141	65	p < 0.001
	Old	0.818 ± 0.050	0.414 ± 0.161	58	p < 0.001
Point years	Young	0.798+/- 0.050	0.276+/- 0.178	53	-
	Old	0.798+/- 0.048	0.318+/- 0.157	55	p < 0.05

4.4. Discussion

To understand the complex forcing of climate on tree growth various statistical approaches are frequently used, such as response function analysis (Briffa and Cook, 1990). Although the validity of the information obtained from these analyses, they are still averaged relationships between climate and tree-ring width, where the climate forcing of single extreme years is not evaluated (Frank et al., 2005), this kind of knowledge can only be obtained through PY analysis.

In our data, *P. pinaster* narrow PY were more frequent than the wide ones. Andreu et al. (2007) working with *Pinus nigra* Arn., *Pinus sylvestris* and *Pinus uncinata* (DC) Ramond in Spain also noticed a higher occurrence of narrow rings related with an increased variability in precipitation. The narrowest PY were the same for both age-classes of *P. pinaster*: 1981 and 2005, which corresponded to very dry years. Martín-Benito et al. (2008) also identified 1981 and 2005 as drought years for *P. nigra* in southeastern Spain. In both age classes, narrow PY were related to years with low winter and spring precipitation and high spring temperature. Winter precipitation is important to refill the water reserves necessary for the next growing season. Severe drought periods in spring may cause inhibition of photosynthesis and/or changes in carbon-allocation in favor of the fine-root system, reducing the carbon available for radial growth (Leuschner et al., 2001; Meier and Leuschner, 2008).

Comparing the frequency of narrow PY, old trees presented less (5) than the young ones (8) (Figures 18 and 19). Probably younger trees are more susceptible to drought due to a less developed root system compared with older trees, thus with less access to deep soil water reserves, making them more dependent on surface water and more susceptible to water stress. This was also confirmed by the fact that two consecutive dry years (1980 and 1981) affected more strongly young trees compared with the old ones, with the occurrence of two narrow PY in the young trees class for those years.

Mediterranean climate is a drought limited environment (Specht, 1981; Cherubini et al., 2003) that is expected to become even drier in the next decades (IPCC, 2008; Nicault et al., 2008). In fact, for the studied period (1940-2006) most of the narrow PY occurred in the last 30 years. Trees may survive to sporadic extreme drought events (Martín-Benito et al., 2008), however the increase frequency of drought years may strongly affect the growth of trees, especially the young ones since they are more dependent on growing season precipitation. Pichler and Oberhuber (2007) studied the effect of a severe drought year, 2003, in *P. nigra* and *Picea abies* (L) Harst. in an inner dry Alpine valley in Austria. Both species experienced an abrupt growth reduction in 2003, accentuated in 2004, showing the dependence of those trees on the previous year precipitation. Other studies reported the negative effect of drought on trees by reducing the trees vigor and increasing its susceptibility to pathogens (Mattson and Haack, 1987; Ogle et al., 2000; Dobbertin, 2005; Bigler et al., 2006).

Although the TRW chronologies showed a stronger climatic signal compared to PY chronologies (Figure 22), PY chronologies can be useful for the reconstruction of extreme climatic events, since less predictors are necessary for that purpose (Touchan et al., 2007).

The predicted increase of drought years in the Mediterranean is already occurring with trees showing a higher frequency of narrow PY in the last 30 years, as recorded in *P. pinaster*. However, it is important to reconstruct a longer chronology of PY in the Mediterranean climate to better understand their frequency and trends. Although Mediterranean forests are adapted to drought conditions, an increase in the frequency of drought events will have major impacts in forest populations because young trees are more susceptible to drought conditions.

4.5. References

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FINAL REMARKS

Final remarks

The information stored in tree-rings is extremely important for climate reconstructions and also to understand what is shifting in the growth of trees under a scenario of climate change in the Mediterranean. From this study we were able to conclude that in *P. pinaster* the climatic response of tree-ring width and IADFs were age-dependent. In young trees IADFs had a better climate resolution than latewood width thus, the inclusion of IADFs in dendroclimatic studies strengths the resolution of the climatic signal in the end of the growing season.

We have also showed that the relation between tree-ring width, IADFs formation and climate has changed over time. The moving response function revealed that climatic factors inducing the formation of tree-rings and anatomical features such as IADFs changed in the last 30 years. Apparently the occurrence of a second growth peak in autumn is becoming more frequent due to milder temperatures and increased precipitation levels, as revealed by the higher IADFs frequency.

Finally, the study of pointer year frequency in both age classes proved to be a good proxy for drought events since PY formation was related with precipitation. Years with above average precipitation produced wider tree-rings whereas dry years were recorded as narrow tree-rings. Young trees presented a higher frequency of narrow PY, revealing a higher susceptibility to drought than old trees. Additionally, the frequency of narrow PY increased in the last 30 years, showing that extreme drought climatic events are becoming more common in the Mediterranean.

The increased frequency of drought events and the instability of the precipitation regime represent a great challenge for Mediterranean forests, especially for young trees.

The present study revealed some ‘fingerprints’ of climate change in the growth of Mediterranean trees.