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Effects of climate change on growth of pine species in Mediterranean environments

DOCTORAL THESIS

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TESIS DOCTORAL:

EFFECTS OF CLIMATE CHANGE ON GROWTH OF PINE SPECIES IN MEDITERRANEAN ENVIRONMENTS

EFECTOS DEL CAMBIO CLIMÁTICO EN EL CRECIMIENTO DE LOS PINOS EN AMBIENTES MEDITERRÁNEOS

Presentada por Jorge Olivar Ruiz para optar al grado de doctor por la Universidad de Valladolid

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NOTE TO READERS

This thesis is based on four original works, which are referred to in the text with Roman numerals (**I**, **II**, **III**, and **IV**), published or under revision in different international journals. Each article constitutes one of the studies or chapters of the thesis. Authors, coauthors, and the stage of the publication are presented below.

Previously to the presentation of each study, the reader will find the outline of the thesis and an abstract of the thesis written in English and Spanish. Then, a summary of the whole thesis which includes an introduction with the general and specific objectives, the main methodologies and analyses employed, the main results and a general discussion. After that, the reader will find the four studies and the general conclusions of the thesis.

LIST OF ORIGINAL WORKS

I Olivar, J., Bogino, S., Spiecker, H., Bravo, F., 2012. Climate impact on growth dynamic and intra-annual density fluctuations in Aleppo pine (*Pinus halepensis*) trees of different crown classes. Dendrochronologia 30, Issue 1: 35-47.

II Olivar, J., Bogino, S., Spiecker, H., Bravo, F., 2013. Changes in climate-growth relationships and IADF formation over time in Mediterranean pine species (*Pinus halepensis, P. pinaster* and *P. sylvestris*) in Spain. Submited to European Journal of Forest Research.

III Olivar, J., Bogino, S., Rathgeber, C.B.K., Bonnesoeur, V., Leporati, J., Ordoñez, C., Bravo, F., 2013. Thinning has a positive effect on growth dynamics and growth-climate relationships in Aleppo pine (*Pinus halepensis*) trees of different crown classes. Accepted with Major Revision in Annals of Forest Science.

IV Olivar, J., Rathgeber, C.B.K., Bravo, F., 2013. Climate impact on wood density in *Pinus halepensis, Pinus pinaster* and *Pinus sylvestris*. Manuscript.

OUTLINE OF THE THESIS

The thesis focuses on evaluating the impact of climate on pine trees growing under Mediterranean conditions. To accomplish these general objective four studies have been developed. First, study I identified relationships between radial growth and climate for different crown classes of Aleppo pine (*Pinus halepensis* Mill.), quantified the presence of intra-annual density fluctuations (IADFs) according to crown class and cambial age and established the relationships between IADFs and climate. Secondly, study II identified and compared radial growth-climate relationships along time for *P. halepensis*, *P. pinaster* and *P.sylvestris* in Spain, quantified and compared the presence of intra-annual density fluctuations (IADFs) on the three species and defined the climatic variables that are associated with the occurrence of IADFs. Study III identified the main climatic drivers of intra-annual growth of Aleppo pine, analysed witch crown class is more affected by water stress and investigated if the reduction of stand density through thinning decreases its vulnerability to water stress. Finally, study IV identified the main climatic driver of the radial growth and wood density patterns for *P. halepensis, P. pinaster* and *P. sylvestris;* and established the relationships between tree-ring width and wood density. Fig. A shows the conceptual model of the thesis topics and studies carried out.

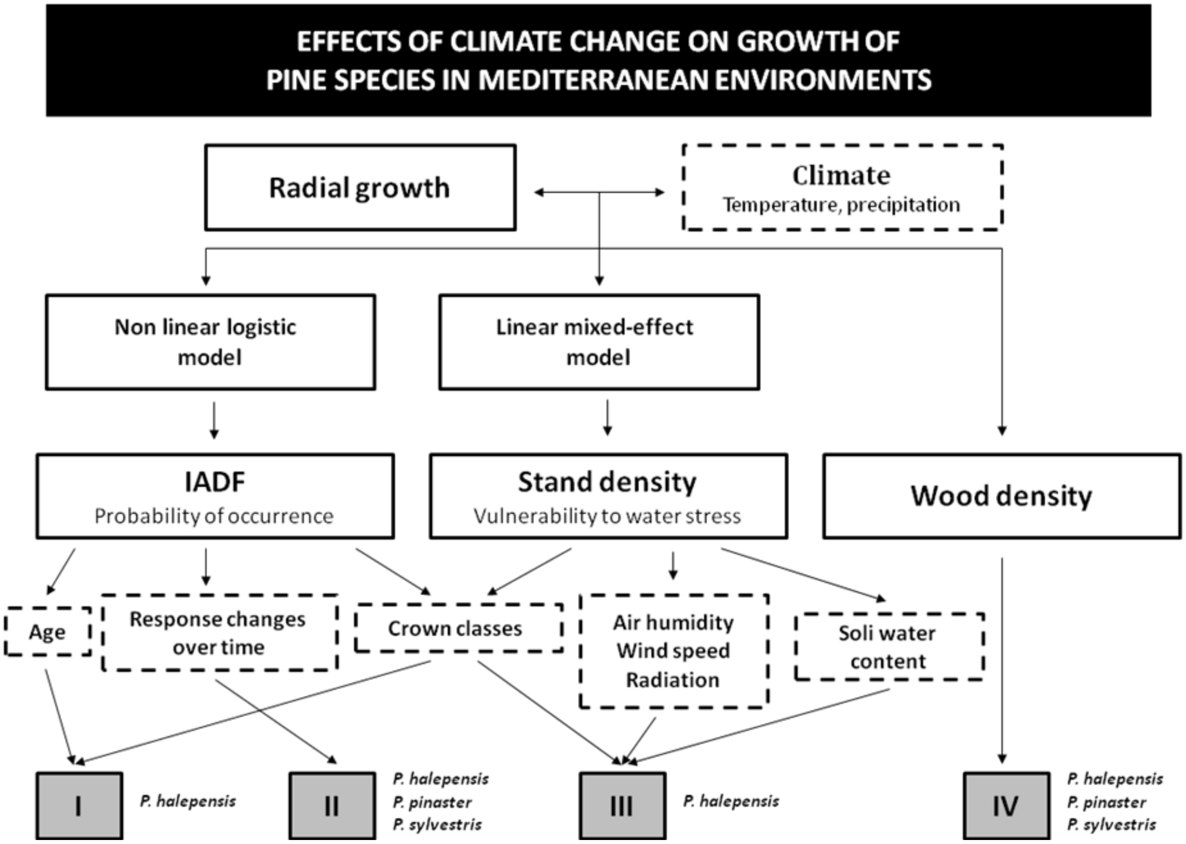

Fig. A: *Conceptual model of the thesis. Key factors (solid squares); main measured variables (discontinuous lines squares); studies (Roman numerals), species and links between them are indicated.*

Abstract

ABSTRACT

Understanding growth-climate relationships is a crucial issue to develop forest management strategies to mitigate climate change impact. During the second half of the 20th century the Mediterranean area has experienced an increase in the mean annual temperature, a decrease in the mean annual precipitation, and an increase in the frequency of severe drought periods. However, in western Mediterranean region winter and spring precipitations have increased while summer precipitation has decreased. Dendroclimatological techniques were applied in 28 sampling sites located in Spain to analyse the association between *Pinus halepensis* (8 sampling sites) *P. pinaster* (9 sampling sites) and *P. sylvestris* (11 sampling sites) and climatic variables in Mediterranean environments. The objectives of this thesis were: a) to identify and compare inter-annual growth-climate relationships along time for *P. halepensis*, *P. pinaster* and *P. sylvestris* in Spain, b) to quantify and compare the presence of intra-annual density fluctuations (IADFs) on the three species and to define the climatic variables that are associated with its occurrence, c) to identify the main climatic drivers of intra-annual growth of *P. halepensis,* d) to analyse crown class differences in the effect of water stress, e) to investigate if the reduction of stand density through thinning decreases its vulnerability to water stress, f) to establish the relationships between climate and wood density for *P. halepensis*, *P. pinaster* and *P. sylvestris*. Results showed that precipitation is the main factor influencing tree growth and its fluctuation determines IADFs occurrence on the three pine species, with *P. pinaster* being the species that had the highest correlation values. Wet periods during winter previous to the growth season and spring induced higher growth rates for *P. halepensis* and *P. pinaster,* while the growth of *P. sylvestris* was mostly influenced by summer precipitation. However, the influence of these climatic variables on the growth of these species changed along the studied period, showing an increase in climate sensitivity at the end of the 20th century. Intra-annual growth of *P. halepensis* is mainly controlled by soil water availability during the growing season. However, relative extractable water in the soil influenced the stem radial increment in a crown class-dependent way. Both crown classes experienced a significant growth release at the most intense thinning treatments. The negative effect of precipitation on wood density is consistent with its positive effect on radial growth. Despite the increasing climate

sensitivity of pine species during the most recent years, the trends in growth and mean density did not change in a 5-year interval. The results of these studies demonstrate that the inclusion of wood density and IADFs detection in chronologies adds new information to ring-width chronologies improving its quality. Besides, they help understanding the effects of stand structure on climate sensitivity and the differential effects of altering stand structure on climate–growth relationships of Mediterranean forest trees, both of which may assist managers promoting forests that are resilient to future climatic conditions.

RESUMEN

Entender las relaciones entre el clima y el crecimiento es un aspecto clave para generar estrategias de mitigación del impacto del cambio climático. Durante la segunda mitad del siglo XX, la región mediterránea ha experimentado un aumento de la temperatura media anual, un descenso de las precipitaciones y un aumento en la frecuencia de los episodios de sequía. Sin embargo, en la parte oeste de la región mediterránea, las precipitaciones durante el invierno y la primavera han aumentado y las precipitaciones durante el verano han disminuido. Se aplicaton técnicas dendrocronológicas en 28 sitios de muestreo situados en España para analizar larelación entre el crecimiento de *Pinus halepensis* (8 sitios de muestreo) *P. pinaster* (9 sitios de muestreo) y *P. sylvestris* (11 sitios de muestreo) y las variables climáticas en ambientes mediterráneos. Los objetivos de la presente tesis son: a) identificar y comparar las relaciones entre el crecimiento inter-anual y el clima a lo largo del tiempo de *P. halepensis*, *P. pinaster* y *P. sylvestris* en España, b) cuantificar y comparar la presencia de fluctuaciones intra-anuales de densidad (IADFs) en las tres especies y definir las variables climáticas asociadas a su presencia, c) identificar las variables climáticas que regulan el crecimiento intra-anual de *P. halepensis*, d) analizar el efecto del estrés hídrico en las diferentes clases de copa, e) investigar si la reducción de la densidad de la masa por medio de claras disminuye la vulnerabilidad de los árboles al estrés hídrico, f) establecer las relaciones entre el clima y la densidad de la madera en *P. halepensis*, *P. pinaster* y *P. sylvestris.* Los resultados muestran que las precipitaciones son el factor más determinante en el crecimiento y el la aparición de IADFs en las tres especies, siendo *P. pinaster* la que muestra las correlaciones más altas. Las precipitaciones durante el invierno previo y la primavera fueron las que afectaron en mayor medida al crecimiento de *P. halepensis* y *P. pinaster*, mientras que el crecimiento de *P. sylvestris* se vio principalmente influido por las precipitaciones en verano. Sin embargo, la influencia de esas variables climáticas en el crecimiento de las tres especies ha variado a lo largo del tiempo, mostrando un aumento de la sensibilidad climática a finales del siglo XX. El crecimiento intra-anual de *P. halepensis* está fundamentalmente controlado por la disponibilidad de agua en el suelo durante el periodo vegetativo. Sin embargo, el agua relativa extraíble del suelo influyó de distinta manera en el crecimiento radial de las distintas clases de copa. Ambas clases de

copa experimentaron un aumento significativo del crecimiento radial tras los tratamientos de clara más intensos. El efecto negativo de las precipitaciones en la densidad de la madera concuerda con su efecto positivo en el crecimiento radial. A pesar del aumento de la sensibilidad climática de los pinos en los últimos años, las tendencias del crecimiento y la densidad media de la madera no cambian en un intervalo de 5 años. Estos resultados demuestran que la inclusión de IADFs en las cronologías añade nueva información mejorando su calidad y enfatizan el impacto de los tratamientos selvícolas en las relaciones entre el clima y el crecimiento. Además, ayudan a enteder los efectos de estructura del rodal en la sensibilidad climática y los efectos de sus cambios en las relaciones clima-crecimiento en los bosques mediterráneos, los cuales promueven bosques con mayor capacidad de resiliencia ante futuras condiciones climáticas.

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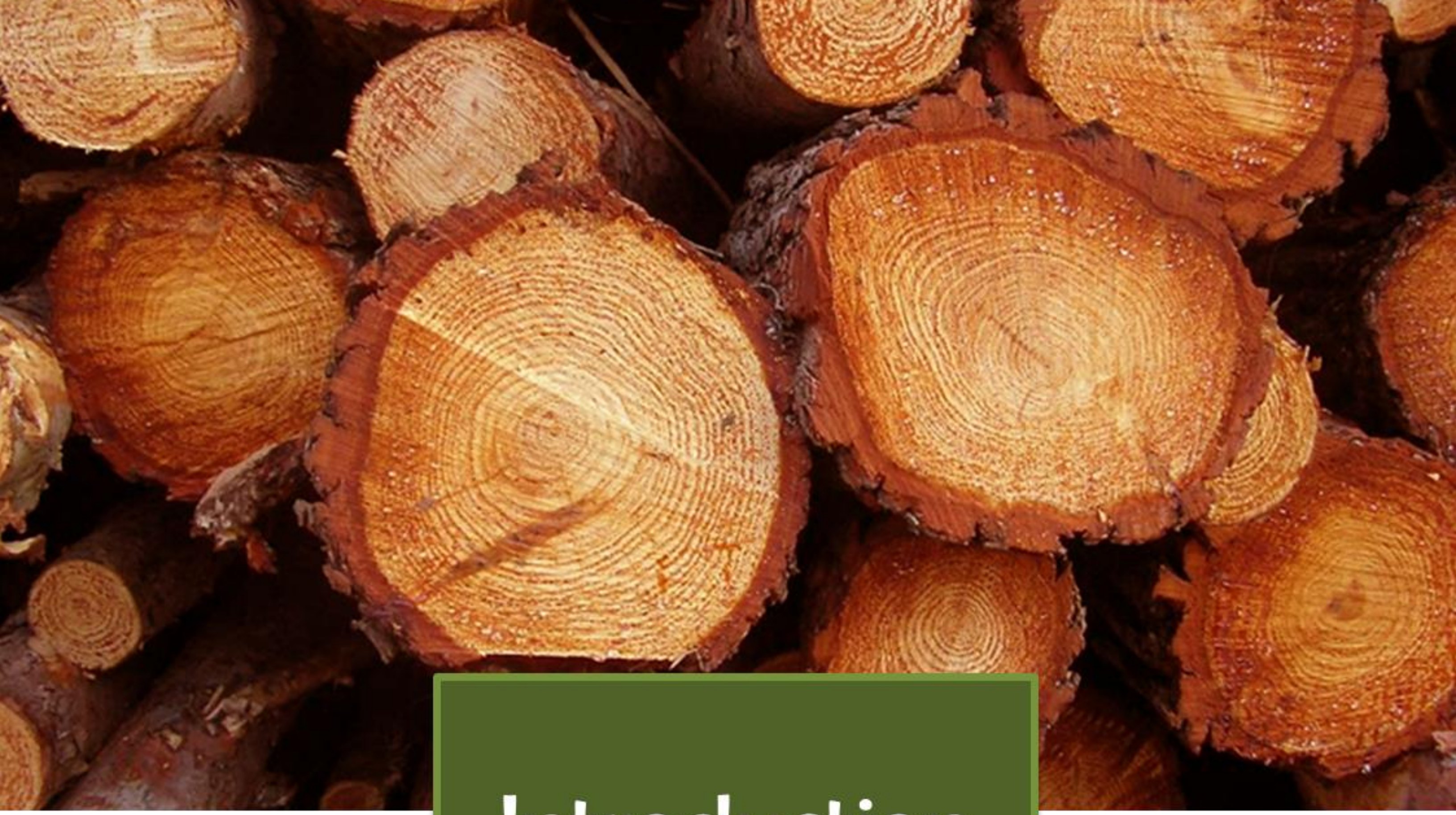


Introduction

1. INTRODUCTION

1.1. Mediterranean environments and climate change

Mediterranean regions, as transitional climate zones between arid and humid regions of the world, are especially interesting for the study of the relationships between climate, tree growth and wood anatomic features. In the Mediterranean region, where rainfall is a major limiting factor for organisms, precipitation has decreased by about 30% during the last century (Petit et al., 2005). Annual temperatures over Europe have increased about 0.8 °C during the 20th century. A larger warming has been observed over the Iberian Peninsula (Houghton et al., 2001), where the 1980–1995 period was characterized by intense droughts, which produced severe damage to several woody species (Peñuelas et al., 2001).

Climate models project rising air temperatures and a decrease in annual mean precipitation over the Mediterranean Basin for the twenty-first century leading to an increase in evapotranspiration (Parry et al., 2007). Although the predicted warming trends are higher in northern and central Europe than in southern Europe (Klein Tank and Können, 2003), most of Europe will have a rather positive water balance, while precipitation in the countries surrounding the Mediterranean Sea are expected to experience the largest decrease on the globe, more than 20%. Therefore, the Mediterranean area is considered to be one of the areas where climatic changes may have the greatest effects (Lavorel et al., 1998). Given the magnitude of forecasted trends, there are great concerns for the particularly rich biodiversity found in the region. Studies on the consequences of past climate shifts on biodiversity represent one of the best sources of data to validate models of the ecological and evolutionary consequences of future changes (Petit et al., 2005).

Precipitation is the main factor influencing tree growth of pine species in semiarid Mediterranean conditions (Raventós et al., 2001). In coastal Mediterranean areas, with mild winters and dry summers, cold season growth dormancy may not occur and, consequently, cambial activity is likely continuous (de Luis et al., 2007). However, in inland areas with continental Mediterranean climate, plants endure double climatic stress, caused by low winter temperatures and summer drought (Mitrakos, 1980). As a result, optimal growth conditions are split into two mild periods with increased rainfall (spring and autumn), which suggest a bimodal pattern of cambial activity (Camarero et al., 2010).

Trees of different sizes and crown classes compete differently for light, water and other resources within a stand (Peet and Christensen, 1987; Orwig and Abrams, 1997). Radial growth responses to climate vary considerably according to local tree density and crown class in areas where water is limiting (Linares et al., 2009; Martín-Benito et al., 2008). Therefore, contrasting growth responses to climate among trees of different crown classes might be particularly relevant for understanding the dynamics of forests dominated by species that are highly sensitive to drought stress (Adams and Kolb, 2004).

1.2. Species studied

1.2.1. Aleppo pine (Pinus halepensis Mill.)

Aleppo pine (*Pinus halepensis*) is one of the most studied Mediterranean species. It plays an important role in the ecology and landscape of the Mediterranean basin. Stands of this species provide a valuable system to explore how growth measured at different time scales is constrained by climate (Pasho et al., 2012). It is considered as a species well adapted to drought by reducing growth as water availability decreases (Rathgeber et al., 2005; de Luis et al., 2007; Camarero et al., 2010).

It is generally found at low elevations, mostly from sea level to 200 metres, but it can grow at an altitude of up to 1000 m in southern Spain. This pioneer and undemanding species is easily regenerated and capable of colonizing very poor and degraded soils. It is an essential component in reforestation strategy for limy soils in the arid or semi-arid climates of the Mediterranean basin, due both to its intrinsic ability to colonize and to its effect in improving soils and microclimates, thus in turn favouring the growth of broadleaved Mediterranean species *(Quercus ilex* L., *Q. coccifera* L. and *Q. faginea* Lamk.*)* within its stands (Quezel, 1986). In Spain, *P. halepensis* occupies 1 million ha in monospecific stands, and 0.5 million ha in mixed stands, of which 0.2 million ha are with *Pinus nigra* Arn. ssp. *salzmanii*, 183,184 ha with *P. pinaster* Ait. and 84,847 ha with *Quercus ilex* (Montero et al., 2001) (Fig. 1a).

1.2.2. Maritime pine (Pinus pinaster Ait.)

Maritime pine (*Pinus pinaster)* is a characteristic species in Mediterranean forests, and is mainly located in the Iberian Peninsula (Blanco et al., 1997). This species shows a wide ecological range (Nicolás and Gandullo, 1967), and it can survive under high or low temperatures, under regular or variable rainfall as well as under severe droughts. It is adapted to the extremely cold winters of the continental climate in central Spain and to the mild, temperate winters of the Atlantic coast in the western Iberian Peninsula (Alía et al., 1996; Blanco et al., 1997).

In Spain, *P. pinaster* constitutes pure and mixed woodlands that are both natural (1 million ha) and planted (0.6 million ha) (DGCN, 2002). These woodlands are mainly spread over the northern Spanish plateau up to 1500 m in elevation. In this area, the climate is characterized by irregular between- and within-year rainfall, and high temperatures in summer. The soils are sandy and rocky and very well drained. Even though the maritime pine had been widely plantated in the Iberian Peninsula, this species grows naturally in some areas, where it is the best adapted woody species (Blanco et al., 1997). Fossil *P. pinaster* cones and pollen dating from the Superior Pliocene have been found in the Iberian Peninsula, suggesting a Pre-Mediterranean origin for this species and evolution in tropical-like environments that gradually incorporated seasonal aridity (Specht, 1981). Some authors suggest that the Iberian System is the natural origin of and centre of distribution for this species (Blanco et al., 1997) (Fig. 1b).

1.2.3. Scots pine (Pinus sylvestris L.)

Scots Pine (*Pinus sylvestris*) plays an important role in Spanish forestry because of its economic, ecological and social relevance. It is the most widely distributed species of pine in the world (Blanco et al., 1997); along with Norway spruce (*Picea abies* L.), it possesses the highest dendrochronological potential in Europe (Schweingruber, 1996). It grows in the cold continental climate of northern Asia as well as in the semiarid climate of southern Europe. Forests of *P. sylvestris* in Spain are ai its southern and western distribution threshold and occupy drier areas than in the other parts of the world (Barbéro et al., 1998).

These dry areas of distribution of this species which usually grows in humid environments are the first places to investigate the effects of increased aridity (Martínez-Vilalta and Piñol, 2002). Besides, in assessing the impact of global warming on ecosystems, any changes in tree growth are likely to occur first in those tree stands located at the ecological boundary of the species (Tessier et al., 1997). In Spain, *P. sylvestris* stands occupy 1.2 million ha, split approximately in 50% pure and 50% mixed stands (DGCN, 2002) (Fig. 1c).

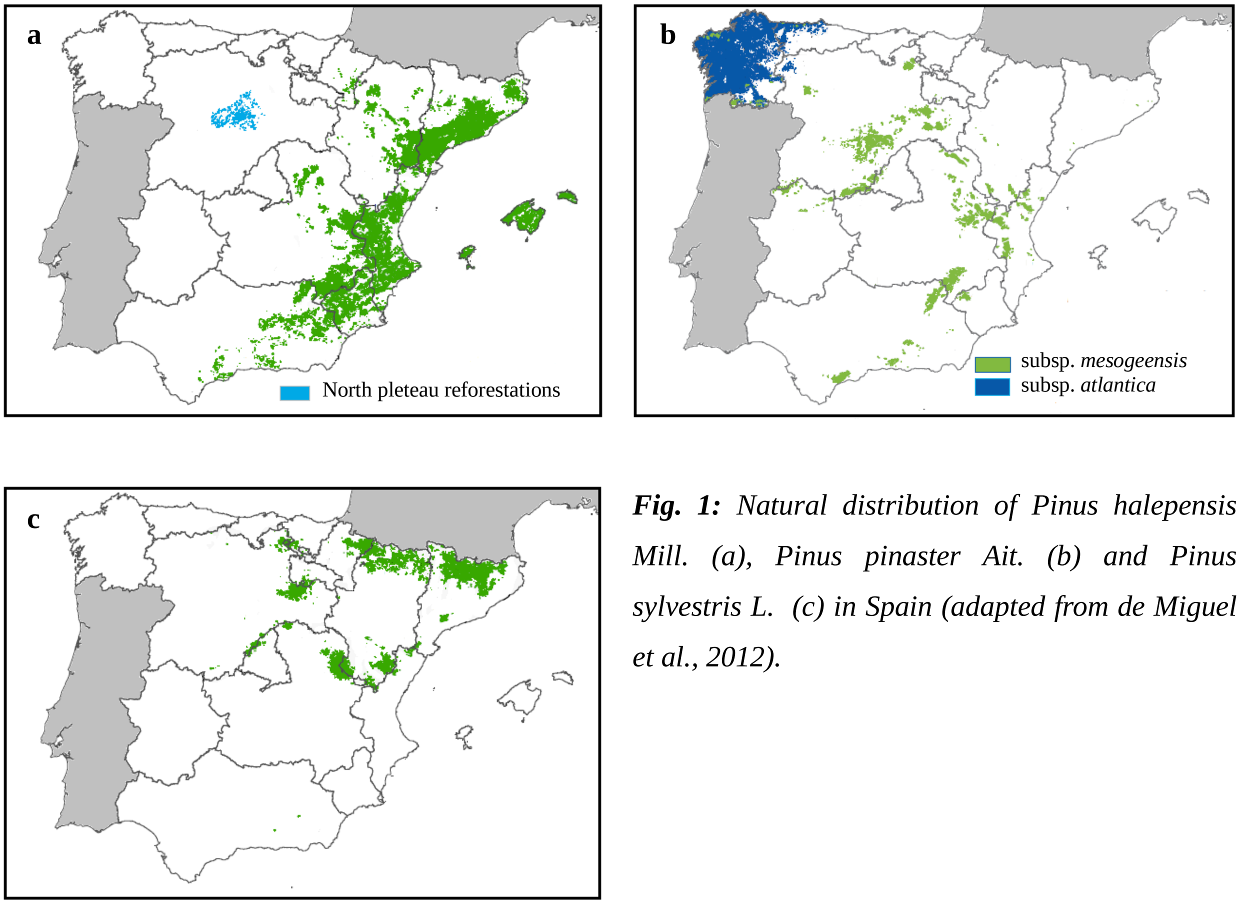


Fig. 1: *Natural distribution of Pinus halepensis Mill. (a), Pinus pinaster Ait. (b) and Pinus sylvestris L. (c) in Spain (adapted from de Miguel et al., 2012).*

1.3. Growth-climate relationships

Kaennel and Schweingruber (1995) definded dendroclimatology as the use of tree rings to study and reconstruct past and present climate. Dendroclimatology starts with site and tree selection and continues with dating, measuring, data quality control, and chronology construction. Tree rings are associated with climate using statistical models that are then evaluated for their full length to reconstruct climate of the past (Sheppard, 2010).

Different methodological approaches can be used to explore tree growth-climate relationships, but dendroclimatological studies are valuable tools in detecting long term changes in radial growth in woody species related to climatic variable response as a result of warmer conditions and precipitation variability (Andreu et al., 2007).

Dendroclimatology generally assumes that climate–growth relationships are age and size independent. However, there is evidence that climate response can be unstable across different species, sites and age/size classes (Tardif et al., 2003; Campelo et al., 2013). Climate change is resulting in both positive and negative trends in tree growth, the latter frequently observed in drought-stressed environments (Camarero et al., 2010). The influence of climatic variables on growth can be modified along time (Andreu et al., 2007) and previous studies showed a changing association between climatic variables and growth of *Pinus* species on the Mediterranean area (Bogino and Bravo, 2008; Vieira et al., 2010; Campelo et al., 2013). Since the mid 20th century, an overall increase of the mean annual temperature, a decrease of the annual precipitation and a higher frequency of severe drought periods have been observed in the Mediterranean area (Martrat et al., 2004; Xoplaki et al., 2006). However, in the western Mediterranean regions, winter and spring precipitation increased and summer precipitation decreased during that period (Bradley et al., 1987; Maheras, 1988; Diaz et al., 1989). Although temperature and precipitation have changed over the last 50 years, it is not clear yet how trees respond to these changes, how these responses vary along time and which anatomical structures, if any, can be used to document tree response to climate change (Vieira et al., 2010).

1.4. Intra-annual density fluctuations (IADFs)

Pine species have a typical conifer wood structure, containing resin canals and clearly distinguishable tree rings with earlywood (EW) and latewood (LW), and a more or less gradual transition between them (Schweingruber, 1993). Wood anatomical features in tree rings have been interpreted as indicators of environmental change (Briffa et al., 2004). However, species growing under Mediterranean climate, with summer droughts and high inter-annual variability in precipitation and temperature, commonly show deviations from such normal structure characterized by abrupt changes in ring width, variable frequency of normal and of traumatic resin canals, and intra annual density fluctuations (IADFs) (de

Luis et al., 2007).

IADFs are defined as “a layer of cells within a tree ring identified by different shape, size and wall thickness” (Kaennel and Schweingruber, 1995). IADFs are easily distinguishable from annual tree ring boundaries by the gradual transition in cell size and wall thickness at the outer borders of these anatomical structures. IADFs are identified by considering the position of the density fluctuation within the ring (Campelo et al., 2007). The IADF type E are characterized by latewood-like cells within earlywood, whereas the IADF type L are considered when earlywood-like cells are within the latewood (study **II**, Fig. 3).

The inclusion of IADFs in dendrochronological studies allows detailed analysis of climatic events within a particular growing season. Different studies of pine species showed a good correlation between IADF formation and climate in the Mediterranean area. IADFs were mainly correlated with precipitation in autumn in *P. pinaster* in Portugal (Vieira et al., 2009) and with precipitation in late winter and spring and higher temperatures in central Spain (Bogino and Bravo, 2009). IADFs were caused by precipitation events early in summer following a water deficit early in the growing season in *P. pinea* in southern Portugal (Campelo et al., 2007).

The consistency of the climatic signal among different pine species and areas suggests that, in the future, a large-scale network of IADFs could be developed in the Mediterranean region to study intra-annual climate variability (Campelo et al., 2013). The analysis of the occurrence of inter-annual density fluctuations and the stabilized IADF frequency in relation to calendar year allows a more detailed analysis of climatic events within affecting tree rings in the growing season.

1.5. Effects of thinning on growth dynamics and growth-climate relationships

The changing demands of today require a widened scope of forest management. Society is asking for sustainable forestry emphasizing biodiversity and naturalistic forest management. It is of great economic and ecological relevance to know on which sites today's forests are most susceptible to climatic and other environmental changes and hazards (Spiecker, 2003).

The response of tree growth to limiting factors can be influenced by silviculture. The effect of different silvicultural scenarios and stand densities on tree growth response to climate is likely to be a key factor for forest preservation, particularly on the driest sites (Gea-Izquierdo et al., 2009). The influence of tree density on their response to climate and drought is not a straightforward issue, since different approaches (e.g. ecophysiological, dendroecological, growth modelling) might give different insights (Misson et al., 2003; Moreno and Cubera, 2008). However, increasing resistance of forests will increase economic and social benefits of forests and reduce the risks by maintaining sustainable forestry (Spiecker, 2003).

Thinning experiment sites are the best option to determine adequate thinning alternatives and density targets. However, stand densities can occur in an almost infinite array of combinations, and as a result, it is unreasonable to expect that all possible combinations could be test in field experiment before apply it in operational forestry (Valbuena and Bravo, 2008). Data on growth dynamics is required to understand growth responses in relation to climatic variability at short time scales, the delayed growth responses to climate and the water budget which may not be detected by coarser assessments based on response functions (Gutiérrez et al., 2011). Dendrometers (Fig. 2) are a valuable tool for these studies since they provide short-term non-destructive measurements of radial fluctuations in relation to climate without disturbing the cambium.

Fig. 2: *Stainless-steel band dendrometer (Dendrometer Increment Sensor DB20 EMS Brno).*

1.6. Climate impact on wood density

Changes in forest productivity in Europe indicate that forest growth has altered considerably during the past decades (Spiecker et al., 1996). Future changes in climate could affect not only tree growth, but also tree-ring structure. Wood density is especially important as it is correlated with various physical, chemical, and anatomical features of the wood and thus yields basic information concerning problems of wood technology, forestry and climatology. Tree-rings are regularly composed of earlywood, in which tracheids have relatively large diameters and thin cell walls and latewood, with small tracheids having thick cell walls. Severe conditions during the growing season, such as water stress, may generate the production of latewood-like cells within earlywood or earlywood-like cells within latewood varying density patterns (Schweingruber et al., 1978; Fritts, 2001).

Previous dendroclimatic studies have shown that wood density is highly sensitive to climate variations at the inter-annual time scale (Parker and Henoch, 1971; Schweingruber et al., 1978; Conkey, 1979; Briffa et al., 2001; Roderick and Berry, 2001; Bouriaud et al., 2005) and intra-annual time scale (Sass and Eckstein, 1995; Horacek et al., 1999; Schmitt et al., 2000; Mäkinen et al., 2003; Bouriaud et al., 2005; Büntgen et al., 2010; van der Maaten et al., 2012), but little is known about intra-annual density-climate relationships in the Mediterranean, where the cambial activity can vary from year to year presenting continuous radial growth or one or two growth interruptions in line with the variability of climatic conditions (de Luis et al., 2007). Previous studies in Spain concluded that the growth of *P. halepensis* and *P. pinaster* was influenced by wet periods during winter previous to the growth season and spring, while the growth of *P. sylvestris* was mostly influenced by summer precipitation (Bogino and Bravo, 2008; Bogino et al., 2009; Olivar et al., 2012). However, there were no previous detailed studies on the impact of climate on wood density in these species.

1.7. Objectives

This thesis has a main objective: to explore the impact of climate on pine trees growing in Mediterranean conditions. This general objective assess through the following specific objectives.

1.7.1. Climate impact on growth dynamic and intra-annual density fluctuations in Aleppo pine (Pinus halepensis) trees of different crown classes: Study I

The knowledge of the growth response of *P. halepensis* to past climate variability can help us to explore how its populations, and the ecosystems they dominate, will behave in the future. Besides reflecting changes in the environmental conditions during the growing season, the inclusion of IADFs detection in chronologies adds new information to ring-width chronologies improving its quality and allows detailed analysis of climatic events within the growing season. The specific objectives of this study were: i) to identify the relationships between radial growth and regional climate for different crown classes of *P. halepensis*, ii) to quantify the presence of intra-annual density fluctuations (IADFs) according to crown class and cambial age and iii) to analyse the relationships between IADFs occurrence and climate.

1.7.2. Changes in climate-growth relationships and IADF formation over time in Mediterranean pine species (Pinus halepensis, P. pinaster and P. sylvestris) in Spain: Study II

The analysis of the responses of Mediterranean pine species to climate change and which anatomical structures can be used to document it will help understand future trends in climate-growth relationships. The specific objectives of this study were: i) to identify and compare radial growth-climate relationships along time for *P. halepensis*, *P. pinaster* and *P. sylvestris* in Spain ii) to quantify and compare the presence of intra-annual density fluctuations (IADFs) on the three species and iii) to define the climatic variables that are associated with the occurrence of IADFs.

1.7.3. Effects of thinning on growth dynamics and growth-climate relationships in Aleppo pine (Pinus halepensis) trees of different crown classes: Study III

Silvicultural practices have an impact on growth-climate relationships. Since a warming trend and a precipitation decline have been predicted for the forthcoming decades, this may have a significant importance on future forest dynamics in Iberian pine forests at a regional scale. The specific objetives of this study were: i) to identify the main climatic driver of intra-annual growth of *P. halepensis* in our site, representing semi-arid Mediterranean environments ii) to analyse which crown class is more affected by water stress and iii) to investigate if the reduction of stand density through thinning decreases its vulnerability to water stress.

1.7.4. Climate impact on wood density in Pinus halepensis, Pinus pinaster and Pinus sylvestris: Study IV

The study of wood formation and its dependence on climatic conditions is of major interest because tree physiological processes, wood density and wood properties are interrelated. The specific objectives of this study were: i) to identify the main climatic drivers of radial growth and density patterns for *P. halepensis*, *P. pinaster* and *P. sylvestris;* and ii) to analyse the relationships between tree-ring width and wood density in the three species.

Materials and Methods

2. MATERIALS AND METHODS

2.1. Study sites

In order to achieve the general and specific objectives of each study, 28 study areas (8 *P. halepensis* sites, 9 *P. pinaster* sites and 11 *P. sylvestris* sites) were selected in Spain (Fig. 3, Table 1).

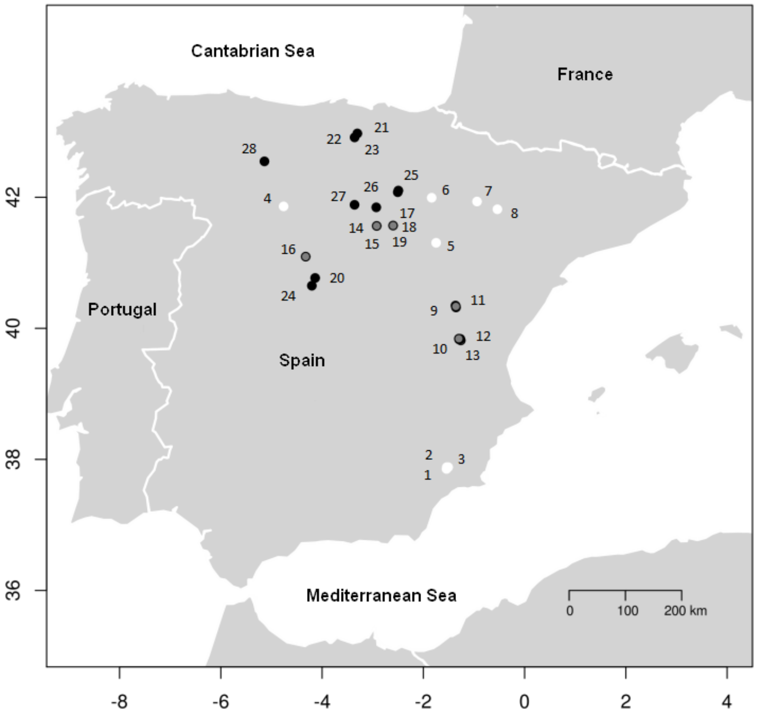


Fig. 3: *Study areas of the three pine species in Spain. White: P. halepensis; grey: P. pinaster; black: P. sylvestris.*

In study **I**, we analysed increment cores from 8 *P. halepensis* sites (tags 1 to 8 in Fig. 3). Fifteen dominant and fifteen suppressed trees were selected in each site. In study **II**, we analysed increment cores from 8 *P. halepensis* sites (tags 1 to 8 in Fig. 3), 10 *P. pinaster* sites (tags 9 to 15 and 17 to 19 in Fig. 3) and 8 *P. sylvestris* sites (tags 20 to 23 and 25 to 28 in Fig. 3). Fifteen dominant trees were selected on each site. In study **III**, a thinning experiment was developed in a *P. halepensis* study site (tag 4 in Fig. 3), where 48 trees were selected (6 dominant and 6 suppressed trees in each of the 4 thinning treatments).

Table 1: *Sampling sites description of P. halepensis, P. pinaster and P. sylvestris in Spain. Precp: Annual precipitation; Temp: Mean annual temperature.*

Species	**Number**	**Location**	**Latitude (N)**	**Longitude (W)**	**Altitude (m)**	**Time span**	**Precip. (mm)**	**Temp. (ºC)**
P. halepensis	1	Murcia	37°52´51´´	1°30´36´´	811	1932-2008	617	12.7
	2	Murcia	37°52´50´´	1°32´15´´	957	1915-2008	617	12.7
	3	Murcia	37°51´13´´	1°32´34´´	1118	1914-2008	617	12.7
	4	Palencia	41°51´36´´	4°45´36´´	849	1975-2008	441	12.1
	5	Zaragoza	41°18´16´´	1°44´52´´	976	1978-2008	344	12.3
	6	Zaragoza	41°59´31´´	1°50´09´´	695	1975-2008	395	13.1
	7	Zaragoza	41°48´58´´	0°32´15´´	535	1919-2007	616	12.4
	8	Zaragoza	41°56´04´´	0°56´25´´	706	1926-2007	587	12.4
P. pinaster	9	Cuenca	39°50´17´´	1°16´11´´	970	1880-2005	901	8.3
	10	Cuenca	39°48´56´´	1°15´36´´	920	1948-2005	901	8.3
	11	Cuenca	39°50´06´´	1°16´37´´	1078	1948-2005	901	8.3
	12	Cuenca	39°49´48´´	1°17´38´´	1010	1978-2005	901	8.3
	13	Cuenca	39°50´28´´	1°17´54´´	1090	1887-2005	901	8.3
	14	Soria	41°34´03´´	2°35´51´´	1059	1918-2005	487	8.3
	15	Soria	41°33´43´´	2°55´17´´	1012	1948-2005	484	10.2
	16	Segovia	41º23’47”	4º28’17”	753	1939-2012	465	11.2
	17	Teruel	40°19´07´´	1°21´18´´	1437	1846-2005	563	10.4
	18	Teruel	40°20´47´´	1°21´54´´	1364	1849-2005	563	10.4
	19	Teruel	40°20´01´´	1°21´26´´	1232	1953-2005	563	10.4
P. sylvestris	20	Avila	40°38´58´´	4°12´7´´	1438	1813-2005	559	7.5
	21	Burgos	42°58´22´´	3°18´12´´	888	1867-2005	632	9.2
	22	Burgos	41°53´4´´	3°21´32´´	1097	1848-2005	487	10.2
	23	Burgos	42°54´46´´	3°21´27´´	814	1935-2005	527	10.3
	24	Segovia	40°46´4´´	4°08´14´´	1440	1891-2005	466	9.7
	25	Soria	42°6´6´´	2°29´36´´	1165	1951-2005	487	10.2
	26	Soria	42°4´36´´	2°30´18´´	1431	1960-2005	487	10.2
	27	Soria	41°50´44´´	2°55´48´´	1659	1946-2005	487	10.2
	28	León	42º48’10”	5º07’31"	975	1989-2012	898	10.0

In study **IV**, we analysed increment cores from one *P. halepensis* site (number 4), one *P.*

pinaster site (number 16) and one *P. sylvestris* site (number 24).

Dominant trees were defined as those standing above all other trees in its vicinity and receiving full light from above, whereas suppressed trees were defined as those growing below the tree canopy. Two cores were extracted at 1.30 m above ground from each selected tree.

Mean monthly air temperatures and total monthly precipitations recorded at the closest meteorological stations were obtained from the "Agencia Estatal de Meteorología, Spain", while daily climatic variables (temperature, precipitation, air humidity, wind speed and radiation) were obtained from *www.inforiego.org* (Table 2).

Table 2: *Description of the meteorological stations. Data provided by Agencia Estatal de Metereología (AEMet) and InfoRiego(IR).*

Meteorological Station	**Time span**	**Location**	**Latitude (N)**	**Longitude (W)**	**Altitude (m)**	**Data**
Moratalla "Benizar"	1934-2008	Murcia	38º16’25”	1º58’59”	899	AEMet
Palencia E.C.A.	1974-2008	Palencia	42º00’30”	4º33’27”	740	AEMet
Fuentes de Nava	2008-2012	Palencia	42º04’59”	4º46’59”	472	IR
Calatayud aguas	1975-2003	Zaragoza	41º19’51”	1º38’49”	600	AEMet
Tarazona	1977-2008	Zaragoza	41º54’28”	1º43’54”	475	AEMet
Sos del Rey Católico	1933-2007	Zaragoza	42º29’34”	1º12’52”	630	AEMet
Cuenca	1956-2005	Cuenca	40º04’35”	2º07’53”	956	AEMet
El Burgo de Osma	1932-2005	Soria	41º35’08”	3º04’17”	895	AEMet
Soria (Observatorio)	1944-2005	Soria	41º45’60”	2º28’00”	1082	AEMet
Villanubla	1939-2011	Valladolid	41º42’41”	4º50’41”	846	AEMet
Pantano de la Toba	1944-2005	Teruel	40º12’60”	1º55’60”	900	AEMet
Aldea del Rey Niño	1935-2005	Ávila	40º34’60”	4º44’60”	1160	AEMet
Miranda de Ebro	1936-2005	Burgos	42º40’49”	2º57’08”	520	AEMet
Villafría	1943-2005	Burgos	42º21’00”	3º36’60”	890	AEMet
Boñar	1990-2006	León	42º45’00”	5º19’22”	975	AEMet

2.2. Dendrochronological analysis (studies I and II)

In studies **I** and **II**, 8 *P. halepensis* sites (15 dominant and 15 suppressed trees per site), 10 *P. pinaster* sites (15 dominant trees per site) and 8 *P. sylvestris* sites (15 dominant trees per site) were selected (Fig. 3, Table 1) and 2 increment cores were extracted from each selected tree. A total of 960 increment cores of *P. halepensis* (420), *P. pinaster* (300) and *P. sylvestris* (240) were analysed. The increment cores were air dried, mounted on wooden supports and dated according to standard dendrochronological techniques (Stokes and Smiley, 1996).

In order to assess the quality of crossdating and measurement accuracy of tree -ring series, the COFECHA program (Holmes, 1992) was applied. This program calculates the Pearson correlation indices between the indexed tree-ring series and a master reference chronology in a series of consecutive, partially overlapped segments of a length specified by the user. All series with potential dating errors or weakly correlated with the master site chronology were corrected when possible or discarded. According to standard dendrochronological methods, tree-ring series exhibiting correlation values with the master chronology below 0.4 were excluded. The proper use of COFECHA adds a high degree of confidence that tree -ring samples have been crossdated correctly and measured accurately, en- suring that the environmental signal is maximized (Grissino-Mayer, 2001).

The turbo ARSTAN program (Cook and Holmes, 1984; Holmes, 1992) was used to eliminate biological trends in tree-ring series and to minimize growth variations that are not shared by most trees. Standardization removes geometrical and ecological trends while preserving inter-annual high-frequency variations that are presumably related to climate. The long-term trend was removed from each time series of ring width measurements by fitting and calculating an index defined as actual ring-width for each year divided by the curve-fit value. Once the individual series were standardized, they were averaged in order to obtain a master chronology at each study site (one for dominant trees and one for suppressed trees in the case of *P. halepensis*). The master chronologies were examined separately to analyse differences in radial growth between species, sites and crown classes.

Chronology quality was evaluated using the following statistical parameters:

- Mean sensitivity (MS): a measure of the mean relative changes between adjacent ring widths (Fritts, 2001).

- Signal-to-noise ratio (SNR): the proportion of the variability explained by climate or other casual factors divided by the residual or unexplained variability (Fritts and Swetnam, 1989).

- Expressed population signal (EPS): the degree to which the particular sample chronology portrays a hypothetically perfect chronology. Values higher than 0.85 imply that the chronologies are representative of tree growth in the stands (Wigley et al. 1984).

The master chronologies were normalized by subtracting the mean and dividing by the standard deviation. Values far from zero mean higher reactions. In the case of *P. halepensis*, the normalized difference index (NDI) was calculated by subtracting the normalized values of the suppressed trees to the normalized values of the dominant trees. Positive NDI values mean stronger reactions from dominant trees, while negative NDI values mean stronger reactions from suppressed trees.

Monthly climatic variables (precipitation sum and mean temperature) measured at the closest meteorological station (Agencia Estatal de Meteorología, Table 2) were divided in climatic seasons: winter previous to the growing season (December-February), spring (March-May), summer (June-August) and autumn (September-November) and regressed against ring-width indices. In order to calculate Pearson correlation coefficients and response functions DENDROCLIM 2002 (Biondi and Waikul, 2004) was applied. Moving correlation function was used to test stationarity and consistency through time with a 20-year interval.

2.3. IADFs occurrence (studies I and II)

The accurately dated cores from studies **I** and **II** were visually examined for IADFs using a stereomicroscope (magnification up to 25x). As opposed to the annual rings, IADFs show a gradual transition boundary between earlywood and latewood cells (Fritts, 2001). IADFs were only considered when present in both cores in the same tree ring, and they were identified by considering the location of the density fluctuation within the ring. Only IADF type E (latewood-like cells within the earlywood) were considered in our study since IADFS type L (earlywood-like cells within the latewood) were rarely present in our sample. As the number of samples changed over time, the relative frequency was calculated with the following formula [1]:

[1] $F = n/N$

where F is the proportion of IADF in a particular year; n the number of trees that formed the IADF and N the total number of trees analysed. The bias in the frequency was assessed by calculating the stabilized IADF frequency (f), according to the formula of Osborn et al. (1997) [2]:

[2] $f = F^{0.5}$

The nonlinear logistic equation form was chosen to model the probability of occurrence of IADFs [3]:

[3] $P = (1.0 + e^{(-z)})^{-1}$

where P is the probability of IADFs and $Z = b0+b1(x1)+b2(x2)+\ldots\ldots+bk(xk) + \varepsilon$; where x1; x2..... xk are the climatic variables and b0; b1; b2 ….. bk are unknown parameters of the model and ε is a normal random error N (0,1); and *e* is the exponential operator. The logistic equation can be formulated to accept a binary variable such as occurrence of IADFs, and the parameters can be estimated by maximum-likelihood methods. The resulting prediction is bounded by 0 and 1. Monthly rainfall and mean monthly temperature were used as explanatory variables. The hydrological year was defined as a period of 12 months, from October of the previous year to September of the current growth year. A stepwise selection method was used to find the best model.

The alternative fits were evaluated on the basis of Akaike information criterion (AIC), the –2*Log Likelihood, the area under the receiver operating characteristic (ROC) curve and the expected behavior - as indicated by the signs of the estimated parameters. ROC curve is displayed for the models and the area underneath was calculated as a value of the accuracy of the model. Value over 0.80 indicates an excellent discrimination (Hosmer et al., 2013). This curve relies on false/true positive/negative tests, and the sensitivity is indicated by the proportion of correctly classified events and the specificity by the proportion of correctly classified non-events (Hair et al., 1998).

Monthly climatic variables (precipitation sum and mean temperature) measured at the closest meteorological station (Agencia Estatal de Meteorología, Table 2) were grouped in climatic seasons: winter previous to the growing season (December-February), spring (March-May), summer (June-August) and autumn (September-November) and regressed against stabilized IADF frequency.

2.4. Effects of thinning on growth dynamics and growth-climate relationships (study III)

Study **III** is based on a thinning experiment conducted in autumn 2010 in a *P. halepensis* plantation located in Ampudia (Palencia) (study site number 4; Fig. 3, Table 1) (study **III**, Fig. 1, Table 1). The thinning experiment consisted of four 40m x 50m stands of similar basal area and stand structure were three thinning intensities were tested: 15% reduction of the total basal area (T15), 30% reduction of the total basal area, (T30) and 45% reduction of the total basal area, (T45) using an unthinned stand as control (T0) (study **III**, Fig. 2).

To estimate intra-annual changes in diameter increment, 48 stainless-steel band dendrometers (Dendrometer Increment Sensor DB20 EMS Brno) were mounted around the stem at 1.3 m height in 12 randomly chosen trees per treatment (6 dominant and 6 suppressed). Dominant trees were defined as those standing above all other trees in its vicinity and receiving full light from above, whereas suppressed trees were defined as those growing below the tree canopy. In most cases, the diameter of dominant trees ranged from 20 to 25 cm and suppressed trees from 10 to 15 cm.

Before the installation of the dendrometers, the dead outermost tissue of the bark was smoothly and evenly removed with a rasp before attaching the dendrometer. The barks were removed in order to reduce swelling and shrinkage as a component of the increment dynamics. Dendrometer readings to the nearest 0.01 mm were done biweekly in the morning to reduce diurnal bias because daytime transpiration causes stem shrinkage (Zweifel et al., 2001). Measurements obtained in the first 3 months after installation were discarded for being within the adjustment period of the band dendrometers (Keeland and

Sharitz, 1993).

Daily climatic variables measured at the closest meteorological station (Table 2) were averaged (or accumulated in the case of precipitation) in 5, 15 and 30 days prior to the growth measurements in order to determine which climatic variables were the main constraints for its growth and at which temporal scales they act. These intervals were chosen to detect delayed growth responses to changes of climate.

In addition to the climatic variables, soil water availability to the trees, which can be characterized by relative extractable water (REW), was included in the analysis. REW can be defined as the daily available water standardized by maximum available water. It was calculated by the daily water balance model Biljou© (https://appgeodb.nancy.inra.fr/biljou/) (Granier et al., 1999). This model requires daily potential evapotranspiration and rainfall as input climatic data. Evapotranspiration was calculated with the Penman potential evapotranspiration equation. Required site and stand parameters are maximum extractable soil water and leaf area index (LAI). Soil conditions are considered homogeneous at the four plots (study **III**, Table 2). LAI controls stand transpiration, forest floor evapotranspiration and rainfall interception (Granier et al., 1999). LAI values differ depending on thinning intensities. The comparison between REW and radial increment on the four plots can be observed in study **III**, Fig. 3. Water stress is assumed to occur when REW drops below a threshold of 0.4 under which transpiration is gradually reduced due to stomatal closure (Granier et al., 1999).

In order to test similarity of growth rates prior to thinning among the four plots, increment cores were extracted from a total of 15 trees in the four plots. As preliminary trials, we fitted a linear model using past growth as response variable and a mixed model including a plot random effect. The relative goodness of fit of the models was assessed using the Akaike information criterion (AIC). Since AIC value (4399) did not change when the plot random effect was included, we concluded that the average growth was not different across the plots.

We fitted a linear mixed-effect model using the lme function with R-Package nlme (Laird and Ware, 1982). The model was fitted by maximizing the restricted log-likelihood:

$$Gr_{ijk} = \beta_0 + \beta_1 * Clim_{ik} + \beta_{2\,im} + \beta_3 * S_{ij} + \beta_4 * (Clim_{ik} * S_{ij}) + \beta_{5im} * S_{ij} + b_{ij} + \varepsilon_{ijk}$$

$$b_{ij} \sim N(0, \sigma^2_{Tree})$$

$$\varepsilon_{ijk} \sim N(0, \sigma^2 \, |Clim_{ik}|^{2\delta})$$

where *Gr* is the radial growth, *Clim* are the mean (or accumulated) values of the bioclimatic variables (temperature, precipitation, evapotranspiration and relative extractable water), *i* is the plot index, *j* is the tree index, *k* is the two-week period index, *m* is the thinning intensity index such that:

m = 1 for the 15% thinning intensity

m = 2 for the 30% thinning intensity

m = 3 for the 45% thinning intensity

S is an indicator variable for the status of the tree (*S* = 0 for suppressed trees and *S* = 1 for dominant trees), b_{ij} is a tree random effect and the variance of the residual error terms ε_{ijk} is modelled as σ^2, multiplied by the power of the absolute value of covariate *Clim*. The parameter δ is also estimated using a maximum likelihood estimator (see Zuur et al. 2009).

The period used for growth and climate analysis should corresponded only with the main period of stem growth (Deslauriers et al., 2007). Therefore, the period between 20 November 2011 and 15 April 2012 was excluded from the analysis in our study. Statistical analyses were performed using R software (R Development Core team, 2011).

Climate sensitivity is defined as the degree of growth response to climate variability. Because our growth data are intra-annual, the coefficient of variation (hereafter abbreviated as CV), which shows the extent of variability in relation to the mean, was calculated in order to assess the climate sensitivity of the radial increment series. CV was calculated for the radial growth during the 2-week growth periods on each tree and analysed for trees within status, plots and years.

2.5. Climate impact on wood density (study IV)

In study **IV**, we analysed increment cores from one *P. halepensis* site (number 4; Fig. 3, Table 1), one *P. pinaster* site (number 16; Fig. 3, Table 1) and one *P. sylvestris* site (number 24; Fig. 3, Table 1). 15 dominant trees were selected at each site and 2 increment cores were extracted at 1.3m from each selected tree. The samples were processed following standard techniques (Stokes and Smiley, 1996), conditioned to a 12% water content and resawn in the transverse plane to a thickness of 2 mm. X-Ray negative photographs of the samples were obtained and the resulting X-ray picture was processed with CERD software (Mothe et al., 1998). The wood density profiles obtained are for each ring and are based on 100 measured positions. Density profiles were produced by averaging the values (XRD, kg/m3) obtained from these segments (Bouriaud et al., 2005; Rathgeber et al., 2006).

To remove non-climatic age trends from the raw measurements, tree-ring width series were detrended (standardized) on a modified negative exponential curve using the package dplR (Bunn, 2008) in R. The standardization removes the geometrical and ecological trends while preserving inter-annual high-frequency variations that are presumably related to climate. The long-term trend was removed from each time series of ring width measurements by fitting and calculating an index defined as actual ring-width for each year divided by the curve-fit value.

Mean monthly temperatures and monthly precipitations recorded at the closest meteorological stations (Agencia Estatal de Meteorología, Table 2) were divided in climatic seasons: winter previous to the growing season (December-February), spring (March-May), summer (June-August) and autumn (September-November) and correlated with tree-ring width, earlywood width, latewood width, mean density, maximum density, earlywood density and latewood density.

Results

3. RESULTS

3.1. Dendrochonological study of *P. halepensis* trees of different crown classes (study I)

Two master chronologies were calculated for each sampling site, one for dominant trees and one for suppressed trees. In sites Za1 and Za2 only a master chronology of the dominant trees was calculated (study **I**, Fig. 3). The master chronologies from Ampudia showed higher mean sensitivity (MS) values (0.40 for dominants and 0.37 for suppressed) than the other locations (study **I**, Table 2). The master chronology of the dominant trees in Ampudia also showed higher SNR and EPS (66.08 and 0.98 respectively) than the rest of the locations. The mean chronology of the suppressed trees showed slightly higher mean sensitivity values (0.30 for dominants and 0.33 for suppressed) and higher SNR values (26.64 for dominants and 12.77 for suppressed) than the mean chronology of the dominant trees. The mean chronology of the dominant trees also showed higher variance and mean correlation values than the mean chronology of the suppressed trees (study **I**, Table 3).

The normalized curves are shown in study **I**, Fig. 3. It can be observed that dominant trees have stronger reactions than suppressed trees in favourable years in Ampudia while in Tarazona suppressed trees react stronger than dominant trees in favourable years, the other sites show no patterns. The Normalized Difference Index (study **I**, Fig. 4) showed that, from 1980 to 2000 suppressed trees reacted stronger in Murcia and Aragón, while since 2000 no clear tendency was found.

3.2. Climate-growth relationships over time in Mediterranean pine species (*P. halepensis, P. pinaster* and *P. sylvestris*) in Spain (study II)

Precipitation proved to be the main factor influencing tree growth on the three species. While *P. halepensis* and *P. pinaster* were influenced by wet periods during winter previous to the growth season and spring, *P. sylvestris* was mostly influenced by summer precipitation. *Pinus pinaster* showed the highest correlations ($p<0.005$) between precipitation and growth (r=0.12 in average) (study **II**, Table 2).

The analysis of the influence of the climatic variables over time also differed between the three species. They experienced a shift in the influence of different climatic variables on its growth at the last third of the 20th century. The positive influence of winter, spring and summer precipitation on *P. halepensis* growth increased from the 1990s decade (study **II**, Fig. 3). During that period, spring temperature shifted its influence from negative to positive, while summer temperature shifted from positive to negative. In the case of *P. pinaster* (study **II**, Fig. 3), the highest changes on the influence of the climatic variables on its growth occurred during the 1970s decade, where spring precipitation became its higher influence followed by summer and winter precipitation. Also during that period, winter temperature increased its positive influence; while the influence of spring, summer and autumn temperature became negative. Summer precipitation had the highest correlation values (0.33) on *P.sylvestris* (study **II**, Fig. 4) and remained essentially stable during the study period. Winter and spring temperature also had a positive influence on its growth, while summer temperature shifted its influence from positive to negative around 1980.

3.3. IADF formation over time in Mediterranean pine species (*P. halepensis, P. pinaster* and *P. sylvestris*) in Spain (studies I and II)

In study **I**, a detailed analysis of the IADF formation in *P. halepensis* was carried out. Samples were grouped according to site location (study **I**, Table 4) age (study **I**, Table 5) and crown class (Table 6, **I**). The percentage of trees with IADFs was rather similar for young and old stands. However, the proportion of IADFs and the mean stabilized IADF was higher for young stands than for old stands. The percentage of trees with IADFs and the percentage of IADFs were both higher for suppressed than dominant trees. Mean stabilized IADF was the same for both crown classes.

In study **II**, *P. pinaster* and *P. sylvestris* were included in the analysis in order to have a more global understanding of IADF formation in pine species growing under Mediterranean climate. The nonlinear logistic equation form was chosen to model the probability of occurrence of IADFs in the three species with monthly precipitation and mean monthly temperature as variables. *Pinus pinaster* had the highest accumulated mean stabilized IADF frequency (0.12), followed by *P. halepensis* (0.03). The occurrence of IADFs was mainly influenced by precipitation on the three species. Precipitation in the winter previous to the growing season and spring were associated with the occurrence of IADFs in *P. halepensis*, while this influence was postponed on time in the case of *P. pinaster*, influenced by spring and early summer precipitation. Both species showed a negative influence of precipitation in July. The IADF frequency *P. sylvestris* was the lowest of the three species (0.004). IADF frequency in relation to calendar year (study **II**, Fig. 5) showed an increase in IADF occurrence in the second half of the century. 1961, 1983, 1995 and 1999 were the years with higher of IADF occurrence, with a stabilized frequency higher than 0.8.

3.4. Effects of thinning on growth dynamics and growth-climate relationships in *P. halepensis* trees of different crown classes (study III)

The thinning experiment performed in study **III** revealed soil water content, represented by relative extractable water (REW) as the main bioclimatic variable which influenced tree radial growth of *P. halepensis*. Nonetheless, its influence on growth differed with crown classes. The results of the linear mixed-effect model applied for each variable (temperature, precipitation, evapotranspiration and REW) confirmed that REW averaged for the previous 15 days was the main bioclimatic variable that influenced tree radial growth at the studied stands (Study **III**, Table 3).

We fitted the model with mean REW of the previous 15 days (study **III**, Table 4) as climatic variable. Results showed that dominant trees and trees in T30 and T45 had significantly higher growth rates. The comparison between real growth and predicted values of the model for T0 and T45 are shown in study **III** Fig. 4. Model predictions are more accurate for dominant than suppressed trees and for T45 than T0.

Study **III**, Fig. 5 describes the growth patterns of the four stands. As it is typical for Mediterranean species, the growth dynamics of *P. halepensis* showed a bimodal growth pattern, with two major growth phases in spring and autumn and low rates in late summer and beginning of autumn. The spring maximum increment rate was higher than the autumn maximum increment rate, except for T0 in 2011. The growth rates of the dominant trees (mean=1.23 mm in 2011 and 0.68 mm in 2012) were significantly higher (p<0.05) than the growth rates of the suppressed trees (mean =0.45 mm in 2011 and 0.18 mm in 2012). Both growth rates decreased in 2012. The analysis of the variance of the growth data (study **III**, Fig. 6) showed that suppressed trees had higher CV values than dominant trees. T45 showed the lowest CV values in both crown classes during the two years of study.

3.5. Climate impact on wood density in *P. halepensis, P. pinaster* and *P. sylvestris* (study IV)

Tree-ring width of *P. halepensis* was positively correlated to precipitation during previous winter and spring (p-values>0.005) and spring temperature (p-value>0.05). Tree-ring width of *P. sylvestris* was positively correlated to summer precipitation (p-value>0.05) *P. pinaster* showed no significant correlations between tree-ring width and climatic variables (study **IV**, Fig. 3). Earlywood width of *P. halepensis* was positively correlated to precipitation during previous winter and spring (p-values>0.005), while its latewood width was positively correlated to spring temperature (p-value>0.05) and winter precipitation (p-value>0.05) (study **IV**, Fig. 3).

Mean and maximum density followed different patterns on the three species, decreasing along time in *P. pinaster* and increasing in *P. halepensis* and *P. sylvestris*. *Pinus halepensis* showed higher values of mean density than the other two species and *P. pinaster* showed lowest values of maximum density than the other two species (study **IV**, Fig. 4 and 5). The analysis of the climate-density relationships showed that mean density of *P. halepensis* was negatively correlated to precipitation during previous winter (p-value>0.05) and spring (p-value>0.005) and to spring temperature (p-value>0.05). Mean density of *P. sylvestris* was positively correlated to autumn temperature (p-value>0.05) (study **IV**, Fig. 6). *Pinus pinaster* showed no significant correlations between mean density and climatic variables. Early wood density was negatively correlated to precipitation during previous winter (p-value>0.05) and spring (p-value>0.005) and to spring temperature (p-value>0.05) in *P. halepensis;* positively correlated to summer temperature in *P. pinaster* and positively correlated to spring and autumn temperature in *P. sylvestris* (study **IV**, Fig. 7).

We calculated the growth and density trends of our sample and compared them to 5-year moving averages (study **IV**, Fig. 8 and 9). A decrease in growth rates was observed in the three species, with high variability of the growth data especially during the most recent years. However, the 5-year moving average followed the decreasing patterns on the three species with lower variability. Mean density rates was showed an increase in sensitivity that did not occur in the 5-years moving averages.

Discussion

4. DISCUSSION

4.1. Dendrochonological study of *P. halepensis* trees of different crown classes (study I)

Pinus halepensis showed good correlations between trees growing at the same site and high expressed population signals which suggest a clear response to environmental factors, proving to be a reliable species for dendrochronological studies. In addition, it confirms the tendency of Mediterranean species to develop special anatomical structures (Schweingruber, 1993). We found common radial growth patterns among dominant and suppressed series in the same site. These results agree with previous studies in the Iberian Peninsula suggesting that pine species growing in the southern dendroecological section could have a common growth response to environmental factors (Richter et al., 1991; Bogino and Bravo, 2008).

The descriptive statistics of the chronologies suggest that the tree-ring series reflects one or more associated factors (including climate), and a strong response of radial growth dynamics to changes in climatic conditions shown by the mean sensitivity values (MS) from 0.21 to 0.40 that are higher than the 0.16 to 0.34 values found in previous studies on pine species (*Pinus sylvestris* L., *Pinus nigra* Arnold, *Pinus pinaster* Ait. and *Pinus mugo* ssp. *uncinata* Turra.) in the Iberian Peninsula (Richter et al., 1991; Bogino and Bravo, 2008, Martín-Benito et al., 2008; Bogino and Bravo, 2009; Bogino et al., 2009; Vieira et al., 2009).

The expressed population signal (EPS) ranging from 0.89 to 0.98 is in all cases higher than the critical level of 0.85 suggested by Wigley et al. (1984), which implies that the chronologies are representative of tree growth in the stands. First eigenvector variance ranges from 39.90 to 74.82%, indicating good homogeneity within the same site. It can be concluded that the fourteen mean chronologies have high MS, SNR, EPS and percentage of the variance accounted for by the first eigenvector, suggesting a strong common signal to related-climatic environmental factors. Ampudia showed higher values of MS, SNR and EPS than the other two locations, especially the dominant trees. However, in the regions of Aragón and Murcia the mean suppressed series showed a higher sensitivity than the mean dominant series. There is evidence that suppressed trees suffer greater drought stress because of greater root competition for soil. However, understory trees receive lower solar radiation and higher wind protection by the influence of neighbouring crowns reducing transpiration rates. Therefore, climate sensitivity may be reduced (Bréda et al., 2006; Martín-Benito et al., 2008). In Mediterranean forests tree density is low and the protection effect from the dominant to the suppressed trees is reduced.

4.2. Climate-growth relationships over time in Mediterranean pine species (*P. halepensis, P. pinaster* and *P. sylvestris*) in Spain (study II)

A change in tree-growth pattern and in the climatic response of the studied species was detected since the mid-20th century and linked to an increase in water stress. These temporal trends were in agreement with the observed increase in warmer conditions and in precipitation variability (Andreu et al., 2007). Precipitation is the main factor influencing tree growth of pine species in semiarid Mediterranean conditions (Raventós et al., 2001). The growth of *P. halepensis, P. pinaster* and *P. sylvestris* is mainly controlled by precipitation at different times of the year. Winter and spring precipitation is related positively with tree-ring growth in *P. halepensis* and *P. pinaster,* while the growth of *P.sylvestris* is mostly influenced by summer precipitation. These results are consistent with those of previous studies in *P. halepensis* in Greece and Spain (Papadopoulos et al., 2009; Olivar et al., 2012), *P. pinaster* in Portugal (Vieira et al., 2010; Campelo et al., 2013) and central Spain (Bogino and Bravo, 2008), and *P. sylvestris* at its southern and western distribution limits (Bogino et al., 2009). However, the influence of these climatic variables on the growth of these species changed along the studied period. The positive influence of winter and spring precipitation on *P. halepensis* growth increased from the 1990s decade and the positive influence of spring precipitation on *P. pinaster* growth increased from the 1970s decade, while the positive influence of summer precipitation on *P. sylvestris* growth remained stable. These results agree with previous reports on pine species in the Mediterranean area (Andreu et al., 2007; Bogino and Bravo, 2008; Vieira et al., 2010; Campelo et al., 2013).

Global studies around the Mediterranean basin indicate that winter and spring precipitation increased and summer precipitation decreased during the second-half of the 20th century

(Bradley et al., 1987; Maheras, 1988; Diaz et al., 1989). Mediterranean pines evolved during the Pliocene under tropical-like climate, before the onset of the Mediterranean climate, as a component of the pre- Mediterranean Arcto-Tertiary flora (Verdú et al., 2003; Petit et al., 2005). These species survived to a past gradual increase of aridity during the transition to Mediterranean conditions, which may have led to its characteristic growth plasticity (Chambel et al., 2007). Mediterranean *Pinus* species are considered as a species well adapted to withstand drought by reducing growth as water availability decreases and increasing growth as conditions become favourable (Rathgeber et al., 2005; de Luis et al., 2007; Camarero et al., 2010; Pasho et al., 2012). This increase of winter and spring precipitation combined with the rising hardening of the climatic conditions during summer may have enhanced the importance of precipitation at the beginning of the growing season on the growth of species subject to higher drought stress conditions during summer, such as *P. halepensis* and *P. pinaster*.

Frequently, climate becomes highly limiting to physiological processes for tree species at the edge of their natural range, because trees on these sites are particularly susceptible to climatic variations (Fritts, 2001). Our study was carried out in Spain with *P. sylvestris* at the edge of its phytogeographical distribution area (southern and western distribution limit). Distribution of the forests in Spain is highly determined by the Atlantic and Mediterranean climatic influences. However, most *P. sylvestris* forests are restricted to the mountainous areas where relief causes important variations to the regional climate and water availability is not limited during the whole year (Blanco et al., 1997; Barbéro et al., 1998). Therefore, *P.sylvestris* did not suffer that shift in the influence of the climatic variables.

4.3. IADF formation over time in Mediterranean pine species (*P. halepensis, P. pinaster* and *P. sylvestris*) in Spain (studies I and II)

We found a higher tendency in young *P. halepensis* stands for developing IADFs. These results corroborate previous dendroecological studies which suggested that IADFs were more frequent in younger tree rings (Villalba and Veblen, 1996; Rigling et al., 2001; Bogino and Bravo, 2009; Vieira et al., 2009). This could be due to a faster response of young trees to changing factors (Villalba and Veblen, 1996) and/or to a longer growing season of young trees (Rossi et al., 2008). Since young trees have a different response to environmental factors than old trees, the incorporation of age-dependent differences on the appearance of special ring features such as IADFs and its association to climatic variables in any dendrochronological study provides a useful proxy for complementing and enhancing the dendroclimatological data. In addition, it can give important clues to predict differences on how young and old trees react to climate change.

IADFs may appear at different development phases of the tree-ring formation depending on the time of the year when the stress conditions occurred. IADF type E is triggered by dry periods during spring and early summer. In contrast, IADF type L is triggered by precipitation during late summer and (or) early autumn (Wimmer et al., 2000). Previous studies in the Mediterranean area showed a high frequency of IADFs in latewood (de Luis et al., 2007; Vieira et al., 2010; Rozas et al., 2011; Campelo et al., 2013; Novak et al., 2013;). However, the low frequency of IADF type L and the high frequency of IADF type E observed in our samples indicate a higher occurrence of water stress episodes inhibiting cell division and enlargement during the first part of the growing season. The ability of species to produce different types and forms of cells in different periods may also be interpreted as an important adaptation of trees for maintaining the balance among the capacity to conduct water, resistance to cavitation and mechanical stability (Novak et al., 2013).

The analysis of the climatic influence in IADF occurrence revealed that the formation of IADFs is triggered by above-average precipitation in the previous winter and spring in *P. halepensis* and in spring and early summer in *P. pinaster*, being both negatively influenced by precipitation in July. These climatic conditions (precipitation at the beginning of the growing season and summer droughts) have been increasingly favoured over the second half of the 20th century, explaining the increasing occurrence of IADFs our study area. This result agrees with previous studies that found an increase in IADF frequencies after 1980 in *P. pinaster* in Spain (Bogino and Bravo, 2009) and Portugal (Vieira et al., 2010; Campelo et al., 2013). Despite being at its southern distribution threshold, where a species that usually grows in humid environments could suffer from the effects of increased aridity (Martínez-Vilalta and Piñol, 2002), *P. sylvestris* showed the lowest IADF frequency of the three species on our sample. As pointed out by Battipaglia et al. (2010), the frequency and the triggering climatic factors promoting different anatomical characteristics may vary among populations, depending on different environmental conditions. As previously pointed out in the case of the swift in the influence of the climatic variables, most *P. sylvestris* forests are restricted to the mountainous areas where relief causes important variations to the regional climate and water availability is not limited during the whole year (Blanco et al., 1997; Barbéro et al., 1998). Therefore, *P.sylvestris* did not suffer the triggering climatic conditions for IADF occurrence.

4.4. Effects of thinning on growth dynamics and growth-climate relationships in *P. halepensis* trees of different crown classes (study III)

The thinning experiment performed in study **III** revealed that the most intense thinning treatments (30 % and 45% removal of the basal area) induced a growth release on both crown classes that did not occur under less intense thinning treatments. 15% treatment was not intense enough to increase growth of dominant trees. Thinning is less effective at dry sites if it is not intense enough, because inter-tree competition for water is stronger, so that site conditions cannot support high-density stands (Moreno and Cubera, 2008; Cotillas et al., 2009; Linares et al., 2009). Thus, a still high competition level that is not sufficiently removed by thinning, coupled with the more limiting climatic conditions, should have obscured the advantages of thinning on less intense thinning treatments (Misson et al., 2003). Growth variability was higher in suppressed than in dominant trees. 45% treatment showed the lowest coefficient of variation (CV) values in both crown classes, which means higher homogeneity of the growth data. These results are in agreement with the idea that in semi-arid environments, suppressed trees usually have more limited access to soil water reserves than dominant trees, due to their shallower and less developed root systems, especially in high density stands. Therefore, their growth rates fluctuate depending on short term water reserves concentrated in surface soil layers, while dominant trees with more developed root systems and suppressed trees released from competition show more homogeneous growth rates.

Adequate stand density levels determination is a difficult task that involves biological, technological, economical and operational factors drive by species, site and management frameworks. Mediterranean species as *P. halepensis* did not have a high productivity, and this implicates than the management costs must be minimized to obtain equilibrate economic balance. Mediterranean pine stands in Spain show high, aesthetic, biodiversity values that are key factors in forest management but have no market price, due to low productivity. Simple, cheap and easy-to-use tools are needed to help managers to fulfil society's demands and management objectives (Valbuena and Bravo, 2008). In addition, with rising living standards, protective, environmental, social and cultural functions became more important. Changing social demands today require a widened scope of forest management and principles of sustainable forest management (Spiecker, 2003).

The reduction of stand density through thinning has been suggested to improve the resistance of individual trees to drought stress and the extent of this differential response changes within the same species along climatic gradients (Misson et al., 2003; Moreno and Cubera, 2008; Gea-Izquierdo et al., 2009). However, this conclusion should not be extrapolated to generalise that open stands are always desirable (Gea-Izquierdo et al., 2009). Forest managers should adequate stand density to mitigate the effect of climatic extremes taking other ecosystem parameters such as regeneration or soil protection into account. Less dense and more diverse stands could be promoted by mixing species with different drought resistance to avoid or limit further growth declines in plantations.

The growth dynamics of *P. halepensis* had two major growth phases during the growth period, one in spring and another in autumn, interrupted by a period with low or no cambial activity during late summer as a result of drought and high temperatures (Cherubini et al., 2003). This pattern of xylem production is responsible for the frequent formation of intra-annual density fluctuations observed in this species (study **I**). Stem growth started at middle March in 2001 and April in 2012, when temperatures were high enough for vegetative growth, and stopped growing at the end of November in both years, when temperatures were too low. The radial growth pattern of *P. halepensis* was asymmetrical with most of the annual growth occurring in spring. The maximum growth rate in spring was the highest of the year except for the control treatment in 2011, where the autumn maximum growth rate was higher. Growth patterns of *P. halepensis* showed high-plasticity in response to climatic conditions during the growing period, which may be an advantage in areas with a Mediterranean climate where the annual rainfall pattern is variable (Gutiérrez et al., 2011).

Pinus halepensis has been characterized as a sensitive Mediterranean species based on the strong response of growth dynamics to changes in climatic conditions (Lev-Yadun, 2000). It has also been suggested that the cambium is able to remain active throughout the whole year when climate conditions are favourable (Liphschitz et al., 1984).

Relative extractable water (REW) was the main bioclimatic variable which influenced tree radial growth at the studied stands. These results add information to studies **I** and **II** that defined precipitation during previous winter and spring as the main climatic driver of its growth. The utilisation of soil moisture, rather than precipitation, is a way to reduce the structural difference between water and temperature variables, and thus to improve the "behaviour" of response functions. Furthermore, soil moisture has more biological meaning than precipitation because it better reflects water availability for trees. These points are particularly relevant for studies performed in Mediterranean regions (Rathgeber et al., 2005).

In the Mediterranean area with a continuous water deficit, at least for *P. halepensis*, wood formation reflects environment conditions for most part of the year, and a precipitation event is generally followed within a time less than a week, by an increase of growth rate (Attolini et al., 1990). Water availability is expected to be the major limiting factor for growth under a Mediterranean climate and its influence decreases with altitude in the Mediterranean region, with temperature showing the opposite trend (Cherubini et al., 2003). There is a general idea that radial growth is primarily dependent on the short-term water availability, which affects the actual tree water status and xylogenesis (Eilmann et al., 2009). In the studied stands, the influence of REW on dominant and suppressed trees was different. In drought stressed environments, suppressed trees usually have shallower and less developed root systems than dominant trees, and less moisture is available to them (Van Lear and Kapeluck, 1995). Therefore, the amount of available water for suppressed trees is limited to the short term water reserves concentrated in surface soil layers; while dominant trees, with more developed root systems can access to deeper soil water reserves accumulated in longer terms (Camarero et al., 2010), inducing higher vulnerability of suppressed trees to water stress.

4.5. Climate impact on wood density in *P. halepensis, P. pinaster* and *P. sylvestris* (study IV)

Mean and maximum wood density levels increased outwards from the pith to the stem surface in *P. halepensis* and *P. sylvestris,* as found earlier by Kellomäki et al. (1999). However, *P. pinaster* showed a more linear decrease in mean and maximum densities. Büntgen et al. (2010) suggested that this decrease might be related to (i) a systematic decrease in the ratio between cell wall size and lumen area with increasing tree age, or alternatively (ii) that the annual lignin content decreases with age.

P. halepensis mean density was inversely correlated to precipitation in the previous winter and spring, the climatic conditions that enhanced its radial growth. Rainfall in the early part of the growing season extends the production of earlywood, resulting in a lower density wood (Wimmer and Downes, 2003). Therefore, the negative effect of precipitation on wood density is accordingly consistent with its positive effect on radial growth. Gilmore (1968) also concluded that factors, which extend the production of earlywood, result in lower wood density wood. During the first part of the growing season, climatic variations affect radial enlargement, whereas during the later part of the growing season, climatic variations only affect the cell wall thickening process (Bouriaud et al., 2005; Büntgen et al., 2010). Detailed experiments showed that maximum tracheid production corresponded to maximum day length, thus allowing cell wall formation and lignification to be completed by the end of summer (Rossi et al., 2006).

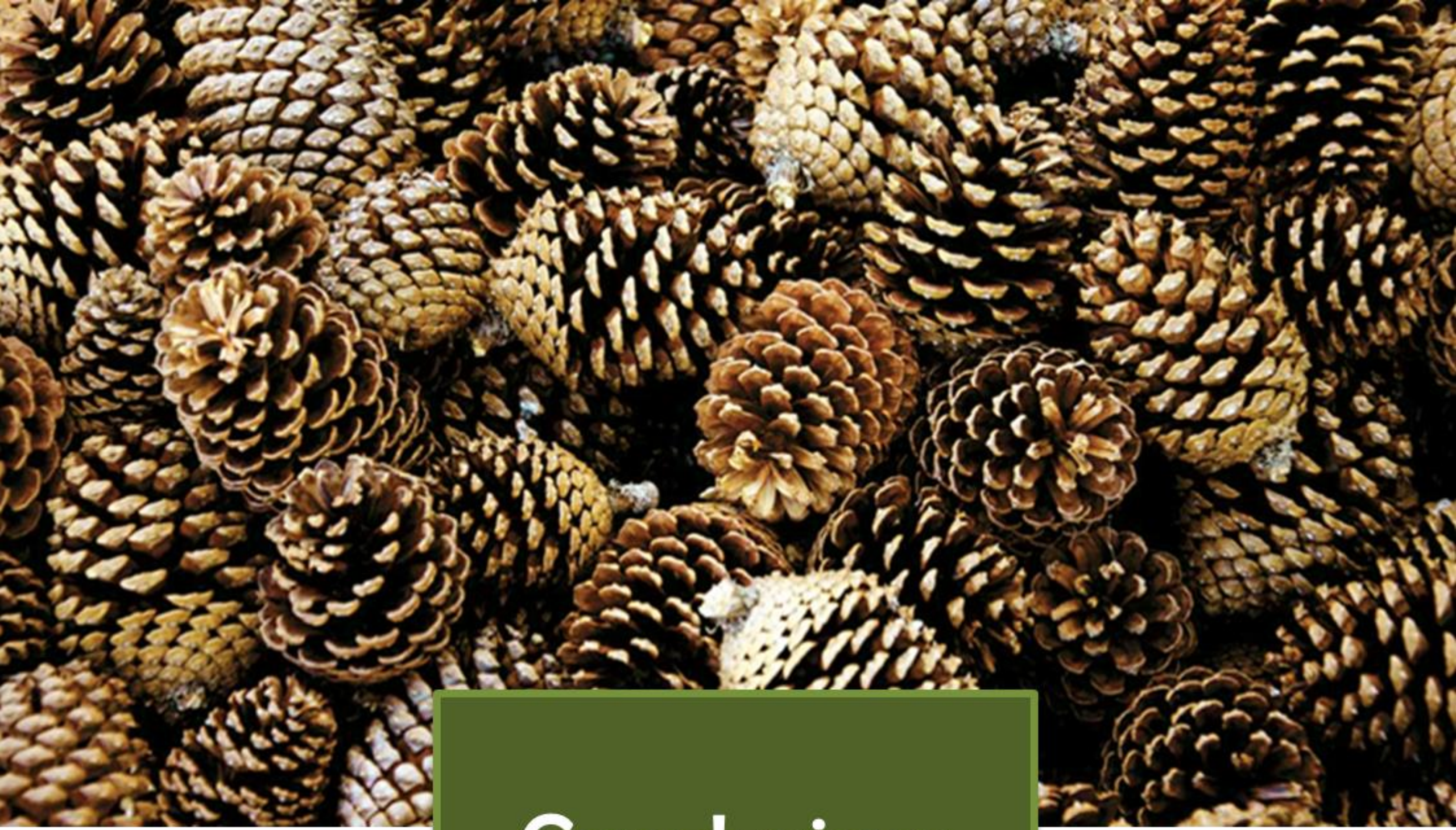
Bouriaud et al. (2005) found a strong positive correlation between soil water deficit and wood density, which they attributed to temperature-mediated reductions in the radial-growth rates due to an increasing soil-water deficit. Cell wall thickness variations play a major role in the wood density variations (Decoux et al., 2004). Rathgeber et al. (2006) suggested that wood density increase is mainly due to cell-wall thickening in tangential and radial directions as well as cell size reduction in radial direction. The reduction in tracheid radial enlargement might also be associated with thicker cell walls (Linares et al., 2009).

There is a general understanding that water stress has a major impact on various aspects of wood formation through direct effects of cell turgor as well as changes in hormonal balances and carbohydrate storage (Kramer, 1964). Similar effects of water availability on wood density were also observed for *Picea abies* (Wimmer and Downes, 2003; Bouriaud et al., 2005), *P. halepensis* and *P. pinaster* (studies **I** and **II**), where wood density was found to increase in association with increased drought stress, creating a false ring. The positive effect of temperature on wood density is a consequence of its negative effects on radial growth rate (D'Arrigo et al., 1992; Yasue et al., 2000; Bouriaud et al., 2005).

Maximum density of *P. halepensis* was positively correlated with climatic conditions favoring tree ring width, agreeing with the idea that favorable years with good climatic conditions at the beginning of the growing season increase the length of the growing period and, therefore, the duration of tracheid maturation, which determines the final cell wall thickness (Wodzicki, 1971; Horacek et al., 1999), and the wall thickness of the latewood cells is the most important factor affecting the tree-ring maximum density (Yasue et al., 2000; Linares et al., 2009).

Especially for coniferous species, relationships between maximum latewood density and climatic variables such as late summer temperature have been established, and are widely used for climate reconstructions (Parker and Henoch, 1971; Schweingruber et al., 1978; Conkey, 1979; D'Arrigo et al., 1992; Briffa et al., 2001; Büntgen et al., 2010). However, these studies were conducted in temperate regions and not in the Mediterranean region. In our study only *P. sylvestris*, growing in higher altitude and having more favorable water conditions showed a positive correlation between maximum latewood density and summer temperature, while *P. halepensis* and *P. pinaster*, subjected to summer water stress showed negative correlations between maximum latewood density and summer temperature. These negative correlations may be due to the low or inexistent growth rates of the trees in that period.

Due to the observed changes in growth-climate associations of pine species during the last part of the 20th century (study **II**) we calculated the variance of the growth and wood density trends of our sample and compared them to a 5-year moving average (the typical modeling horizon) and a 5-year moving weighted average respectively. An increase in the climate sensitivity was observed in both trends during the last years, producing high irregularity in tree ring width and mean density. However, this irregularity is not observed in the 5-year moving interval, where low growth rates of some years are compensated with high growth of the surrounding years. These results indicate that, despite the increasing climate sensitivity of pine species during the most recent years, the trend in growth and mean density does not change in a 5-year interval. However, these inter-annual variability could have an effect on wood properties producing a loss of wood quality (Persson et al., 1995).



Conclusions

5. CONCLUSIONS

The following conclusions can be drawn from the studies that compose this thesis:

- *Pinus halepensis* showed good correlations between trees growing at the same site, high expressed population signals and accurate statistical values which indicate a clear response to environmental factors, proving to be a reliable species for dendrochronological studies.

- The growth of *P. halepensis, P. pinaster* and *P. sylvestris* is mainly controlled by precipitation at different times of the year. Winter and spring precipitation is related positively with tree-ring growth in *P. halepensis* and *P. pinaster*, while the growth of *P. sylvestris* is mostly influenced by summer precipitation.

- The formation of IADFs is triggered by above-average precipitation in the previous winter and spring in *P. halepensis* and in spring and early summer in *P. pinaster*, being both negatively influenced by precipitation in July.

- A change in tree-growth pattern and in the sensitivity of climatic response of *P. halepensis* and *P. pinaster* has been detected since the mid-20th century and linked to an increase in water stress. *Pinus sylvestris*, restricted to the mountainous areas where water availability is not limited during the whole year, did not suffer that shift in the influence of the climatic variables.

- Radial growth of *P. halepensis* was mainly controlled by soil water availability during the growing season and differed across the crown class.

- The most intense thinning treatments (30 % and 45 % removal of the basal area) reduced the effects of water limitations on individual tree growth.

- Climatic conditions promoting the increase in radial growth produced low mean wood density values in *P. halepensis*. However, the same climatic conditions produced high maximum wood density values.

- *Pinus sylvestris*, growing in higher altitude and having more favorable water conditions showed a positive correlation between maximum latewood density and summer temperature; while *P. halepensis* and *P. pinaster*, subjected to summer water stress producing low or inexistent growth rates of the trees in that period, showed negative correlations.

- Despite the increasing climate sensitivity of pine species during the most recent years, the trend in growth and mean density does not change in a 5 year interval (the typical modeling horizon) so modeling predictions are not affected. However, these inter-annual variability could have an effect on wood properties producing a loss of wood quality.

5. CONCLUSIONES

A continuación se presentan las conclusiones más importantes derivadas de cada uno de los estudios realizados:

- *Pinus halepensis* mostró altas correlaciones entre árboles del mismo rodal, altos valores de la señal de población expresada y valores estadísticos que indican una clara respuesta a factores ambientales, demostrando ser una especie con alto potencial para estudios dendrocronológicos.

- El crecimiento de *P. halepensis, P. pinaster* y *P. sylvestris* está fundamentalmente controlado por las precipitaciones en distintos periodos del año. Las precipitaciones durante el invierno previo y la primavera están positivamente correlacionadas con el crecimiento en *P. halepensis* y *P. pinaster*, mientras que el crecimiento de *P. sylvestris* está fundamentalmente influenciado por las precipitaciones durante el verano.

- La formación de IADFs está asociada a altas precipitaciones durante el invierno previo y la primavera en *P. halepensis,* y a las precipitaciones en primavera y el principio del verano en *P. pinaster*, siendo negativamente influenciadas por las precipitaciones en el mes de Julio en ambas especies.

- Se ha detectado un cambio en los patrones de crecimiento y en la sensibilidad climática de *P. halepensis* y *P. pinaster* a partir de 1980 ligado a un aumento del estrés hídrico. *Pinus sylvestris*, restringido a áreas de montaña donde la disponibilidad de agua es constante durante todo el año, no ha sufrido ese cambio.

- El crecimiento radial de *P. halepensis* estuvo fundamentalmente controlado por la disponibilidad de agua en el suelo durante el period vegetativo, pero su efecto varia dependiendo de la clase de copa.

- Los tratamientos de clara más intensos (eliminación del 30 % y 45 % del área basimétrica) disminuyeron el efecto del estrés hídrico en el crecimiento radial de los árboles.

- Las condiciones climáticas que fomentaron un aumento en el crecimiento radial de P.

halepensis también produjeron un descenso de su densidad media. Sin embargo, las mismas condiciones climáticas produjeron un aumento de su densidad máxima.

- Pinus sylvestris, al crecer en mayor altitud y tener unas condiciones hídricas más favorables que las otras dos especies, mostró una correlación positiva entre la densidad máxima de la madera tardía y la temperatura del verano; mientras que *P. halepensis* y *P. pinaster*, con bajos niveles de crecimiento en verano debido al estrés hídrico, mostraron correlaciones negativas.

- A pesar del aumento de la sensibilidad climática durante los últimos años, los patrones de crecimiento y densidad no varían en un intarvalo de 5 años (el típico horizonte de modelización) por lo que las predicciones de los modelos no se ven afectadas. Sin embargo, esas variaciones inter-anuales pueden afectar las propiedades de la madera produciendo un descenso de su calidad.

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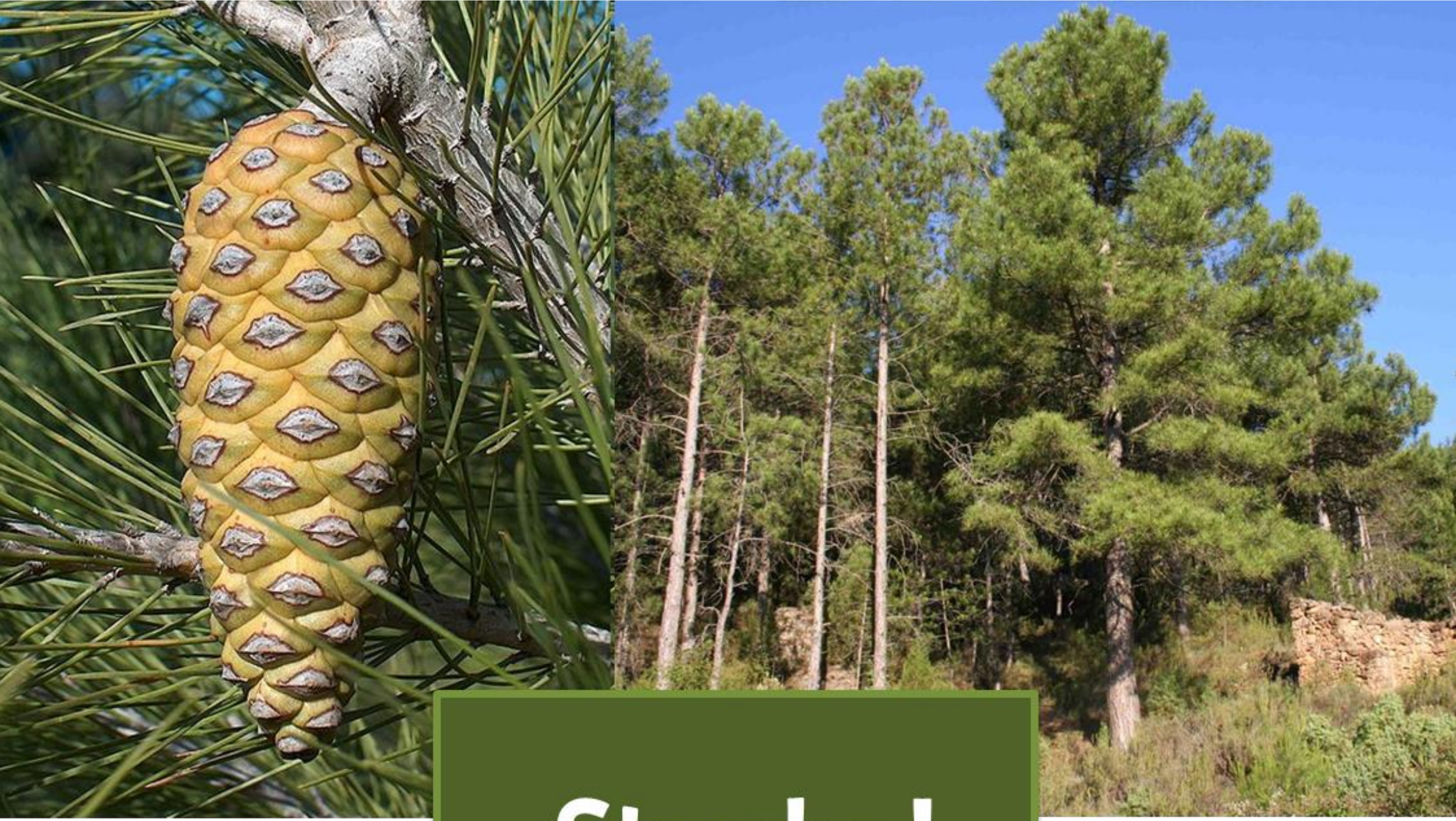
Studies

Study I: Climate impact on growth dynamic and intra-annual density fluctuations in Aleppo pine (*Pinus halepensis*) trees of different crown classes

Study II: Changes in climate-growth relationships and IADF formation over time in Mediterranean pine species (*Pinus halepensis, P. pinaster* and *P. sylvestris*) in Spain

Study III: Effects of thinning on growth dynamics and growth-climate relationships in Aleppo pine (*Pinus halepensis*) trees of different crown classes

Study IV: Climate impact on wood density in *Pinus halepensis, Pinus pinaster* and *Pinus sylvestris*



Study I

Climate impact on growth dynamic and intra-annual density fluctuations in Aleppo pine (*Pinus halepensis*) trees of different crown classes

* Olivar, J., Bogino, S., Spiecker, H., Bravo, F., 2012. Climate impact on growth dynamic and intra-annual density fluctuations in Aleppo pine (*Pinus halepensis*) trees of different crown classes. Dendrochronologia 30 Issue 1: 35-47.

Climate impact on growth dynamic and intra-annual density fluctuations in Aleppo pine (Pinus halepensis) trees of different crown classes

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Abstract

Mediterranean environments are of special interest for the study of the relationships between climate, growth and anatomic features. Dendrochronological techniques were applied at eight sampling sites that were selected throughout the natural distribution area of *Pinus halepensis* in Spain. The objectives of this paper were: i) to identify relationships between radial growth and climate for different crown classes of Aleppo pine (*Pinus halepensis* Mill.) ii) to quantify the presence of intra-annual density fluctuations (IADFs) according to crown class and cambial age and iii) to establish the relationships between IADFs and climate. In the more mesic sites, dominant trees showed higher climate sensitivity than suppressed trees, while in the more xeric sites suppressed trees showed higher sensitivity than dominant trees. Tree-ring growth of both crown classes correlated positively with precipitation during and prior to the growing season. IADFs were more frequent in young than in old stands without differences between crown classes. Precipitation in April and December was positively correlated to the occurrence of IADFs, while precipitation in July correlated negatively. A higher frequency in IADFs occurred in the last fifty years, which coincides with the increase in drought events in Spain

Key words

Mediterranean climate, dendrochronology, Spain, tree-ring growth.

Introduction

Tree radial-growth models are valuable for simulating the impacts of climate change on the future growth of forest species. Understanding how forest growth responds to climate is a key element for a deeper knowledge of forest dynamics in a changing environment. Trees growing in extreme conditions respond strongly to climate variations. Mediterranean regions, as transitional climate zones between arid and humid regions of the world, are areas where climatic changes may have the greatest effects (Lavorel et al., 1998).

Different Mediterranean pine species have been analysed to detect relationship between climatic trends and tree growth. Growth of pine species is positively correlated to precipitation in the Iberian Peninsula: *Pinus pinea* in southern Portugal (Campelo et al., 2007), *Pinus pinaster* in central Portugal (Vieira et al., 2009) and in central Spain (Bogino and Bravo, 2008), *Pinus nigra* in southeastern Spain (Martin-Benito et al., 2008) and *Pinus sylvestris* in its southern and western distribution threshold (Bogino et al., 2009). In southern Italy, growth rate of *Pinus hapepensis* is sensitive mainly to temperature variations during the wet season and to soil humidity variations during the dry season (Attolini et al., 1990). In France, Rathgeber et al. (2005) concluded that *Pinus halepensis* growth is mainly controlled by soil water availability during the growing season. In Greece, the growth of Aleppo pine was related positively with the winter and spring precipitations and negatively with the temperatures of the spring months (Papadopoulos et al., 2008).

Wood anatomical features in tree rings have been interpreted as indicators of environmental change (see for instance Briffa et al., 2003). Species growing under Mediterranean climate, with summer droughts and high inter-annual variability in precipitation and temperature, commonly show special anatomical characteristics in tree rings (Schweingruber, 1993). Intra-annual density fluctuations (IADFs) are defined as “a layer of cells within a tree ring identified by different shape, size and wall thickness” (Kaennel and Schweingruber, 1995). Their inclusion of IADFs in dendrochronological studies allows detailed analysis of climatic events within the growing season. Different studies of pine species showed a good correlation between IADF formation and climate in the Mediterranean area. IADFs were mainly correlated with precipitation in autumn in *P. pinaster* in Portugal (Vieira et al.,

2009) and with precipitation in late winter and spring and higher temperatures in central Spain (Bogino and Bravo, 2009). IADFs were caused by precipitation events early in summer following a water deficit early in the growing season in *Pinus pinea* in southern Portugal (Campelo et al., 2007). Until now, the information about the impact of climate on IADFs of *Pinus halepensis* is scarce.

The Aleppo Pine (*Pinus halepensis* Mill.) is a native pine of the Mediterranean region, where it is one of the main species in the present landscape. Therefore, the study of the impact of climatic variables (temperature and precipitation) on its radial growth becomes of major interest. The objectives of the present study were: i) to identify relationships between radial growth and climate for different crown classes of Aleppo pine (*P. halepensis* Mill.) ii) to quantify the presence of intra-annual density fluctuations (IADFs) according to crown class and cambial age and iii) to establish the relationships between IADFs and climate.

Materials and Methods

Sites

Eight sampling sites were selected throughout the natural distribution area of *Pinus halepensis* in Spain (Fig. 1; Table 1).

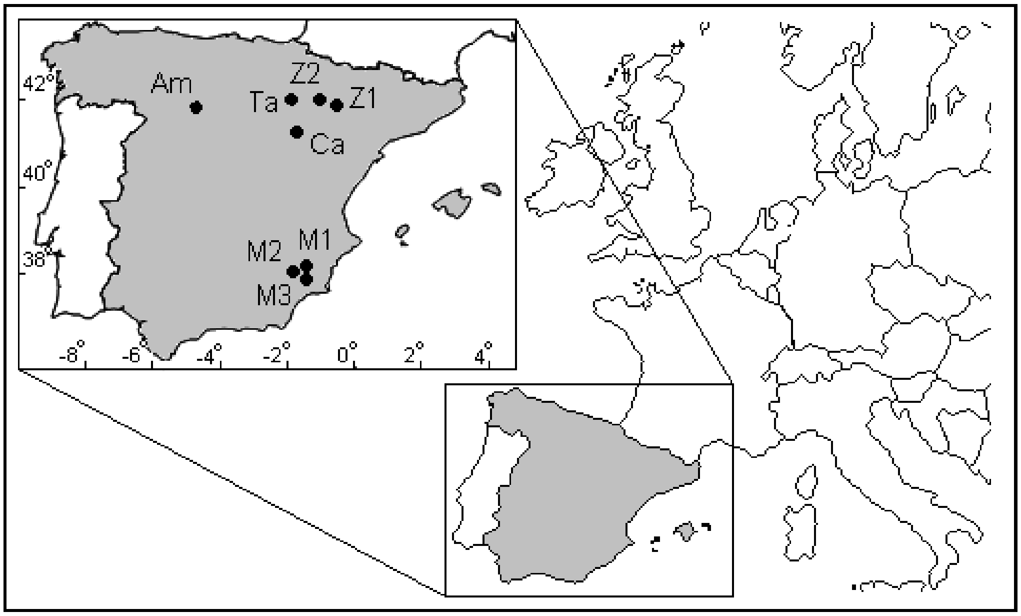


Fig. 1: *Study areas of Pinus halepensis in Spain.*

The site index, a relative measure of forest site quality based on the height of the dominant trees at a specific age, was determined by using site index curves. The site index was defined as the top height (m) at age 80 (Montero et al., 2001).

Table 1: *Sampling sites and meteorological stations.*

Site	Location	Latitude	Longitude	Altitude (m)	Site Index	Met. Station	Longitude	Latitude
Am	Palencia	41°51´36´´	4°45´36´´	849	Q17	Palencia E.C.A	42º00’30”	4º33’27”
Ca	Aragón	41°18´16´´	1°44´52´´	976	Q14	Calatayud aguas	41º19’51”	1º38’49”
Ta	Aragón	41°59´31´´	1°50´09´´	695	Q20	Tarazona	41º54’28”	1º43’54”
Za1	Aragón	41°48´58´´	0°32´15´´	535	Q11	Sos del rey catolico	42º29’34”	1º12’52”
Za2	Aragón	41°56´04´´	0°56´25´´	706	Q11	Sos del rey catolico	42º29’34”	1º12’52”
M1	Murcia	37°52´51´´	1°30´36´´	811	Q11	Moratalla "benizar"	38º16’25”	1º58’59”
M2	Murcia	37°52´50´´	1°32´15´´	957	Q20	Moratalla "benizar"	38º16’25”	1º58’59”
M3	Murcia	37°51´13´´	1°32´34´´	1118	Q17	Moratalla "benizar"	38º16’25”	1º58’59”

Chronologies

Fifteen trees from each crown class (dominant and suppressed) were selected on each sampling site. Dominant trees were defined as those standing above all other trees in its vicinity and receiving full light from above, whereas suppressed trees were defined as those growing below the tree canopy. In sites Za1 and Za2 only dominant trees were sampled. Therefore, a total of fourteen tree-ring chronologies (eight dominant and six suppressed) were analysed. On each tree two cores were extracted at 1.30 m above ground. The increment cores were air dried and mounted on wooden supports and dated according to standard dendrochronological techniques (Stokes and Smiley, 1968). The preparation of the samples was done by use of the diamond flycutter (Kugler, F500). This machine was designed specifically for single point diamond flycutting of plan surfaces requiring an optical quality surface finish, precise flatness and exacting parallelism.

The v6.06P COFECHA program (Holmes, 2001; Grissino-Mayer, 2001; available at www.ltrr.arizona.edu) was applied to assess measurement and dating accuracy. This program calculates the Pearson correlation indices between the indexed tree-ring series and a master reference chronology in a series of consecutive, partially overlapped segments of a length specified by the user. Absolute dating is essential for any dendroclimatological study, and it is impossible to compare climatic variables in one specific year with tree-ring growth if the individual tree-ring series are not dated correctly. According to standard methods in dendrochronology trees exhibiting correlation values with the master chronology below 0.4 were excluded.

To eliminate biological trends in tree-ring series and to minimize growth variations that are not shared by most trees, the v6.05P ARSTAN program (Cook and Holmes, 1984; Holmes, 2001; available at www.ltrr.arizona.edu) was used. Standardization removes geometrical and ecological trends while preserving inter-annual high-frequency variations that are presumably related to climate. To obtain a master chronology at each study site and crown class, the standardized series were averaged.

The fourteen master chronologies were calculated separately to analyse differences in radial growth. Series from dominant and suppressed trees were averaged in two separate general chronologies. Chronology quality was evaluated using the mean sensitivity (MS), which is a measure of the mean relative changes between adjacent ring widths (Fritts, 2001); the signal-to-noise ratio (SNR), the proportion of the variability explained by climate or other casual factors divided by the residual or unexplained variability (Fritts and Swetnam, 1989); and the expressed population signal (EPS), which indicates the degree to which the particular sample chronology portrays a hypothetically perfect chronology (Wigley et al., 1984). The master chronologies were normalized by subtracting the mean and dividing by the standard deviation. Values far from cero mean higher reactions. The Normalized Difference Index was calculated by subtracting the normalized values of the suppressed trees to the normalized values of the dominant trees. Positive NDI values mean stronger reactions from dominant trees, while negative NDI values mean stronger reactions from suppressed trees.

Relationships between climatic data and tree-ring widths

Mean monthly air temperatures and total monthly precipitations were recorded at the closest meteorological stations (Agencia Estatal de Meteorología, Spain) (Table 1). Climatic data were grouped in climatic seasons: winter (December, January and February), spring (March, April and May), summer (June, July and August) and fall (September, October and November). These seasonal data were regressed against ring-width indices in order to assess climate-growth relationships. DENDROCLIM 2002 (Biondi and Waikul, 2004) was applied to calculate correlation and response functions by bootstrapped error estimates (Guiot, 1991).

Intra-annual density fluctuations

The correctly dated cores were visually examined for IADFs. IADFs show a non-sharp transition in opposite to the annual rings boundary (Fritts, 2001). Because of the variability of IADFs tangentially and vertically within the tree ring along the stem the IADFs were only considered when present in both cores, in the same tree ring. As the number of samples changed over time, the relative frequency was calculated with the following formula [1]:

[1] $F = n/N$

where F is the relative frequency of IADF in a particular year; n the number of trees that formed the IADF and N the total number of trees analysed. The bias in the frequency was assessed by calculating the stabilized IADF frequency (f), according to the formula of Osborn et al (1997) [2]:

[2] $f = F^{0.5}$

The nonlinear logistic equation form was chosen to model the probability of occurrence of IADFs [3]:

[3] $P = (1.0 + e^{(-z)})^{-1}$

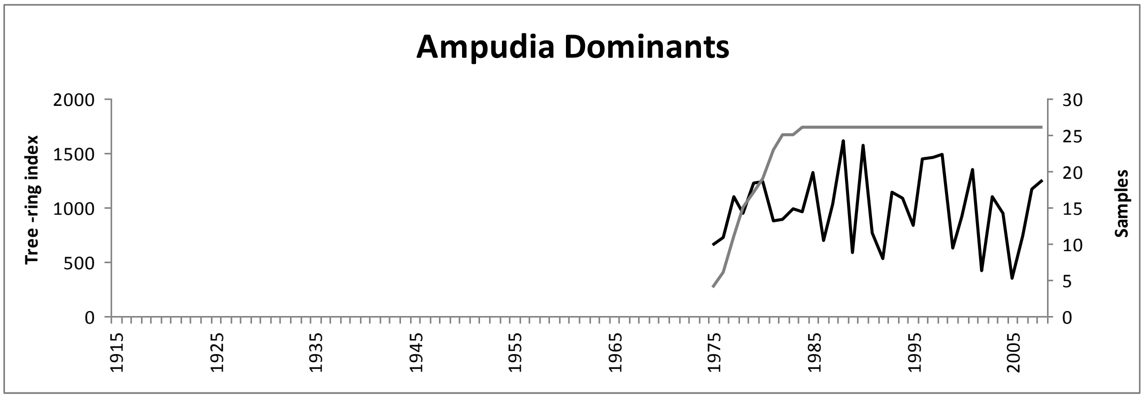
where P is the probability of IADFs and $Z = b0+b1(x1)+b2(x2)+\ldots\ldots+bk(xk) + \varepsilon$; where x1; x2..... xk are the climatic variables and b0; b1; b2 ….. bk are unknown parameters of the model and ε is a normal random error N (0,1); and *e* is the exponential operator. The logistic equation can be formulated to accept a binary variable such as occurrence of IADFs, and the parameters can be estimated by maximum-likelihood methods. The resulting prediction is bounded by 0 and 1. Monthly rainfall and mean monthly temperature were used as explanatory variables. The hydrological year was defined as a period of 12 months, from October of the previous year to September of the current growth year. A stepwise selection method was used to find the best model.

The alternative fits were evaluated on the basis of Akaike information criterion (AIC), the –2*Log Likelihood, the area under the receiver operating characteristic (ROC) curve and the expected behavior - as indicated by the signs of the estimated parameters. ROC curve is displayed for the models and the area underneath was calculated as a value of the accuracy of the model. Value over 0.80 indicates an excellent discrimination (Hosmer and Lemeshow, 2000). This curve relies on false/true positive/negative tests, and the sensitivity is indicated by the proportion of correctly classified events and the specificity by the proportion of correctly classified non-events (Hair et al., 1998). This model was successfully used to estimate the probability of occurrence of IADFs in *Pinus pinaster* subsp. *mesogenesis* in Spain (Bogino and Bravo, 2009a). PROC LOGISTIC of SAS 9.1 (SAS Institute Inc. 2004) was used to fit the model. Samples were first grouped according to site location (Palencia, Aragón and Murcia), age (younger than 80 years and older than 80 years) and crown class (dominant and suppressed).

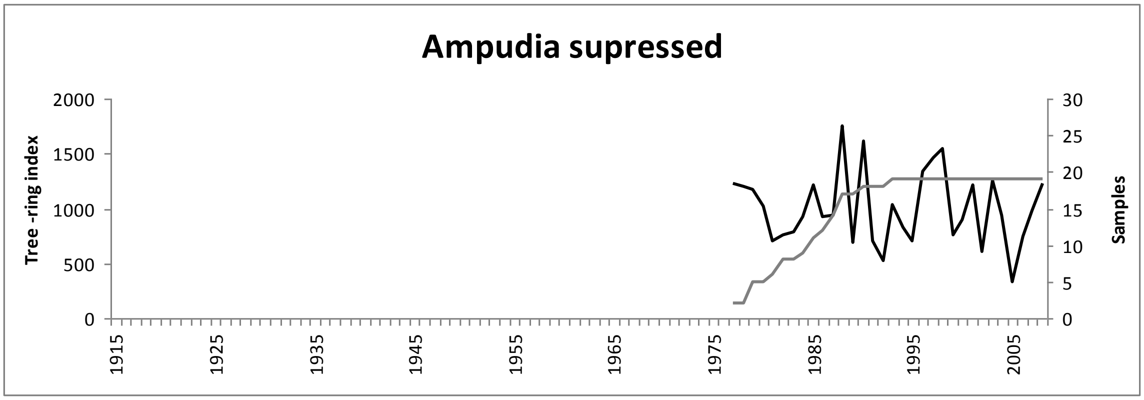
Results

Chronologies

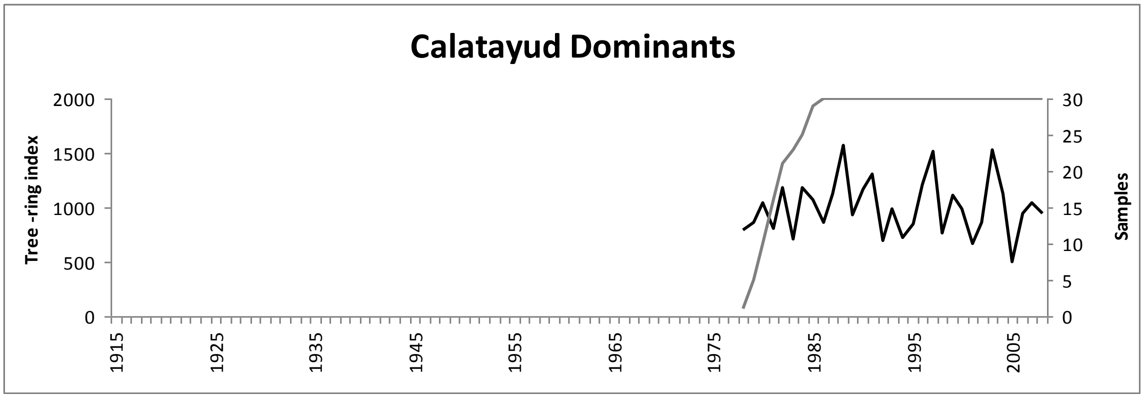
The master chronologies of *Pinus halepensis* and the number of samples used at each sampling site are shown in Fig. 2.



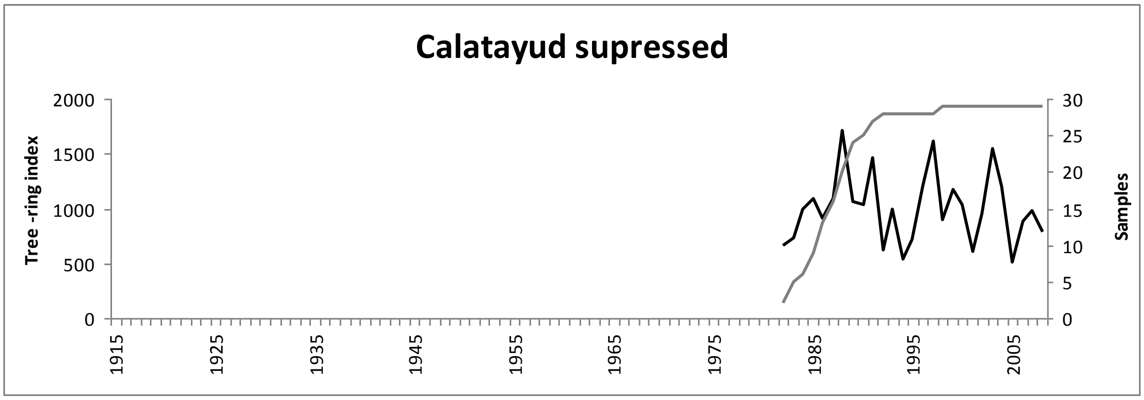
Ampudia Dominants



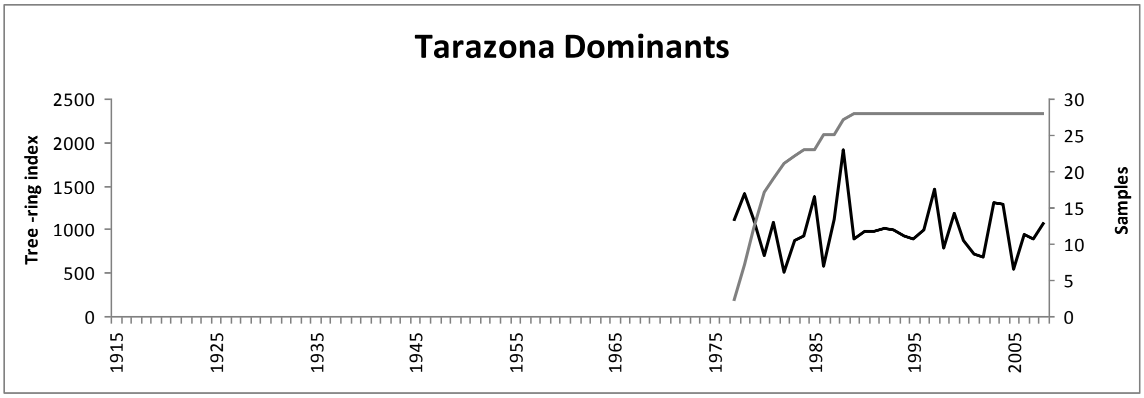
Ampudia supressed



Calatayud Dominants



Calatayud supressed


Tarazona Dominants
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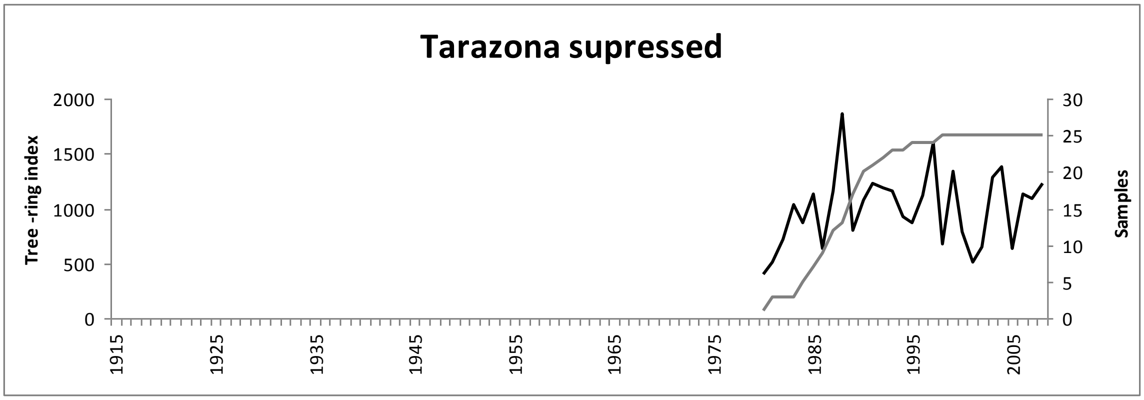
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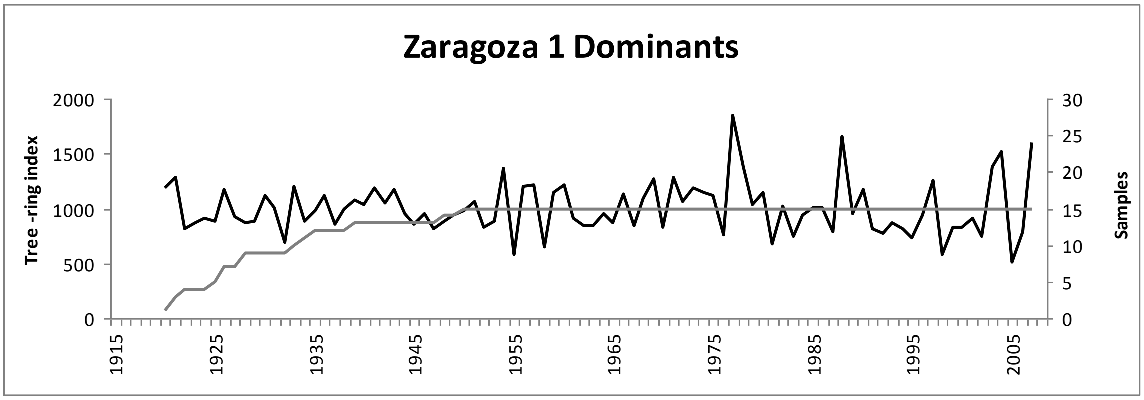
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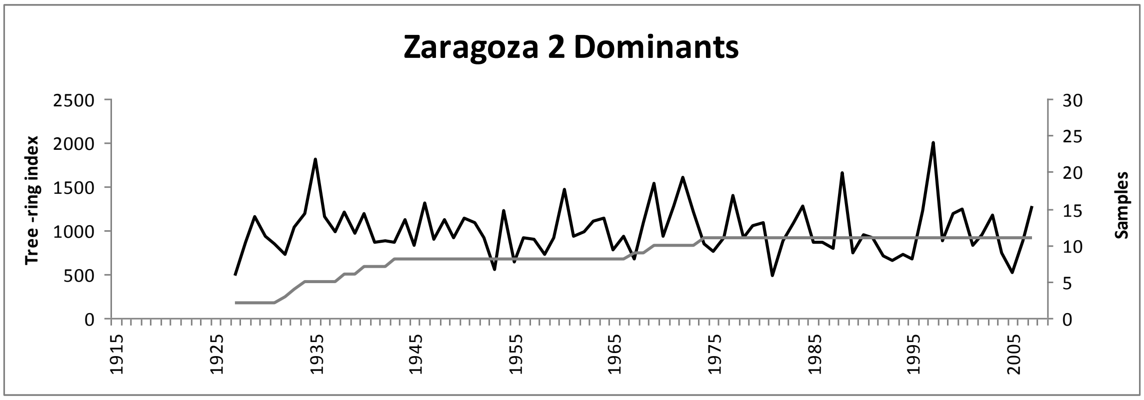
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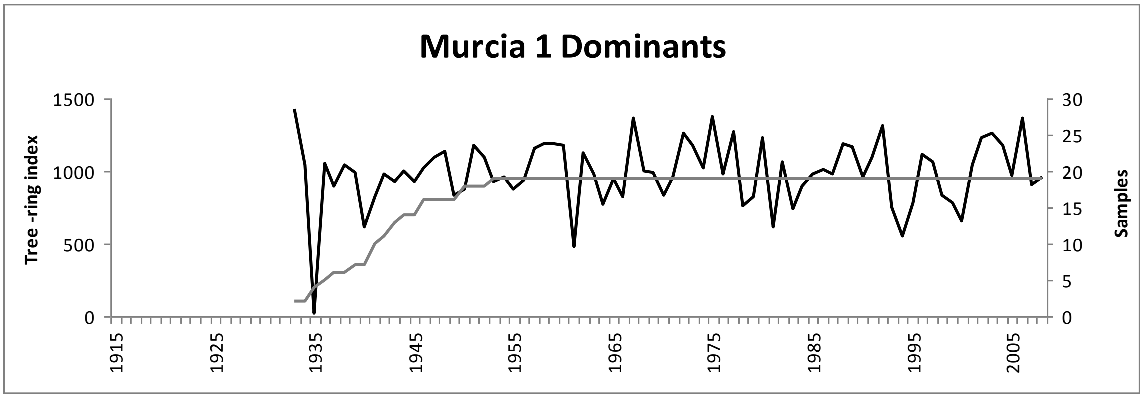
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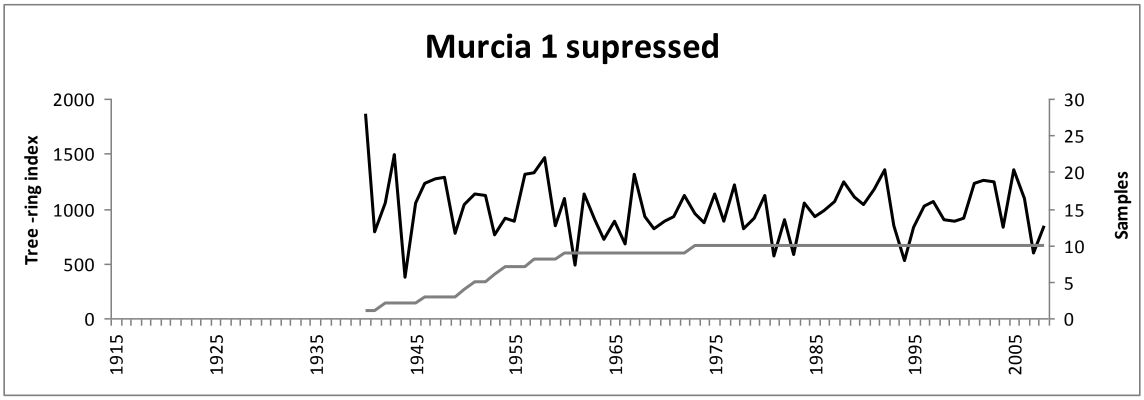
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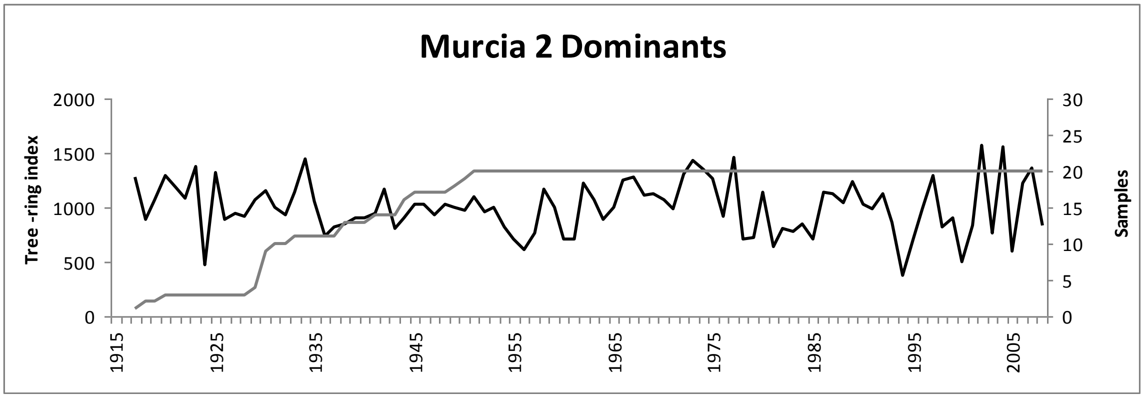
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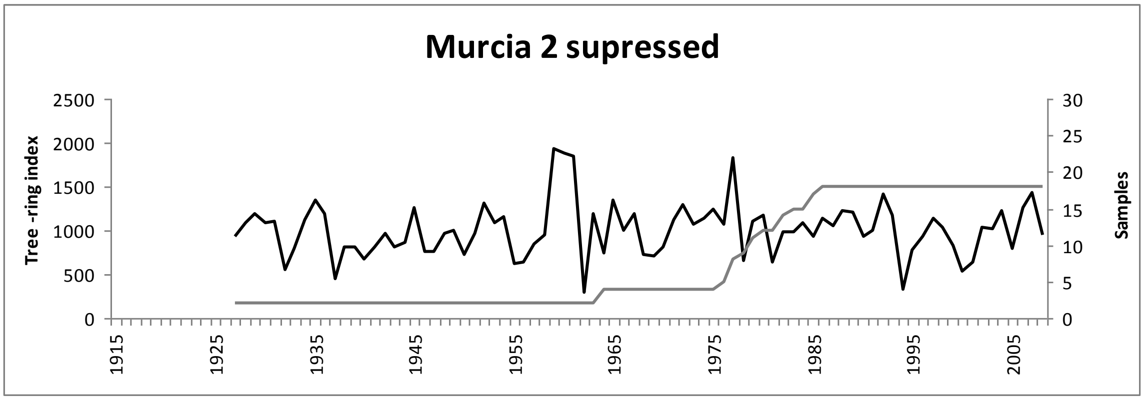
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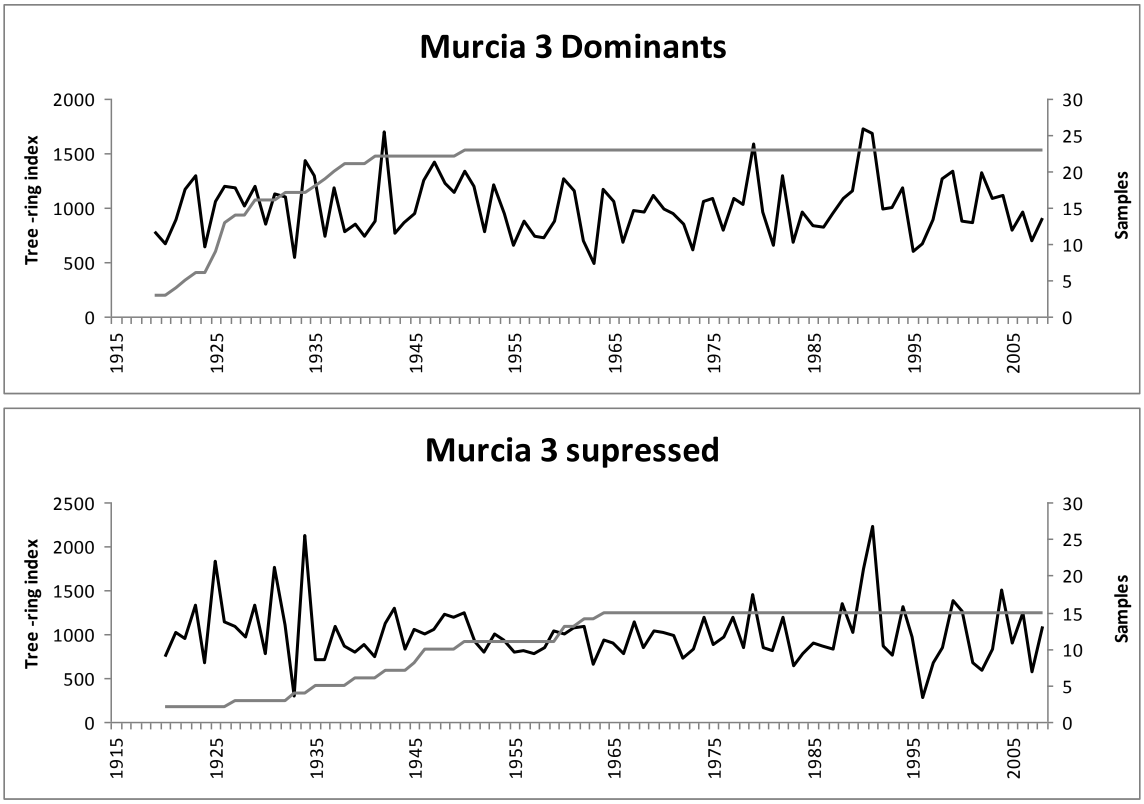


Fig. 2: *Standardized chronologies of Pinus halepensis along the natural distribution in Spain. The black line shows the tree-ring index through time and the grey line shows the number of samples used in each chronology.*

The master chronologies from Ampudia showed higher mean sensitivity values (0.40 for dominants and 0.37 for suppressed) than the other locations. The master chronology of the dominant trees in Ampudia also showed higher SNR and EPS (66.08 and 0.98 respectively) than the rest of the locations (Table 2).

Table 2: *Descriptive statistics of the master chronologies of Pinus halepensis. SD: standard deviation; MS: mean sensitivity; SNR: signal to noise ratio; EPS: expressed population signal; Var: variance in first eigenvector; and Mean corr.: mean correlation among trees.*

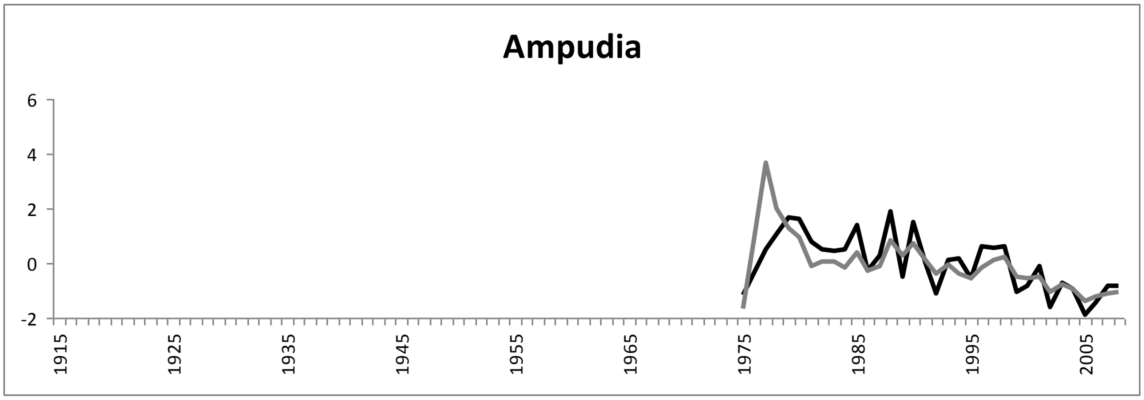
Code	Crown class	Location	Time span	Core num.	SD	MS	SNR	EPS	Var.	Mean corr.
AmD	Dom.	Palencia	1974-2008	27	0.32	0.40	66.08	0.98	74.82%	0.861
Ams	Suppr.	Palencia	1976-2008	19	0.32	0.37	14.30	0.93	57.34%	0.722
CaD	Dom.	Aragón	1977-2008	30	0.25	0.33	28.70	0.96	70.28%	0.819
Cas	Suppr.	Aragón	1981-2008	29	0.31	0.36	15.57	0.94	63.16%	0.755
TaD	Dom.	Aragón	1976-2008	28	0.29	0.33	39.87	0.97	71.00%	0.831
Tas	Suppr.	Aragón	1979-2008	25	0.34	0.35	20.33	0.95	64.38%	0.784
Z1	Dom.	Aragón	1919-2007	15	0.24	0.25	8.94	0.90	48.40%	0.679
Z2	Dom.	Aragón	1926-2007	11	0.28	0.30	8.31	0.89	55.18%	0.724
MD1	Dom.	Murcia	1932-2008	19	0.20	0.21	12.86	0.93	44.45%	0.656
Ms1	Suppr.	Murcia	1939-2008	10	0.25	0.28	3.94	0.98	39.90%	0.596
MD2	Dom.	Murcia	1915-2008	21	0.24	0.25	18.20	0.95	51.00%	0.707
Ms2	Suppr.	Murcia	1921-2008	18	0.33	0.31	8.10	0.96	57.25%	0.751
MD3	Dom.	Murcia	1915-2008	23	0.30	0.32	30.20	0.97	62.68%	0.778
Ms3	Suppr.	Murcia	1917-2008	15	0.34	0.32	14.35	0.94	55.35%	0.731

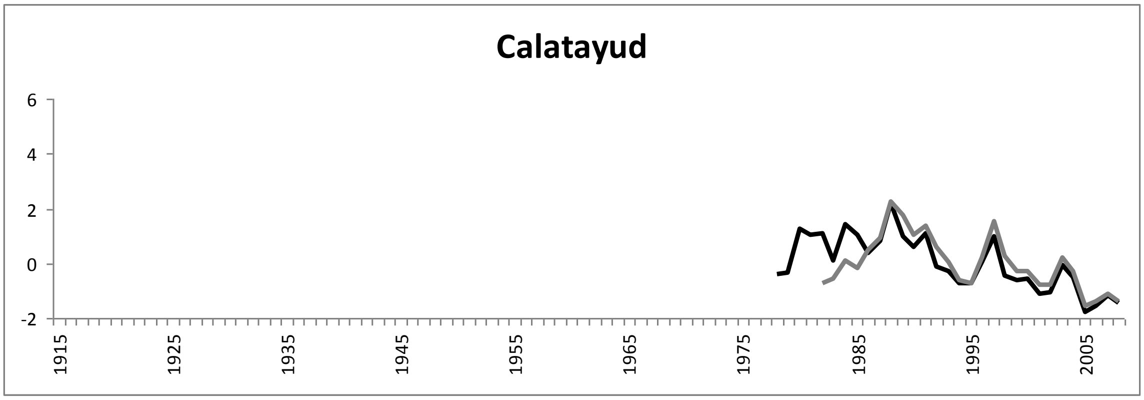
The mean chronology of the suppressed trees showed slightly higher mean sensitivity values (0.30 for dominants and 0.33 for suppressed) and higher SNR values (26.64 for dominants and 12.77 for suppressed) than the mean chronology of the dominant trees. The mean chronology of the dominant trees also showed higher variance and mean correlation values than the mean chronology of the suppressed trees (Table 3).

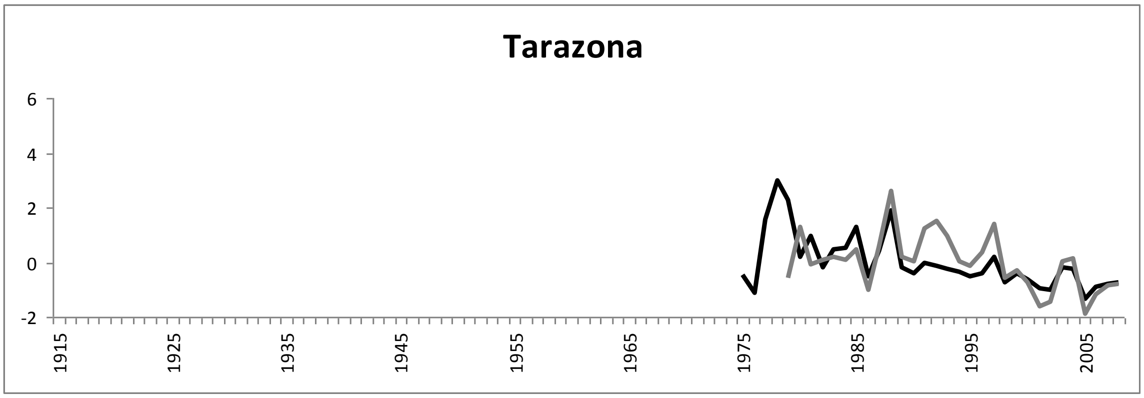
Table 3: *Descriptive statistics of the mean dominant and suppressed chronologies. SD: standard deviation; MS: mean sensitivity; SNR: signal to noise ratio; EPS: expressed population signal; Var: variance in first eigenvector; and Mean corr.: mean correlation among trees.*

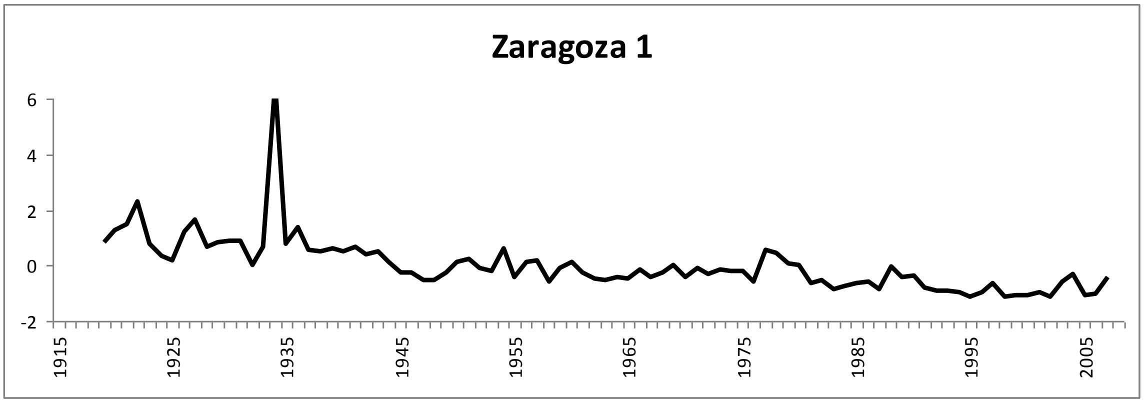
Social class	Time span	Av.core num.	Av.ring num.	Age range	SD	MS	SNR	EPS	Var.	Mean corr.
Dom.	1915-2008	22	1139	95-27	0.27	0.30	26.64	0.94	0.60	0.70
Suppr.	1917-2008	19	732	92-20	0.32	0.33	12.77	0.95	0.56	0.63

Looking at the normalized curves (Fig. 3) it can be observed that dominant trees react stronger than suppressed trees in favourable years in Ampudia while in Tarazona suppressed trees react stronger than dominant trees in favourable years, the other sites show no patterns.

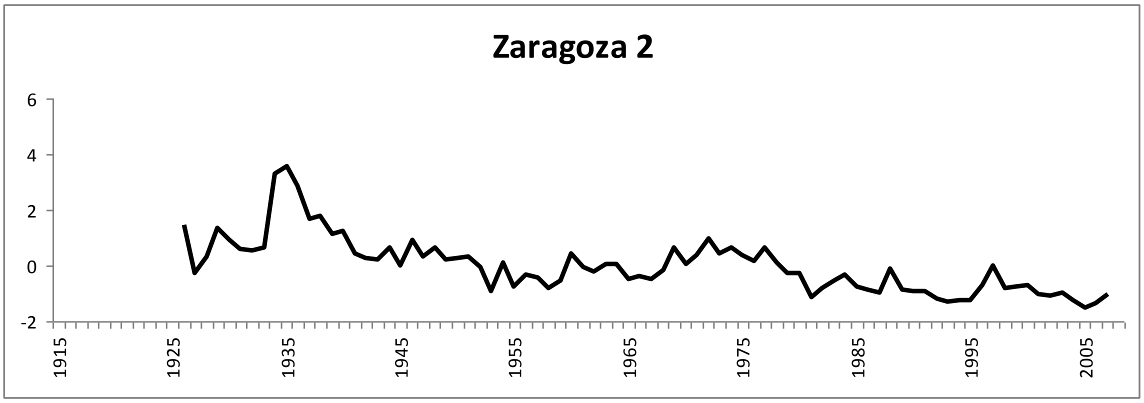








Zaragoza 1



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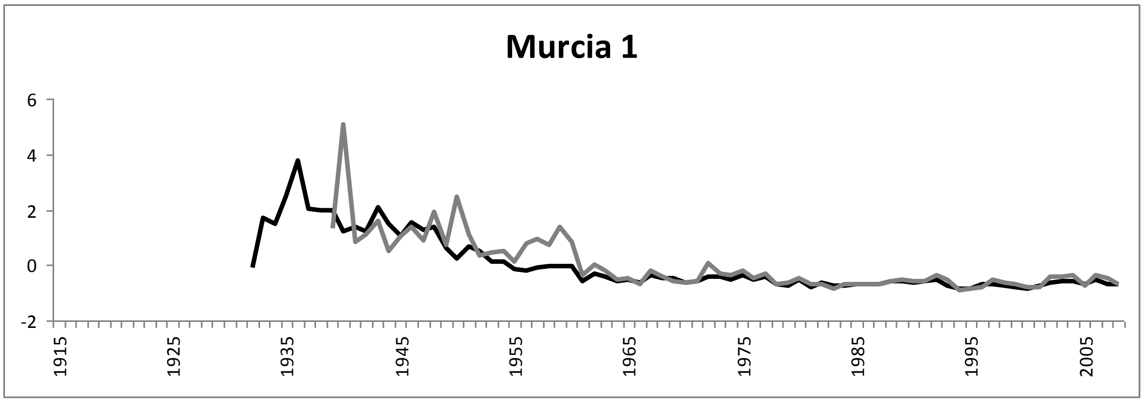
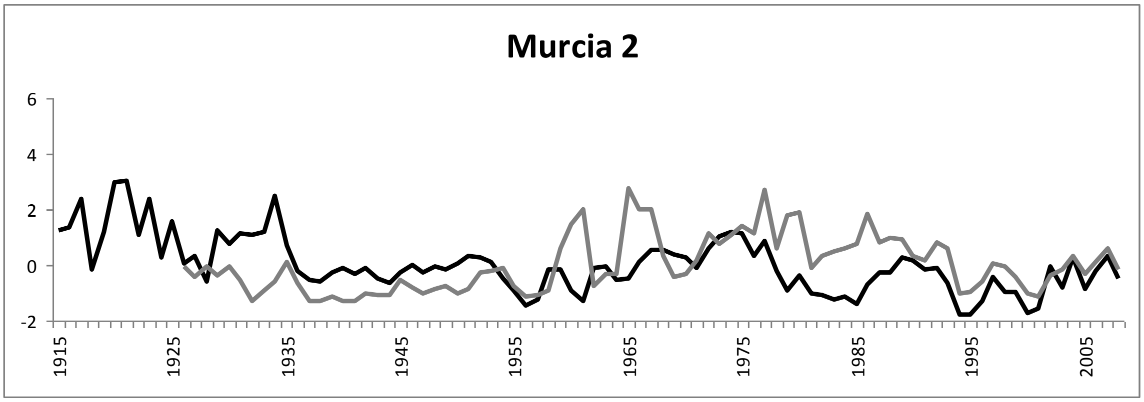


Murcia 1



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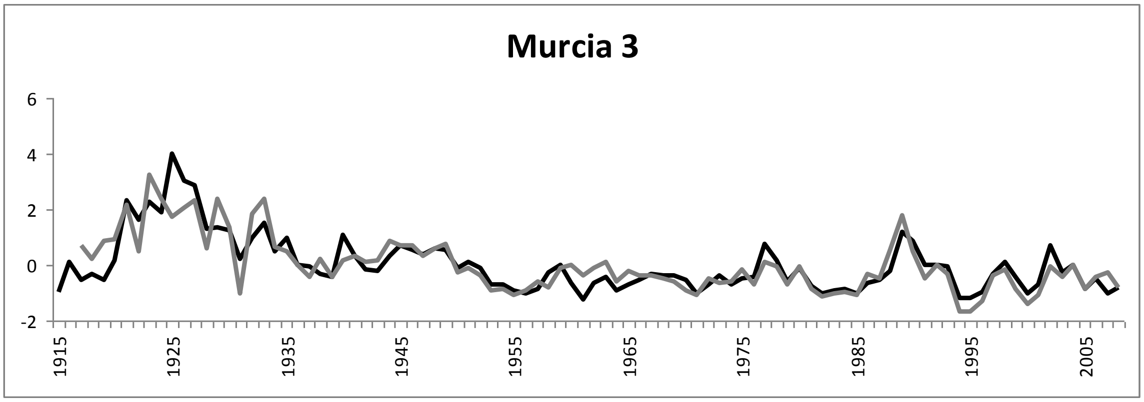

Fig. 3: *Normalized chronologies of Pinus halepensis. The black line shows the mean dominant series and the grey line shows the mean suppressed series.*

The Normalized Difference Index (Fig. 4) showed that, from 1980 to 2000 suppressed trees reacted stronger in Murcia and Aragón, while since 2000 no clear tendency was found.

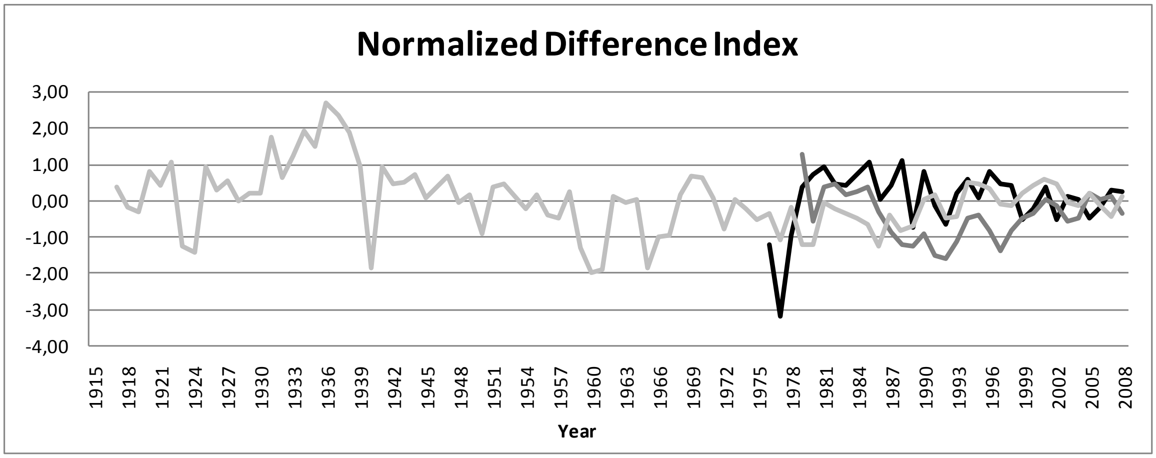

Fig. 4: *Normalized Difference Index of Palencia (black line), Aragón (dark grey line) and Murcia (light grey line).*

Relationships between climatic data and tree-ring widths

Relationships between mean seasonal temperatures mean seasonal precipitation and radial growth of the different crown classes in the eight sampling sites are presented in Figs. 5 and 6.

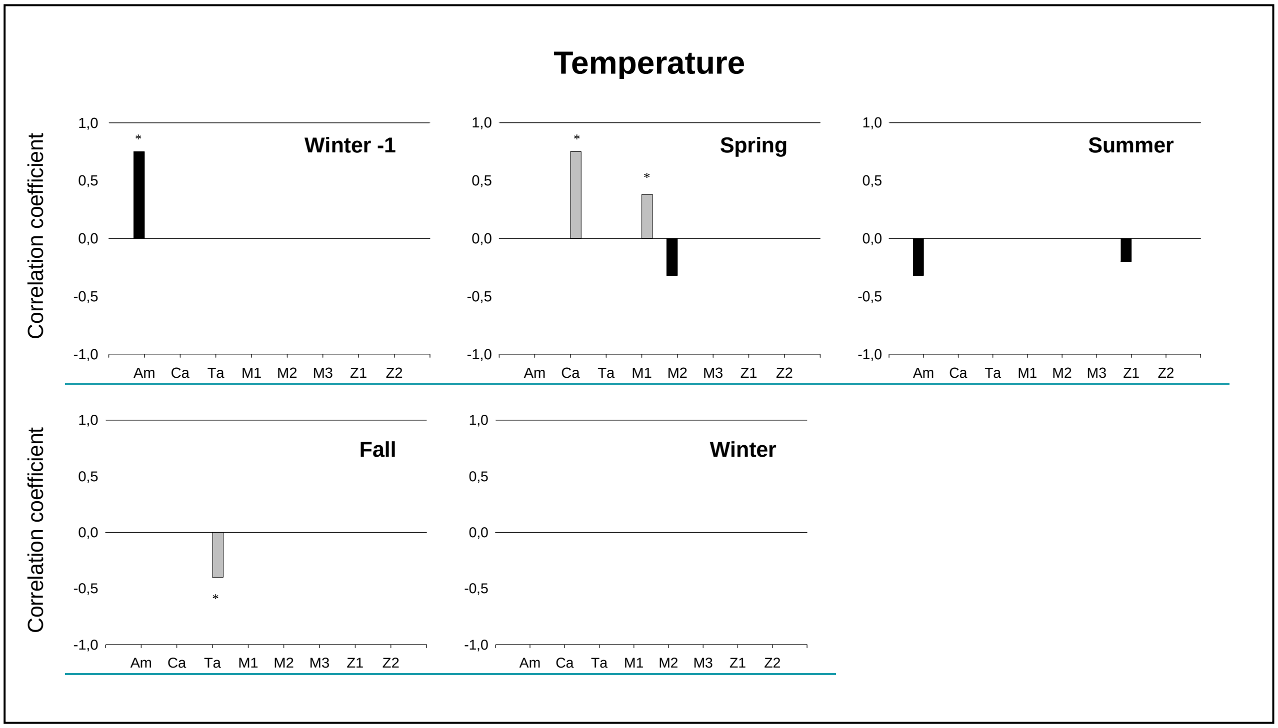

Fig. 5: *Correlation coefficients between tree-ring width and temperature. Black bars represent dominant trees and grey bars represent suppressed trees (* p<0.05).*

Precipitation appeared to be the main factor influencing tree growth with significant values in all seasons, while temperature showed weak correlation values showing significant values in only two of the five seasons. Spring precipitation showed the most significant positive correlations followed by summer and winter previous to the growing season.

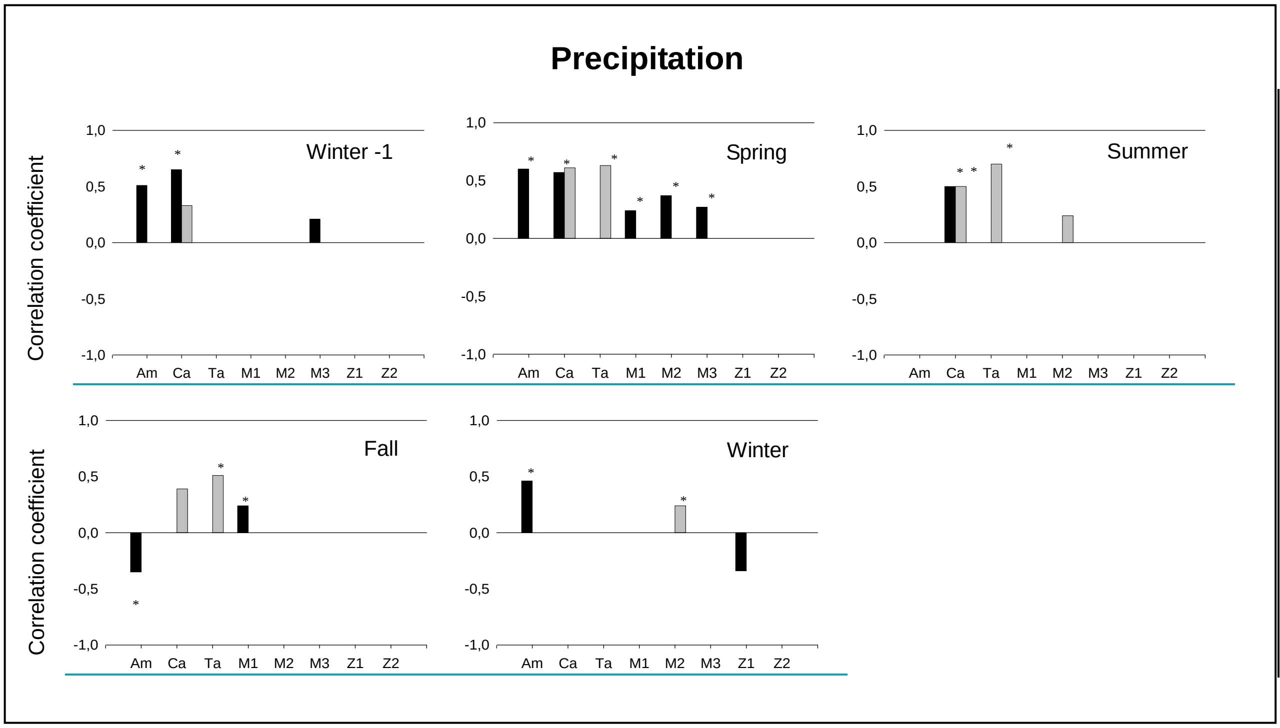

Fig. 6: *Correlation coefficients between tree-ring width and precipitation. Black bars represent dominant trees and grey bars represent suppressed trees (* p<0.05).*

A total of 13502 tree rings were analysed from trees from the eight sampling sites and a total of 107 IADFs were found. Samples were grouped according to age and crown class (Table 4).

***Table 4:** Descriptive statistics of the IADFs in Pinus halepensis according to age (younger than 80 years and older than 80 years) and crown class (dominant and suppressed).*

	Age		**Crown class**	
	<80	**>80**	**Dom**	**Suppr**
Number of trees	79	66	87	58
Period	1974-2008	1915-2008	1915-2008	1917-2008
Trees with IADF	28	33	32	30
Trees with IADF (%)	41,77	43,94	36,78	51,72
Tree rings in total	4183	9319	9112	4390
Tree rings with IADFs	67	40	64	43
Proportion of IADF	0.016	0.004	0.007	0.010
Mean stabilized IADF	0.65	0.55	0.61	0.61

The percentage of trees with IADFs was rather similar for young and old stands. However, the percentage of IADFs and the mean stabilized IADF was higher for young stands than for old stands. The percentage of trees with IADFs and the percentage of IADFs were both higher for suppressed than dominant trees. Mean stabilized IADF was the same for both crown classes.

Intra-annual density fluctuations

The nonlinear logistic equation form was chosen to model the probability of occurrence of IADFs with monthly precipitation and mean monthly temperature as variables (Table 5).

Table 5: *Stepwise selection (pp: monthly precipitation; tt: mean monthly temperature; AIC: Akaike's Information Criterion; L: likelihood).*

Variables included in the model	**AIC**	**-2*Log L**
pp-October	519.728	515.728
pp-July, pp-October	505.353	499.353
pp-June, pp-July, pp-October	491.281	483.281
tt-May, pp-June, pp-July, pp-October	478.292	468.292
tt-September, tt-May, pp-June, pp-July, pp-October	460.197	448.197
pp-May, tt-September, tt-May, pp-June, pp-July, pp-October	453.722	439.722
pp-August, pp-May, tt-September, tt-May, pp-June, pp-July, pp-October	450.977	434.977
pp-October, pp-August, pp-May, tt-September, tt-May, pp-June, pp-July, pp-October	445.196	427.196
pp-October, pp-August, tt-September, tt-May, pp-June, pp-July, pp-October	445.241	429.241
pp-November, pp-October, pp-August, tt-September, tt-May, pp-June, pp-July, pp-October	440.880	422.880
tt-July, pp-November, tt-October, tt-July, pp-October t, pp-August, tt-September, tt-May, pp-June, pp-July, pp-October	433.910	413.910
pp-March, tt-July, pp-November, tt-October, tt-July, pp-October, pp-August, tt-September, tt-May, pp-June, pp-July, pp-October	426.127	404.127

The logistic function estimated that 10 monthly climatic variables out of 24 had a significant effect on predicting future IADFs (Tables 6 and 7).

Table 6: *Climatic variables with a significant effect on the probability of IADFs in Pinus halepensis (pp:monthly precipitation; tt:mean monthly temperature).*

Parameter	**AmD**	**Ams**	**CaD**	**Cas**	**TaD**	**Tas**	**Z1**	**Z2**	**M1D**	**M1s**	**M2D**	**M2s**	**M3D**	**M3s**
pp-Oct.												** -		
pp-Jan.		* -	*** +											
pp-Mar.												*** -		
pp-Apr.									* -					
pp-May.			*** +		*** +									
pp-July.	* -		** -	*** -										
pp-Aug.			*** +											
pp-Sept.			* -											
tt-Oct.				** +										
tt-Feb.			** +											
tt-Mar.												** -		
tt-May.						*** -				* -				
tt-June.											* +			
tt-July.				** -							* +			
tt-Aug.											* -			
tt-Sept.				* +										

**p<0.05, **p<0.01, ***p<0.001, the absence of an asterisk shows that there was no significant association.*

The model showed that, without considering crown classes, precipitations in October, December, March, April, June and mean monthly temperatures in June and September had a positive impact on the formation of IADFs, while precipitations in November and July and mean monthly temperatures in May had a negative impact on the formation of IADFs. Precipitations in December and April had a positive impact on the formation of IADFs in both dominant and suppressed trees, while precipitations in July had a negative impact.

Table 7: *Climatic variables with a significant effect on the probability of IADFs in Pinus halepensis (pp:monthly precipitation; tt:mean monthly temperature).*

Parameter	**All**				**Dominant**				**Suppressed**			
	Estim.	**St. Error**	**Wald Chi^2**	**Pr>Chi^2**	**Estim.**	**St. Error**	**Wald Chi^2**	**Pr>Chi^2**	**Estim.**	**St. Error**	**Wald Chi^2**	**Pr>Chi^2**
Intercept	8.05	3.22	6.25	0.01	8.98	3.55	6.41	0.01	5.98	3.82	2.45	0.12
pp-Oct.	-0.03	0.01	17.26	<.0001								
pp-Nov.	0.04	0.01	14.67	0.00								
pp-Dec.	-0.07	0.01	38.20	<.0001	-0.08	0.01	29.14	<.0001	-0.09	0.02	21.90	<.0001
pp-Jan.									0.12	0.04	9.53	0.00
pp-Mar.	-0.02	0.01	9.01	0.00								
pp-Apr.	-0.02	0.01	8.64	0.00	-0.02	0.01	8.08	0.00	-0.07	0.03	6.08	0.01
pp-May.					-0.03	0.01	7.11	0.01				
pp-June.	-0.06	0.01	22.72	<.0001	-0.06	0.02	17.27	<.0001				
pp-July.	0.03	0.01	23.08	<.001	0.02	0.01	4.69	0.03	0.07	0.02	14.13	0.00
tt-Nov.									1.75	0.53	10.88	0.00
tt-Dec.									-1.40	0.45	9.48	0.00
tt-May.	0.72	0.15	23.20	<.0001					0.67	0.29	5.35	0.02
tt-June.	-0.50	0.15	10.66	0.00	-0.40	0.16	6.50	0.01				
tt-Sept.	-0.51	0.14	12.94	0.00					-1.69	0.52	10.39	0.00

The area underneath ROC curve was 0.918 for all trees, 0.917 for dominant trees and 0.943 for suppressed trees. The accuracy of the model is also sufficient to use it to predict occurrence of IADFs. IADF frequency in relation to calendar year (Fig. 7) showed an increase in IADFs from the 1980s to the present. 1983, 1989, 1995 and 1999 were the years with more IADFs, with a stabilized frequency higher than 0.2.

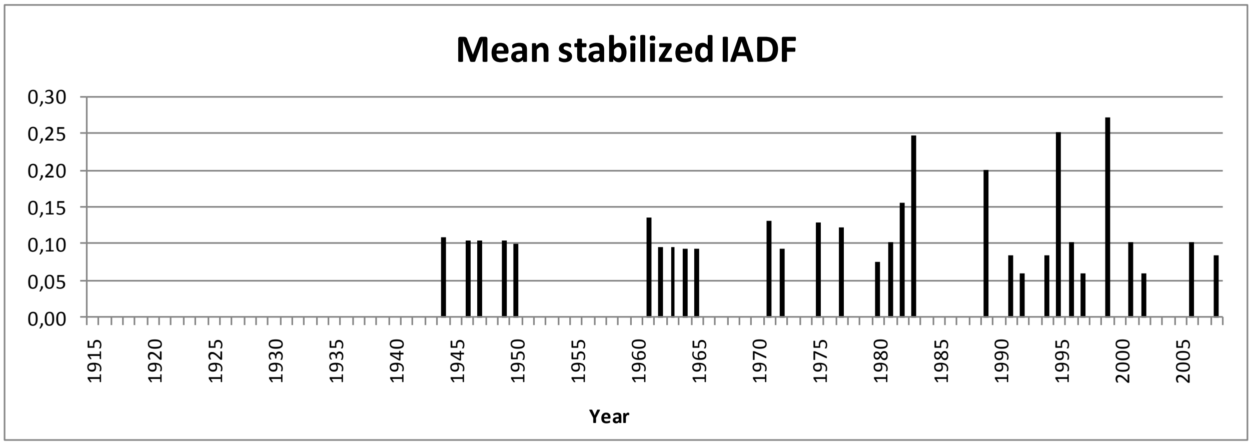

Fig. 7: *Mean stabilized IADF in relation to calendar year.*

Discussion

Pinus halepensis appears as a reliable species for dendrochronological studies, showing good correlations between trees growing at the same site, high expressed population signals and accurate statistical values which suggest a clear response to environmental factors. In addition, it confirms the tendency of Mediterranean species to develop special anatomical structures (Schweingruber, 1993). We found common radial growth patterns among dominant and suppressed series in the same site. These results agree with previous studies in Spain suggesting that pine species growing in the southern dendroecological section could have a common growth response to environmental factors (Richter et al., 1991; Bogino and Bravo, 2008).

The descriptive statistics of the chronologies suggest that the tree-ring series reflects one or more associated factors (including climate), and a strong response of radial growth dynamics to changes in climatic conditions shown by the mean sensitivity values (MS) from 0.21 to 0.40 that are higher than the 0.16 to 0.34 values found in previous studies on pine species (*Pinus sylvestris* L., *Pinus nigra* Arnold, *Pinus pinaster* Ait. and *Pinus mugo* ssp. *uncinata* Turra.) in the Iberian Peninsula (Richter et al., 1991; Bogino and Bravo, 2008; Martin-Benito et al., 2008; Bogino and Bravo, 2009; Bogino et al., 2009; Vieira et al., 2009).

The expressed population signal (EPS) ranging from 0.89 to 0.98 is in all cases higher than the critical level of 0.85 suggested by Wigley et al. (1984), which implies that the chronologies are representative of tree growth in the stands. First eigenvector variance ranges from 39.90 to 74.82%, indicating good homogeneity within the same site. It can be concluded that the fourteen mean chronologies have high MS, SNR, EPS and percentage of the variance accounted for by the first eigenvector, suggesting a strong common signal to related-climatic environmental factors. Ampudia showed higher values of MS, SNR and EPS than the other two locations, especially the dominant trees. However, in the regions of Aragón and Murcia the mean suppressed series showed a higher sensitivity than the mean dominant series. There is evidence that suppressed trees suffer greater drought stress because of greater root competition for soil. However, understory trees receive lower solar radiation and higher wind protection by the influence of neighbouring crowns reducing transpiration rates. Therefore, climate sensitivity may be reduced (Bréda et al., 2006; Martin-Benito et al., 2008). In Mediterranean forests stand density is low and the protection effect from the dominant to the suppressed trees gets reduced.

The results show that *P. halepensis* growth is mainly controlled by precipitation. Previous studies reported that growth of Aleppo pine is controlled by soil water availability (Rathgeber et al., 2005) and precipitation is the main factor influencing tree growth of pine species in semiarid Mediterranean conditions (Raventós et al., 2001). Winter previous to the growing season and spring precipitation are related positively with tree-ring growth. The same results were found in the Attica basin (Greece) (Papadopoulos et al., 2008). Other Mediterranean pine species showed similar results: growth of *Pinus pinea* in a dry Mediterranean area in Portugal, *Pinus pinaster* in central Spain and *Pinus sylvestris* at its southern distribution limits were positively correlated with rainfall (Campelo et al., 2007; Bogino and Bravo, 2008; Bogino et al., 2009). Growth of *Pinus nigra* in central Spain and *Pinus pinaster* in Portugal were mainly influenced by May precipitation (Martin-Benito et al., 2008; Vieira et al., 2009).

We found a higher tendency in young stands for developing IADFs. These results corroborate previous dendroecological studies which suggested that IADFs were more frequent in younger tree rings (Rigling et al., 2001; Villalba and Veblen, 1994; Vieira et al., 2009; Bogino and Bravo, 2009). 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). Since young trees have a different response to environmental factors than old trees, the incorporation of age-dependent differences on the appearance of special ring features such as IADFs and its association to climatic variables in any dendrochronological study provides a useful proxy for complementing and enhancing the dendroclimatological data. In addition, it can give important clues to predict differences on how young and old trees react to climate change.

The occurrence of IADFs in *Pinus halepensis* was positively correlated with precipitation in December and April and negatively correlated with precipitation in July. These results are consistent with those of previous studies in *Pinus pinaster* in central Spain, where IADFs were mainly correlated with rainfall pulses in late winter and spring (Bogino and Bravo, 2009). IADFs correlated positively with precipitation events early in summer following a water deficit early in the growing season in *Pinus pinea* in southern Portugal (Campelo et al., 2007), which is consistent with the present results that showed precipitation in July to have a negative effect on IADFs. Favourable climatic conditions in winter and spring as well as water deficits early in the growing season followed by rainfall indicate an increase in the probability of the occurrence of IADFs. This corroborates that growth may temporarily stop, but is always ready to resume activity as soon as climatic conditions become favourable.

Winter precipitation that precedes the formation phase of the tree-rings as well as the spring rainfall at the beginning of the tree-growth play a prevailing role to the development of wider tree rings in P. halepensis (Papadopoulos et al., 2008). These climatic conditions coincide with the favourable conditions for the formation of IADFs in our study. Therefore we can agree with previous studies that have shown that IADFs are more frequent in wider tree rings (Vieira et al., 2009; Rigling et al., 2001; Villalba and Velben, 1994). As it was previously reported (Bogino and Bravo, 2009) a higher frequency in IADFs occurred in the last fifty years. The increase in drought events in Spain (IPCC 2007) may explain the higher IADF frequency during this period.

Conclusions

Pinus halepensis is an accurate species for tree-ring analysis with good correlations between trees growing at the same site and a clear response to environmental factors. Suppressed trees showed higher sensitivity than dominant trees, with greater growth rates during favourable years except for Ampudia, where dominant trees showed higher sensitivity than suppressed trees. Precipitation was the main factor influencing tree-ring growth. IADFs were more frequent in young than in old stands with no clear differences according to crown classes. The probability model used, showed that high precipitation in spring and winter indicates an increase in the probability of the occurrence of IADFs, while high precipitation in July indicates a decrease in the probability of the occurrence of IADFs. A higher frequency in IADFs occurred in the last fifty years, which coincides with the increase in drought events in Spain.

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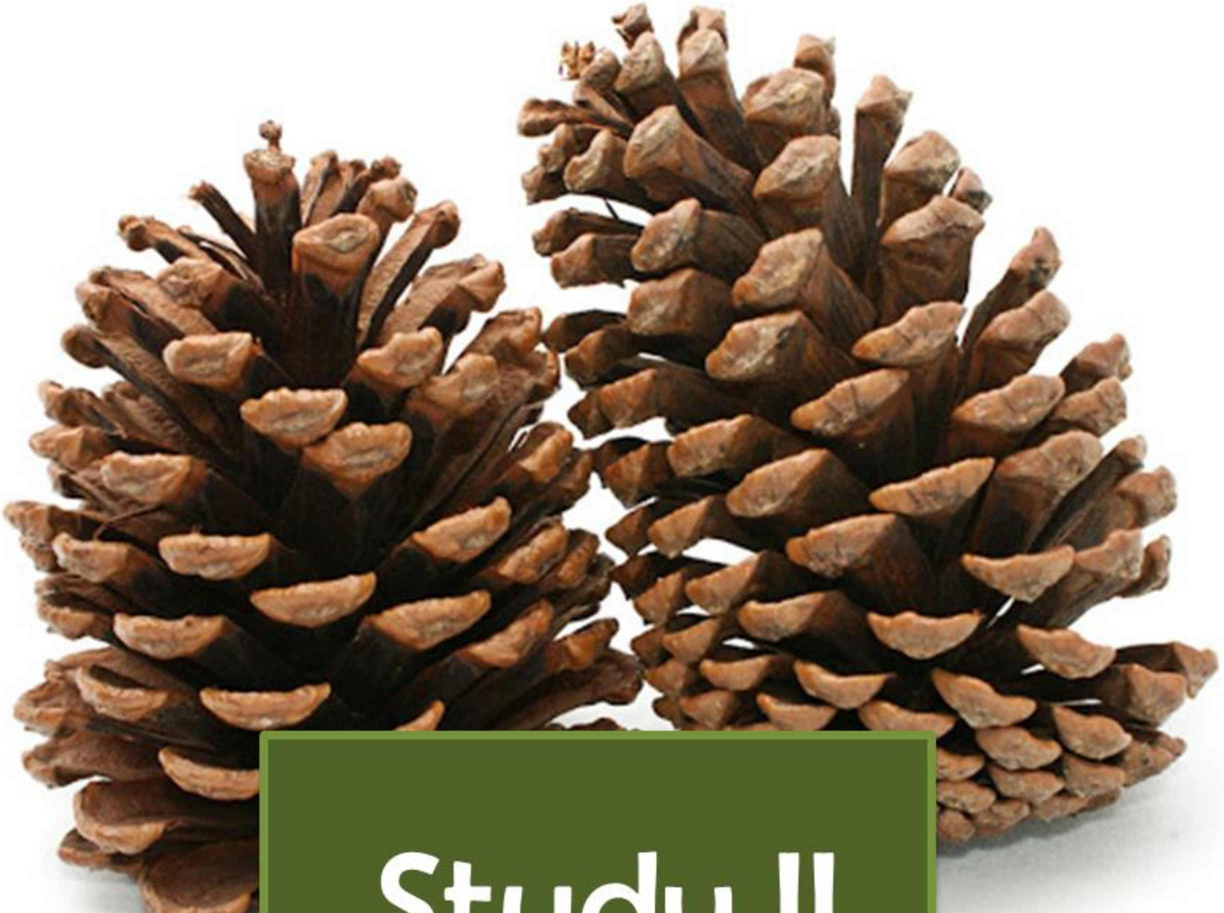

Study II

Changes in climate-growth relationships and IADF formation over time in Mediterranean pine species (*Pinus halepensis, P. pinaster* and *P. sylvestris*) in Spain

* Olivar, J., Bogino, S., Spiecker, H., Bravo, F., 2013. Changes in climate-growth relationships and IADF formation over time in Mediterranean pine species (*Pinus halepensis, P. pinaster* and *P. sylvestris*) in Spain. Submited to European Journal of Forest Research.

Changes in climate-growth relationships and IADF formation over time in Mediterranean pine species (*Pinus halepensis*, *P. pinaster* and *P. sylvestris*) in Spain

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Abstract

The Mediterranean area has experienced an increase in the mean annual temperature, a decrease in the mean annual precipitation, and an increase in the frequency of severe drought periods during the second half of the 20th century. However, winter and spring precipitation has increased and summer precipitation has decreased in western Mediterranean region. The objectives of the present study were: i) to identify and compare changes in climate-growth relationships along time for *Pinus halepensis*, *P. pinaster* and *P. sylvestris* in Spain ii) to quantify and compare the presence of intra-annual density fluctuations (IADFs) on the three species and iii) to define the climatic variables that are associated with the occurrence of IADFs. Dendrochronological techniques were applied at 26 sampling sites (8 for *P. halepensis,* 8 for *P. pinaster* and 10 for *P. sylvestris)* in Spain. Results showed that precipitation is the main factor influencing tree growth and its fluctuation determines IADF occurrence in the three pine species, *P. pinaster being* the species that had the highest correlation values. Wet periods during winter previous to the growth season and spring induced higher growth rates on *P. halepensis* and *P. pinaster*, while the growth of *P. sylvestris* was mostly influenced by summer precipitation. However, the influence of these climatic variables on the growth of these species changed over the studied period. The increase of winter and spring precipitation combined with increasingly harsh climatic conditions during summer in the second half of the 20th century may have enhanced the importance of precipitation at the beginning of the growing season on the growth of species subject to higher drought stress conditions during summer, such as *P. halepensis* and *P. pinaster* and increased the IADF occurrence. Besides reflecting changes in the environmental conditions during the growing season, the inclusion of IADF detection in chronologies adds new information to ring-width chronologies, thereby improving its quality.

Key words

Aleppo pine, maritime pine, scots pine, dendroclimatology, IADFs.

Introduction

Mediterranean environments, as transitional climate zones between arid and humid regions of the world, are of special interest for the study of the relationships between climate, tree growth and wood anatomic features. In addition, the Mediterranean is one of the areas where climatic changes may have the greatest effects (Lavorel et al., 1998). Mediterranean forests are the most important ecological infrastructure of the region, providing goods and services and acting as a key for the resilience and adaptability. *Pinus halepensis, P. pinaster* and *P. sylvestris* are native pines in the Mediterranean region and dominate the current forested landscape. Previous studies on *P. halepensis* concluded that its growth rate is mainly controlled by soil water availability (Rathgeber et al., 2005). *Pinus halepensis* has been the subject of several dendroecological and dendroclimatological studies in some Mediterranean regions such as Spain (de Luis et al., 2007; de Luis et al., 2011; Moreno-Gutiérrez et al., 2012; Olivar et al., 2012; Novak et al., 2013), Greece (Papadopoulos et al., 2008), Israel (Lev-Yadun et al., 1981), Morocco (Chbouki et al., 1995) and Algeria (Safar, 1994). Radial growth of *P. pinaster* is positively correlated with precipitation in Portugal (Vieira et al., 2010; Campelo et al., 2013) and central Spain (Bogino and Bravo, 2008). This fact was also reported for *P. sylvestris* in its southern and western distribution threshold in Spain (Bogino et al., 2009).

Climatic influences on tree growth are unstable, species specific and site dependent (Tardif et al., 2003). Climate change is resulting in both positive and negative trends in tree growth, the latter frequently observed in drought-stressed environments (Camarero et al., 2010). The influence of climatic variables on growth can be modified over time (Andreu et al., 2007) and previous studies showed a changing association between climatic variables and growth of *Pinus* species in the Mediterranean area (Bogino and Bravo, 2008, Vieira et al., 2010; Campelo et al., 2013). During the second half of the 20th century, an overall increase of the mean annual temperature, a decrease of the annual precipitation and a higher frequency of severe drought periods have been observed in the Mediterranean area (Martrat et al., 2004; Xoplaki et al., 2006). However, in the western Mediterranean regions, winter and spring precipitation increased and summer precipitation decreased during that period (Bradley et al., 1987; Maheras, 1988; Díaz et al., 1989).

The analysis of temporal and seasonal dynamics of intra-annual cell formation and wood density profiles is a relevant topic in the literature of the last years (Edmondson, 2010; Bender et al., 2012; Harley et al., 2012). Species growing under Mediterranean climate, with summer droughts and high inter-annual variability in precipitation and temperature, commonly show special anatomical characteristics in tree rings (Schweingruber, 1993). Intra-annual density fluctuations (IADFs) are defined as a layer of cells within a tree ring identified by different shape, size and wall thickness (Kaennel and Schweingruber, 1995). Different studies in conifer species: *P. halepensis* (de Luis et al., 2007; de Luis et al., 2011; Moreno-Gutiérrez et al., 2012; Olivar et al., 2012; Novak et al., 2013) *P. pinaster* (de Micco et al., 2007; Bogino and Bravo, 2009; Vieira et al., 2010; Rozas et al., 2011; Campelo et al., 2013) and P.sylvestris (Panayotov et al., 2013), and broadleaved species (Battipaglia et al., 2010; de Micco et al., 2012) have shown good correlations between IADF formation and climate around the Mediterranean. The consistency of the climatic signal among different pine species and areas suggests that, in the future, a large-scale network of IADFs could be developed in the Mediterranean region to study intra-annual climate variability (Campelo et al., 2013). A more detailed analysis of climatic events may detect effects on inter-annual density fluctuations as determined by a logistic model that includes the stabilized IADF frequency assessed in relation to calendar year.

In order to understand the responses of Mediterranean pine species to climate change and which anatomical structures can be used to document it, the present work investigates: i) radial growth-climate relationships over time for *P. halepensis*, *P. pinaster* and *P. sylvestris* in Spain, ii) the presence of intra-annual density fluctuations (IADFs) on the three species and, iii) the climatic variables that are associated with the occurrence of IADFs.

Materials and Methods

Twenty-six sampling sites (8 *P. halepensis* sites, 8 *P. pinaster* sites and 10 *P. sylvestris* sites) were selected in their distribution area in Spain (Fig. 1; Table 1). *Pinus halepensis* sampling sites consist of an upper storey of *P. halepensis* and an understorey formed by broadleaved Mediterranean species (*Quercus ilex* L., *Q. coccifera* L. y *Q. faginea* Lamk.). Silviculture in the sampling area of *P. pinaster* is traditionally based on natural regeneration following a seed tree system and focused on multifunctional uses (timber, resin, recreation, etc). *Pinus sylvestris* sampling sites are at its southern and western distribution threshold. These dry areas of distribution of this species which usually grows in humid environments are the first places to investigate the effects of increased aridity (Martínez Vilalta and Piñol, 2002). Besides, in assessing the impact of global warming on ecosystems, any changes in tree growth are likely to occur first in those tree stands placed at the ecological boundary of the species (Tessier et al., 1997). At each sampling site, 15 dominant trees were selected. Two cores were extracted at 1.30 m above ground from each selected tree. The increment cores were air dried, mounted on wooden supports and dated according to standard dendrochronological techniques (Stokes and Smiley, 1968).

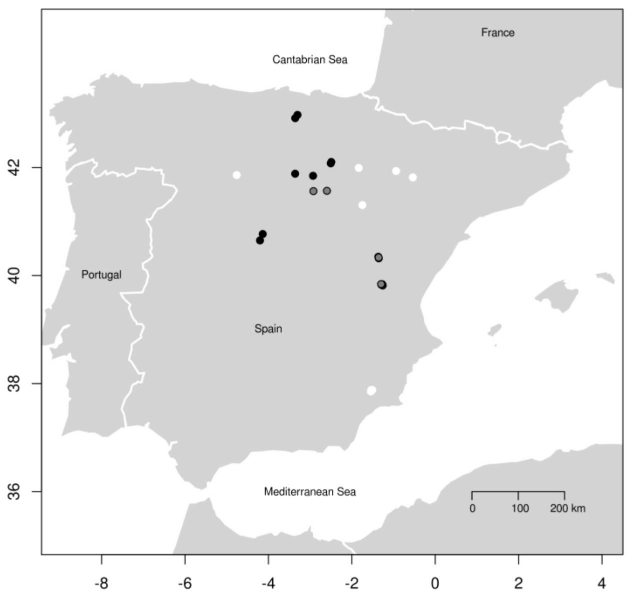


Fig. 1: *Study areas of the three pine species in Spain. White: Pinus halepensis; grey: P. pinaster; black: P. sylvestris.*

Absolute dating is essential for any dendroclimatological study, and it is impossible to compare climatic variables in one specific year with tree-ring growth if the individual tree-ring series are not dated correctly (Fritts, 2001). To assess measurement and dating accuracy, the v6.06P COFECHA program (Holmes, 2001; Grissino-Mayer, 2001; available at www.ltrr.arizona.edu) was applied. This program calculates the Pearson correlation indices between the indexed tree-ring series and a master reference chronology in a series of consecutive, partially overlapped segments of a length specified by the user. According to standard dendrochronological methods, tree-ring series exhibiting correlation values with the master chronology below 0.4 were excluded.

Standardization removes geometrical and ecological trends while preserving inter-annual high-frequency variations that are presumably related to climate. To eliminate biological trends in tree-ring series and to minimize growth variations that are not shared by most trees, the v6.05P ARSTAN program (Cook and Holmes, 1984; Holmes, 2001; available at www.ltrr.arizona.edu) was used. The long-term trend was removed from each time series of ring width measurements by fitting and calculating an index defined as actual ring-width for each year divided by the curve-fit value. The standardized series were averaged in order to obtain a master chronology at each study site.

Table 1: *Sampling sites description of Pinus halepensis, P. pinaster and P. sylvestris in Spain.*

Species	**Code**	**Altitude (m)**	**Time span**	**Annual precipitation (mm)**	**Mean monthly Temperature (ºC)**
P. halepensis	H30101	811	1932-2008	617	12.7
	H30102	957	1915-2008	617	12.7
	H30103	1118	1914-2008	617	12.7
	H34001	849	1975-2008	441	12.1
	H50009	976	1978-2008	344	12.3
	H50001	695	1975-2008	395	13.1
	H50101	535	1919-2007	616	12.4
	H50102	706	1926-2007	587	12.4
P. pinaster	P16106	970	1880-2005	901	8.3
	P16108	920	1948-2005	901	8.3
	P16201	1078	1948-2005	901	8.3
	P16202	1010	1978-2005	901	8.3
	P16208	1090	1887-2005	901	8.3
	P42002	1059	1918-2005	487	8.3
	P42201	1012	1948-2005	484	10.2
	P44002	1437	1846-2005	563	10.4
	P44005	1364	1849-2005	563	10.4
	P44204	1232	1953-2005	563	10.4
P. sylvestris	S05006	1438	1813-2005	559	7.5
	S09005	888	1867-2005	632	9.2
	S09209	1097	1848-2005	487	10.2
	S09501	814	1935-2005	527	10.3
	S40006	1440	1891-2005	466	9.7
	S42415	1165	1951-2005	487	10.2
	S42504	1431	1960-2005	487	10.2
	S42505	1659	1946-2005	487	10.2

The accurately dated cores were visually examined for IADF using a stereomicroscope (magnification up to 25x). In contrast to the annual rings, IADFs show a non-sharp transition boundary between earlywood and latewood cells (Fritts, 2001). IADFs were only counted when present in both cores in the same tree ring, and they were identified by considering the position of the density fluctuation within the ring. Only IADF type E (latewood-like cells within the earlywood) were considered for our study since IADF type L (earlywood-like cells within the latewood) were rarely present in our sample (Fig. 2).

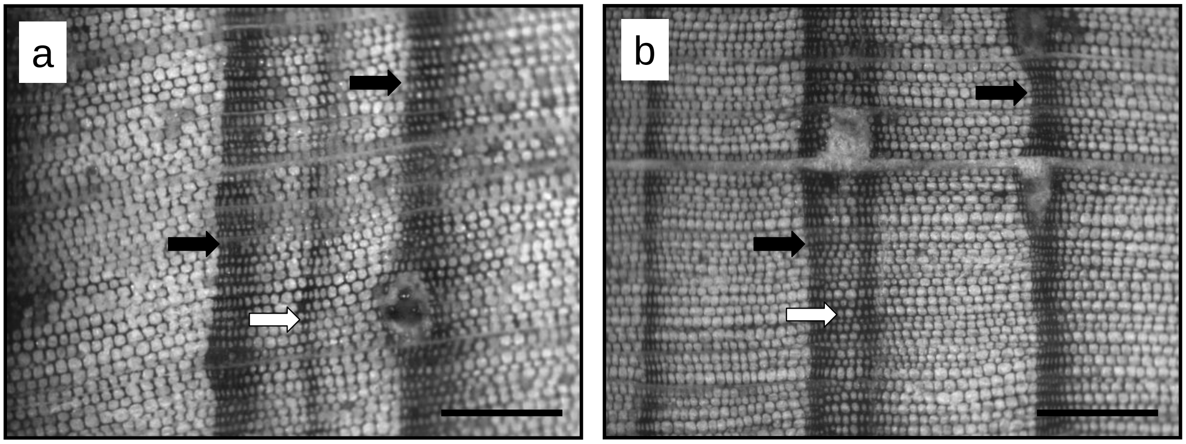


Fig. 2: *Intra-annual density fluctuations (IADF) in Pinus pinaster (Bogino and Bravo, 2009). IADF type E (a): Latewood-like tracheids within the earlywood. IADF type L (b): Earlywood-like tracheids within the latewood. Annual tree rings grew from left to right. Black arrows indicate the true tree-ring boundary and white arrows the IADFs. Scale bars 1 mm.*

As the number of samples changed over time, the relative frequency was calculated with the following formula [1]:

[1] $F = n/N$

where F is the relative frequency of IADF in a particular year; n the number of trees that formed the IADF and N the total number of trees analysed. The bias in the frequency was assessed by calculating the stabilized IADF frequency (f), according to the formula of Osborn et al. (1997) [2]:

[2] $f = F^{0.5}$

The nonlinear logistic equation form was chosen to model the probability of occurrence of IADFs [3]:

[3] $P = (1.0 + e^{(-z)})^{-1}$

where P is the probability of IADFs and $Z = b0+b1(x1)+b2(x2)+\ldots\ldots+bk(xk) + \varepsilon$; where x1; x2..... xk are the climatic variables and b0; b1; b2 ….. bk are unknown parameters of the model and ε is a normal random error N (0,1); and *e* is the exponential operator. The logistic equation can be formulated to accept a binary variable such as occurrence of IADFs, and the parameters can be estimated by maximum-likelihood methods. The resulting prediction is bounded by 0 and 1. Monthly rainfall and mean monthly temperature were used as explanatory variables. The hydrological year was defined as a period of 12 months, from October of the previous year to September of the current growth year. A stepwise selection method was used to find the best model.

The alternative fits were evaluated on the basis of Akaike information criterion (AIC), the –2*Log Likelihood, the area under the receiver operating characteristic (ROC) curve, and the expected behavior - as indicated by the signs of the estimated parameters. ROC curve is displayed for the models and the area underneath was calculated as a value of the accuracy of the model. Value greater than 0.80 indicates an excellent discrimination (Hosmer and Lemeshow, 2000). This curve relies on false/true positive/negative tests, and the sensitivity is indicated by the proportion of correctly classified events and the specificity by the proportion of correctly classified non-events (Hair et al., 1998). Logistic regression was previously successfully used to estimate the probability of occurrence of IADFs in *P. pinaster* subsp. *mesogenesis* and *P. halepensis* in Spain (Bogino and Bravo, 2009; Olivar et al., 2012). PROC LOGISTIC of SAS 9.1 (SAS Institute Inc. 2004) was used to fit the model.

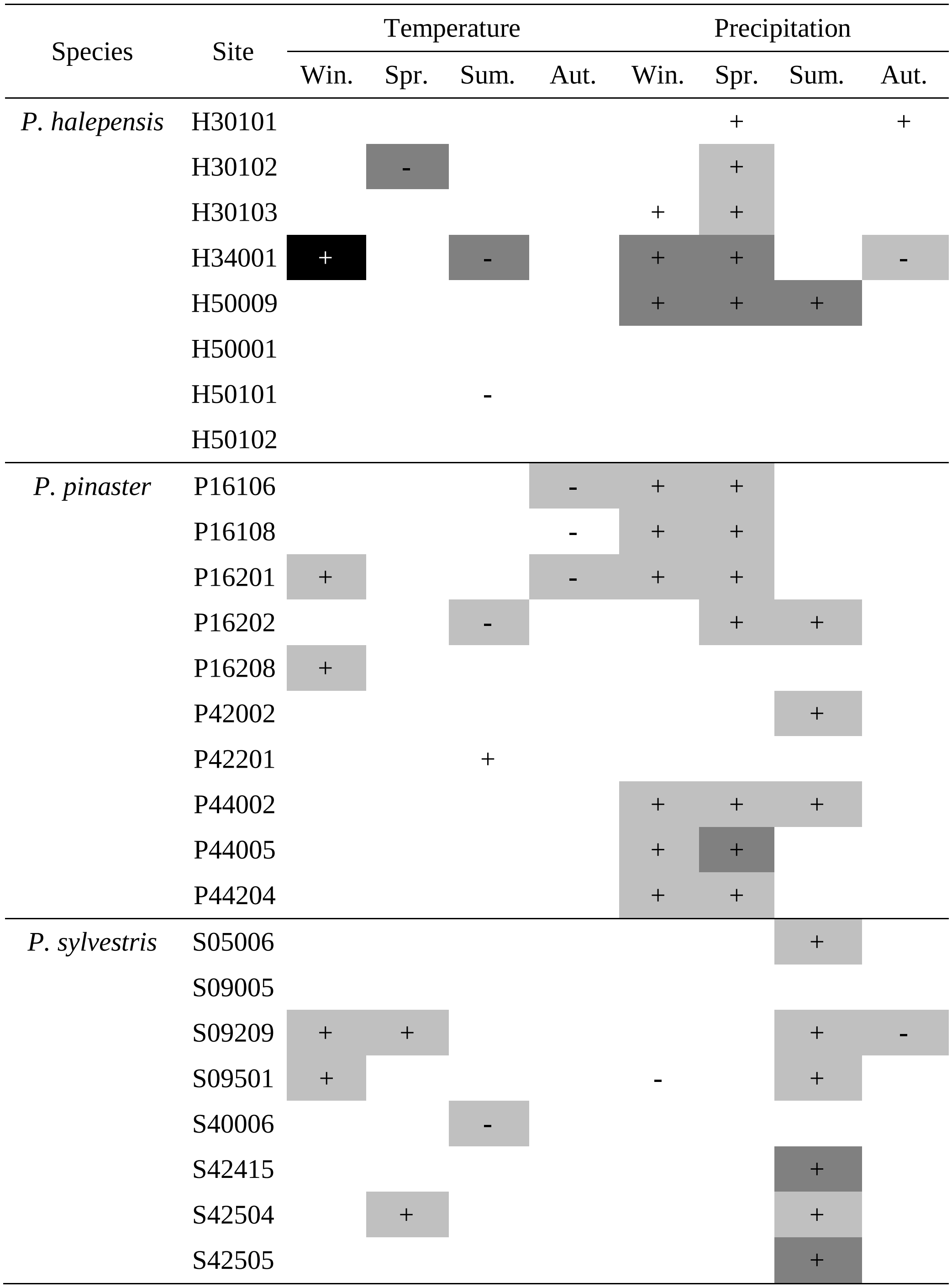
We grouped the climatic variables (monthly precipitation and mean monthly temperature) recorded at the closest meteorological stations (Agencia Estatal de Meteorología, Spain) in climatic seasons: winter previous to the growing season (December-February), spring (March-May), summer (June-August) and autumn (September-November). The climatic data were regressed against ring-width indices and the stabilized IADF frequency. In order to calculate Pearson correlation coefficients and response functions we used DENDROCLIM 2002 (Biondi and Waikul, 2004). Moving correlation function was used to test stationarity and consistency through time with a 20-year interval.

Results

Precipitation is the main factor influencing tree growth of the three Mediterranean tree species. Bootstrap correlation significant values ($p<0.5$) between radial growth of *P. halepensis, P. pinaster* and *P. sylvestris* and seasonal climate is shown in Table 2. Despite site variability, there was a correspondence between the higher correlation values and seasonal climate indicating that wet periods during winter previous to the growth season and spring induced high growth rates on *P. halepensis* and *P. pinaster*, while the growth of *P. sylvestris* was mostly influenced by summer precipitation. *Pinus pinaster* showed the highest correlations ($p<0.005$) between precipitation and growth ($r= 0.12$ in average).

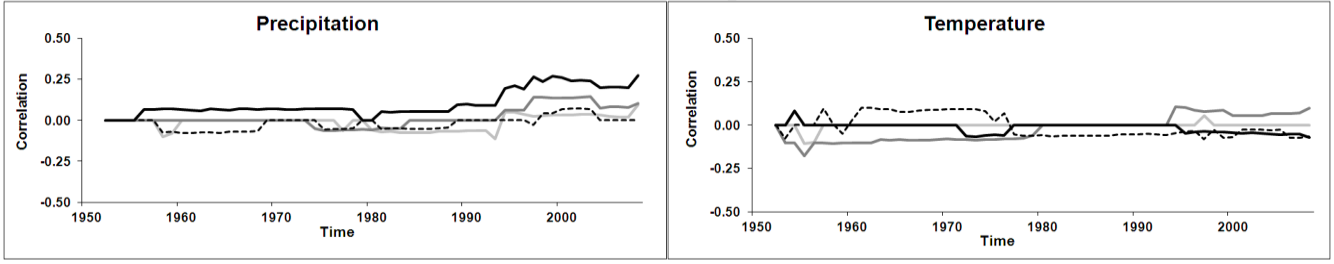
The analysis of the influence of the climatic variables over time on *P. halepensis* shows that this positive influence of winter, spring and summer precipitation on its growth began increasing in the 1990s decade. During that period, spring temperature shifted its influence from negative to positive, while summer temperature shifted from positive to negative (Fig. 3a). In the case of *P. pinaster,* the greatest increase in the influence of the climatic variables on growth occurred during the 1970s, when spring precipitation became the dominant influence followed by summer and winter precipitation. Also during that period, winter temperature increased its positive influence, while the influence of spring, summer and autumn temperature became negative (Fig. 3b). Summer precipitation had the highest correlation values (0.33) with *P. sylvestris*, and they remained essentially stable during the study period. Winter and spring temperature also had a positive influence on its growth, while summer temperature shifted its influence from positive to negative around 1980 (Fig. 3c).

Table 2: *Bootstrap correlation significant values (p<0.5) between radial growth of Pinus halepensis, P. pinaster and P. sylvestris and seasonal climate (mean temperature and precipitation). White: 0-0.24; light grey: 0.25-0.49; dark grey: 0.5-0.74; black: 0.75-1.*

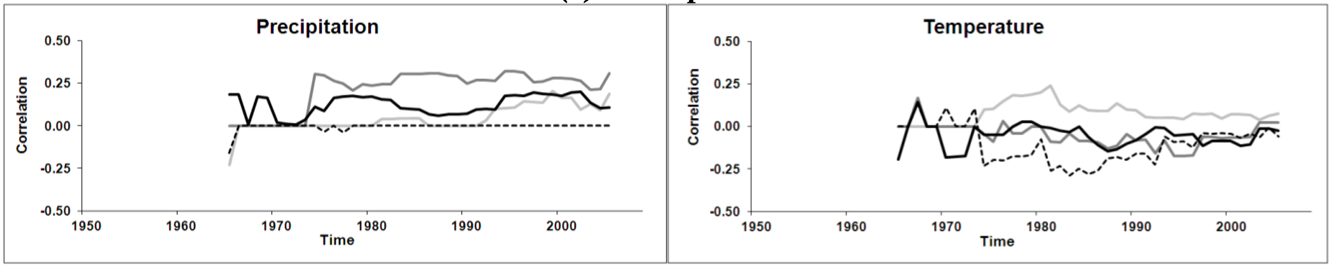


Species	Site	Temperature				Precipitation			
		Win.	Spr.	Sum.	Aut.	Win.	Spr.	Sum.	Aut.
P. halepensis	H30101						+		+
	H30102		-				+		
	H30103					+	+		
	H34001	+		-		+	+		-
	H50009					+	+	+	
	H50001								
	H50101			-					
	H50102								
P. pinaster	P16106				-	+	+		
	P16108				-	+	+		
	P16201	+			-	+	+		
	P16202			-			+	+	
	P16208	+							
	P42002							+	
	P42201			+					
	P44002					+	+	+	
	P44005					+	+		
	P44204					+	+		
P. sylvestris	S05006							+	
	S09005								
	S09209	+	+					+	-
	S09501	+				-		+	
	S40006			-					
	S42415							+	
	S42504		+					+	
	S42505							+	

(a) Pinus halepensis



(b) Pinus pinaster



(c) Pinus sylvestris

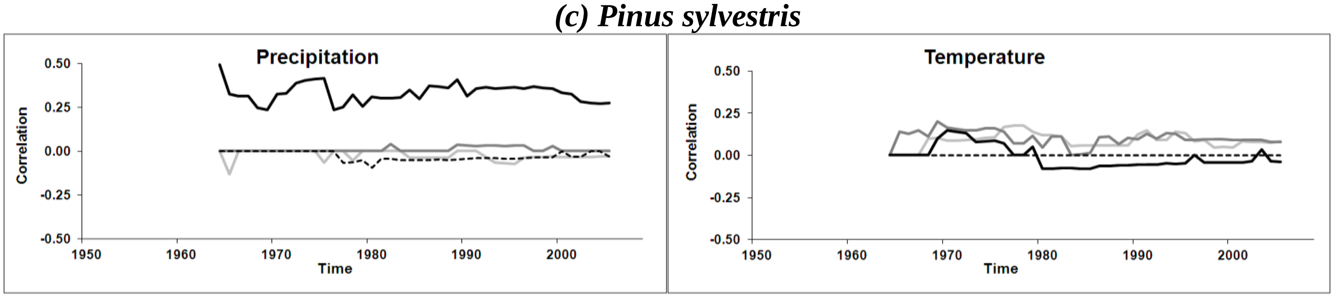


Fig. 3: *Influence of the climatic variables (precipitation and mean temperature) over time on radial growth of Pinus halepensis, P. pinaster and P. sylvestris ($p<0.005$). Light grey: winter; dark grey: spring; black: summer; dashed line: autumn.*

P. pinaster had the highest accumulated mean stabilized IADF frequency (0.12), followed by *P. halepensis* (0.03). The logistic function estimated that the occurrence of IADFs is mainly influenced by precipitation on the three species. Precipitation in the winter previous to the growing season and spring was associated with the occurrence of IADFs in *P. halepensis*, while this influence was delayed in the case of *P. pinaster*, influenced by spring and early summer precipitation. Both species showed a negative influence of precipitation in July. The IADF frequency of *P. sylvestris* was the lowest of the three species (0.004). IADF frequency in relation to calendar year (Fig. 4) showed an increase in IADFs in the second half of the century. The years 1961, 1983, 1995 and 1999 had a higher occurrence of IADFs, with a stabilized frequency higher than 0.8.

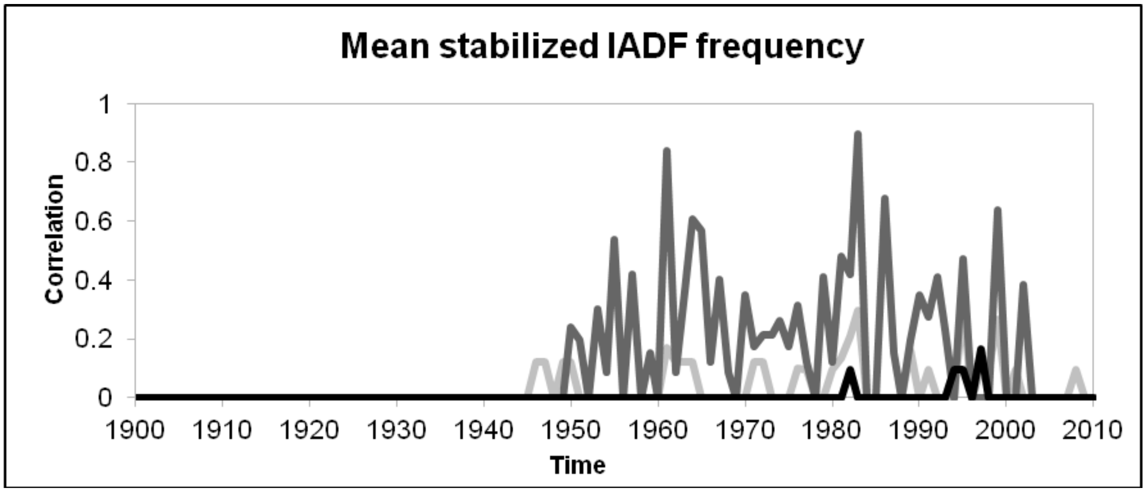


Fig. 4: *Accumulated mean stabilized IADF frequency in relation to calendar year of the three species. White: Pinus halepensis (1914-2008), grey: P. pinaster (1880-2005), black: P. sylvestris (1813-2005).*

Discussion

Climate-growth relationship along time

Precipitation is the main factor influencing tree growth of pine species in semiarid Mediterranean conditions (Raventós et al., 2001). In our study sites, the growth of *P. halepensis*, *P. pinaster* and *P. sylvestris* is mainly controlled by precipitation at different times of the year. Despite the lack of biological significance of some low correlation values and the site variability, higher correlation values between seasonal climatic conditions and species reflect differences in the influence of climatic conditions between species. Winter and spring precipitation is related positively with tree-ring growth in *P. halepensis* and *P. pinaster*, while the growth of *P. sylvestris* is mostly influenced by summer precipitation. These results are consistent with those of previous studies in *P. halepensis* in Greece and Spain (Papadopoulos et al., 2008; Olivar et al., 2012), *P. pinaster* in Portugal (Vieira et al., 2010; Campelo et al., 2013) and central Spain (Bogino and Bravo, 2008), and *P. sylvestris* at its southern and western distribution limits (Bogino et al., 2009).

However, the influence of these climatic variables on the growth of these species changed over the studied period. The positive influence of winter and spring precipitation on *P. halepensis* growth increased beginning in the 1990s and the positive influence of spring precipitation on *P. pinaster* growth increased beginning in the 1970s, while the positive influence of summer precipitation on *P. sylvestris* growth remained stable. These results agree with previous reports on pine species in the Mediterranean area, which suffered a change in growth response to climatic conditions in the second half of the 20th century (Andreu et al., 2007; Bogino and Bravo, 2008; Vieira et al., 2010; Campelo et al., 2013).

Global studies around the Mediterranean basin indicate that winter and spring precipitation increased and summer precipitation decreased during the second-half of the twentieth century (Bradley et al., 1987; Maheras, 1988; Díaz et al., 1989). Mediterranean pines evolved during the Pliocene under tropical-like climate, before the onset of the Mediterranean climate, as a component of the pre-Mediterranean Arcto-Tertiary flora (Verdú et al., 2003; Petit et al., 2005). This species survived to a past gradual increase of aridity during the transition to Mediterranean conditions, which may have led to its characteristic growth plasticity (Chambel et al., 2007). Mediterranean *Pinus* species are considered well adapted to withstand drought by reducing growth as water availability decreases and increasing growth as conditions become favourable (Rathgeber et al., 2005; De Luis et al., 2007; Camarero et al., 2010; Pasho et al., 2012). This increase of winter and spring precipitation combined with the increasingly harsh climatic conditions during summer may have enhanced the importance of precipitation at the beginning of the growing season on the growth of species subject to higher drought stress conditions during summer, such as *P. halepensis* and *P. pinaster*. On the other hand, *P. sylvestris*, growing under higher water availability during the whole year, didn’t suffer that swift in the influence of the climatic variables.

IADF occurrence

The occurrence of IADFs is mainly influenced by precipitation in these species. IADFs may appear at different development phases of the tree-ring formation depending on the time of the year when the stress conditions occurred. IADF type E is triggered by dry periods during spring and early summer. In contrast, IADF type L is triggered by precipitation during late summer and (or) early autumn (Wimmer et al., 2000). Previous studies in the Mediterranean area showed a high frequency of IADFs in latewood (de Luis et al., 2007; Vieira et al., 2010; Rozas et al., 2011; Campelo et al., 2013; Novak et al., 2013). However, the low frequency of IADF type L and the high frequency of IADF type E observed in our samples indicate a higher occurrence of water stress episodes inhibiting cell division and enlargement during the first part of the growing season. The ability of species to produce different types and forms of cells in different periods may also be interpreted as an important adaptation of trees for maintaining the balance among the capacity to conduct water, resistance to cavitation and mechanical stability (Novak et al., 2013).

The formation of IADFs is triggered by above-average precipitation in the previous winter and spring in *P. halepensis* and in spring and early summer in *P. pinaster* and negatively influenced by precipitation in July. These climatic conditions (precipitation at the beginning of the growing season and summer droughts) have been increasingly favoured over the second half of the 20th century, explaining the increasing occurrence of IADFs our study area. This result agrees with previous studies that found an increase in IADF frequencies after 1980 in *P. pinaster* in Spain (Bogino and Bravo, 2009) and Portugal (Vieira et al., 2010; Campelo et al., 2013). Despite being at its southern distribution threshold, where a species that usually grows in humid environments could suffer from the effects of increased aridity (Martínez Vilalta and Piñol, 2002), *P. sylvestris* showed the lowest IADF frequency of the three species on our sample. As pointed out by Battipaglia et al., 2010, the frequency and the triggering climatic factors promoting different anatomical characteristics may vary among populations, depending on different environmental conditions.

Conclusions

Precipitation is the main factor influencing tree growth and its fluctuation determines IADF occurrence in the three pine species. *Pinus pinaster* showed the highest correlations between precipitation and growth. Wet periods during winter previous to the growth season and spring induced higher growth rates in *P. halepensis* and *P. pinaster*, while the growth of *P. sylvesteris* was mostly influenced by summer precipitation. Precipitation in the winter previous to the growing season and spring was associated with the occurrence of IADFs in *P. halepensis,* while this influence was delayed in the case of *P. pinaster,* influenced by spring and early summer precipitation. However, the influence of these climatic variables on the growth of these species changed over the studied period. The increase of winter and spring precipitation combined with the increasingly harsh climatic conditions during summer in the second half of the 20th century may have enhanced the importance of precipitation at the beginning of the growing season on the growth of species subject to higher drought stress conditions during summer, such as *P. halepensis* and *P. pinaster* and the occurrence of IADFs increased. The incorporation of special ring features such as IADFs and their association with climatic variables in any dendrochronological study provides a useful proxy for complementing and enhancing the dendroclimatological data.

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Study III

Thinning has a positive effect on growth dynamics and growth-climate relationships in Aleppo pine (*Pinus halepensis*) trees of different crown classes

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Thinning has a positive effect on growth dynamics and growth-climate relationships in Aleppo pine (*Pinus halepensis)* trees of different crown classes

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Abstract

· ***Context*** Modification of stand density by thinning may buffer the response of tree growth and vigor to changes in climate by modulating soil water availability.

· ***Aims*** We tested the impact of thinning intensity on cambial growth of Aleppo pine (*Pinus halepensis* L.) under semi-arid, mediterranean conditions.

· ***Methods*** A multiple thinning experiment was established on an Aleppo pine plantation in Spain. We analysed the stem growth dynamics of two different crown classes under four different thinning intensities (15%, 30% and 45% removal of the basal area) for two years based on biweekly band dendrometer recordings. Local relative extractable soil water was derived from the use of a water balance model Biljou© and used as an explanatory variable.

· **Results** Radial growth was mainly controlled by soil water availability during the growing season and differed by crown class. The growth rates of dominant trees were significantly higher than the growth rates of suppressed trees. 30% and 45% removal of the basal area produced a growth release in both dominant and suppressed trees that did not occur under less intense thinning treatments.

· ***Conclusions*** Soil water availability was the main driver of radial growth during the growing season. Forest management confirmed its value for ameliorating the effects of water limitations on individual tree growth. These results may help managers with understanding how altering stand dynamics will differentially affect climate-responses of Aleppo pine trees, promoting forests that are resilient to future climatic conditions.

Key words

Forest management, stand density, dendrometer, dominant, suppressed.

Introduction

In coastal Mediterranean areas, with mild winters and dry summers, cold season growth dormancy may not occur and, consequently, cambial activity is likely continuous (de Luis et al. 2007). However, in inland areas with continental Mediterranean climate, plants endure double climatic stress, caused by low winter temperatures and summer drought (Mitrakos 1980). As a result, optimal growth conditions are split into two mild periods with higher rainfall (spring and autumn), which suggest a bimodal pattern of cambial activity (Camarero et al. 2010). Pine stands are the most extensive coniferous forests in the Mediterranean zone, and Aleppo pine (*Pinus halepensis* Mill.) is one of the dominant tree species in the Western Mediterranean Basin and the most ecologically important species in semi-arid woodlands (Néeman and Trabaud 2000). Growth rates of *P. halepensis* may be expected to follow the bimodal pattern described above for trees growing in a continential Mediterranean climate, with maximum values in spring and autumn when precipitation is high and temperatures are mild.

In the Mediterranean climate, water is considered to be the most limiting resource for plant growth (Specht 1981). Nevertheless, trees of different sizes and crown classes compete differently for light, water and other resources within a stand (Peet and Christensen 1987; Orwig and Abrams 1997). Radial growth responses to climate vary considerably according to local stand density and crown class in areas where water is limiting (Linares et al. 2009). Therefore, contrasting growth responses to climate among trees in different crown classes might be particularly relevant for understanding the dynamics of forests dominated by species that are highly sensitive to drought stress (Adams and Kolb 2004).

Climate change models project a decrease in annual mean precipitation and rising air temperatures over the Mediterranean Basin for the twenty-first century leading to an increase in evapotranspiration (Parry et al. 2007). If water is a limiting resource, as it is predicted to be, one of the most important measures that forest managers have to moderate the influence of climate on tree growth within stands is to thin to residual stand densities that make more water available for the remaining trees (Martín-Benito et al. 2010).

Although forest managers rely heavily on the use of silvicultural treatments that manipulate stand structure and stand dynamics to modify responses to climate change, few studies have directly assessed the effects of stand structure or canopy position on climate–growth relationships (Carnwath et al. 2012). The effect of different silvicultural treatments and stand densities on tree growth response to climate is likely to be a key aspect for influencing forest preservation, particularly in the driest sites (Gea-Izquierdo et al. 2009). The influence of stand density on tree response to climate and drought is not a straightforward issue, since different approaches (e.g. ecophysiological, dendroecological, growth modelling) might give different insights (Misson et al. 2003; Moreno and Cubera 2008).

Short term data on growth dynamics are required to understand growth responses in relation to climatic variability at short time scales. Dendrometers are a valuable tool for these studies since they provide short-term measurements of radial fluctuations in relation to climate without disturbing the cambium. Here, we analysed the stem growth dynamics of *P. halepensis* of two different crown classes under four different thinning intensities for two years based on biweekly band dendrometer recordings. We expected that short-term variations in climatic factors and water availability were reflected in the entire growth profile. The following hypothesis were tested: (i) soil water content is the main climatic driver of intra-annual variation in growth of *P. halepensis* at our site, representing semi-arid Mediterranean environments, (ii) suppressed trees are more affected by water stress than dominant trees and (iii) the reduction of stand density through thinning stimulates diameter growth responses that offset declines expected under more severe water limitation.

Materials and Methods

Species and study site

P. halepensis is one of the most studied Mediterranean trees. Forests dominated by this species provide a valuable system to explore how growth measured at different time scales is constrained by climate (Pasho et al. 2012) and it is considered well adapted to withstand drought by reducing growth as water availability decreases (Rathgeber et al. 2005; de Luis et al. 2007; Camarero et al. 2010; Pasho et al. 2012). The study site is located in a 60-year-old *P. halepensis* plantation in the north of the Spanish Meseta (Fig. 1). The area is characterized by a continental Mediterranean climate, with low winter temperatures and summer droughts. Mean annual temperature is 11.3 ºC and average annual precipitation is 418 mm for the last thirty years. The climatic conditions in 2011 and 2012 were among the driest in the late 20th century in Spain. Total annual rainfall was 245 and 244 mm respectively (40% lower than the average).

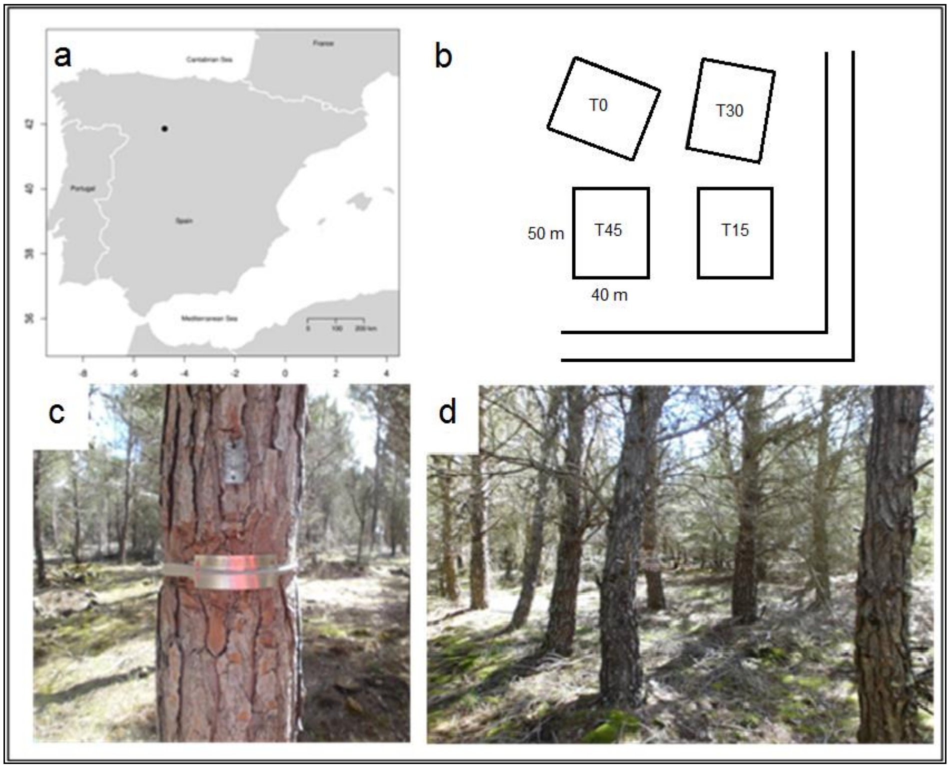

Fig. 1: *(a) Study area of Pinus halepensis in Spain. (b) Large scale map of the thinning experiment.*

(c) Band dendrometer (Dendrometer Increment Sensor DB20 EMS Brno). (d) T0: unthinned stand.

Thinning treatments

Thinning treatments were conducted in autumn 2010 in three stands of similar basal area and stand structure (Table 1). The stand sizes were 40m x 50m. Three thinning intensities were considered: 15% reduction of the basal area (T15), 30% reduction of the basal area (T30) and 45% reduction of the basal area (T45), using an unthinned stand as control (T0) (Fig. 2). The reduction of the basal area was based on initial basal area of each stand.

Table 1 *Stand parameters of the four plots. BA = Basal area; DBH = Diameter at breast height;* H_0 *= Dominant height (mean height of the hundred largest trees per hectare); LAI = Leaf area index (projected needle area per unit ground area).*

Stand	Thinning intensity	H_0 (m)	Initial BA (m^2/ha)	Final BA (m^2/ha)	Initial Mean DBH (cm)	Final Mean DBH (cm)	Initial LAI	Final LAI
T0	0%	10.6	22.6	22.6	18.6	18.6	1.90	1.90
T15	15%	10.1	27.6	23.5	18.2	18.9	2.19	1.90
T30	30%	9.4	20.0	14.0	17.1	18.5	1.97	1.45
T45	45%	9.1	20.5	11.2	17.6	21.2	1.80	1.00

The thinning treatments conducted on pine species in this area usually follow a 30% reduction of the total basal area, and the Marginal Thinning Intensity (MTI), defined as maximum intensity that can be maintained without loss of volume production (Kula, 1988), might be close to a 45% removal of the total basal area.

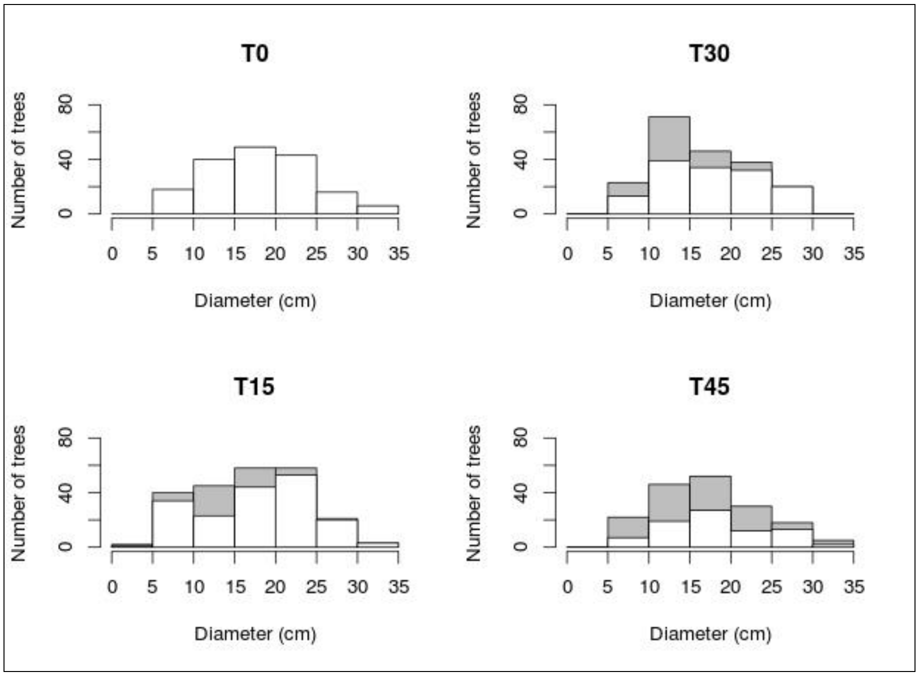

Fig. 2: *Thinning treatments of Pinus halepensis at the four plots. Grey: extracted trees. T0: unthinned stand; T15: 15% reduction of the total basal area; T30: 30% reduction of the total basal area; T45: 45% reduction of the total basal area.*

Dendrometer measurements

To estimate the changes in stem increment, 48 stainless-steel band dendrometers (Dendrometer Increment Sensor DB20 EMS Brno) were mounted around the stem at 1.3 m height in 12 randomly chosen trees per treatment: 6 dominant trees (trees with crowns receiving full light from above and partly from the side) and 6 suppressed (trees with crowns receiving no direct light either from above or from the sides) in each plot. In most cases, the diameter of dominant trees ranged from 20 to 25 cm and suppressed trees from 10 to 15 cm. However, some trees were chosen as dominant or suppressed being out of these ranges if their relative position in relation to its neighbours gave them the dominant or suppressed status.

Dead outermost tissue of the bark was smoothly and evenly removed with a rasp before attaching the dendrometer. Removing the bark allowed us to reduce swelling and shrinkage from the bark as a component of the increment dynamics. Dendrometer readings to the nearest 0.01 mm were performed biweekly over a two-year period. Readings were done in the morning to reduce diurnal bias because daytime transpiration causes stem shrinkage (Zweifel et al. 2001). Following Keeland and Sharitz (1993), measurements obtained in the first 3 months after installation were discarded for being within the adjustment period of the band dendrometers.

Statistical analyses

Daily climatic data were measured at the closest meteorological station (data provided by InfoRiego and the Spanish Meteorological Agency AEMET). Soil water availability to the trees, which can be characterized by relative extractable water (hereafter abbreviated as REW), was included in the analysis. REW is the daily available water standardized by maximum available water. Water stress is assumed to occur when REW drops below a threshold of 0.4 under which transpiration is gradually reduced due to stomatal closure (Granier et al. 1999). REW was calculated by the daily water balance model Biljou© (https://appgeodb.nancy.inra.fr/biljou/) (Granier et al. 1999). This model requires daily potential evapotranspiration and rainfall as input climatic data. Evapotranspiration was calculated with the Penman potential evapotranspiration equation (Granier et al. 1999). Required site and stand parameters are maximum extractable soil water and leaf area index (LAI). Soil properties were measured at the site are considered homogeneous at the four plots (Table 2). LAI controls stand transpiration, forest floor evapotranspiration and rainfall interception (Granier et al. 1999). LAI was calculated acording with López-Serrano et al. (2000):

$$L_t = 0.0536 * D^{2.05}$$

where L_t is the leaf area of individual tree (m2) and D is the DBH (cm2), R^2 = 85.53% (Table 1). The bioclimatic variables (temperature, precipitation, evapotranspiration and REW) were averaged (or accumulated in the case of precipitation) in 5, 15 and 30 days prior to prior to each diameter measurement in order to determine which climatic variables were the main constraints for growth and at which temporal scales they act. These weather intervals were selected under the assumption that growth responded to weather or climatic variables that prevailed just prior to each growth period.

Table 2: *Soil characteristics of the study area of Pinus halepensis included in the model Biljou© (Granier et al., 1999).*

Variables	Horizon 1	Horizon 2
Dept (cm)	0-30	30-200
Water reserve (mm)	36	48
Roots (%)	75	25
Stones (%)	1	80
Humidity (pF 4.2)	0.0158	0.0158
Apparent density	1.46	1.46

In order to test similarity of growth rates prior to thinning among the four plots, increment cores were extracted from a total of 15 trees in the four plots. As preliminary trials, we fitted a linear model using past growth as response variable and a mixed model including a plot random effect. The relative goodness of fit of the models was assessed using the Akaike information criterion (AIC). Since AIC value (4399) did not change when the plot random effect was included, we concluded that the average growth was not different across the plots.

We fitted a linear mixed-effect model using the lme function with R-Package nlme (Laird and Ware, 1982). The model was fitted by maximizing the restricted log-likelihood:

$$Gr_{ijk} = \beta_0 + \beta_1 * Clim_{ik} + \beta_{2\,im} + \beta_3 * S_{ij} + \beta_4 * (Clim_{ik} * S_{ij}) + \beta_{5im} * S_{ij} + b_{ij} + \varepsilon_{ijk}$$

$$b_{ij} \sim N(0, \sigma^2_{Tree})$$

$$\varepsilon_{ijk} \sim N(0, \sigma^2\, |Clim_{ik}|^{2\delta})$$

where *Gr* is the radial growth, *Clim* are the mean (or accumulated) values of the bioclimatic variables (temperature, precipitation, evapotranspiration and relative extractable water), *i* is the plot index, *j* is the tree index, *k* is the two-week period index, *m* is the thinning intensity index such that:

m = 1 for the 15% thinning intensity

m = 2 for the 30% thinning intensity

m = 3 for the 45% thinning intensity

S is an indicator variable for the status of the tree (*S* = 0 for suppressed trees and *S* = 1 for dominant trees), b_{ij} is a tree random effect and the variance of the residual error terms ε_{ijk} is modelled as σ^2, multiplied by the power of the absolute value of covariate *Clim*. The parameter δ is also estimated using a maximum likelihood estimator (see Zuur et al. 2009).

The period used for growth and climate analysis corresponded only with the main period of stem growth (Deslauriers et al. 2007). Based on our records, the period between 20 November 2011 and 15 April 2012 was excluded from the analysis. Statistical analyses were performed using R software (R Development Core Team 2011). Initial stand density was similar between sites. However, there were some differences due to microsite effect prevailing in Mediterranean environments. In order to probe similarity of growth rates prior to thinning among the four plots, past radial growth (40 years) was tested through increment cores extracted from 15 trees belonging to the four plots. We fitted a linear model using past growth as a response variable and a mixed model including the plot random effect. The relative goodness of fit of the models was assessed using the Akaike information criterion (AIC). Since AIC value (4399) did not change when the plot random effect was included, we can conclude that there are no significant differences on previous growth between plots.

Climate sensitivity is defined as the degree of growth response to climate variability. Because our growth data are intra-annual, coefficient of variation (hereafter abbreviated as CV), which shows the extent of variability in relation to the mean, was calculated in order to assess the climate sensitivity of the radial increment series. CV was calculated for the radial growth during the 2-week growth periods on each tree and analysed for trees within status, plots and years.

Results

The results of the linear mixed-effect model applied for each variable (temperature, precipitation, evapotranspiration and REW) confirmed that REW was the main bioclimatic variable that influenced tree radial growth at the studied stands (Table 3).

Table 3: *Comparison of the linear mixed-effect model for the different bioclimatic variables (mean or accumulated of the previous 5, 15 and 30 days). AIC = Akaike information criterion; RMSE = Root Mean Square Error; ETP = Evapotranspiration; T = Mean temperature; PP = Accumulated precipitation; REW = Relative extractable water (daily available water standardized by maximum available water).*

Variable	Delay (days)	AIC	RMSE
ETP	5	4565	0.016
	15	4539	0.015
	30	4463	0.014
T	5	4226	0.014
	15	4155	0.013
	30	4009	0.012
PP	5	4165	0.013
	15	4196	0.014
	30	4165	0.013
REW	5	3745	0.011
	15	3734	0.011
	30	3978	0.011

Mean REW of the previous 15 days showed the lowest AIC values. The comparison between mean REW of the previous 15 days and radial increment of the four plots can be observed in Fig. 3. Growth increased with thinning intensity.

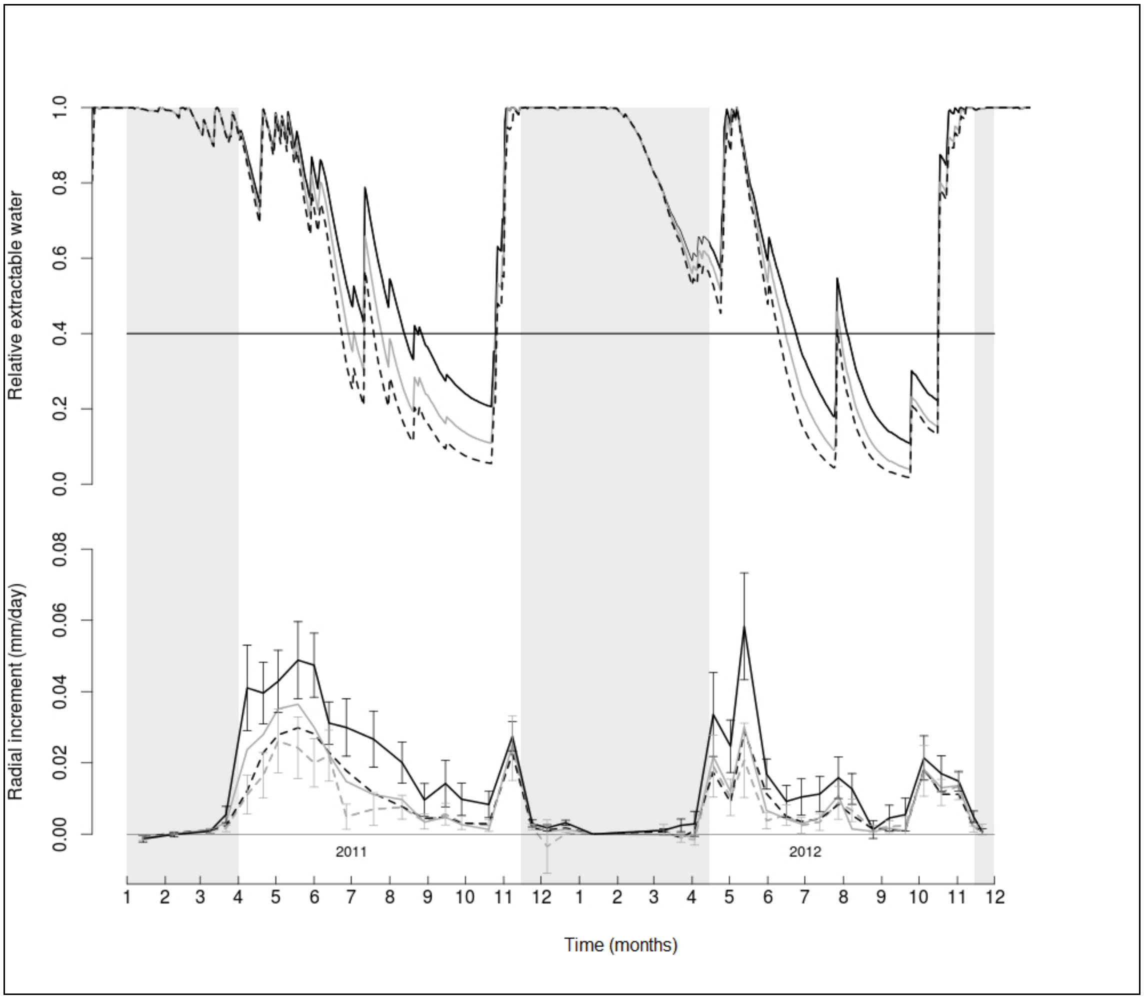

Fig. 3: *Relative extractable water (daily available water standardized by maximum available water) and radial increment of Pinus halepensis at the four plots. T45: black solid line, T30: grey solid line, T15: black dashed line. T0: black dashed line for REW and grey dashed line for radial increment. Water stress is assumed to occur when REW drops below a threshold of 0.4 (Granier et al. 1999). Grey areas show periods not considered for the analysis.*

We fitted the model with mean REW of the previous 15 days (Table 4) as climatic variable. Results showed that dominant trees and trees in T30 and T45 had significantly higher growth rates.

Table 4: *Results of the linear mixed-effect model for REW15. Relative extractable water (REW) is the daily available water standardized by maximum available water. T0: unthinned stand; T15: 15% reduction of the total basal area; T30: 30% reduction of the total basal area; T45: 45% reduction of the total basal area.*

Parameter	Value	Std. Error	P-value
Intercept	-0.0004	0.0017	0.834
Dominant	0.0049	0.0025	0.055
REW	0.0081	0.0018	<0.0001
T15	-0.0014	0.0025	0.587
T30	-0.0006	0.0026	0.793
T45	-0.0032	0.0026	0.223
Dominant * REW	0.0233	0.0018	<0.0001
T15 * REW	0.0055	0.0023	0.017
T30 * REW	0.0093	0.0024	<0.0001
T45 * REW	0.0248	0.0026	<0.0001
Dominant * T15	0.0005	0.0036	0.896
Dominant * T30	-0.0042	0.0036	0.253
Dominant * T45	-0.0014	0.0036	0.708
$\sigma^2 Tree$	0.0042		

The comparison between real growth and predicted values of the model for T0 and T45 are shown in Fig. 4. Model predictions are more accurate for dominant than suppressed trees and for T45 than T0.

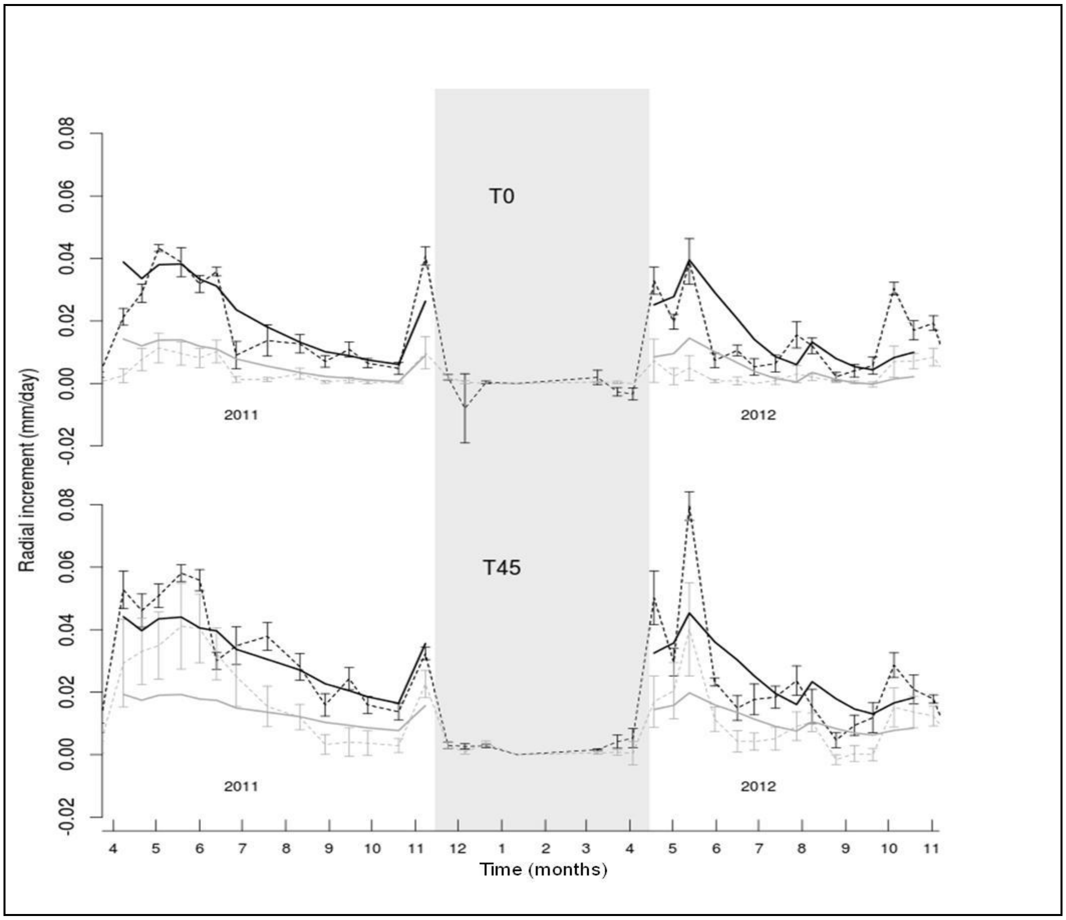

Fig. 4: *Real growth of Pinus halepensis (dashed lines) and growth predictions from the model (solid line). Black: dominant trees (trees with crowns receiving full light from above and partly from the side); grey: suppressed trees (trees with crowns receiving no direct light either from above or from the sides). T0: unthinned stand. T45: 45% reduction of the total basal area. Grey areas show periods not considered for the analysis.*

Growth patterns of the four stands are described in Fig. 5. The growth dynamics of *P. halepensis* showed a bimodal growth pattern, with two major growth phases in spring and autumn and low rates in late summer and beginning of autumn. The spring maximum increment rate was higher than the autumn maximum increment rate, except for T0 in 2011.

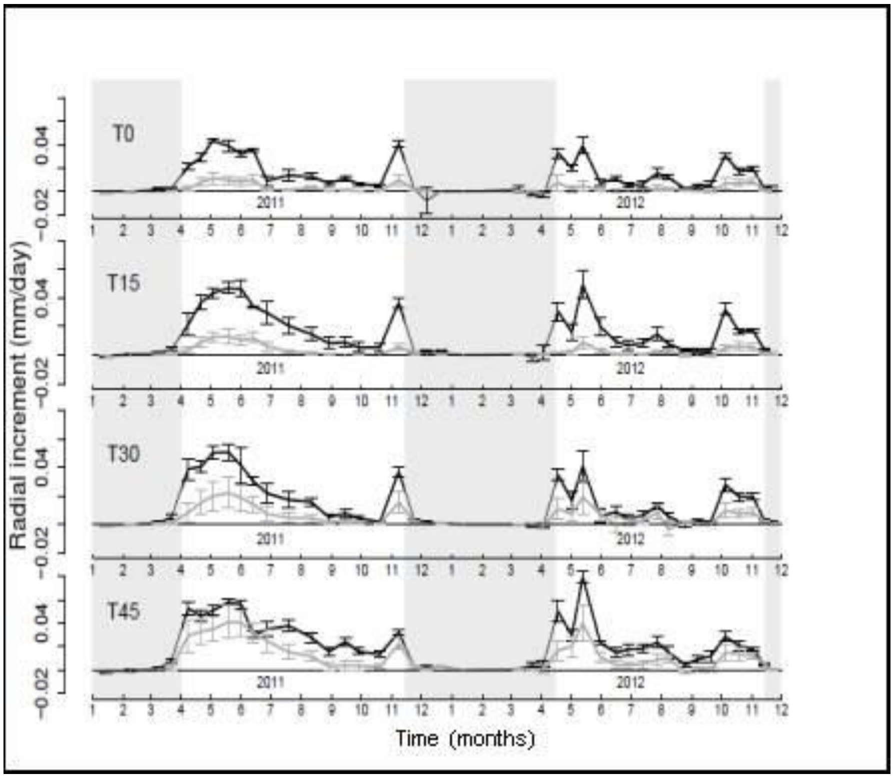

Fig. 5: *Growth dynamics of Pinus halepensis at the study site. Black line: dominant trees (trees with crowns receiving full light from above and partly from the side); grey line: suppressed trees (trees with crowns receiving no direct light either from above or from the sides). Grey areas show periods not considered for the analysis.*

The analysis of the variance of the growth data (Fig. 6) showed that suppressed trees had higher CV values than dominant trees. T45 showed the lowest CV values in both crown classes during the two years of study.

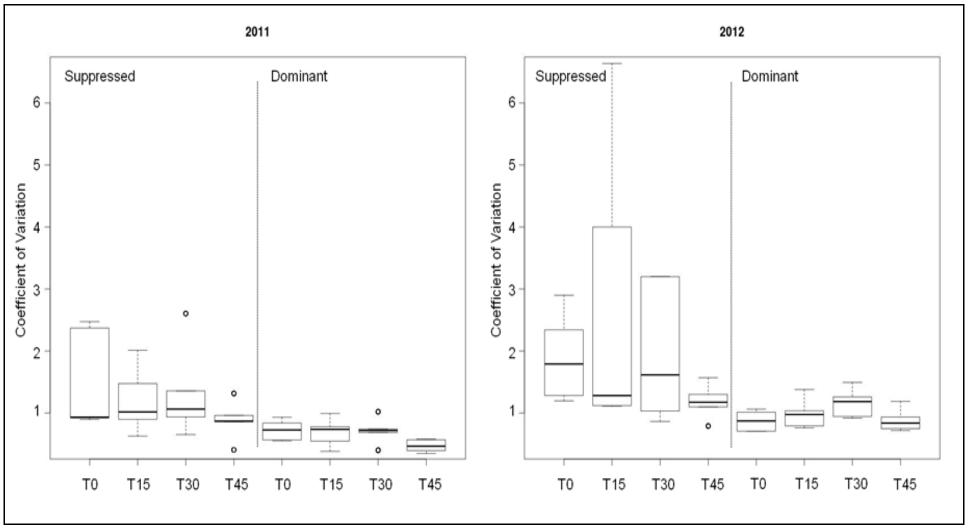

Fig. 6: *Coefficient of variation of the growth data of Pinus halepensis at the four plots: unthinned stand (T0); 15% reduction of the total basal area (T15); 30% reduction of the total basal area (T30) and 45% reduction of the total basal area (T45). Dominant trees: trees with crowns receiving full light from above and partly from the side. Suppressed trees: trees with crowns receiving no direct light either from above or from the sides.*

Discussion

Dendrometers have been criticized when used to record cambial activity with a high temporal resolution because of the reversible stem shrinking and swelling (de Luis et al. 2007). These criticisms are, to some extent, justified when dendrometers are used to identify cambial growth onset and ending or differentiate growth from stem water content variation in slow-growing species (Deslauriers et al. 2007). However, several studies have been published in recent years describing stem growth phenology and/or assessing growth–climate relationships (Bouriaud et al. 2005; de Luis et al. 2007; Gea-Izquierdo et al. 2009; Linares et al. 2009; Camarero et al. 2010; Gutierrez et al. 2011). In our study, removing the bark and the fixing interval of the day during that stem radius variation was measured allowed us to improve precision by eliminating effects of bark irregularities and subtle sloughing and to reduce swelling and shrinkage as a component of the increment dynamics (Zweifel et al. 2001).

REW is the main bioclimatic variable which influences tree radial growth at the studied stands. These results add information to previous inter-annual studies on the same stands (Olivar et al. 2012) that defined monthly rainfall as the main climatic driver of tree growth. The utilisation of soil moisture rather than rainfall is less susceptible to confounding between the effects of water and temperature in the growth response function. Furthermore, soil moisture has more biological meaning than precipitation because it better reflects water availability for trees. These points are particularly relevant for studies performed in Mediterranean regions (Rathgeber et al. 2005).

In the Mediterranean area with a continuous water deficit, at least for *P. halepensis*, wood formation reflects environment conditions for most of the year, and a precipitation event is generally followed by an increase in growth within a week after the event (Attolini et al. 1990). Water availability is expected to be the major limiting factor for growth under a Mediterranean climate and its influence decreases with altitude in the Mediterranean region, with temperature showing the opposite trend (Cherubini et al. 2003). There is a general idea that radial growth is primarily dependent on the short-term water availability, which affects the actual tree water status and xylogenesis (Eilmann et al. 2009). In the studied stands, the influence of REW on dominant trees was significantly higher than on suppressed trees. In drought stressed environments, suppressed trees usually have shallower and less developed root systems than dominant trees, and less moisture is available to them (Van Lear and Kapeluck 1995). Therefore, the amount of available water for suppressed trees is limited to the short term water reserves concentrated in surface soil layers; while dominant trees, with more developed root systems can access to deeper soil water reserves accumulated in longer terms (Camarero et al. 2010), inducing higher vulnerability of suppressed trees to water stress.

The growth dynamics of *P. halepensis* had two major growth phases during the growing season, one in spring and another in autumn, interrupted by a period with low or no cambial activity during late summer as a result of drought and high temperatures (Cherubini et al. 2003). This pattern of xylem production is responsible for the frequent formation of intra-annual density fluctuations observed in this species (Olivar et al. 2012). Stem growth started at middle March in 2001 and April in 2012, when temperatures were high enough for vegetative growth, and stopped growing at the end of November in both years, when temperatures were too low. The radial growth pattern of *P. halepensis* was asymmetrical with most of the annual growth occurring in spring. The maximum growth rate in spring was the highest of the year except for T0 in 2011, where the autumn maximum growth rate was higher. Growth patterns of *P. halepensis* showed high-plasticity in response to climatic conditions during the growing season, which may be an advantage in areas with a Mediterranean climate where the annual rainfall pattern is variable (Gutiérrez et al. 2011). *P. halepensis* has been characterized as a sensitive Mediterranean species based on the strong response of growth dynamics to changes in climatic conditions (Lev-Yadun 2000). It has also been suggested that the cambium is able to remain active throughout the whole year when climate conditions are favourable (Liphschitz et al. 1984).

Growth rates prior to thinning probed to be not significantly different among the experimental units, suggesting that the thinning intensities were sufficient to account for residual stand density. Therefore, we can conclude that T30 and T45 thinning treatments were able to induce a growth release on both crown classes that did not occur under less intense thinning treatments. Thinning is less effective at dry sites if it is not intense enough, because inter-tree competition for water is stronger and drier sites cannot support stands of sufficiently high density to warrant thinning (Cotillas et al. 2009; Linares et al. 2009; Moreno and Cubera 2008). Thus, a still high competition level that is not sufficiently reduced by thinning, coupled with the more limiting climatic conditions, should have obscured the advantages of thinning on less intense thinning treatments (Misson et al. 2003).

Growth variability was higher in suppressed than in dominant trees. T45 showed the lowest CV values in both crown classes, which means higher homogeneity of the growth data. Tree growth responses to climate are highly context-dependent (Carnwath et al. 2012). However, these results are in agreement with the idea that in semi-arid environments, suppressed trees usually have more limited access to soil water reserves than dominant trees, due to their shallower and less developed root systems, especially in high density stands. Therefore, their growth rates fluctuate depending on short term water reserves concentrated in surface soil layers, while dominant trees with more developed root systems and suppressed trees released from competition show more homogeneous growth rates.

The reduction of stand density through thinning has been suggested to improve the resistance of individual trees to drought stress and the extent of this differential response changes within the same species along climatic gradients (Misson et al. 2003; Moreno and Cubera 2008; Gea-Izquierdo et al. 2009). However, this conclusion should not be extrapolated to generalise that open stands are always desirable (Gea-Izquierdo et al. 2009). Forest managers should retain adequate stand density to mitigate the effect of climatic extremes taking other ecosystem parameters such as regeneration or soil protection into account. Less dense and more diverse stands could be promoted by mixing species with different drought resistance to avoid or limit further growth declines in plantations.

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Study IV

Climate impact on wood density in *Pinus halepensis, Pinus pinaster* and *Pinus sylvestris*

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Climate impact on wood density in *Pinus halepensis*, *Pinus pinaster* and *Pinus sylvestris*

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Abstract

During the second half of the 20th century the Mediterranean area has experienced an increase in the mean annual temperature, a decrease in the mean annual precipitation, and an increase in the frequency of severe drought periods. As a consequence, pine species suffered a swift in the climate-growth relationships and increased its climate sensitivity. Understanding growth-climate relationships of pine species growing under Mediterranean climate is a key issue nowadays. However, wood features such as wood density are also affected by recent climatic changes producing an effect on wood structure and quality. In order to to identify the main climatic drivers of the radial growth and wood density patterns for *Pinus halepensis*, *P. pinaster* and *P. sylvestris* and to establish the relationships between tree-ring width and wood density in the three species, three sampling sites were selected throughout their natural distribution area in Spain. The results showed that precipitation influenced tree radial growth and wood density of the three species at different times of the growth period. Mean and maximum density followed different patterns on the three species, decreasing along time in *P. pinaster* and increasing in *P. halepensis* and *P. sylvestris*. Climatic conditions increasing radial growth reduced mean density and increased maximum density in *P. halepensis*. *Pinus sylvestris* showed a positive correlation between maximum latewood density and summer temperature, while *P. halepensis* and *P. pinaster* showed negative correlations. Despite the increasing climate sensitivity of pine species during the most recent years, the trend in growth and mean density does not change in a 5 years

interval. However, these inter-annual variability could have an effect on wood properties producing a loss of wood quality.

Key words

Aleppo pine, maritime pine, scots pine, mean wood density, maximum wood density.

Introduction

Global climate simulation studies predict that trees in Europe and especially in the Mediterranean region, which has been defined as a major climate change hotspot, are likely to experience more severe droughts and higher summer temperatures in the future (Parry et al., 2007). These changes could affect not only tree growth, but also tree-ring structure. Wood density is especially important as it is correlated with various physical, chemical, and anatomical features of the wood and thus yields basic information concerning problems of wood technology, forestry and climatology (Schweingruber et al., 1978; Fritts, 2001). Tree-rings are regularly composed of earlywood, in which tracheids have relatively large diameters and thin cell walls and latewood, with small tracheids having thick cell walls. Severe conditions during the growing season, such as water stress, may generate the production of latewoodlike cells within earlywood or earlywood-like cells within latewood (Schweingruber, 1978; Fritts, 2001) varying wood density patterns.

Dendroclimatic studies have shown that wood density is highly sensitive to climate variations at the inter-annual time scale (Parker and Henoch, 1971; Schweingruber et al., 1978; Conkey 1979; Briffa et al., 2001; Roderick and Berry, 2001; Bouriaud et al., 2005). Relationships between climate and wood anatomy or ring development have been also studied at intra-annual time scales in various tree species including conifers like norway spruce (*Picea abies*) (Horacek et al., 1999; Mäkinen et al., 2003; Bouriaud et al., 2005), silver fir (*Abies alba*), scots pine (*Pinus sylvestris*) and mountain pine (*Pinus uncinata*) (Büntgen et al., 2010), and broad leaved species like black locust (*Robinia pseudacacia*) (Schmitt et al., 2000) and beech (*Fagus sylvatica*) (Sass and Eckstein, 1995; Schmitt et al., 2000; van der Maaten et al., 2012), but little is known about intra-annual density-climate relationships in the Mediterranean, where the cambial activity can vary from year to year presenting continuous radial growth or one or two growth interruptions in line with the variability of climatic conditions (de Luis et al., 2007).

Previous studies in Spain concluded that the growth of *P. halepensis* and *P. pinaster* was influenced by wet periods during winter previous to the growth season and spring, while the growth of *P. sylvestris* was mostly influenced by summer precipitation (Bogino and Bravo, 2008; Bogino et al., 2009; Olivar et al., 2012). However, there were no previous studies on the impact of climate on wood density in these species. We hypothesize that: i) precipitation is the main climatic driver of the radial growth and wood density patterns for *P. halepensis*, *P. pinaster* and *P. sylvestris; and* ii) tree-ring width and mean density will be inversely correlated. The results will be valuable for increasing the understanding on the effects of changing environmental conditions on wood formation

Materials and Methods

The sampling sites were selected throughout the natural distribution area of *Pinus halepensis*, *P. pinaster and P. sylvestris* in Spain (Fig. 1). On each sampling site, 15 dominant trees were selected. Two cores were extracted at 1.30 m above ground from each selected tree. The samples were processed following standard techniques (Stokes and Smiley, 1968), conditioned to a 12% water content and resawn in the transverse plane to a thickness of 2 mm. X-Ray negative photographs of the samples were obtained and the resulting X-ray picture was digitized and processed with CERD software (Mothe et al., 1998). The wood density profiles obtained are for each ring and are based on 100 measured positions. Wood density profiles were produced by averaging the values (XRD, kg/m3) obtained from these segments (Bouriaud et al., 2005; Rathgeber et al., 2006).

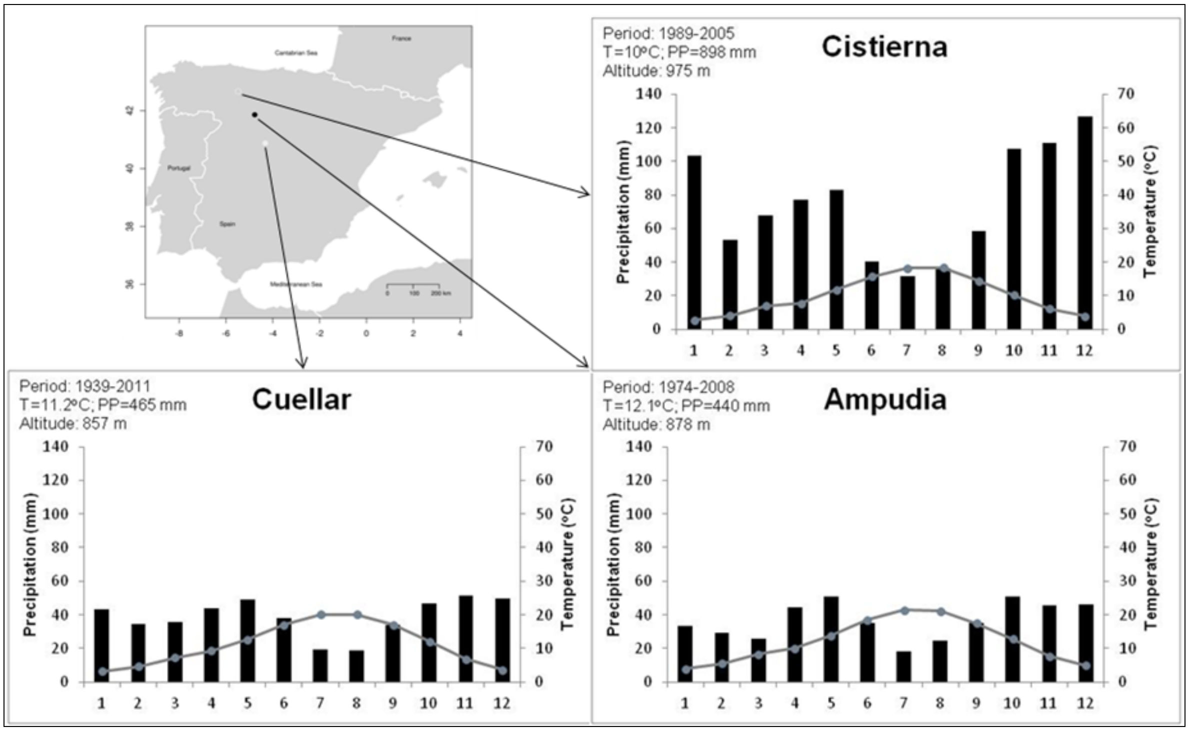

Fig. 1: *Location of the three study sites in Spain. Black: Ampudia (P. halepensis); grey: Cuellar (P. pinaster); white: Cistierna (P. sylvestris).*

Mean monthly temperature and monthly precipitation recorded at the closest meteorological stations (Agencia Estatal de Meteorología, Spain) were grouped in climatic seasons (winter previous to the growing season, spring, summer and autumn) and correlated with tree-ring width, earlywood width, latewood width, mean density, maximum density, earlywood density and latewood density.

To remove non-climatic age trends from the raw measurements, tree-ring width series were detrended (standardized) on a modified negative exponential curve using the package dplR (Bunn, 2008) in program R. Standardization removes geometrical and ecological trends while preserving inter-annual high-frequency variations that are presumably related to climate. The long-term trend was removed from each time series of ring width measurements by fitting and calculating an index defined as actual ring-width for each year divided by the curve-fit value.

Results

The relationships between tree- ring width, wood density and climate was analysed for the three species.

Tree-ring width of *P. halepensis* was positively correlated to precipitation during previous winter and spring (*p-values>0.005)* and spring temperature (*p-value>0.05*). Tree-ring width of *P. sylvestris* was positively correlated to summer precipitation (*p-value>0.05*) *P. pinaster* showed no significant correlations between tree-ring width and climatic variables (Fig. 2).

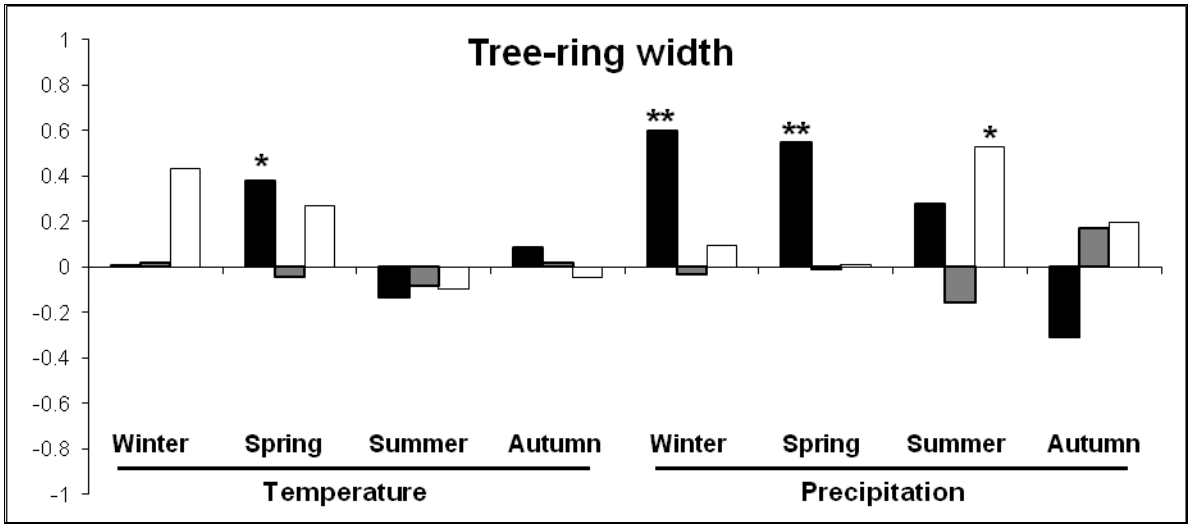

Fig. 2: *Influence of the climatic variables (precipitation and mean temperature) over time on tree ring width of P. halepensis (black), P. pinaster (grey) and P. sylvestris (white). *: p-value>0.05; **: p-value>0.005.*

Earlywood width of *P. halepensis* was positively correlated to precipitation during previous winter and spring (*p-values>0.005)*, while its latewood width was positively correlated to spring temperature (*p-value>0.05*) and winter precipitation (*p-value>0.05*). Earlywood width and latewood width of *P. sylvestris* were positively correlated with summer precipitation (Fig. 3).

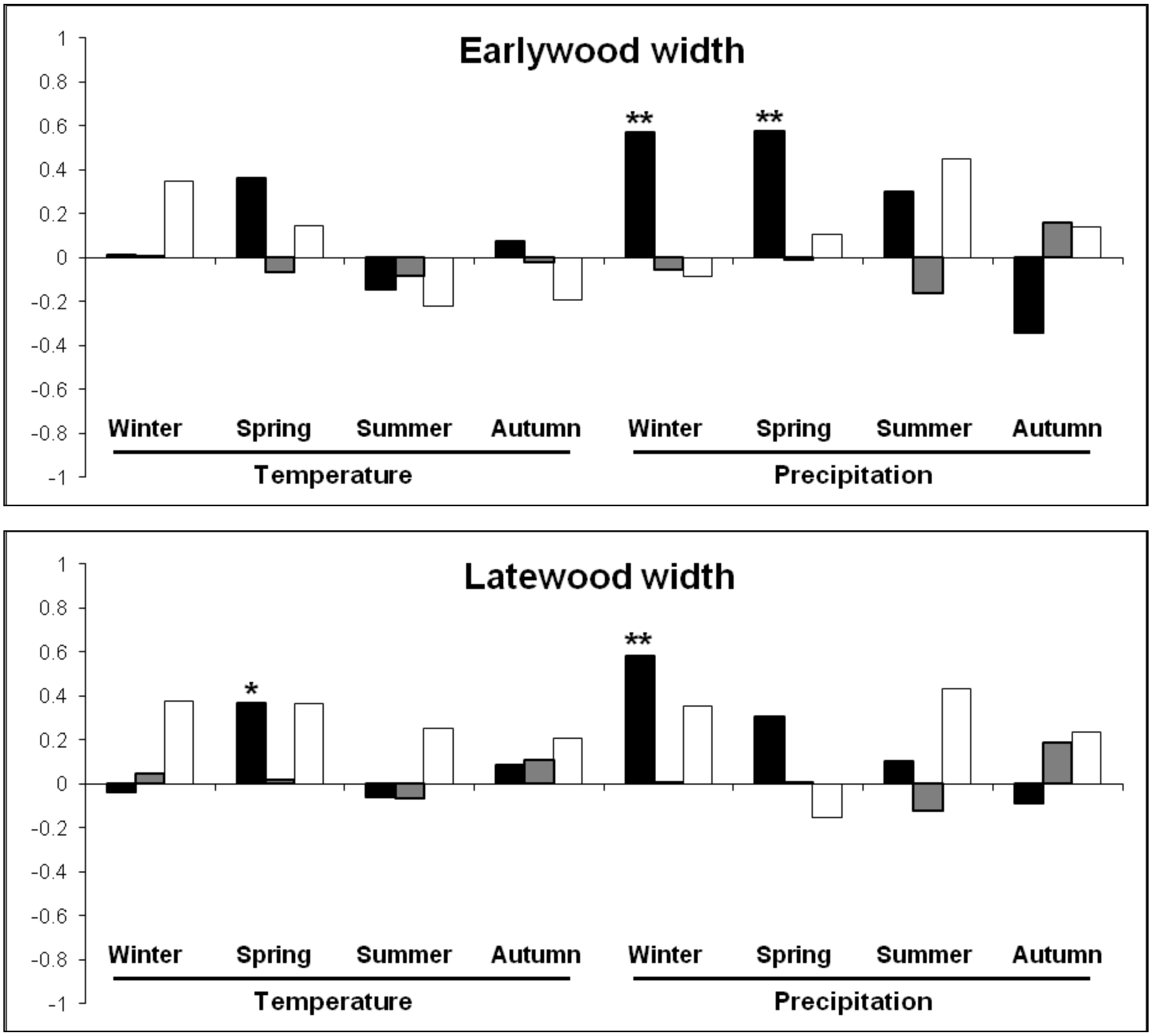

Fig. 3: *Influence of the climatic variables (precipitation and mean temperature) over time on early wood and late wood width of P. halepensis (black), P. pinaster (grey) and P. sylvestris (white). *: p-value>0.05; **: p-value>0.005.*

Mean and maximum density followed different patterns on the three species, decreasing along time in *P. pinaster* and increasing in *P. halepensis* and *P. sylvestris*. *Pinus halepensis* showed higher values of mean density than the other two species and *P. pinaster* showed lowest values of maximum density than the other two species. *Pinus pinaster* experienced an abrupt decrease in the mean density pattern around 1970 and two moderate decreases en the maximum density pattern around 1970 and 1990. (Fig. 4).

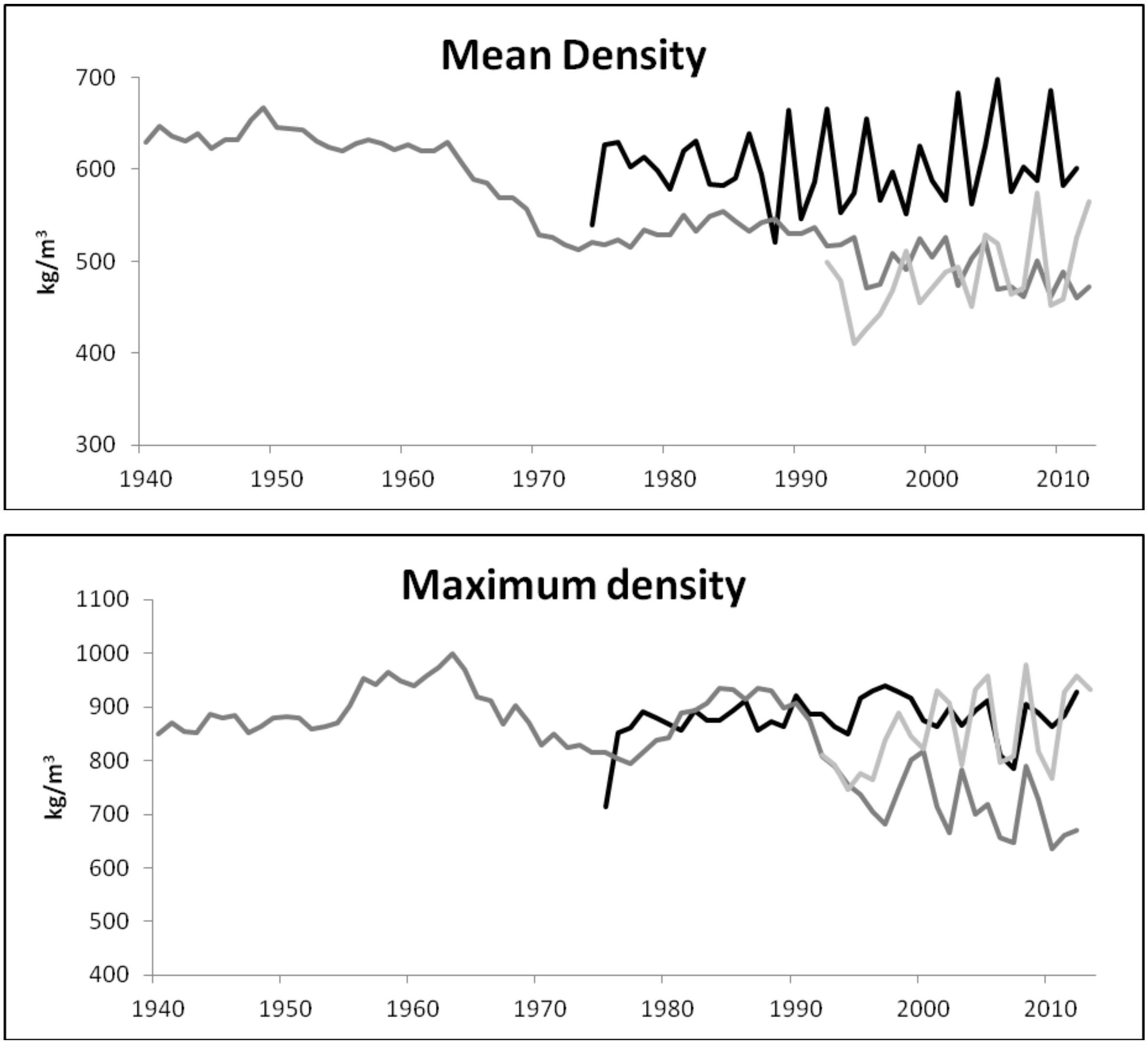

Fig. 4: *Mean and maximum density patterns over time on P. halepensis (black), P. pinaster (dark grey) and P. sylvestris (light grey).*

The analysis of the climate-density relationships showed that mean density of *P. halepensis* was negatively correlated to precipitation during previous winter (*p-value>0.05*) and spring (*p-value>0.005)* and to spring temperature (*p-value>0.05*). Mean density of *P. sylvestris* was positively correlated to autumn temperature (*p-value>0.05*) (Fig. 5). *Pinus pinaster* showed no significant correlations between mean density and climatic variables.

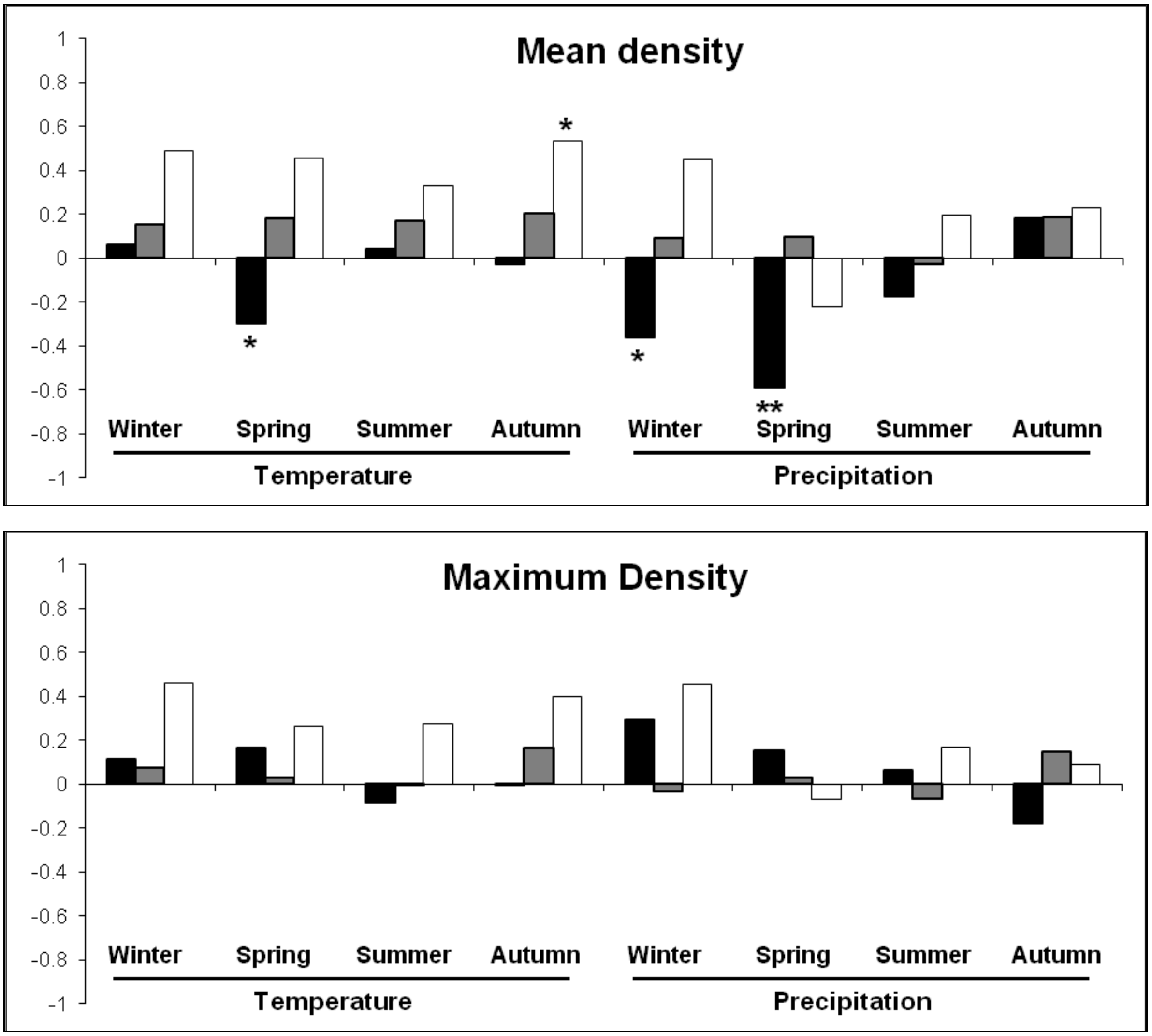

***Fig. 5:** Influence of the climatic variables (precipitation and mean temperature) over time on mean and maximum density of P. halepensis (black), P. pinaster (grey) and P. sylvestris (white). *: p-value>0.05; **: p-value>0.005.*

Early wood density was negatively correlated to precipitation during previous winter (*p-value>0.05*) and spring (*p-value>0.005)* and to spring temperature (*p-value>0.05*) in *P. halepensis;* positively correlated to summer temperature in *P. pinaster* and positively correlated to spring and autumn temperature in *P. sylvestris* (Fig. 6). We calculated the growth and wood density trends of our sample and compared them to 5-year moving averages.

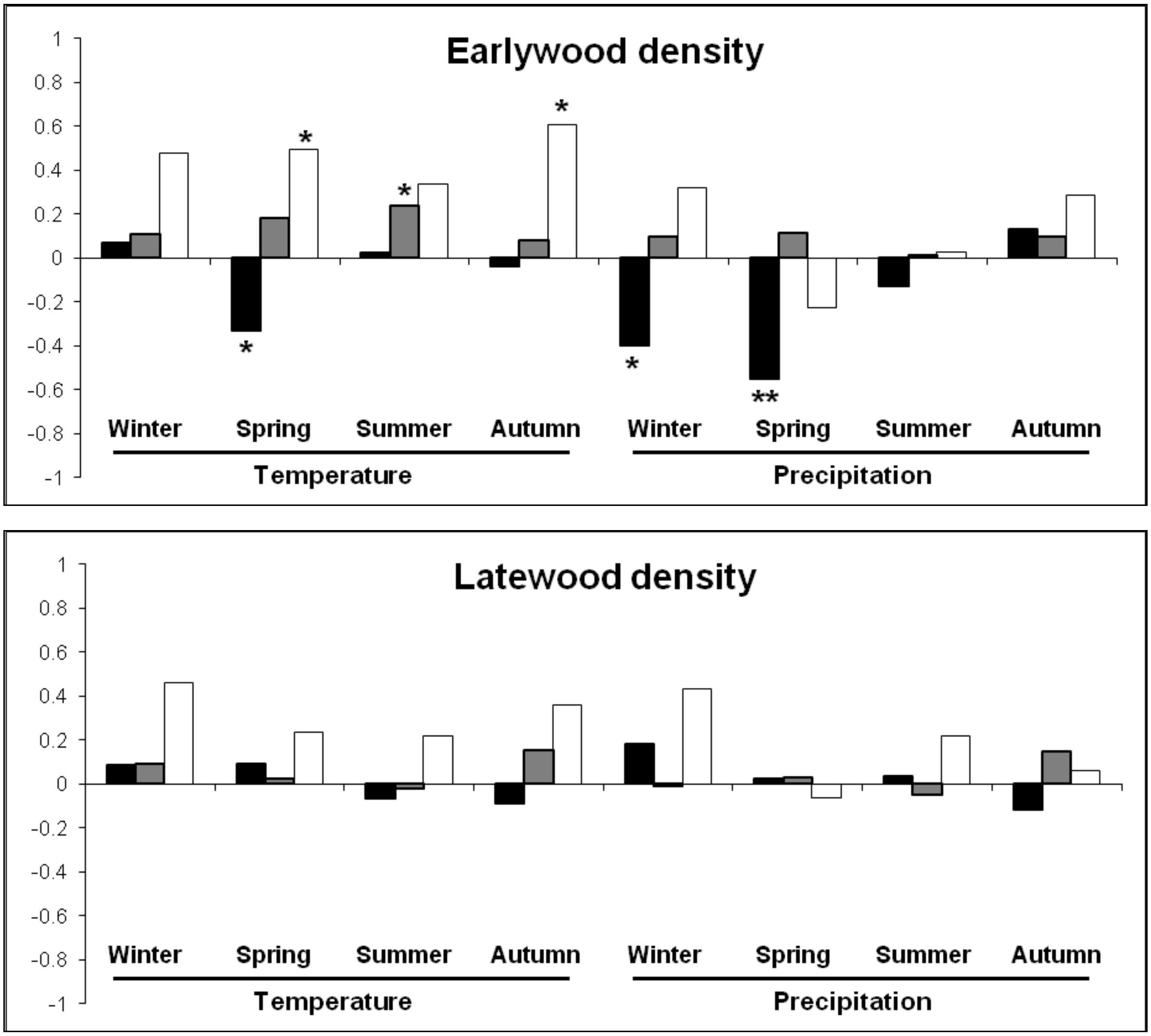

Fig. 6: *Influence of the climatic variables (precipitation and mean temperature) over time on early wood and late wood density of P. halepensis (black), P. pinaster (grey) and P. sylvestris (white). *: p-value>0.05; **: p-value>0.005.*

A decrease in growth rates was observed in the three species (Fig. 7), with high variability of the growth data especially during the most recent years (Standard deviation = 1.3; 0.7 and 1 mm for *P. halepensis*, *P. pinaster* and *P. sylvestris* respectively). *Pinus pinaster* showed an irregular growth trend, with two maximum growth rates on the 1960s and the 1980s and a general decrease along time, especially during the most recent years.

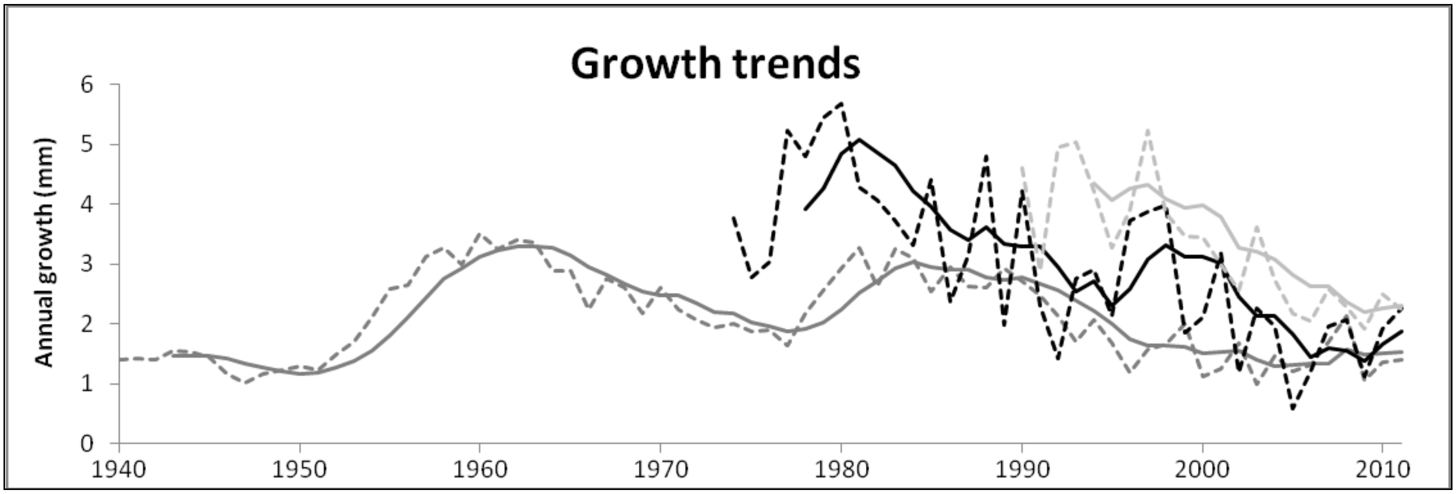

Fig. 7: *Growth trends (dashed lines) of P. halepensis (black), P. pinaster (dark grey) and P. sylvestris (light grey). Full lines: 5-years moving averages.*

However, the 5-year moving average followed the decreasing patterns on the three species with lower variability (Standard deviation = 1; 0.6 and 0.8 mm for *P. halepensis*, *P. pinaster* and *P. sylvestris* respectively).

Mean density rates showed an increase in sensitivity during the most recent years (Fig. 8) (Standatd deviation = 41; 60 and 42 kg/m^3 for *P. halepensis*, *P. pinaster* and *P. sylvestris* respectively). The 5-year moving weighted averages followed the same increasing (*P. halepensis* and *P. sylvestris*) and decreasing (*P. pinaster*) patters with lower variability of the data (Standatd deviation = 12; 57 and 37 kg/m^3 for *P. halepensis*, *P. pinaster* and *P. sylvestris* respectively).

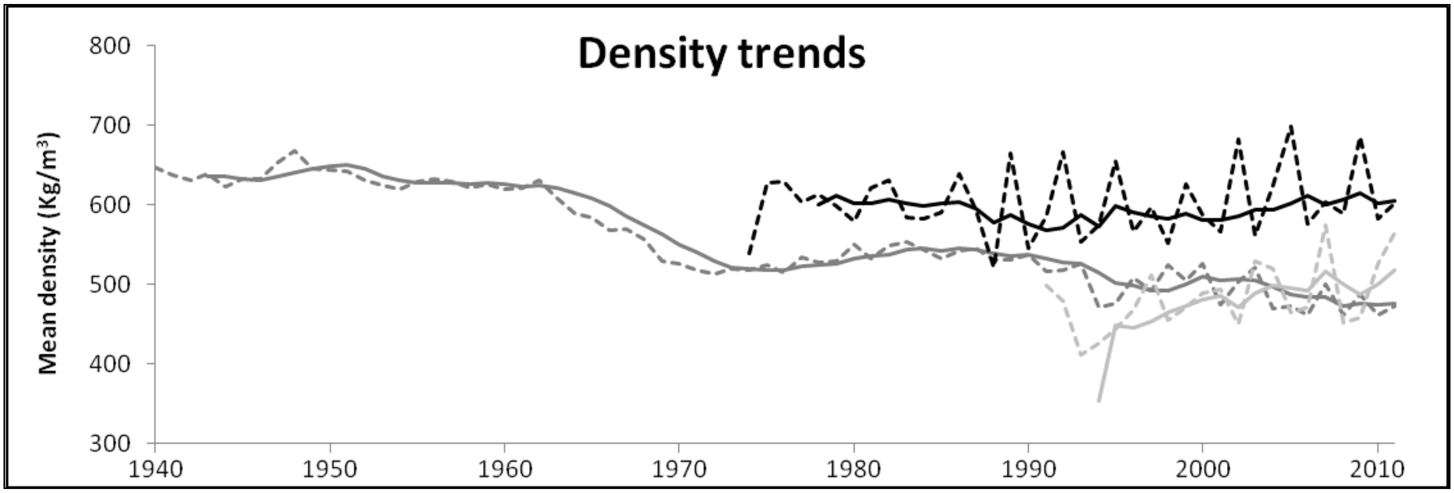

Fig. 8: *Mean density trends (dashed lines) of P. halepensis (black), P. pinaster (dark grey) and P. sylvestris (light grey). Full lines: 5-years moving weighted averages.*

Discussion

Precipitation is the main factor influencing tree growth of pine species in semiarid Mediterranean conditions (Raventós et al., 2001). Previous studies in Spain reported that tree-ring growth of *P. halepensis* was related positively to precipitations during winter previous to the growing season and spring (Olivar et al., 2012), integrating effects from previous year climatic and ecological conditions. Such physiological induced autocorrelation reflects the utilization of abundant carbohydrates stored towards the end or even after the growing season (Kozlowski and Pallardy, 1997; Büntgen et al., 2010). *Pinus pinaster* showed no significant correlations between tree-ring width and climatic variables. Previous studies reported that its growth is influenced by precipitations during winter previous to the growing season and spring in Spain (Bogino and Bravo, 2008) and Portugal (Vieira et al., 2009). However, the site selected for the present study is located at a more xeric site than the previously mentioned. Therefore, the relationships between growth and climate may vary. Radial growth of *P.sylvestris* was mostly influenced by summer precipitations agreeing with previous studies in Spain (Bogino et al., 2009).

Mean and maximum density levels increased outwards from the pith to the stem surface in *P. halepensis* and *P. sylvestris*, as found earlier by Kellomäki et al. (1999). However, *P. pinaster* showed a linear decrease in mean and maximum densities. Büntgen et al. (2010) suggested that this decrease might be related to (i) a systematic decrease in the ratio between cell wall size and lumen area with increasing tree age, or alternatively (ii) that the annual lignin content decreases with age.

Mean density was inversely correlated to precipitation in the previous winter and spring on *P. halepensis*. Rainfall in the early part of the growing season extends the production of earlywood, resulting in a lower density wood (Wimmer and Downes, 2003). Therefore, the negative effect of precipitation on wood density is accordingly consistent with its positive effect on radial growth. Gilmore (1968) also concluded that factors, which extend the production of earlywood, result in lower wood density wood. During the first part of the growing season, climatic variations affect radial enlargement, whereas during the later part of the growing season, climatic variations only affect the cell wall thickening process (Bouriaud et al., 2005; Büntgen et al., 2010). Detailed experiments showed that maximum tracheid production corresponded to maximum day length, thus allowing cell wall formation and lignification to be completed by the end of summer (Rossi et al., 2006).

Bouriaud et al. (2005) found a strong positive correlation between soil water deficit and wood density, which they attributed to temperature-mediated reductions in the radial-growth rates due to an increasing soil-water deficit. Cell wall thickness variations play a major role in the wood density variations (Decoux et al., 2004). Rathgeber et al. (2006) suggested that wood density increase is mainly due to cell-wall thickening in tangential and radial directions as well as cell size reduction in radial direction. The reduction in tracheid radial enlargement might also be associated with thicker cell walls (Linares et al., 2009).

There is a general understanding that water stress has a major impact on various aspects of wood formation through direct effects of cell turgor as well as changes in hormonal balances and carbohydrate storage (Kramer, 1964). Similar effects of water availability on wood density were also observed for *Picea abies* (Wimmer and Downes, 2003, Bouriaud et al., 2005) and *Pinus halepensis* (Olivar et al., 2012), where wood density was found to increase in association with increased drought stress, creating a false ring. The positive effect of temperature on wood density is a consequence of its negative effects on radial growth rate (D’Arrigo et al., 1992; Yasue et al., 2000; Bouriaud et al., 2005).

Maximum density of *P. halepensis* was positively correlated with climatic conditions favouring tree ring width, agreeing with the idea that favourable years with good climatic conditions at the beginning of the growing season increase the length of the growing period and, therefore, the duration of tracheid maturation, which determines the final cell wall thickness (Wodzicki, 1971; Horacek et al., 1999). The wall thickness of the latewood cells is the most important factor affecting the tree-ring maximum density (Yasue et al., 2000; Linares et al., 2009).

Especially for coniferous species, relationships between maximum latewood density and climatic variables such as late summer temperature have been established, and are widely used for climate reconstructions (Parker and Henoch, 1971, Schweingruber, 1978; Conkey, 1979; D'Arrigo et al., 1992; Briffa et al., 2001). However, these studies were conducted at temperate regions and not in the Mediterranean region. In our study only *P. sylvestris*, growing in higher altitude and having more favorable water conditions during summer showed a positive correlation between maximum latewood density and summer temperature, while *P. halepensis* and *P. pinaster,* subjected to summer water stress showed negative correlations between maximum latewood density and summer temperature. These negative correlations may be due to the low or inexistent growth rates of the trees in that period.

Due to the observed changes in growth-climate associations of pine species during the last part of the 20th century (Bogino and Bravo, 2008; Vieira et al., 2010; Campelo et al., 2013) we calculated the variance of the growth and wood density trends of our sample and compared them to a 5-year moving average (the typical modeling horizon) and a 5-year moving weighted average respectively. An increase in the climate sensitivity was observed in both trends during the last years, producing high irregularity in tree ring width and mean density. However, this irregularity is not observed in the 5-year moving interval, where low growth rates of some years are compensated with high growth of the surrounding years. These results indicate that, despite the increasing climate sensitivity of pine species during the most recent years, the trend in growth and mean density does not change in a 5-year interval. However, these inter-annual variability could have an effect on wood properties producing a loss of wood quality (Persson et al., 1999)

Conclusions

Precipitation is the main climatic driver of the radial growth and wood density patterns for *P. halepensis*, *P. pinaster* and *P.sylvestris* in Spain. Climatic conditions increasing radial growth of *P. halepensis* reduced its mean density and increased its maximum density. *Pinus sylvestris*, growing in higher altitude and having more favorable water conditions during summer showed a positive correlation between maximum latewood density and summer temperature; while *P. halepensis* and *P. pinaster*, subjected to summer water stress producing low or inexistent growth rates of the trees in that period, showed negative correlations. Despite the increasing climate sensitivity of pine species during the most recent years, the trend in growth and mean density does not change in a 5-year interval. However, these inter-annual variability could have an effect on wood properties producing a loss of wood quality.

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