Universidad de Valladolid

ESCUELA TÉCNICA SUPERIOR DE INGENIERÍAS AGRARIAS

Departamento de Producción Vegetal y Recursos Forestales

CLIMATE AND FOREST GROWTH IN MEDITERRANEAN ENVIRONMENTS: *PINUS PINASTER* AND *PINUS SYLVESTRIS* IN SPAIN

Presentada por **Stella Marys Bogino** para optar por el título de Doctora

Dirigida por

Dr. Felipe Bravo Oviedo
Dra. María José Fernández Nieto

Palencia, España. Junio de 2008

UNIVERSIDAD DE VALLADOLID

ESCUELA TÉCNICA SUPERIOR DE INGENIERÍAS AGRARIAS

Departamento de Producción Vegetal y Recursos Forestales

CLIMATE AND FOREST GROWTH IN MEDITERRANEAN ENVIRONMENTS: *PINUS PINASTER* AND *PINUS SYLVESTRIS* IN SPAIN

Stella Marys Bogino
Ingeniera Agrónoma
Especialista en Docencia Universitaria
Magíster en Ciencias Agropecuarias

Tesis doctoral

Directores

Dr. Felipe Bravo Oviedo
Dra. María José Fernández Nieto

Palencia, España. Junio de 2008

El Dr. **Felipe Bravo Oviedo** y la Dra. **María José Fernández Nieto**, Profesores Titulares de la Universidad de Valladolid,

CERTIFICAN:

Que la Ingeniera Agrónoma (M.Sc.) **Stella Marys Bogino** ha realizado bajo nuestra dirección el trabajo que, para optar al Grado de Doctor, presenta con el título "Climate and forest growth in Mediterranean environments: *Pinus pinaster* and *Pinus sylvestris* in Spain".

Por lo que mediante este Trabajo de tesis, se solicita el reconocimiento del Doctorado europeo de la doctoranda, quien realizó una estancia de cuatro meses en la *Georg-August Universität, Göttingen*, Alemania, bajo la supervisión del Dr. Christoph Klein (Profesor titular de dicha Universidad).

Y para que así conste a los efectos lo firmamos en Palencia, a 30 de Junio de 2008.

Vº.bº	Vº.bº
Dr. Felipe Bravo Oviedo	Dra. María José Fernández Nieto

ABSTRACT

Climate change will affect Mediterranean forests by modifying driving factors related to different processes and functions. Relationship between tree growth and climate is crucial to develop forestry strategies to mitigate climate change impact. Dendroclimatological techniques were applied in sampling sites placed in central Spain to analyse the association between *Pinus pinaster* and *Pinus sylvestris* and climatic variables in Mediterranean environments. The objectives of this thesis were: a) To analyse the relationship between *P. sylvestris* and *P. pinaster* tree-ring width and climatic variables (precipitation and temperature). b) To explore possible shifts in the association between climate variables and tree growth over time. c) To investigate the effects of drought and pulse and interpulse water events on radial growth of *P. sylvestris*. d) To explore the age effect on climate-growth relationship of *P. sylvestris*. e) To estimate the potentiality of Intra-annual density fluctuation (IADFs) in *P. pinaster* as dendroclimatological proxy. f) To estimate the climatic variables that drive the carbon thirteen variability ($\delta^{13}C$) in tree ring of *P. sylvestris* and *P. pinaster*. g) To analyse the variability of $\delta^{13}C$ related to water use efficiency in both species. Results showed that radial growth of *P. pinaster* and *P. sylvestris* was positively correlated with rainfall during and prior to the growing season. Mean temperature effect varied according to site altitude. *P. pinaster* has a changing association between radial growth and climate variables, initiated in the 1980s. A climatic response according to the age was also found in *P. sylvestris*: young stands have higher variability than old stands. Correlation between successive years was also detected in old stands. There was a significant association between radial growth of *P. sylvestris* and drought index (DRI). The periodicity related to pulse and interpulse events was two or two and a half years suggesting the strong impact of the interannual precipitation variation on radial growth. IADFs in *P. pinaster* showed: an increase in IADF frequency from the 40 decade to the present; the mean frequency of IADF was higher in younger than in older trees; a negative correlation between radial growth and IADFs and the IADFs may be predicted using a logistic model and monthly rainfall and temperature as independent variables. The values of $\delta^{13}C$ in *P. pinaster* and *P. sylvestris* were affected by moisture, rainfall, maximum temperature and solar radiation. *P. pinaster* and *P. sylvestris* growing in Mediterranean environments are accurate species for dendroclimatological studies to analyse tree-growth association with climate and for studying plant behaviour under global change conditions.

RESUMEN

El cambio climático afectará los bosques mediterráneos pues altera los factores que controlan sus diferentes funciones y los procesos que tienen lugar en ellos. La relación existente entre el crecimiento de los árboles y el clima es esencial para aplicar y desarrollar estrategias forestales cuyo objeto sea mitigar el impacto del cambio climático. En este contexto, se aplicaron distintas técnicas dendroclimatológicas en varios sitios de muestreo emplazados en el centro de España, para analizar la relación entre *Pinus pinaster* y *Pinus sylvestris* y las variables climáticas en los ambientes Mediterráneos. Los objetivos de esta tesis fueron: a) Analizar la relación entre el ancho de los anillos de crecimiento y las variables climáticas (precipitación y temperatura). b) Explorar posibles cambios en la asociación entre las variables climáticas y el crecimiento de los árboles a través del tiempo. c) Investigar los efectos de la sequía y de los pulsos e interpulsos de agua sobre el crecimiento radial de *P. sylvestris* d) Explorar el efecto de la edad de los árboles en la relación clima-crecimiento de *P. sylvestris*. e) Estimar la potencialidad de las variaciones interanuales de la densidad (FIAD) en *P. pinaster* como herramienta dendroclimatológica. f) Estimar las variables climáticas que controlan la variabilidad del carbono trece ($\delta^{13}C$) en los anillos de crecimiento de *P. sylvestris* y *P. pinaster*. g) Analizar la variabilidad del $\delta^{13}C$ en relación con el uso del agua, en las dos especies. Los resultados demostraron que el crecimiento radial de *P. pinaster* y *P. sylvestris* se correlaciona positivamente con la precipitación, tanto antes como durante la estación de crecimiento. El efecto de la temperatura media varió de acuerdo con la altitud del sitio de muestreo. La relación entre el crecimiento radial de *P. pinaster* y las variables climáticas mostró una variación a través del tiempo que se inició en la década del ochenta. *P. sylvestris* manifestó una respuesta climática en función de la edad: los árboles jóvenes tienen más variabilidad de su crecimiento radial explicada por el clima, que los árboles viejos. En los árboles viejos se detectó correlación entre los anillos de crecimiento de años sucesivos. Hay una correlación significativa entre el crecimiento radial de *P. sylvestris* y el índice de sequía relativa. La periodicidad relacionada con los pulsos e interpulsos de agua fue de dos y dos años y medio, lo que sugiere un fuerte impacto de la variación interanual de la precipitación sobre el crecimiento radial. Las variaciones intraanuales de la densidad mostraron: un aumento de la frecuencia de FIAD en *P. pinaster* desde la década de los cuarenta hasta la actualidad; la frecuencia media de FIAD fue más alta en los árboles jóvenes que en los viejos y también se detectó una correlación negativa entre el crecimiento radial y las FIAD. Las FIAD podrían predecirse usando un modelo logístico que tiene a la precipitación y la temperatura mensual como variables independientes. La variabilidad del isótopo carbono trece ($\delta^{13}C$) en *P. pinaster* y *P. sylvestris* está afectada por la humedad, la precipitación, la temperatura máxima y la radiación solar. En los ambientes Mediterráneos, *P. pinaster* y *P. sylvestris* son especies óptimas para estudios dendroclimáticos que analicen la asociación entre el crecimiento de los árboles y el clima, así como para estudiar el comportamiento de las bosques mediterráneos en un entorno de cambio climático.

AGRADECIMIENTOS

A mi director Felipe Bravo Oviedo y Co-Directora María José Fernández Nieto, por su apoyo permanente.

A las Universidades de Valladolid, España, y *Georg-August Universität, Göttingen,* Alemania, en las que desarrollé este programa de doctorado.

Al Programa Alβan, Programa Europeo de Becas de Alto Nivel para América Latina, beca # E05D049920AR; al Programa de Becas *Erasmus* de la Universidad de Valladolid y a los proyectos de la Junta de Castilla y León, España, Código VA096A05, y del Ministerio de Educación y Ciencia, España, Códigos AGL2004-07094-C02-02 y AGL2007-65795-C02-01 por la ayuda económica.

A la Agencia Estatal de Meteorología, España, por haberme facilitado los datos climáticos.

A la Universidad Nacional de San Luis, Argentina, y a la Escuela Remedios Escalada de San Martín, Argentina, por la licencia otorgada durante estos tres años para la realización del presente doctorado.

Un especial agradecimiento a Vicente Rozas, Esteban Jobbágy y Mariano Morales por los inestimables comentarios realizados a esta tesis. A María Laura Cangiano por las correcciones del texto en Inglés, a Iñaki Etxebeste Larrañaga y Reinhard Langel por la inestimable ayuda en los capítulos de densidad e isótopos, respectivamente, y a Cristóbal Ordóñez por la ayuda en el trabajo de campo.

A Wilson Lara, Iñaki Etxebeste Larrañaga y Javier Castaño, por la edición final de la tesis.

A Celia Redondo por su ayuda incondicional con la documentación, siempre que la he necesitado.

A aquellas personas que me han hecho sentir la vida un poco más fácil durante estos tres años lejos de mi querido país: en España: Encarna Rodríguez García y familia, Antonio Sanz, María Gómez, Antonio Urchaga, Pilar Valbuena, Claudia Escudero, Jorge Martín García y familia, Iñaki Etxebeste Larrañaga, Leticia Botella, Yésica Palavecini, Celia Herrero de Aza y familia, Patricia Recio, Lucía de Soto Suárez, Darío Martín Benito, José Reque y familia, Wilson Lara, Javier Castaño, Amelework Kassa, Luis Fernando Osorio, Gonzalo Álvarez Baz, Laura Benito y Charo Litardo y familia. En Argentina: Elena Riccio, María Victoria Vega, Mirta Gómez, Zunilda Furlán, Hernando Casagrande, Liliana Gabutti, Edgardo Guerrero, Graciela Ardú, Emilio Lotto, Ana María Bogino, Andrés Bogino, Daniel Bogino, Anselmo Avila, María Pachoud, Patricia Pagnone, Lautaro Barbaglia, Agostina Serrano, Rosita Giraudo, Zoraide Fernández Montero, Salvador Micali, Ana María Careaga, Claudia Dustchazky, Olga Callovi, Iván Niveyro, Patricia Vidal, María Eugenia Moreno, Maximiliano Dellacassa y Nedda Rimondi. En Alemania: Hardy Schilling, Frank Bräuer, Michael Barthel, Lena Hollmann, Frank Stein, Anja Sölter, Nicola Köberl and Benjamin Jung.

A mis alumnos de la Escuela y de la Universidad en Argentina, por todo lo que me han enseñado durante estos 20 años. Al estudiante Juan Politano quien dio su vida luego de salvar a mucha gente en la explosión de la Universidad de Río Cuarto el 5 de diciembre de 2007, mi dolor y mi gratitud.

A mi abuela, María Adelia Perassi, siempre estarás en mis recuerdos.

A mis padres y maestros, Manuela Juana Sacco y José Antonio Bogino.

A mi querido país, Argentina, por todo y siempre.

ACKNOWLEDGEMENTS

Thanks to my director Felipe Bravo Oviedo and Co-Director María José Fernández Nieto for their permanent support.

To the *Universidad de Valladolid* (Spain) and *Georg-August Universität, Göttingen* (Germany) where I did this PhD Program.

To the Programme Alβan, the European Union Programme of High Level Scholarships for Latin America, scholarship # E05D049920AR; the Erasmus Scholarship of the *Universidad de Valladolid* and the research projects from the Regional *Castilla y León* government (Spain), Project Code VA096A05 and the *Ministerio de Ciencia y Educación* (Spain), Project Code AGL2004-07094-C02-02 and AGL2007-65795-C02-01 for economical support.

To the *Agencia Estatal de Meteorología* (National Meteorological Agency, Spain) for providing meteorological data.

To the *Universidad Nacional de San Luis* (Argentina) and the *Escuela Remedios Escalada de San Martín* (Argentina) for the extended leave they gave me along these three years.

Special thanks to Vicente Rozas, Esteban Jobbágy and Mariano Morales for the very useful comments made to this work. Thanks are also extended to María Laura Cangiano for improving the English version, to Iñaki Etxebeste Larrañaga and Reinhard Langel for helping me with the density and isotope chapters, respectively, and to Cristobal Ordoñez for field assistance.

Thank are also extended to Wilson Lara, Iñaki Etxebeste Larrañaga and Javier Castaño for the final edition of the thesis.

To Celia Redondo for helping me with my documents whenever I needed it.

To those people who have supported me throughout these three years far away from my beloved land. In Spain: Encarna Rodríguez García and family, Antonio Sanz, María Gómez, Antonio Urchaga, Pilar Valbuena, Claudia Escudero, Jorge Martín García and family, Iñaki Etxebeste Larrañaga, Leticia Botella, Yesica Palavecini, Celia Herrero de Aza and family, Patricia Recio, Lucía de Soto, Darío Martín Benito, José Reque and family, Wilson Lara, Javier Castaño, Amelework Kassa, Laura Benito, Charo Litardo and family, Gonzalo Álvarez Baz and Luis Fernando Osorio. In Argentina: María Victoria Vega, Mirta Gómez, Zunilda Furlán, Hernando Casagrande, Liliana Gabutti, Edgardo Guerrero, Graciela Ardú, Emilio Lotto, Ana María Bogino, Andrés Bogino, Daniel Bogino, Anselmo Avila, María Pachoud, Patricia Pagnone, Lautaro Barbaglia, Agostina Serrano, Rosita Giraudo, Zoraide Fernández Montero, Salvador Micali, Elena Riccio, Edith Rojas, Ana María Careaga, Claudia Dustchazky, Olga Callovi, Iván Niveyro, Patricia Vidal, María Eugenia Moreno, Maximiliano Dellacassa and Nedda Rimondi. In Germany: Hardy Schilling, Frank Bräuer, Michael Barthel, Lena Hollmann, Frank Stein, Anja Sölter, Nicola Köberl and Benjamin Jung.

To my school and university students for everything you have taught me along these years. My sorrow and gratitude to the student Juan Politano, who died after saving the life of many people in the explosion at the *Universidad Nacional de Río Cuarto* the 5th of December 2007.

To my grandmother María Adelia Perassi, you will be always in my memories.

To my parents and teachers, Manuela Juana Sacco and José Antonio Bogino.

To my dearly loved land Argentina, for everything.

Con sólo pensarte, soy feliz, por eso esta tesis es para vos,
mi dulce y amada hija María Laura.

Si piensan en las cualidades que una persona perfecta debería tener,
tal vez puedan comprender cómo es ella.

I think about you and I am very happy. This thesis is for you,
my sweet and beloved daughter María Laura.

If you think about the gifts that a perfect person should have,
you may understand how she is.

CONTENTS

.

TABLA DE CONTENIDOS

1. INTRODUCTION

1.1. CLIMATE CHANGE AND MEDITERRANEAN ENVIRONMENTS

Climate change will affect Mediterranean forests by modifying driving factors related to different processes and functions. Complex interactions between climate and biotic and abiotic factors are leading to more complex forest management in the future.

Mediterranean environments in the Iberian Peninsula, which are water limited ecosystems, are characterised by summer droughts and high interannual variability of precipitation and temperature. In summer months, when temperature is favourable for growth, moisture is limiting; whereas in winter, when moisture is available, low temperature limits further growth (Mooney and Dunn, 1970). In arid and semi-arid ecosystems, where water is a limiting resource, water availability experiences two phases: pulse, when the resource is available, and interpulse, when water availability is too low for plant use (Noy-Meir, 1973; Goldberg and Novoplansky, 1997).

Projections of climate change impact on the European distribution of higher plants in 2050 suggest that the Iberian Peninsula could be one of the most vulnerable areas for species lost (Bakkenes et al., 2002). Climate shifts have already taken place in the region, as exemplified by the reduction of rainy days by 50 and 30% over the southern coast of Spain and the Pyrenees in the last century, respectively (Esteban-Parra et al., 1998; Rodrigo et al., 2000). On the other hand, the mean annual temperature has increased about 1.6º C in the Iberian Peninsula over the last century (Parry, 2000; IPCC, 2007).

Global change effect on tree growth is essential to understand tree growth and productivity response to the warming environment, and the impact that this response could have on global warming and on the other hand, is crucial to understand and model the carbon sequestration process in forests and to develop forestry strategies to mitigate climate change impact.

1.2. SPECIES STUDIED

1.2.1. *Pinus pinaster* Ait.

The Mediterranean Maritime pine (*Pinus pinaster* Ait.) 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 (Nicolas and Gandullo, 1967), and 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 (Figure 1)].

In Spain, the Mediterranean Maritime pine constitutes pure and mixed woodlands that are both natural (1 million ha) and planted (0.6 million ha) (DGCN, 1998; 2002). These woodlands are mainly spread over the northern Spanish plateau up to 1500 m. In this area, the climate is characterised by irregular rainfall, between and within years, and high temperatures in summer. The soils are sandy and rocky and very well drained. Even though the Mediterranean Maritime pine had been used widely on plantations 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 (Di Castri, 1981). Authors suggest that the Iberian System is the natural origin of and centre of distribution for this species (Blanco et al., 1997).

The Mediterranean Maritime pine has rarely been used for Dendrochronology and Dendroclimatology because stands older than 100 years are very difficult to find. In addition, trees from the lower mountain level often have false rings and most trees have been damaged by resin harvest in the past (Schweingruber, 1993).

1.2.2. *Pinus sylvestris* L.

Scots Pine (*Pinus sylvestris* L) covers a very wide ecological spectrum: across Eurasia, together with other species, it dominates forest landscapes from boreal region in northern Europe and Russia to the western Mediterranean mountains in southern Europe.

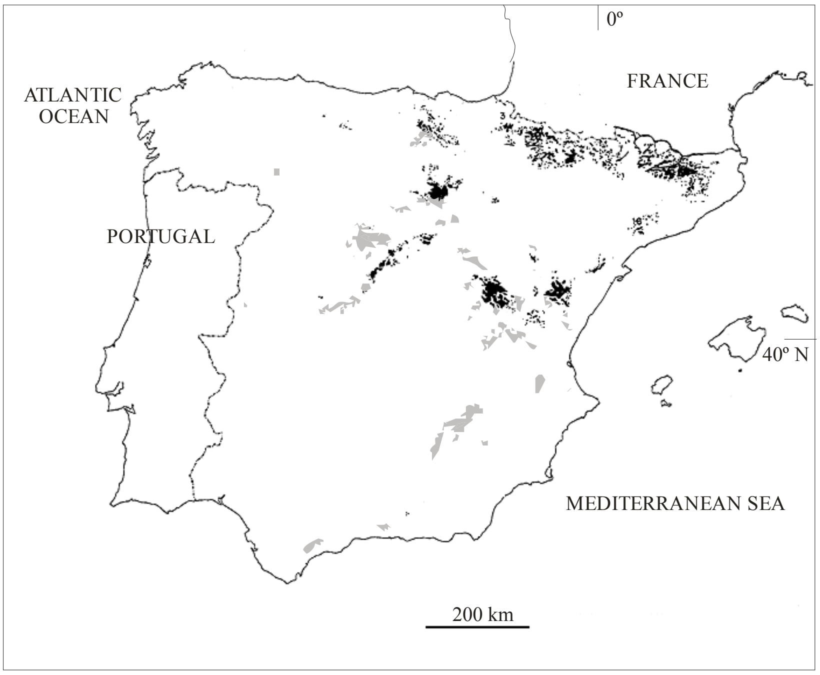


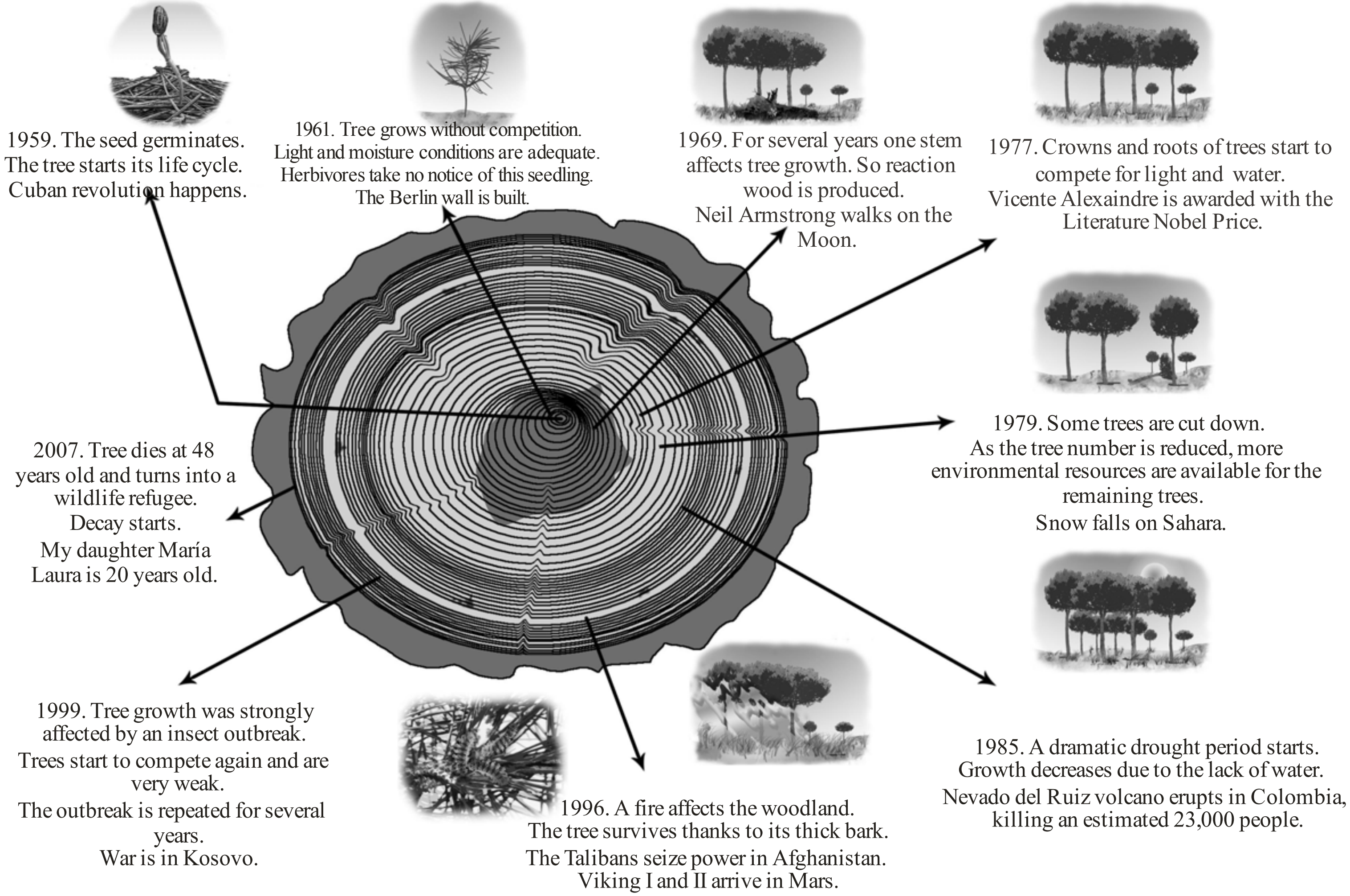
Figure 1. Distribution of *P. pinaster* (gray), and *P. sylvestris* (black) in Spain (Adapted from *www.mma.es*).

It grows in the cold continental climate of northern Asia as well as in the semiarid climate of southern Europe. On northern European sites, temperatures in January average around –15ºC (the minimal temperature is around –40ºC), in southern Europe, around 8ºC. On the northern forest border, temperatures in July are about 10ºC, while in the Mediterranean region they can reach 22ºC. In Western Europe, annual precipitation is 2500 mm, but in southern Europe only 400 mm. On all natural sites, Scots pine grows up to 300 year old and it could reach 600 years on dry sites.Scots Pine is the most widely distributed species of pine in the world (Blanco et al., 1997); along with Norway spruce [*Picea abies* (L.) H. Karst.], it possesses the highest dendrochronological potential in Europe (Schweingruber, 1996). Forests of *P. sylvestris* in Spain are the southern and western distribution threshold worldwide of that species and occupy drier areas than in the other parts of the world (Barbéro et al., 1998; Figure 1). 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). In Spain, *P. sylvestris* stands occupy 1 210 000 ha, split approximately in 50% pure and 50% mixed stands (DGCN, 1998; 2002).

1.3. DENDROCHRONOLOGICAL TOOLS AVAILABLE FOR CLIMATE GROWTH RELATIONSHIP ANALYSIS

Dendrochronology is the science of dating tree rings of woody species. Due to the cambial activity, a tree ring is formed every year and the analysis of tree rings provides useful information about both the structure of dated rings and applications to environmental and historical questions (Kaennel and Schweingruber, 1995). Dendroecology is a sub field of Dendrochronology which utilizes dated tree rings to study ecological problems and the environments, for example: endogenous disturbances due to interactions between trees; exogenous disturbances as for example: climate impact, fire, insect outbreaks, etc. (Figure 2).

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).



1959. The seed germinates.
The tree starts its life cycle.
Cuban revolution happens.

1961. Tree grows without competition.
Light and moisture conditions are adequate.
Herbivores take no notice of this seedling.
The Berlin wall is built.

1969. For several years one stem affects tree growth. So reaction wood is produced.
Neil Armstrong walks on the Moon.

1977. Crowns and roots of trees start to compete for light and water.
Vicente Alexandre is awarded with the Literature Nobel Price.

1979. Some trees are cut down.
As the tree number is reduced, more environmental resources are available for the remaining trees.
Snow falls on Sahara.

1985. A dramatic drought period starts.
Growth decreases due to the lack of water.
Nevado del Ruiz volcano erupts in Colombia, killing an estimated 23,000 people.

1996. A fire affects the woodland.
The tree survives thanks to its thick bark.
The Talibans seize power in Afghanistan.
Viking I and II arrive in Mars.

1999. Tree growth was strongly affected by an insect outbreak.
Trees start to compete again and are very weak.
The outbreak is repeated for several years.
War is in Kosovo.

2007. Tree dies at 48 years old and turns into a wildlife refugee.
Decay starts.
My daughter María Laura is 20 years old.

Figure 2. Dendrochronological history of a tree life and related historical events (Adapted from Schweingruber, 1988 and redrawn by A. Muñoz)

1.3.1. Tree-ring growth

Tree-ring widths of conifers offer some of the best climatological reconstructions of semiarid regions (Schweingruber, 1996), as in the case of annual precipitation estimates in Mediterranean climates (Tessier et al., 1997). On the other hand, different climatic variables (mainly derived from precipitation and temperatures but also derived from atmospheric pressure balance) can be used to express climatic oscillation and to derive relationships between tree growth parameters and climatic signal (D'Arrigo and Jacoby, 1992; Jones et al., 1997).

Tree-ring growth is influenced by several simultaneous environmental factors: solar radiation, temperature, water precipitation, soil nutrient content, etc. Depending on conditions and species, one or more of these factors can become limiting for tree growth (Fritts, 1976). Climate variability often explains past tree growth (Hughes, 2002), helping in the projection of future growth responses in the context of climate change (Yeh and Wensel, 2000).

1.3.2. Intra-annual density fluctuations (IADFs)

Intra-annual density fluctuations (IADFs), which include false rings, growth bands, double rings and multiple rings, are anomalies in ring growth that are formed by latewood-like cells within the earlywood, or earlywood-like cells within the latewood (Fritts, 1976).

When IADFs are properly identified, intra-annual structures from trees growing in xeric environments may be used to develop records of growing seasons characterised by early drought and followed by moist conditions (Villalba and Veblen, 1996). Species growing under a Mediterranean climate, which is characterised by summer drought and high inter-annual variability in precipitation and temperature, commonly show special anatomical characteristics in tree rings (Schweingruber, 1993). Consequently, the importance of incorporating intra-annual features or anomalies in radial growth may be useful for ecological and climatological interpretation (Tessier et al., 1997).

1.3.3. Isotopes

The isotopic composition of carbon stored in the growth rings of trees may represent a record of variations in $^{13}C/^{12}C$ as a result of physiological responses to environmental changes (Francey and Farquhar, 1982). This ratio is expressed as $\delta^{13}C$, the proportional deviation of the $^{13}C/^{12}C$ ratio from the international accepted PDB carbonate standard (Craig, 1957). Since cellulose is not transferred between annual growth rings, intra and interannual seasonal events are recorded permanently in $\delta^{13}C$ signal in tree ring (Tans et al., 1978).

Water use efficiency (WUE), the amount of carbon assimilated per unit leaf area per unit time at per unit cost of water (Ehleringer et al., 1993) and $\delta^{13}C$ are positively related to each other (Farquhar et al., 1989). The crucial interdependence between carbon and water relations of plants can be particularly well studied by the use of stable carbon isotopes. Determination of $\delta^{13}C$ was suggested as a sensitive long-term monitoring of physiological changes (Francey and Farquhar, 1982).

1.4. OBJECTIVES

This thesis has a main objective: to explore the impact of climate on pine trees growth under Mediterranean conditions. This general objective is asses by the following specific objectives.

1.4.1. Tree-ring growth-climate relationship

The relationship between tree-ring growth and climatic variables is essential to predict the future growth trend of *P. pinaster* and *P. sylvestris*. The knowledge of the growth response of these species to past climate variability can help us to explore how its populations, and the ecosystems they dominate, will behave in the future.

Objective 1: To analyse the relationship between *P. pinaster* and *P. sylvestris* tree-ring width and climatic variables (precipitation and temperature), and to explore possible shifts in the association between these climate variables and tree growth over time as a result of changing environmental conditions.

1.4.2. Tree-ring growth-atmospheric indexes relationship

There are no previous studies made on the impact of the SOI and NAO indexes on conifers growing in the Iberian Peninsula. As many scientists argue about both indexes have global impact on the earth’s surface, it could be an excellent opportunity to analyse the relationship between these indexes and the growth of woody species.

Objective 2. To analyse the relation between the *P. pinaster*‘s tree-ring width and the NAO and SOI atmospheric indexes in Eastern Spain.

1.4.3. Tree-ring growth-water availability relationship

In the Iberian Peninsula, *P. sylvestris* grows in its southern and western distribution threshold worldwide and under water limited conditions. Therefore, dendroclimatological studies may provide valuable information about both, the association with climatic variables and the interannual water availability effect (pulse and interpulse) on radial growth.

Objective 3. To investigate the drought effect and pulse and interpulse water events on radial growth of *P. sylvestris* and to analyse cambial age effect on climate-growth relationship.

1.4.4. Intra-annual density fluctuations

As conifers growing in dry environments tend to produce false rings, and as dendroclimatological studies concluded that *P. pinaster* is very susceptible to rainfall during the growing season [especially in spring and early summer (Paper 1)], the study of IADFs in *P. pinaster* may be a useful tool for enhancing dendroclimatological studies based on tree-ring width fluctuations, and for reconstructing past intra-annual climate events.

Objective 4. To estimate the potentiality of intra-annual density fluctuation (IADFs) features in *P. pinaster* as dendroclimatological proxy.

1.4.5. Isotope study

There is not information about stable carbon isotope in tree rings of *P. pinaster* and *P. sylvestris* growing under Mediterranean climate conditions. Stable carbon isotope variability may provide very valuable information about the climatic variables which affect this variability and WUE as a result of globally changed conditions.

Objective 5. To estimate the climatic variables that determine the $\delta^{13}C$ signal in *P. sylvestris* and *P. pinaster* tree rings, and to analyse the variability in $\delta^{13}C$ in relation to water use efficiency.

2. MATERIALS AND METHODS

Dendroclimatological tools (tree-ring width, IADFs and carbon isotopes) were applied on *Pinus pinaster* and *Pinus sylvestris*, two species that constitute pure and mixed forests in Mediterranean ecosystems in the Iberian Peninsula. Dendroclimatology, analysing tree ring and climate association, give information about past growth-climate relationship which may provide very valuable information about future tree growth on changing climatic conditions. Linear and non-linear models were applied to estimate this association between climatic conditions and tree ring. Figure 3 summarizes the main facts of the thesis.

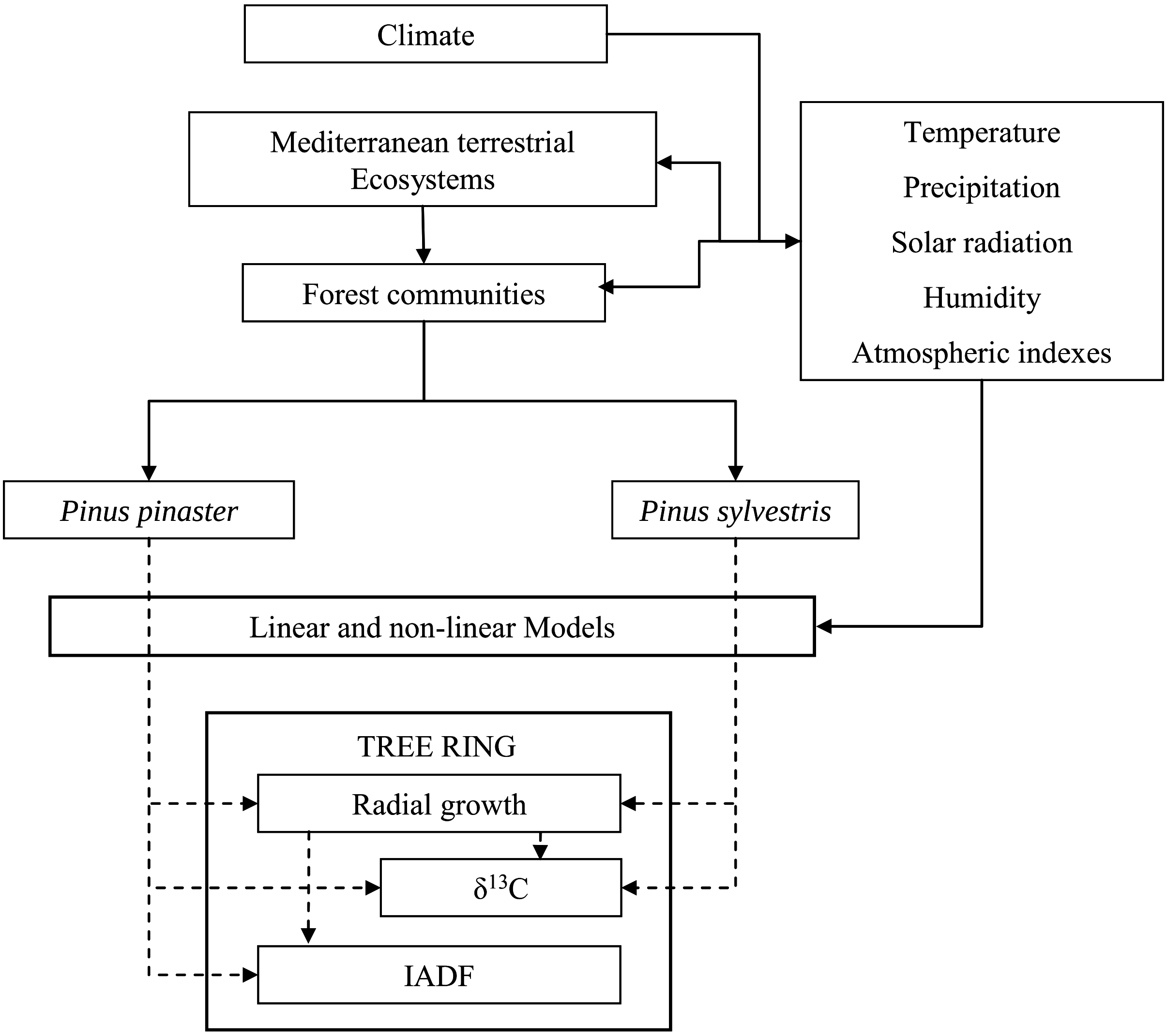


Figure 3. Main facts of the thesis that relate the parts involved in the dendroclimtological study of *P. pinaster* and *P. sylvestris* in the Iberian Peninsula. Dark lines mean incoming variables and dashed lines are modeled variables.

2.1. STUDY SITES

Ten and six sampling sites for *P. pinaster* and *P. sylvestris*, respectively, were selected from different bioclimatological ecoregions where these species grow in the Iberian Peninsula. Each ecoregion has common physiographic, climatic and lithological characteristics (Elena Roselló et al., 1997; Table 2.1).

Table 2.1. Ecoregion classification, geographical position and mean basal area of ten *P. pinaster* (*Pp*) and six *P.sylvestris* (*Ps*) sampling sites across its natural distribution area in the Iberian Peninsula.

Site	**Eco-region**	**Sp.**	**Lat. N**	**Long. W**	**Alt. (m)**	**BA (m^2 ha^{-1})**
P42201	*Duriense*	*Pp*	41º 33' 43”	02º 55' 17”	1012	53.84
P42002	*Duriense*	*Pp*	41º 34' 03”	02º 35' 51”	1059	34.84
P16201	*Catalano-Aragonesa*	*Pp*	39º 50' 06”	01º 16' 37”	1078	45.83
P44002	*Catalano*	*Pp*	40º 19' 07”	01º 21' 18”	1437	40.17
P44005	*Catalano*	*Pp*	40º 20' 47”	01º 21' 54”	1364	45.73
P44204	*Catalano*	*Pp*	40º 20' 01”	01º 21' 26”	1232	34.33
P16008	*Litoral-Mediterranea*	*Pp*	39º 48' 56”	01º 15' 36”	920	51.44
P16106	*Litoral-Mediterranea*	*Pp*	39º 50' 17”	01º 16' 11”	970	36.66
P16202	*Machega*	*Pp*	39º 49' 48”	01º 17' 38”	1135	41.69
P16208	*Machega*	*Pp*	39º 50' 28”	01º 17' 54”	1090	34.74
Miñon	*Galaico-Cantábrica*	*Ps*	42º 54' 46”	03º 21' 27”	860	37.18
Oña	*Galaico-Cantábrica*	*Ps*	42º 58' 22”	03º 18' 12”	760	32.25
El Espinar	*Machega*	*Ps*	40º 38' 58”	04º 12' 07”	1426	19.19
Molino	*Catalano-Aragonesa*	*Ps*	42º 04' 36”	02º 30' 18”	1676	38.66
El amogable	*Duriense*	*Ps*	41º 50' 44”	02º 55' 48”	1134	56.88
Arauzo de miel	*Duriense*	*Ps*	41º 53' 04”	03º 21' 32”	1081	41.55

2.2. DENDROCHRONOLOGICAL METHODOLOGY

2.2.1. Fiel and laboratory work

During the summer of 2006, two cores were extracted, at 1.30 m above ground level, from fifteen dominant and co-dominant trees at each sampling site. As growth of tapped trees may be affected by resin extraction, such trees were avoided. Cores were glued on to channelled wood, dried for two weeks and polished with progressively thinner sandpaper. In order to detrmine the calendar year in which a tree ring was formed, tree rings were dated by use of a binocular microscope Nikon SMZ1000 (20X), according to standard dendrochronological techniques (Stokes and Smiley, 1968; Fritts, 1976; Cook and Kairiukstis, 1990). The cores in transverse sections were scanned at high resolution (2.000 dpi) with an Epson Expression 1640 XL scanner (0.01-mm accuracy), and rings were measured by use of the WinDENDRO programme (Regent Instrument Inc., 2002). The v6.06P COFECHA programme (Holmes, 2001; Grissino-Mayer, 2001; available at www.ltrr.arizona.edu) was applied to assess measurement and dating accuracy. COFECHA allows calculation of the Pearson's correlation indices for the indexed tree-ring series and a master reference chronology in a series of consecutive, partially overlapping segments of a length specified by the user. 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.

2.2.2. Climate-growth relationship

To eliminate biological trends in tree-ring series and to minimize growth variations that are not shared by most trees (Fritts, 1976), the v6.05P ARSTAN programme (Cook and Holmes, 1984; Holmes, 2001; available at www.ltrr.arizona.edu) was used. Standardisation removes geometrical and ecological trends while preserving interannual high-frequency variations that are presumably related to climate. To obtain a master chronology at each study site, the standardized series were averaged. These temporal series or master chronologies expressed the annual variations in *P. pinaster* and *P. sylvestris* radial growth at the population level in each sampling site.

Chronology quality was evaluated using mean sensitivity (MS), is the degree to which a tree reacts to environmental factors or the measure of the year-to-year variability (Schweingruber 1996); signal-to-noise ratio (SNR), the proportion of the variability explained by climate or other causal factors divided by the residual or unexplained variability (Fritts and Swetnam, 1989); and expressed population signal (EPS), which describes how a finite sample estimates the hypothetical infinite population (Wigley et al., 1984, Briffa, 1995). Chronology was considered confident with an EPS value higher than 0.85.

2.2.2.1. Climate-growth relationship at regional scale

The common variance between all chronologies was analysed using a Principal Component Analysis for the common growth period 1952-2005 (Sokal and Rohlf, 1995) using Infostat V.2 (Di Rienzo et al., 2002). The variance explained by the first principal component (PC1) was used as an indicator of the similarity among the chronologies. PC1 was used as a regional chronology of *P. pinaster* in central Spain.

To determine the climatic variables that control *P. pinaster* radial growth, the average of the meteorological station data were compared with the regional chronology (PC1).

2.2.2.2. Climate-growth relationship at local scale

The local chronologies were compared with the meteorological stations closest to the site analysed. The period explored was from the previous June to September of the current growth year. The v 5.17 PRECON programme (Fritts, 1999; available at www.ltrr.arizona.edu) was used to compute the response functions of tree growth to climate by means of a multiple stepwise regression. Coefficients were considered significant at $^{*}p<0.05$ and $^{**}p<0.01$. A bootstrapped analysis was also applied to improve the statistical significance of the correlation coefficients. In this analysis, 1000 bootstrap interactions were made.

2.2.2.3. Climate-growth relationship over time

The Kalman filter, which estimates regression models by time-varying coefficients, was applied to determine if climatic variable effect changed over time (Fritts, 1998). The output of this analysis included predicted tree-ring indexes and the confidence interval (95% level); therefore, when the interval did not include a zero value, the regression coefficient was considered as significant (Kalman filter, V5.17 PRECON programme).

2.2.2.4. Drought index effect and pulse and interpulse analysis

The drought index (DRI) of Thornthwaite (1948) was applied to detect pulse-interpulse effect on radial growth of *P. sylvestris*. The DRI at each sampling site was calculated from the months that at the response function analysis showed a significant association between precipitation and radial growth (May and June at *Miñon*, *Oña* and *Arauzo de Miel* sites; June and July at *El Espinal* site and July and August at *Molino Piqueras* and *Amogable* sites). Monthly DRI was calculated by the formula,

$$DRI = P - PET \tag{2.1}$$

where DRI is the drought index, P = monthly precipitation and PET = the potential evapotranspiration estimated from the monthly mean temperature and the geographical position of the meteorological station.

The Pearson’s correlation coefficient was applied between DRI and tree-ring width at each sampling site (Sokal and Rohlf, 1995). A time series analysis was performed to detect both autocorrelation between consecutive tree-ring index and periodicity in the radial growth fluctuation due to pulse and interpulse frequency (Box and Jenkins, 1976). Tree-ring index autocorrelation of the six sampling sites was estimated using the autocorrelation function (ACF). The lag number changed according to the sampling site due to the fact that the series length was different. All lags were shorter than 25% of the total tree-ring series at each sampling site. The ACF provided information about the correlation between a specific year and the previous one. Coefficients statistically significant were those higher than the lines at a confidence level of 95%. Period-grams of the tree-ring index series were also calculated. The occurrence and periodicity of cycles was determined using the inverse of the peak of highest intensity which results from the spectral density due to the frequency. In order to eliminate the high peak in the lower frequency that may hide other cycles, the original series were transformed in a first order integrated series using a first difference transformation (Box and Jenkins, 1976), Infostat V.2 (Di Rienzo et al., 2002).

2.2.3. Intra-annual density fluctuations analysis

Once the cores were dated accurately, they were re-examined in a binocular microscope Nikon SMZ1000 (20X). Differentiation between a true tree ring and an IADF was established by the clear boundary in the true annual ring and the progressive and gradual transition in cell size and wall thickness in the IADFs (Masiokas and Villalba, 2004).

Photographs were obtained with a digital camera Leica DFC290 with a binocular microscope Leica S8APO. As the number of samples changed over time, the relative frequency was calculated with the following formula:

$$F = \frac{n}{N}, \qquad (2,2)$$

where F is the number of IADFs per year; n the number of trees that formed the IADF and N the total number of trees analysed. As the number of samples changed over time, the bias in the frequency was assessed by calculating the stabilized IADF frequency (*f*), according to the formula of Osborn et al. (1997), so

$$f = F^{0.5}. \qquad (2,3)$$

The Pearson’s correlation between stabilized IADFs was applied to detect common patterns in IADFs at different sites. ANOVA and Fisher’s test were used to detect significant differences in stabilized IADF between sites and over time. To determine the variability in IADFs over time, sampling sites of more than 100 years old were subdivided into two periods (between 1886 and 1939 and between 1940 and 2005). The Pearson’s correlation between the frequency of IADFs and the residual tree-ring series (Paper 1) was also calculated to analyse the relationship between IADFs and radial growth (Sokal and Rohlf, 1995). Infostat V.2 software (Di Rienzo et al., 2002) was used to statistical analysis.

The nonlinear logistic equation form was chosen to model the probability of occurrence of IADFs in *P. pinaster* rings,

$$P = \left(1.0 + e^{-z}\right)^{-1} \qquad (2,4)$$

where P is the probability of IADFs and $Z = b_0 + b_1(x_1) + b_2(x_2) + \ldots + b_k(x_k) + \varepsilon$; where x_1; x_2..... x_k are the climatic variables and b_0; b_1; b_2 ….. b_k are unknown parameters of the model, 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. 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 area under the receiver operating characteristic (ROC) curve and the expected behavior - as indicated by the signs of the parameters estimates. The area under the ROC curve can be considered as an estimator of accuracy. This curve, which is widely used in health sciences but not in Dendrochronology, 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). PROC LOGISTIC of SAS 9.1 (SAS Institute Inc., 2004) was used to fit the model.

2.2.4. Isotope analysis

The $\delta^{13}C$ isotope was determined on whole wood (Schleser et al., 1999a; Babour et al., 2001). Cellulose was not extracted from the wood as these two components were shown to yield highly correlated signals (Borella and Leuenberger, 1998) and even higher climatic signal can be detected in the untreated material (Loader et al., 2003). Powdered material was obtained for each tree ring with a Micromot 40E instrument with a 0.5 mm thick needle. The samples were obteined after identification of the previously dated tree ring by use of a binocular microscope. Each sample was analysed individually, the material was weighted, and for each tree ring, 0.2-0.3 mg of powered material was placed into tin capsules. The stable carbon isotope was measured with an NA 2500 elemental analyser (CE Instruments, Rodano, Italy), with an isotope ratio mass spectrometer (Finningan MAT Delta plus, Bremen, Germany).

The isotopic composition ($\delta^{13}C$) of samples was determined with the formula,

$$\delta^{13}C(‰) = \frac{\left({}^{13}C/{}^{12}C\right)_{sample}}{\left({}^{13}C/{}^{12}C\right)_{PDB-1}} \times 10^3 \qquad (2,5)$$

where $\delta^{13}C$ (‰) is the proportional deviation from the international Peedee belemite (PDB) carbonate standard (Craig, 1957)

As a previous study showed a changing association between climatic variables and growth of *P. pinaster* (Paper 1) from the 1980s onwards, and phenological changes have been reported over the last 25 years (Peñuelas et al., 2002), the period analysed was between 1975 and 1999. The same period was considered for *P. sylvestris*.

Pearson's correlation analysis was applied to all trees of the same species to detect common patterns in $\delta^{13}C$, and between $\delta^{13}C$ and the residual tree-ring chronologies of both species (data from Papers 1 and 3) to detect any significant association between growth and isotope variability. Pearson's correlation analysis was also used for $\delta^{13}C$ and monthly climatic variables (monthly mean maximum temperature, monthly precipitation, monthly air moisture and monthly hours of solar radiation) in order to estimate which environmental variables were statistically significant. The monthly climatic variables that were best correlated with $\delta^{13}C$ (***$p<0.001$) were grouped to construct simple linear regression models, as follows (Sokal and Rohlf, 1995):

$$y = a + bx + \varepsilon , \qquad (2,6)$$

where y is $\delta^{13}C$; x is the sum of climatic variables; a and b are unknown parameters of the model and ε is a normal random error N(0,1).

Statistic analysis was carried out with the Infostat programme (Di Rienzo et al., 2002).

2.3. CLIMATIC DATA

Monthly precipitation and mean monthly temperature by the *Agencia Estatal de Meteorologia,* Spain, were used to detect climate-growth relationship. The meteorological data provided belong to meteorological stations placed within 30 km of the sampling sites (Table 3). The data recorded cover a period of, at least, 50 years. The data from four meteorological stations were averaged to obtain a regional climatic record to be applied in the regional dendroclimatic analysis of *P. pinaster*.

Table 3. Meteorological station data from the *Agencia Estatal de Meteorología*, Spain

Location	**Lat. (N)**	**Long. (W)**	**Alt. (m)**	**Rain. (mm)**	**Temp. (ºC)**	**Period**
El burgo de Osma	41º 35' 10"	03º 04' 02"	895	529.5	10.54	1932-2005
Cuenca	40º 04' 00"	02º 08' 17"	956	541.42	12.43	1956-2005
Pantano de la Toba	40º 13' 19"	01º 55' 33"	1154	764.68	10.22	1944-2005
Observatoriode Soria	41º 46' 00"	02º 28' 00"	1082	418.34	10.66	1944-2005
Miranda del Ebro	42º 40' 42"	02º 57' 20"	520	529.97	12.08	1936-2005
Villafría	42º 21' 22"	03º 37' 57"	890	564.67	10.15	1943-2005
Aldea del rey Niño	41º 34' 35"	04º 42' 02"	1160	522.24	9.17	1935-2005
Yemeda	39º 45' 40"	01º 43' 17"	868	405.13	12.41	1950-2000
Cella	40º 27' 20"	01º 17' 27"	1023	370.81	12.01	1939-2006

The HOM component (Homogeneity of Meteorological Data) of the Directory Programme Library for Dendrochronology (Holmes, 1983; available at www.ltrr.arizona.edu) was used to determine the homogeneity of the climatic variables.

For the atmospheric indexes and radial growth analysis the NAO and SOI values were obtained from www.cru.uea.ac.uk/cru/data/nao.htm and www.cru.uea.ac.uk/cru/data/soi.htm (Jones et al., 1997). Also, monthly air moisture and hours of solar radiation were used to detect $\delta^{13}C$ variability.

3. RESULTS

3.1. CHRONOLOGY DESCRIPTION

One chronology of *Pinus pinaster* was not included in the analysis because its time span was only 30 years and it would have limited the period analyzed of all series.

The nine and six chronologies of *P. pinaster* and *Pinus sylvestris*, respectively had high SNR, EPS and percentage of the variance accounted for the first eigenvector; this suggests that they reflect a strong common signal, presumably related to climatic factors. The descriptive statistics showed that mean sensitivity varied from 0.1857 to 0.3179 and from 0.1556 to 0.2669, standard deviation varied from 0.1650 to 0.3108 and from 0.1858 to 0.3527, for *P. pinaster* and *P. sylvestris*, respectively. SNR varied from 27.615 to 68.444 and from 10.676 to 24.747 and EPS values varied from 0.958 to 0.986 and from 0.914 to 0.961, for *P. pinaster* and *P. sylvestris*, respectively. All chronologies analysed have high SNR (values always over 10) and EPS (always over 0.914), while the variance accounted by the first eigenvector is over 35 %; these facts suggest a strong common signal related to environmental climatic factors (Tables 3, I and III).

In *P. pinaster*, despite the diversity of tree ages and the difference in bioecological regions, a clear common macroclimatic signal expressed by the first principal component (PC1) of the PCA was found. PC1 explained 56% of the total variance of indexed tree-ring growth among the nine chronologies. All the chronologies had a positive correlation with PC1, indicating they shared a common variance (Figure 4 and Table 4, I).

3.2. CLIMATE GROWTH ASSOCIATION: MULTIPLE STEPWISE AND BOOTSTRAPPED ANALYSIS

Radial growth, at both regional and local scales, in *P. pinaster* was strongly associated with climatic variables, especially rainfall during and prior to the growing season in all the sites analysed. Mean temperature effect changed according to the sampling site, from insignificant at the highest altitudinal sites to positively significant in winter at the lowest altitudinal position sites (Figure 6, I).

As a result of the PCA analysis, PC1 was considered indicative of the interannual high-frequency variation of tree-ring growth at regional level for the common period 1952-2005. This chronology was correlated with regional climatic variables, precipitation and temperature, and the results showed that more than 60.8% of the total variance in radial growth was explained by climate (Figure 5, I).

3.3. CLIMATE GROWTH ASSOCIATION OVER TIME: THE KALMAN FILTER TECHNIQUE IN *P. PINASTER*

Results suggested a changing association between growth and climatic variables from insignificant to significant (*$p<0.05$) at six of the sampling sites.

The change in association between growth and climatic conditions took place during the 1980s. During this period, precipitation changed from insignificant to positively significant at four sampling sites. This change occurred in winter prior to the growing season or at the beginning of the growing season. Temperature also showed a changing association with growth at two sampling sites. Temperature became negatively significant on radial growth in springtime (May) at one sampling site; this change in association may be related to the intense drought and the warming that characterised the 1980s in the peninsula. Finally a positive change was found in the other sampling site in relation to winter temperature (February) (Table 5, I).

3.4. NAO AND SOI EFFECT ON RADIAL GROWTH

The total variance explained by atmospheric indexes varied from 8.95 to 37.46%.

The total variance explained by the NAO and SOI indexes is higher in the sites at higher positions. In these places there is a significant negative association between the NAO index and growth during January and March or December and March, but only March is significant in the bootstrapped analysis. Only one place showed a positive association with NAO values in September prior to the growing season, but this association was not significant in the bootstrapped analysis. The association with the SOI was positive in all the analysed sites, but it was not significant, in the correlation coefficient and the in the bootstrapped response function. Only one site showed a negative association with the SOI, shown by the bootstrap coefficient during March previous to the growing season (Figure 2, II).

3.5. CLIMATE AND RADIAL GROWTH OF *P. SYLVESTRIS*

Tree-ring width was positively correlated with rainfall in the growing season at all sites. Mean temperature effect varied according to site. A climatic response according to age was also found: young stands had higher variability explained by climatic variables than old stands (Figure 3, III).

Because the six sites analysed showed a clear association with rainfall in the growing season, the months where this association was significant at each sampling site were selected for DRI calculation. The Pearson’s correlation coefficient between tree-ring index and DRI showed a significant association in all sampling sites (* $p < 0.05$; **$p < 0.01$;***$p < 0.001$) (Table 4 and Figure 4, III).

The ACF of the six chronologies performed to detect autocorrelation between successive tree-ring growth values suggested that autocorrelation coefficients in young stands (less than 100 years old) were not significant. However, this association changed in old stands (more than 100 year old), where a significant association between current year tree growth with that of one, two or three years before was detected (Figure 5, III).

The time series analysis used to detect periodicity in fluctuation in tree-ring growth relating to pulse and interpulse water availability events suggested that the strong intensity variation in DRI between years determined a clear variation in tree growth showed by a periodicity of two or two and a half years, except for only one sampling site (Table 5 and Figure 6, III).

3.6. IADFS AND CLIMATE RESULTS

P. pinaster is characterised by different anatomical features. A total of 11 930 tree rings were analysed and a total of 1 038 IADFs were detected in the trees, 8.7% of the tree ring analysed (Table 3, IV). Even though the species showed different anatomical IADFs they were rather easy to identify by correct preparation of the samples. Samples showed an increase in IADFs from the 1940s to the present, except in only one site (Table 6, IV). The absence of IADFs at this site may be attributed to the extreme thinness of the tree rings (less than 0.3mm), which made it impossible to detect IADFs (Figure 4, IV). There were IADFs at all sampling sites in 1961 and 1983, with a stabilized frequency higher than 3. A low frequency of IADFs was recorded in the 1970s at all sampling sites (Figure 3, IV).

The Pearson's correlation between stabilized IADFs was positive and significant for all comparisons between sampling sites (*$p<0.05$ and ***$p<0.001$) (Table 4, IV).

The results of the ANOVA and Fisher's test performed to detect significant differences between IADFs at different sampling sites (from the common growth period 1953-2005) showed that younger sampling sites have higher IADFs than older sampling sites. However, Fisher's test does not enable complete separation of young and old stands. The higher mean IADFs also coincided with the highest site index, but as these sites are also the youngest in terms of cambial age, it was not possible to ensure that the higher IADFs are related to site index (Table 5, IV).

Significant differences were found in the last 120 years in the stabilized IADFs. The Pearson's correlation coefficients showed a negative significant correlation (*$p<0.05$; *** $p<0.001$) between tree-ring growth and stabilised IADF at all sampling sites. The results suggest that IADFs restrict growth at all sampling sites (Table 7, IV).

The logistic function used to predict the possibility of the occurrence of IADF estimated that 18 monthly climatic variables out of 24 had a significant effect on predicting future IADFs. Drought events in May and July had a positive impact on IADF while wet periods in April and June also promoted IADF. Such pulses in precipitation (rainy months follow by dry months) are typical of Mediterranean areas. Increases in temperatures also had a generally positive impact on IADFs (Table 8, IV). The value of the area under ROC curve (0.84) shows that the accuracy of model is good enough to use it to predict occurrence of IADFs (Figure 5, IV).

3.7. CARBON ISOTOPES AND CLIMATE RESULTS

The mean values that characterising each tree-ring $\delta^{13}C$ in both species in each year analysed showed that the highest and the lowest values of $\delta^{13}C$ (-22.62‰ and -25.87‰) corresponded to *P. pinaster*, whereas the corresponding values for *P. sylvestris* varied between -23.85‰ and –25.8‰. (Figure 3, V).

A negative significant correlation between $\delta^{13}C$ and radial growth was found for the 1975-1999 period. The Pearson's correlation coefficient for the residual tree-ring chronologies and the mean $\delta^{13}C$ was r: -0.83 (***$p < 0.001$) for *P. pinaster* and r:-0.41 (*$p < 0.05$) for *P. sylvestris*.

Pearson's correlation coefficient for climatic variables and $\delta^{13}C$ in *P. pinaster* showed that moisture is a driving factor affecting the variability of $\delta^{13}C$ between winter and summer (January to July). Rainfall also had a negative effect on $\delta^{13}C$ between winter and spring although the effect was only statistically significant in April and May. Monthly mean maximum temperature in March had a positive significant effect on $\delta^{13}C$ and monthly hours of solar radiation in January, April and May (Figure 4, V). Pearson's correlation coefficient for climatic variables and $\delta^{13}C$ of *P. sylvestris* showed that moisture is a driving factor affecting $\delta^{13}C$ variability in summer (July) and in autumn (October). Rainfall in October also had a negative effect on $\delta^{13}C$. Monthly mean maximum temperature in summer (June and July) had a positive significant effect on $\delta^{13}C$ as well as hours of solar radiation in October (Figure 5, V).

For *P. pinaster* RH from January to July, rainfall from January to May, maximum mean temperature from April to July and hours of solar radiation of January, April and May were selected to construct four different simple linear regression models that relate each variable and $\delta^{13}C$ (Figure 6, V). For *P. sylvestris* RH in July and October months, rainfall in July and October, maximum temperature in June and July and hours of solar radiation in October were selected to construct four different simple linear regression models. All variables were significantly correlated with $\delta^{13}C$ in both species (Figure 7, V).

4. DISCUSSION

Pinus pinaster is a reliable species for dendrochronological studies, showing good correlation between trees growing at the same sampling site, high signal related to total noise and accurate statistical values that mean clear response to environmental factors. *P. pinaster* also shows accurate performance in studying the association between tree growth and global change showed by a changing relationship with climatic variables over time.

A common growth pattern among all series has been detected. PCA analysis suggests a clear strong common variance among all the sampling sites (explaining over 56% of variability) and a positive correlation (**p<0.01 and ***p< 0.001) with the PC1 axis. Although the sampling sites included trees of different ages and trees from different eco-regions, all the series can be considered to share a common variance related to causal factors.

The total variance explained by average climatic variables and regional radial growth index of *P. pinaster* (60%) can be considered higher than the most common findings obtained in dendroclimatic studies worldwide. Tree rings rarely cover more than 60% of the variance registered in instrumental records, and 40 to 50% is quite a common level (Fritts, 1991; Jones et al., 1998). Richter et al. (1991) found 68% of the total variance explained by precipitation and temperature in pine species growing in the Iberian Peninsula.

Rainfall is the dominant climatic variable that has a significant association with *P. pinaster* growth, as the stepwise correlation analysis indicated. Rainfall effect constitutes the significant climatic variable in both, the regional and local analysis.

Temperature showed a changing significant effect on tree growth that varied according to the sampling site. No effect was recorded at the sites placed at the highest positions and a negative effect was recorded at five sampling sites. The difference found out in this study is that temperature effect could not be simplified to a common response in all the sampling sites because local issues determine the association between this variable and radial growth.

Although *P. pinaster* shows a strong association with precipitation and the temperature impact is lower than that of rainfall, its increase might raise evaporation, limit environmental moisture and, consequently, restrict growth. However, the predicted temperature trend until 2080 will probably not lead to an unsuitable environment for *P. pinaster* (Harrison et al., 2006). As *P. pinaster* is a pre-Mediterranean species that has suffered different environmental changes [from subtropical environments to Mediterranean ones (DiCarlo, 1931)], it can also deal with very atypical severe droughts, such as that of 1994 (Peñuelas et al., 2002), and it shows a better water-use efficiency than *P. sylvestris* (Martínez-Vilalta and Piñol, 2002). These facts indicate that *P. pinaster* might be well adapted to summer rainfall reductions, as IPCC forecasts in the Iberian Peninsula.

The climate effect over time suggests changing results, and some variables that were not significant 30 years ago have been stated to be significant since the 1980s.

The climatic warming of the 1980-1995 period was characterised by intense droughts that produced severe damage in woody species (Peñuelas et al., 2001). Our findings indicate that some climatic variables (May temperature and April-May rainfall) have changed from insignificant to significant in the last 30 years (from 1980 until now). This coincides with previously-reported phenological changes in plant life (related to temperature) in the Mediterranean region. In addition, these changes started during the 1980s, which could mean a changing association with climatic factors (Peñuelas et al., 2001; 2002).

It is difficult to find a simple linear correlation between radial growth of *P. pinaster* and atmospheric indexes because their global effects and their impact on regional climatic variables are not yet completely understood.

In these results, the total variance explained by NAO and SOI indexes suggested that the signal is weak if it is compared with regional climatic variables. However, the negative correlation with NAO in winter in two sites, and the changing effect of SOI index over time in one site, offer new information about the association between atmospheric indexes and coniferous species growing in the Iberian Peninsula.

Although atmospheric indexes explain less variability than other regional climatic variables, these results emphasised that these indexes effects could be recorded on tree ring and they could have a sensible effect in growth of woody species, even if their action centres are located too far away from the analysed sites.

Pinus sylvestris is a useful species in dendrochronological studies showing accurate statistical values that mean clear response to environmental causal factors. The six chronologies have high mean sensitivity (MS), expressed population signal (EPS), signal to noise ration (SNR) and percentage of the variance accounted for the first eigenvector, suggesting a strong common signal to related-climatic environmental factors.

The association between growth and climate shows that rainfall in the growing season has a positive significant effect on radial growth among all sites, no matter what their altitudinal location, geographical position or cambial age are. Differences in rainfall effect within the growing season were found: at sites located at the lowest altitudinal position, this effect happened in spring (May and June); in contrast, with sites placed at a higher altitudinal position, the association occurred in late spring and summer. This different association may be due to temperature. Sites located at the lowest altitudinal positions are warmer; consequently, the growing season starts early, and the spring rainfall is a driving factor that affects growth. However, sites located at the highest altitudinal position are colder, the growing season starts later and growth depends on summer rainfall. These results emphasised how important the within-season precipitation dynamic is; it can be equal to or more important than the seasonal or annual total for plant growth (Fay et al., 2000; Knapp et al., 2002).

The significant association between radial growth and DRI suggests that drought is a key factor affecting *P. sylvestris* growth in its southern and western distribution area. The effect of consecutive year growth was indicated by the autocorrelation analysis: young stands did not show correlation between successive tree rings, and old stand tree ring growth is affected by previous growth. Young stands have higher variability explained by climatic variables in the response function analysis (from 53 to 69%) and are not vulnerable to previous growth; in contrast, old stands have a lower percentage of the variability explained by climatic variables (from 31 to 39%) but are vulnerable to previous growth. Since ecosystems have the capacity to store water, they clearly have a "memory" of past precipitation events. As a result of such a "memory" effect, it is not correct to understand rainfall effect as a single seasonal or annual event (Schwinning et al., 2004). This study confirms the "memory" of past events when autocorrelation analysis is performed, suggesting an association between present growth with previous growth years in old *P. sylvestris* stands. When growth periodicity is analysed to determine pulse and interpulse periods in *P. sylvestris* growth, it is clear that the strong DRI variability and the significant effect that this index has on tree-ring growth result in a strong variability between successive years that is reflected as periods of two or two and a half years in the time series analysis. Even though, depending on the sampling site, other cycle periods were detected, two-year periodicity is stronger than other long-period cycles.

P. pinaster showed nine different anatomical structures that confirm the tendency of Mediterranean species, and this species in particular, to develop special anatomical structures (Schweingruber, 1993). Even though IADFs, which occur in all series at all sampling sites, may previously have limited dendroclimatological studies in *P. pinaster* (Schweingruber, 1993), when the correct date is obtained, the significant association between IADFs and radial growth or climatic variables provides a useful proxy for complementing and enhancing dendroclimatological data (Paper 1).

Latewood is formed from carbohydrates produced during photosynthesis which is the result of water stress and temperature (Kozlowski et al., 1991). IADFs of *P. pinaster* growing in Tuscani Italy had a higher $^{13}C/^{12}C$ isotope ratio in latewood than earlywood, which suggests a better water use efficiency (De Micco et al., 2007). The present results emphasised the impact of drought events on IADFs (significant effect of rainfall in the growing season) and suggest a physiological response of *P. pinaster* to unfavourable climatic conditions and the development of anatomical structures that lead to better water use efficiency. Isotope analysis in the Iberian Peninsula, showed that *P. pinaster* makes more efficient use of water under severe drought events than *P. sylvestris* and *Pinus nigra* Arnold (Martinez Vilalta and Piñol, 2002).

Previous studies that relate IADFs to growth of *P. sylvestris* in Switzerland determined a positive association between tree-ring growth and IADFs (Rigling et al., 2001); these results contrast with the present results in which a significant negative relationship between radial growth and IADFs was found, suggesting that the environmental variables that produce IADFs also limit growth.

The effect of climate on IADFs of *P. pinaster* is determined by both the significant correlation between stabilised IADFs at all sampling sites -suggesting the impact of the driving factor (climate)- and the accurate results of the logistic function.

Climate change scenarios developed by IPCC (2007) show that irregularity in intra-annual rainfall and trends of increasing temperature should be expected during the next decades. According to the present results, this predicted future climatic situation will lead to a higher occurrence of IADFs.

In the present study, the frequency of stabilized IADFs in *P. pinaster* was higher in more productive sites (higher site index), thus demonstrating the importance of the incorporation of intra-annual features in dendroecological and dendroclimatological studies, which is highly recommended because it enables differentiation of site types (Rigling et al. 2001; 2002). Extensive studies are needed to provide further insight in this finding.

The $\delta^{13}C$ is a useful tool that provides both reliable information about climatic variables that affect the physiology of *P. pinaster* and *P. sylvestris* and WUE of these species growing under Mediterranean climatic conditions.

This high $\delta^{13}C$ between trees suggests an individual tree response in relation to fractionation [the ratio of carbon isotope ratios in reactant and products (Farquhar et al., 1989)]. The values of $\delta^{13}C$ data for all trees of *P. pinaster* were significantly correlated which suggests that this variability is driven by a strong environmental effect. The $\delta^{13}C$ values for individual *P. sylvestris* trees were also significantly correlated but the coefficient were lower than in *P. pinaster* and two trees did not show a significant association.

The highly significant inverse correlation between $\delta^{13}C$ and with tree-ring width in *P. pinaster* and *P. sylvestris* emphasise the potential usefulness of this type of studies for analysing the environmental factors that affect growth of *P. pinaster* and *P. sylvestris* under Mediterranean climatic conditions. The results are consistent with previous statements that suggest that species growing in variables environments such as Mediterranean environments show a changing ratio of $\delta^{13}C$ in each tree ring as a result of the variables climatic conditions (high temperature and low precipitation) and that $\delta^{13}C$ provide a strong indicator of the severity of these climate variables (McCarroll and Loader, 2004).

The $\delta^{13}C$ was significantly correlated with moisture, rainfall, maximum temperature and solar radiation. The negative effect of moisture and rainfall prior to and throughout the growing season, and the positive effect of maximum temperature and solar radiation throughout the same period confirm the hypothesis that $\delta^{13}C$ in conifers worldwide is an indicator of drought stress in dry climates (Warren et al., 2001).

In *P. pinaster* the $\delta^{13}C$ accounted for rainfall from Jannuary to May and RH between January and July was 44% and 52%, respectively. On the other hand, rainfall and humidity in July and October on *P. sylvestris* accounted for 34% and 22% of the $\delta^{13}C$, respectively. These results underlines the importance of water availability as a driving factor for isotope fluctuation in both arid and semiarid environments and in pines species (Warren et al., 2001).

Although temperature had a significant effect on drought (the higher the temperature, the greater the effect of drought), the results showed that each climatic variable analysed may have a significant effect on $\delta^{13}C$ in these Mediterranean environments. In the present study, maximum mean temperature was the only climatic variable that was more more closely correlated with $\delta^{13}C$ in *P. sylvestris* than in *P. pinaster* (61% and 57%, respectively). This association was highly significant in both species and the significant effect varied according to the period analysed: summer temperature were significant for *P. sylvestris* (June and July) and spring temperature (March) for *P. pinaster*. Part of this signal may be due to the fact that hot summer are strongly correlated with high evaporation rates and thus, with $\delta^{13}C$.

Considering that *P. sylvestris* in the Iberian Peninsula grows within the limit of the worldwide distribution, and Schleser et al., (1999b) reported that under these conditions, $\delta^{13}C$ tends to respond to one atmospheric parameter, these results are also consistent with those of McCarroll and Pawellek (2001) who concluded that $\delta^{13}C$ response is complex.

It was reviouly concluded that the same species may have different isotopic response and in different environments (Sternberg and DeNiro, 1983; Leavitt and Long, 1984), and *P. pinaster* growing under maritime climatic conditions in Italy did not show any strong variation in $\delta^{13}C$ in different years, even though there was a severe drought during the period analysed (summer of 2001) and the authors commented on the limitation of the application of stable carbon analysis in assessing the severity of drought in environments characterised by seasonal aridity (De Micco et al., 2007). Even though we analysed the same species, the present results showed that *P. pinaster* is an accurate tool for studying climatic conditions, as shown by the significant correlation coefficients that underlined the recommendation made by Leavitt and Long (1986) that results for $\delta^{13}C$ in one species should not be extrapolated to other environments.

When WUE is analysed, the results suggest the importance of this studies for understanding the physiological changes in trees related to general changing environmental conditions. Phenological changes and the increased WUE in woody species in the Iberian Peninsula have been already reported (Peñuelas et al., 2002).

Regarding the results, it is difficult to agree with Harrison et al. (2006) who suggested an unsuitable environment for these species from the year 2050 because the $\delta^{13}C$ studies revealed a clear adaptable capacity relate to a better WUE.

5. CONCLUSIONS

The conclusive association between climatic variables and different dendroclimatological tools (tree-ring width, IADFs and carbon isotopes) in *Pinus pinaster* and *Pinus sylvestris* may provide an excellent tool to study the climate-growth relationship in woody species and to understand the dynamic of the species under changing climatic conditions in Mediterranean environments. Even thought in same cases isotopes may have a better correlation with climatic variables than tree-ring width; one proxy does not limit the use of others.

P. pinaster radial growth was strongly associated with water supply, which means that it may be an excellent tool for reconstructing past weather conditions in the Iberian Peninsula, especially the temporal fluctuations of rainfall. Mean temperature effect varied according to site altitude, from insignificant at the highest sites to positively significant during winter at the lowest. Growing season temperature also had a negative effect. On the other hand, a changing association between growth and climatic variables has shown that this species is an accurate tool for studying global change effect on tree growth.

P. sylvestris showed a relationship that changes depending on the climatic variable analysed: rainfall in the growing season was the driving climatic variable that controls growth in all the sites analysed, while the association with temperature changed according to the site and could be positive or negative. The variability explained by climate variables is higher in the young trees than in the older. DRI was an accurate tool to explain radial growth of this species. Correlation between successive tree-ring growth changed according to the cambial age of the stand. Growth periodicity of *P. sylvestris* relate to pulse and intepulse water events was two or two and a half years, which coincided with DRI variability.

IADFs in *P. pinaster* were a useful tool in the application of dendrochronological techniques to date samples. IADFs were determined by cambial age and had increased in frequency in the last sixty years. Finally, the probability model used showed that rainfall pulses in late winter and spring and higher temperatures will lead to a more frequent occurrence of intra-annual density fluctuations in *P. pinaster* trees growing under Mediterranean climate conditions.

Finally, $\delta^{13}C$ of *P. pinaster* and *P. sylvestris* growing in Mediterranean environments showed a strong correlation with climatic conditions which suggests that they are a very valuable tool for studying the effect of climate change.

6. CONCLUSIONES

La asociación clara entre las variables climáticas y los distintos métodos dendroclimatológicos analizados en este trabajo, a saber: el ancho de los anillos de crecimiento, las variaciones interanuales de la densidad de los anillos de crecimiento y del carbono trece; proporcionarían una herramienta muy adecuada para estudiar la relación del crecimiento de las especies leñosas con el clima así como para entender la dinámica de estas especies bajo condiciones variables del clima en los ambientes Mediterráneos.

A pesar de que en algunos casos los isótopos podrían tener una correlación mejor con las variables climáticas que el ancho de los anillos de crecimiento, un método dendroclimatológico no limita el uso y la aplicación de otros.

El crecimiento radial de *Pinus pinaster* está fuertemente asociado a la disponibilidad de agua, lo que implica que podría ser una herramienta muy adecuada para la reconstrucción de condiciones meteorológicas pasadas en la Península Ibérica, en particular las variaciones acaecidas en cuanto a la precipitación. El efecto de la temperatura varía de acuerdo con la altitud del sitio de muestreo, siendo no significante en los sitios más altos y positivamente significantivo durante el invierno en las estaciones de menor altitud. La variabilidad del crecimiento explicada por el clima es mayor en los árboles jóvenes que en los añejos. La temperatura durante la estación de crecimiento tiene un efecto negativo sobre el crecimiento radial de la especie. Por otra parte, la relación cambiante entre el crecimiento radial y las variables climáticas demuestran que esta especie conforma una herramienta adecuada para estudiar el efecto del cambio climático sobre el crecimiento de los bosques.

Pinus sylvestris muestra una relación con el clima que cambia de acuerdo con la variable climática estudiada: la lluvia durante la estación de crecimiento es la variable climática que controla el crecimiento en todos los sitios analizados, mientras que la asociación con la temperatura cambia de acuerdo con el lugar de muestreo pudiendo ser negativa ó positiva. El índice de sequía es una herramienta adecuada para explicar el crecimiento radial de esta especie. La correlación entre anillos de crecimiento sucesivos, cambia de acuerdo con la edad de los árboles. La periodicidad del crecimiento es de dos a dos años y medio y está relacionada con los eventos de pulsos e interpulsos de agua.

La interpretación de los cambios interanuales de la densidad de *P. pinaster* son una herramienta muy útil en la aplicación de técnicas dendrocronológicas para datar muestras. Las fluctuaciones en la densidad interanual están determinadas por la edad y se ha incrementado en los últimos 60 años. Por ultimo, el modelo probabilístico usado en este estudio determinó que los pulsos de lluvia en el invierno tardío y en primavera, al igual que sucede con las altas temperaturas, ocasionarían una mayor ocurrencia de fluctuaciones interanuales de la densidad de *P. pinaster* en ambientes mediterráneos.

Finalmente, *P. pinaster* and *P sylvestris* mostraron una correlación significativa entre $\delta^{13}C$ y las condiciones climáticas, lo que supone que son unas especies muy adecuadas para el estudio del clima.

REFERENCES

Alía M., Martín S., De Miguel J., Galera R., Agúndez D., Gordo J., Catalán G., Gil L. 1996. Regiones de procedencia *P. pinaster* Aiton., Dirección general de Conservación de la Naturaleza, Madrid, 75p.

Andreu L., Gutiérrez E., Macias M., Ribas M., Bosch O., Camarero J. 2007. Climate increases regional tree-growth variability in Iberian pine forest. Glob. Change Biol.13 (4), 804-815.

Bakkenes M., Alkemade J.R.M., Ihle F., Leemansand R., Latour J.B. 2002. Assessing effects of forecasted climate change on the diversity and distribution of European higher plants for 2050. Glob. Change Biol. 8 (4), 390-407.

Barbéro M., Loisel R., Quezel P. 1998. Pines of the Mediterranean Basin. Pinus. Ecology and Biogeography of Pinus (ed. D.M. Richardson), Cambridge University Press, Cambridge, pp. 153-170.

Barbour M., Andrews J., Farquhar, G., 2001. Correlations between oxygen isotope ratios of wood constituents of Quercus and Pinus samples from around the world. Australian Journal of Plant Physiology 28(5) 335 - 348

Beerling D. 1994. Predicting leaf exchange and δ^{13}C responses to the past 30000 years of global environmental change. New Phytologist 128, 425-433.

Blanco E., Casado M., Costa M., Escribano R., Gracía Antón M., Génova M., Gómez Manzaneque G., Gómez Manzanaque F., Moreno J., Morla C., Regato P., Sainz Ollero H. 1997. Los bosques ibéricos, Editorial Planeta, Barcelona, España, 572p.

Bogino S., Bravo F., 2008. Growth response of *P. pinaster* Ait. to climatic variables in central Spanish forests. In press.

Bogino S., Fernández M., Bravo F. Drought index and radial growth of *Pinus sylvestris* L. in its southern and western distribution threshold. Journal of Arid environments. Submitted

Borella S., Leuenberger M., 1998. Reducing uncertainties in d13C analysis of tree rings: pooling, milling and cellulose extraction. Journal of Geophysical Research 103, 19519–19526.

Box G.E.P., Jenkins G.M. 1976. Time Series Analysis, Forecasting and Control, Revised ed. Holden-Day.San Francisco.CA.

Briffa K.R. 1995. Interpreting high-resolution proxy climate data-the example of dendroclimatology. In: von Storch, H.; Navarra, A. (Eds), Analysis of climate data variability, application of statistical techniques, New York, Springer, pp.77–94.

Cook E.R., Holmes R.L. 1984. Program ARSTAN users manual. Lab. Tree Ring. Res. Univ. of Arizona, Tucson, Arizona, EUA.

Cook E. R., Kairiuskstis L. A. 1990. Methods of Dendrochronology, Applications in the Environmental Sciences. Kluwer Academic Publishers, 393 p.

Craig H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. Geochim. Cosmochim. Acta 12:133-49.

D'Arrigo R.D., Jacoby G.C. 1992. A tree-ring reconstruction of New Mexico winter precipitation and its relation to El Niño/Southern Oscillation events. In Diaz, H.F.; Markgraf, V. (Eds), El Niño. Historical and paleoclimatic aspects of the Southern Oscillation. Cambridge, University Press, pp. 243-258.

De Micco V., Saurer M., Aronne G., Tognetti R., Cherubini P. 2007. Variations of wood anatomy and $\delta^{13}C$ within-tree rings of coastal *P. pinaster* showing intra-annual density fluctuations IAWA J. 28 (1): 61–74.

DGCN 1998. Segundo Inventario Forestal Nacional 1986-1996. Ed. Ministerio de Medio Ambiente, España.

DGCN 2002. Plan Forestal Español. Ed. Ministerio de Medio Ambiente, España.

Di Castri F. 1981. Mediterranean-type shrublands of the world. In di Castri F., Goodall D., Specht R.L. (Eds.), Mediterranean-type shrub lands, Elsevier Scientific Publishing Amsterdam, The Netherlands, 643p.

Di Rienzo J., Balzarini M., Casanoves F., Gonzalez L., Tablada E., Robledo C. 2002. Infostat Software Estadístico versión 2. Grupo infoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Ehleringer J. R., Hall A. E., and Farquhar G. D. (eds) (1993) Stable Isotopes and Plant Carbon-Water Relations. Academic Press. San Diego, CA. 555 p.

Elena-Roselló R. 1997. Clasificación Biogeoclimática de España Peninsular y Balear Madrid: Ministerio de Agricultura, Pesca y Alimentación, Madrid, 100p.

Esteban-Parra M., Rodrigo F., Castro Diéz Y. 1998. Spatial and temporal patterns of precipitation in Spain for the period 1880-1992. Int. J. Climatol. 18: 1557-1574.

Farquhar G., Ehleringer J., Hubick K. 1989. Carbon isotope discrimination and photosynthesis Annu. Rev. Plant Physiol. Plant Mol. Biol. 40:503-537.

Fay P.A., Carlisle J.D., Knapp A.K., Blair J.M., Collins S.L. 2000. Altering rainfall timing and quantity in a mesic grassland ecosystem: design and performance of rainfall manipulation shelters. Ecosystems 3, 308-319.

Francey R., Farquhar G.1982. An explanation of 13C/12C variations in tree rings. Nature 297, 28-31.

Fritts H. 1976. Tree Ring and Climate, Academic Press Inc, London, 567p.

Fritts H. 1991.Reconstructing Large-scale Climatic Patterns from Tree-Ring Data, The University of Arizona Press, Tucson, USA, 286p.

Fritts H.C. 1998. Factors preconditioning growth with Kalman filter: an empirical model of the tree-ring response to monthly variations in climate. Laboratory of tree-ring research, University of Arizona, Tucson, USA.

Fritts, H.C. 1999. PRECON version 5.17, http://www.arizona.edu/webhome/hal/dlprecon.html.

Fritts H.C., Swetnam T. 1989. Dendroecology: a tool for evaluating variations in past and present forest environments. Adv. Ecol. Res. 19: 111-188.

Gagen M., McCarroll D., Edouard J. 2004. Latewood Width, Maximum Density, and Stable Carbon Isotope Ratios of Pine as Climate Indicators in a Dry Subalpine Environment, French Alps. Arctic, Antarctic, and Alpine Research 36(2) 166–171.

Goldberg D., Novoplansky A. 1997. On the relative importance of competition in unproductive environments. J. Ecol 85, 409-418.

Grissino-Mayer H.D. 2001. Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. Tree-Ring Res. 57: 205–221.

Hair J. E., Anderson R.E., Tatham R. L., Black, W.C. 1998. Multivariate data analysis. 5th ed. Prentice Hall. Upper Saddle River, NJ, USA.

Harrison P., Berry P., Butt N., New M. 2006. Modelling climate change impacts on species' distributions at the European scale: implications for conservation policy. Environ. Sci. Policy 9: 116-128.

Holmes R.L. 1983. Computer-assisted quality control in tree-ring dating and measurement. Tree-ring Bull. 43, 69-78.

Holmes R.L 2001. Dendrochronology Program Library. Available from the Laboratory of Tree-Ring Research, University of Arizona, Tucson, USA.

Hughes M. 2002. Dendrochronology in climatology – the state of the art. Dendrochronologia 20: 95-116.

IPCC 2007. Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jones P.D., Jónsson T., Wheeler D. 1997. Extension to the North Atlantic Oscillation using early instrumental pressure observations from Gibraltar and South-West Iceland. Int. J. Climat.17: 1433-1450.

Jones P.D., Briffa K.R., Barnett T.P., Tett S.F.B. 1998. High-resolution palaeoclimatic records for the last millennium: interpretation, integration and comparison with General Circulation Model control-run temperatures. The Holocene 8: 455-471.

Kaennel M., Scheweingruber F.H. 1995. Multilingual Glossary of Dendrochronology. Terms and Definitions in English, German, French, Spanish Italian, Portuguese, and Russian. Birmensdorf, Swiss Federal Institute for Forest, Snow and Landscape Research. Berne, Stuttgart, Vienna, Haupt. 467 pp.

Knapp A.K., Fay P.A., Blair J.M., Collins S.L., Smith M.D., Carlisle J.D., Harper C.W., Danner B.T., Lett M.S., Mc Carron J.K. 2002. Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. Science 298: 2202-2205.

Kozlowski T.T., Kramer P.J., Pallardy S.G. 1991. The physiological ecology of woody plants, Academic Press, New York, EUA.

Leavitt S. 1993. Seasonal C13/C12 changes in tree-ring species and site coherence, and a possible drought influence. Can. J. For. Res.23: 210:218.

Leavitt S., Long A. 1984. Sampling strategy for stable carbon isotope analysis of tree-rings in pine. Nature 311:145-147.

Leavitt S., Long A. 1986. Stable carbon isotope variability in tree foliage and wood. Ecology 67 (4) 1002-1010.

Loader N.J., Robertson I., McCarroll D. 2003. Comparison of stable carbon isotope ratios in the whole wood cellulose and lignin of oak tree-rings. Palaeogeography, Palaeoclimatology, Palaeoecology, 196: 395–407.

Martínez-Vilalta J., Piñol, J. 2002. Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. For. Ecol. Manage.161: 247-256.

Masiokas M., Villalba R. 2004. Climatic significance of intra-annual bands in the wood of Nothofagus pumilio in southern Patagonia. Trees 18: 698-704.

McCarroll D., Pawellek F. 2001.Stable carbon isotope ratios of *Pinus sylvestris* from northern Finland and the potential for extracting a climate signal from long Fennoscandian chronologies. The Holocene 11:(5) 517-526.

McCarroll D., Loaded N. 2004. Stable isotopes in tree rings. Quaternary Science Reviews 23: 771–801.

Mooney C.Z., Duval R.D. 1993. Bootstrapping: a nonparametric approach to statistical inference. Sage University Paper series on Quantitative Applications in the Social Sciences, 07-095. Sage, Newbury Park.

Nicolas A.; Gandullo J. 1967. Ecología de los pinares españoles. 1, *P. pinaster* Ait. Ministerio de Agricultura, Madrid, España, 311p.

Noy-Meir I. 1973. Desert ecosystems: environment and producers. Annu. Rev. Ecol. Syst. 4: 25-41.

Parry M. 2000. Assessment of potential effects and adaptations for Climate change in Europe: The Europa ACACIA Projet. Jackson Environments Institute, University of East Anglia, Norwich, UK.

Ogaya R., Peñuelas J. 2007. Tree growth, mortality, and above-ground biomass accumulation in a holm oak forest under a five-year experimental field drought. Plant Ecol. 189: 291–299.

Peñuelas J., Lloret F., Montoya R. 2001. Severe drought effects on Mediterranean Woody Flora in Spain. For. Sci. 47(2): 214-218.

Peñuelas J., Fillela I., Comas P. 2002 Change plant and animal life cycles from 1952 to 2000 in the Mediterranean region. Glob. Change Biol. 8: 531-544.

Regent Instrument Inc. 2002. Windendro TM v.2002a. Québec, Qc.

Richter K., Eckstein D., Holmes R.L. 1991. The dendrochronological signal of pine trees (Pinus spp.) in Spain. Tree-Ring Bull. 51: 1-13.

Rigling A., Waldner P., Forster T., Bräker O., Pouttu A. 2001. Ecological interpretation of tree-ring width and intraannual density fluctuations in *Pinus sylvestris* on dry sites in the central Alps and Siberia. Can.J.For.Res. 31(1): 18-31.

Rigling A., Bräker O., Schneiter G., Schweingruber F. 2002. Intra-annual tree-ring parameters indicating differences in drought stress of *Pinus sylvestris* forests within the Erico-Pinion in the Valais (Switzerland). Plant ecology 163: 105-121.

Rodrigo F., Esteban-Parra M., Pozo-Vázquez D., Castro-Diéz Y. 2000. Rainfall variability in southern Spain on decadal to centennial time scales. Int. J. Climatol. 20: 221-732.

SAS Institute Inc. 2004. SAS/STAT versión 9.1, User's Guide. Cary, NC, USA.

Schleser G.H., Frielingsdorf J., and Blair A. 1999a. Carbon isotope behaviour in wood and cellulose during artificial aging. Chem. Geol. 158: 121-130.

Schleser, G.H., Helle, G., Lücke, A. and Vos, H. 1999b. Isotope signals as climate proxies: the role of transfer functions in the study of terrestrial archives. Quat. Sci. Rev. 18: 927-943.

Schweingruber F.H. 1988. Tree rings. Basics and applications of dendrochronology. Kluwer Dordrecht.

Schweingruber F.H. 1993. Trees and wood in Dendrochronology, Springer series in Wood Science, Springer-Verlag, 474 p.

Schweingruber F.H. 1996. Tree rings and environment: Dendroecology, Berne, Sttutgart,Vienna:Paul Haupt Publisher, 602p.

Schwinning S., Sala O., Loik M., Ehleringer J. 2004. Thresholds, memory, and seasonality: understanding pulse dynamics in arid/semi-arid ecosystems. Oecologia 141: 191-193.

Sokal R.R., Rohlf F.J. 1995. Biometry: the principles and practice of statistics in biological research, 3rd edition, WH Freeman and Co., New York, UEA, 358p.

Sternberg L., DeNiro J. 1983. Isotopic composition of cellulose from C3, C4 and CAM plants growing near one another. Science 220: 947-949.

Stokes M., Smiley T. 1968. An Introduction to Tree-Ring Dating, University of Arizona Press, Tucson, UEA, 120 p.

Tans P.,DE Jong A., Mook W. 1978. Chemical pre-treatment and radial flow of 14C in tree rings. Nature 271: 234-235.

Tessier L., Guibal F., Schweingruber F. 1997. Research strategies in dendroecology and dendroclimatology in mountain environments. Clim. Change 36: 499-517.

Thornthwaite C.W. 1948. An approach toward a rational classification of climate. Geogr. Rev. 38: 55-94.

Villalba R., Veblen T. 1996. A tree-ring record of dry spring-wet summer events in the forest-steppe ecotone, northern Patagonia, Argentina. In Proceeding of International Conference: Tree rings, environment and humanity, 17-21 May 1994, Tucson, Arizona. Edited by Dean, JS, Meko, DM, Swetnam, TW. Department of Geosciences, University of Arizona, Tucson, EUA. Pp. 107–116.

Warren C., McGrath J., Adams M. 2001. Water availability and carbon isotope discrimination in conifers. Oecologia 127 :476–486.

Wigley T.M.L., Briffa K.R., Jones P.D. 1984. On the average value of correlated time series, with applications in dendroclimatology and hydrometeorology. J. Clim. Appl. Met. 23: 201-213.

Yen H.; Wensel L. 2000. The relationship between tree diameter growth and climate for coniferous species in northern California. Can. J. For. Res. 30, 1463-1471.

LIST OF ORIGINAL ARTICLES

Bogino S., Bravo F. 2008. Growth response of *Pinus pinaster* Ait. to climatic variables in central Spanish forests. Annals of Forest Sciences 65: 506-518.

Bogino S., Bravo F. 2007. SOI and NAO impacts on *Pinus pinaster* Ait. growth in Spanish forests. In Proceeding of the Dendrosymposium 2007. May 3rd – 6th 2007, Riga, Latvia. Scientific Technical Report STR08/05. Edited by Didzis E., Brumelis G., Gärtner H., Helle G., Schleser G. Pp. 21–26.

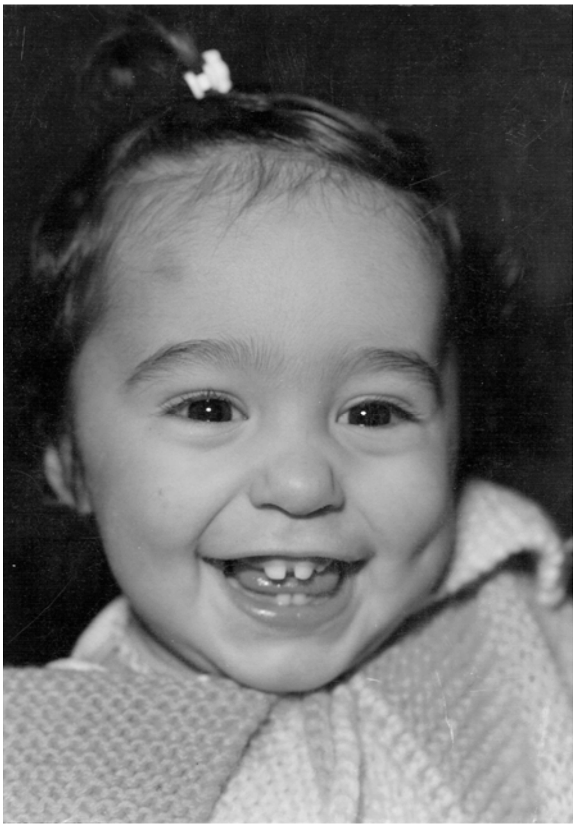
Bogino S., Fernández Nieto M., Bravo F. Drought and climate effect on radial growth of *Pinus sylvestris* L. in its southern and western distribution threshold. Journal of Vegetation Sciences. Submitted.

Bogino S., Bravo F. Climate and intra-annual density fluctuations in *Pinus pinaster* in Spanish woodlands. Canadian Journal of Forest Research. Submitted.

Bogino S., Bravo F. Stable carbon isotope ^{13}C in *Pinus pinaster* and *Pinus sylvestris* tree rings: climatic signals and water use efficiency in Mediterranean environments. Annals of Forest Sciences. Submitted.

I

This article was accepted to be published in Annals of Forest Sciences; Vol. 65 (2008), Bogino, S., Bravo, F. Growth response of Pinus pinaster Ait. to climatic variables in central Spanish forest, pages 506-518, © INRA, EDP Sciences, 2008, and is reprinted with kind permission of INRA, EDPSciences.



Growth response of *Pinus pinaster* Ait. to climatic variables in central Spanish forests

RESUMEN

Se estudió el efecto de las variables climáticas (temperatura y precipitación) sobre el crecimiento radial del pino marítimo (*Pinus pinaster* Ait.) usando técnicas dendrocronológicas. Se construyeron diez cronologías de ancho de anillo de crecimiento a partir de material de sitios de muestreo en el área central de distribución de esta especie en la Península Ibérica. La variabilidad de las cronologías se analizó usando el análisis de componentes principales (ACP) para el período 1952-2005. El primer componente principal (PC1) explicó el 56% de la variabilidad del crecimiento. La relación entre el ancho de los anillos y el clima se analizó en escalas regional y local usando el coeficiente de correlación de Pearson y la función de respuesta de remuestreo *bootstrapped*. El crecimiento radial en ambas escalas se correlacionó positivamente con la precipitación durante y antes de la estación de crecimiento en todos los sitios, y con la lluvia de verano antes de la estación de crecimiento en cinco sitios. El efecto de la temperatura media varió de acuerdo con la altitud de los sitios de muestreo, desde insignificante en los sitios más altos a positivamente significante en invierno en los sitios más bajos. La temperatura en la estación de crecimiento también tuvo un efecto negativo. El filtro de Kalman se aplicó para estimar la asociación cambiante entre el crecimiento y el clima a través del tiempo. Los resultados sugieren un cambio, de no-significativa a significativa (*p <0,05), en la relación del clima con el crecimiento, que se inició en la década del ochenta en seis de los sitios estudiados. A partir de estos resultados es posible concluir que *P. pinaster* es una especie apta para el análisis de la relación del crecimiento con el clima y para estudiar el comportamiento de los bosques en un contexto de cambio climático.

Ann. For. Sci. 65 (2008) 506
© INRA, EDP Sciences, 2008
DOI: 10.1051/forest:2008025

Original article

Growth response of *Pinus pinaster* Ait. to climatic variables in central Spanish forests

Stella M. Bogino[1,2]*, Felipe Bravo[1]

[1] Departamento de Producción Vegetal y Recursos Forestales, Universidad de Valladolid, Avda. de Madrid 44, 34004 Palencia, Spain
[2] Departamento de Ciencias Agropecuarias. Facultad de Ingeniería y Ciencias Económico-Sociales, Universidad Nacional de San Luis,
Avda. 25 de Mayo 384, 5730 Villa Mercedes, San Luis, Argentina

(Received 29 January 2008; accepted 2 April 2008)

Abstract –
- The effect of climatic variables (temperature and precipitation) on radial growth of the Mediterranean Maritime pine (*Pinus pinaster* Ait.) was studied using dendrochronological techniques in the Iberian Peninsula.
- Ten tree-ring width chronologies, along the central distribution area of the species, were built. Chronology variability was analysed using Principal Component Analysis (PCA) for the period 1952–2005.
- The first principal component (PC1) explained 56% of tree-growth variability. Tree-growth association with climate was analysed at regional and local scales using correlation coefficient and bootstrapped response functions.
- Radial growth at both scales was positively correlated with rainfall during and prior to the growing season at all sites, and with summer rainfall before the growing season at five sites. Mean temperature effect changed according to the sampling site, from non-significant at the highest sites to significant (positive relationship in winter) at the lowest sites. Growth season temperature also had a negative effect.
- The Kalman filter was applied to estimate changing association between growth and climate over-time. Results suggested a change in association, initiated in the 80s, from non-significant to significant ($^{*}p < 0.05$) at six of the sampling sites.
- *Pinus pinaster* is an accurate species for analysing tree-growth association with climate and for studying plant behaviour under global change conditions.

dendroclimatology / Mediterranean Maritime pine / tree-ring / response function / Kalman filter

Résumé – L'influence des variables climatiques sur la croissance radiale de *Pinus pinaster* Ait. dans les forêts de l' Espagne centrale.
- L'influence des variables climatiques (températures et précipitations) sur la croissance radiale du pin maritime (*Pinus pinaster* Ait.) a été étudiée en utilisant des techniques dendrochronologiques dans la péninsule Ibérique.
- Dix chronologies de largeur de cerne ont été établies tout au long de la zone centrale de distribution de l'espèce. La variabilité des chronologies a été étudiée par une Analyse en Composantes Principales (PCA) pour la période 1952–2005.
- La première composante principale (PC1) a expliqué 56 % de la variabilité de la croissance des arbres. L'association entre la croissance des arbres et le climat a été analysée à l'échelle locale et régionale en utilisant un coefficient de corrélation et une méthode de rééchantillonnage.
- La croissance radiale à ces deux échelles a montré une corrélation positive avec les précipitations survenues pendant et avant la saison de croissance dans tous les sites et avec les précipitations estivales survenues avant la saison de croissance dans cinq sites. L'effet de la température moyenne a varié en fonction de l'altitude des sites, en étant non significatif dans les sites les plus élevés et positivement significatif en hiver dans les sites les plus bas. La température de la saison de croissance a également eu un effet négatif.
- On a appliqué le filtre de Kalman pour estimer les variations temporelles de l'association variable entre la croissance et le climat. Les résultats ont suggéré que cette association est passée, dans les années 80, de non significative à significative ($^{*}p < 0,05$) dans six sites d'échantillonnage.
- *Pinus pinaster* est une espèce adéquate pour analyser l'association entre la croissance des arbres et le climat, et pour étudier le comportement de l'espèce dans des conditions de changement global.

dendrochronologie / pin maritime méditerranéen / cerne / fonction de réponse / filtre de Kalman

1. INTRODUCTION

Understanding the growth and productivity response to climate change is a key issue in forest modelling and forestry. It is also crucial in enhancing past climate reconstructions made using dendrochronological techniques. Tree ring growth is influenced by several simultaneous environmental factors: solar radiation, temperature, water precipitation, soil nutrient content, etc. Depending on conditions and species, one or more of these factors can become limiting for tree growth (Fritts, 1976). Climate variability often explains past tree growth (Hughes, 2002), helping in the projection of future growth responses in the context of climate change (Yeh and Wensel, 2000).

---

* Corresponding author: sbogino@fices.unsl.edu.ar

Table I. Ecoregion classification, geographical position and mean basal area of ten *Pinus pinaster* sampling sites across its natural distribution area in the Iberian Peninsula.

Site	Code	Ecoregion	Latitude	Longitude	Altitude (m)	BA ($m^2.ha^{-1}$)
P42201	2a	Duriense	41° 33' 43"	02° 55' 17"	1012	53.84
P42002	2b	Duriense	41° 34' 03"	02° 35' 51"	1059	34.84
P16201	3a	Catalano-Aragonesa	39° 50' 06"	01° 16' 37"	1078	45.83
P44002	3b	Catalano-Aragonesa	40° 19' 07"	01° 21' 18"	1437	40.17
P44005	3c	Catalano-Aragonesa	40° 20' 47"	01° 21' 54"	1364	45.73
P44204	3d	Catalano-Aragonesa	40° 20' 01"	01° 21' 26"	1232	34.33
P16008	4a	Litoral-Mediterránea	39° 48' 56"	01° 15' 36"	920	51.44
P16106	4b	Litoral-Mediterránea	39° 50' 17"	01° 16' 11"	970	36.66
P16202	6a	Manchega	39° 49' 48"	01° 17' 38"	1135	41.69
P16208	6b	Manchega	39° 50' 28"	01° 17' 54"	1090	34.74

Tree-ring widths of conifers offer some of the best climatological reconstructions of semiarid regions (Schweingruber, 1996), as in the case of annual precipitation estimates in Mediterranean climates (Tessier et al., 1997). On the other hand, different climatic variables (mainly derived from precipitation and temperatures but also derived from atmospheric pressure balance) can be used to express climatic oscillation and to derive relationships between tree growth parameters and climatic signal (D'Arrigo and Jacoby, 1992; Jones et al., 1997).

The Mediterranean climate is characterised by summer drought and high inter-annual variability of precipitation and temperature. Projections of climate change impact on the European distribution of higher plants in 2050 suggest that the Iberian Peninsula could be one of the most vulnerable areas for species lost (Bakkenes et al., 2002). Climate shifts have already taken place in the region, as exemplified by the reduction of rainy days by 50 and 30% over the southern coast of Spain and the Pyrenees in the last century, respectively (Esteban-Parra et al., 1998; Rodrigo et al., 2000). The mean annual temperature has increased about 1.6 °C in the Iberian Peninsula over the last century (IPCC, 2007; Parry, 2000).

The Mediterranean Maritime pine (*Pinus pinaster* Ait.) is a characteristic species in Mediterranean forests, with its main populations located in the Iberian Peninsula (Blanco et al., 1997). This species shows a wide ecological range (Nicolas and Gandullo, 1967), being able to 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 ones of the Atlantic coast in the western Iberian Peninsula (Alía et al., 1996; Blanco et al., 1997).

In Spain, *Pinus pinaster* constitutes pure and mixed woodlands that are both natural (1 million ha) and planted (0.6 million ha) (DGCN, 1998; 2002). These woodlands are mainly spread over the northern Spanish plateau up to 1500 m. In this area, the climate is characterised by irregular rainfall, between and within years, and high temperatures in summer. The soils are sandy and rocky and very well drained. Even though *Pinus pinaster* pine had been used widely on plantations in the Iberian Peninsula, this species grows naturally in some areas, where it is the best adapted woody species (Blanco et al., 1997). Fossil *Pinus 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 (Di Castri, 1981). Authors suggest that the Iberian System is the natural origin and centre of distribution for this species (Blanco et al., 1997).

Pinus pinaster has rarely been used for dendrochronology and dendroclimatology studies because stands older than 100 years are very difficult to find. In addition, trees from the lower mountain level often have false rings and most trees have been damaged by resin harvest in the past (Schweingruber, 1993).

The relationship between growth indices and climatic variables is essential for predicting the future growth trend of *Pinus pinaster*. Given the high vulnerability of Iberian plant communities to climate change and the importance of *Pinus pinaster* forests in this region, understanding the growth response of this species to past climate variability can help us to explore how its populations, and the ecosystems they dominate, will behave in the dramatic climatic shifts expected in future. The objectives of this paper are to analyse the relationship between *Pinus pinaster* tree ring width and climatic variables (precipitation and temperature) at the (a) regional and (b) local scales, and (c) to explore possible shifts in the association between these climate variables and tree growth over time as a result of changing environmental conditions.

2. MATERIALS AND METHODS

2.1. Study sites

Ten sampling sites were selected from four different bioclimatological ecoregions where *Pinus pinaster* grows in the Iberian Peninsula. Each ecoregion has common physiographic, climatic and lithological characteristics (Elena Roselló et al., 1997) (Tab. I and Fig. 1).

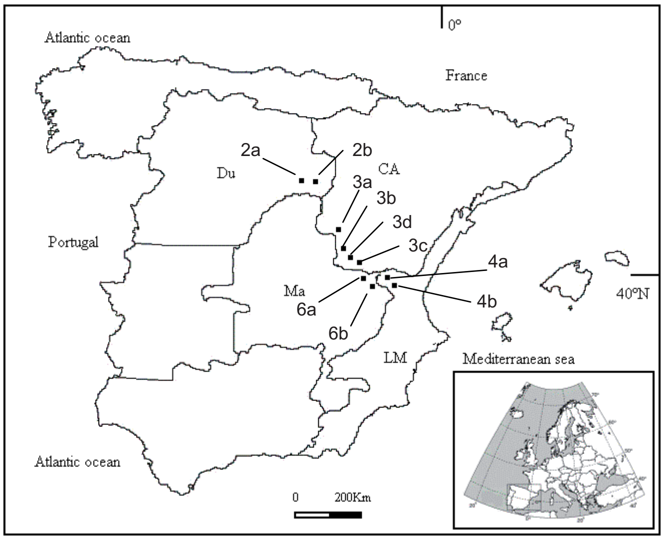


Figure 1. Sampling sites set up across the natural distribution area of *Pinus pinaster* in the Iberian Peninsula. Du: *Duriense*; CA: *Catalano-Aragonesa*; Ma: *Manchega* and LM: *Litoral-Mediterránea* bioclimatological ecoregions (Elena Roselló et al., 1997).

Table II. Meteorological station data from the *Agencia Estatal de Meteorología* (Spain) used in this study. Rainfall: Annual precipitation; Temp.: Mean monthly temperature; Period: Time with data available. Ecoregion: CA: *Catalano-Aragonesa*; LM: *Litoral-Mediterránea;* Ma: *Manchega* and Du: *Duriense* bioclimatological ecoregions.

Meterological station	Latitude	Longitude	Altitude (m)	Rainfall (mm)	Temp. (°C)	Period	Ecoregion
El Burgo de Osma	41° 35' 10"	03° 04' 02"	895	529.5	10.54	1932–2005	CA
Cuenca	40° 04' 00"	02° 08' 17"	956	541.42	12.43	1956–2005	LM
Pantano de la Toba	40° 13' 19"	01° 55' 33"	1154	764.68	10.22	1944–2005	Ma
Soria (Observatorio)	41° 46' 00"	02° 28' 00"	1082	418.34	10.66	1944–2005	Du

2.2. Field work and laboratory methods

In the summer of 2006, two cores were extracted at 1.30 m above ground level from fifteen dominant and co-dominant trees at each sampling site. Tapped trees were avoided because their growth was likely to have been affected by resin extraction, confounding possible climatic signals. Cores were glommed on channelled wood, dried for two weeks and polished with progressively thinner sandpaper. Tree-rings were dated to establish correctly the calendar year in which a tree-ring was formed. Dating was achieved using a binocular microscope following standard dendrochronological techniques (Cook and Kairiukstis, 1990; Fritts, 1976; Stokes and Smiley, 1968;). The cores in transverse sections were scanned at high resolution (2 000 dpi) with an Epson Expression 1640 XL scanner with 0.01-mm accuracy, and rings were measured using the WinDENDRO program (Regent Instruments Inc., 2002).

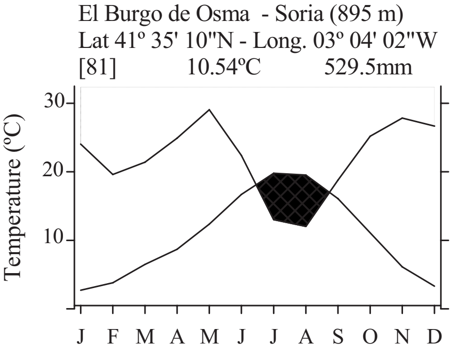


Figure 2. Climate diagram of *El Burgo de Osma* meteorological station (1932–2005).

2.3. Climatic data

Monthly precipitation and mean monthly temperature, provided by the *Agencia Estatal de Meteorología* (Spain), were selected to assess the climate-growth relationship. Meteorological data from four meteorological stations placed within 30 km of the sampling sites have been used (Tab. II). The climate-diagram of the *El Burgo de Osma* meteorological station is provided as an example (Fig. 2). The data recorded cover a period of at least 50 years. The data from the four meteorological stations were averaged to obtain a regional climatic record to be applied in the regional dendroclimatic analysis. The HOM component (Homogeneity of Meteorological Data) of the Directory Program Library for Dendrochronology

(Holmes, 1983, available at www.ltrr.arizona.edu) was used to determine the homogeneity of the climatic variables.

2.4. Statistical analysis

The v6.06P COFECHA program (Grissino-Mayer, 2001; Holmes, 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.

To eliminate biological trends in tree-ring series and to minimize growth variations that are not shared by most trees (Fritts, 1976), the v6.05P ARSTAN program (Cook and Holmes, 1984; Holmes, 2001) (available at www.ltrr.arizona.edu) was used. Standardisation 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, the standardised series were averaged. These temporal series or master chronologies expressed the annual variations in *Pinus pinaster* radial growth at the population level in each sampling site.

Chronology quality was evaluated using mean sensitivity (MS), which is the degree by which one or more casual factors are reflected by a tree-ring series (Schweingruber, 1996); 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 expressed population signal (EPS), which describes how a finite sample estimates the hypothetical infinite population (Briffa, 1995; Wigley et al., 1984). Chronology was considered confident with an EPS value higher than 0.85. The common variance between all residual chronologies was analysed using a Principal Component Analysis for the common growth period 1952-2005 (Sokal and Rohlf, 1995) using Infostat V.2 (Di Rienzo et al., 2002). The variance explained by the first principal component (PC1) was used as an indicator of the similarity among the chronologies. PC1 was used as a regional chronology of *Pinus pinaster* in central Spain.

To determine the climatic variables that control *Pinus pinaster* radial growth, mean monthly temperature and monthly rainfall were compared with the regional chronology (PC1) and the local chronologies at each sampling site. PC1 was related with the average of the meteorological station data. Each site chronology was compared with the meteorological station closest to the analysed site. The period explored was from the previous June to September of the current growth year. The v 5.17 PRECON program (Fritts, 1999) (available at www.ltrr.arizona.edu) was used to compute the response functions of tree growth to climate by means of a multiple stepwise regression. Coefficients were considered significant at $^{*}p < 0.05$ and $^{**}p < 0.01$. A bootstrapped analysis was also applied to improve the robustness of the correlation coefficients. In this analysis, 1000 bootstrap interactions were made. The Kalman filter, which estimates regression models by time-varying coefficients, was applied to determine if climatic variable effect changed over time (Fritts, 1998). The output of this analysis included predicted tree-ring indexes and the confidence interval (95% level); therefore, when the interval did not include a zero value, the regression coefficient was considered significant (Kalman filter, V5.17 PRECON program).

3. RESULTS

3.1. Chronology description

Chronology 6a was not included in the analysis because its time span was only 30 years and it would have limited the period analysed of all series. The nine chronologies of *Pinus pinaster* growing in central Spain are shown in Figure 3. The chronologies had high SNR, EPS and percentage of the variance accounted for the first eigenvector; this suggests that they reflect a strong common signal, presumably related to climatic factors. The descriptive statistics showed that mean sensitivity varied from 0.1857 to 0.3179 and standard deviation varied from 0.1650 to 0.3108, depending on the sampling site. SNR varied from 27.615 to 68.444 and EPS values varied from 0.958 to 0.986 (Tab. III). All chronologies analysed have high SNR (values always over 22) and EPS (always over 0.95), while the variance accounted by the first eigenvector is over 48%; these findings suggest a strong common signal related to environmental climatic factors (Tab. III). Despite the diversity of tree ages and the difference in bioecological regions, a clear common growth signal expressed by the first principal component (PC1) of the PCA was found. PC1 explained 56% of the total variance of the nine residual chronologies. All the residual chronologies had a positive correlation with PC1, indicating they shared a common variance (Tab. IV and Fig. 4).

3.2. Climate growth association: multiple stepwise and bootstrapped analysis

Radial growth, at both regional and local scales, was strongly associated with climatic variables, especially rainfall during and prior to the growing season in all the sites analysed. Mean temperature effect changed according to the sampling site, from non-significant at the highest sites to significant (positive relationship in winter) at the lowest sites.

As a result of the PCA analysis, PC1 was considered indicative of the inter-annual high-frequency variation of tree-ring growth at regional level for the common period 1952–2005. This chronology was correlated with regional climatic variables, precipitation and temperature, and the results showed that more than 60.8% of the total variance in radial growth was explained by climate (Fig. 5).

The total variance explained by both climatic variables (monthly rainfall and mean monthly temperature) in the *Duriense* ecoregion varied from 42 to 50%. Both chronologies

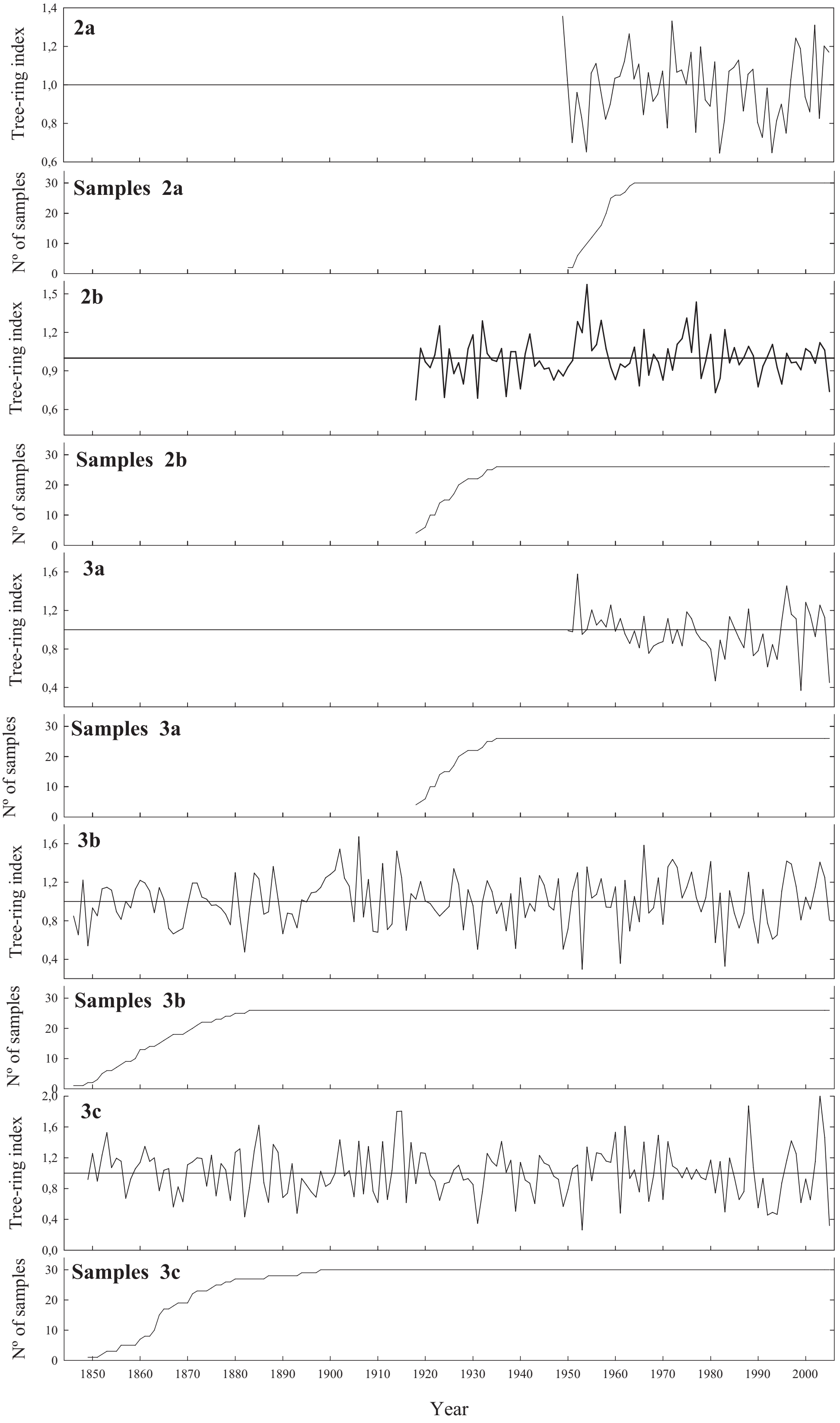


Figure 3. Standardized chronologies of *Pinus pinaster* along the natural distribution area in Central Spain. The upper part of each figure show the tree-ring indexes through time and the bottom part the number of samples used in each chronology.

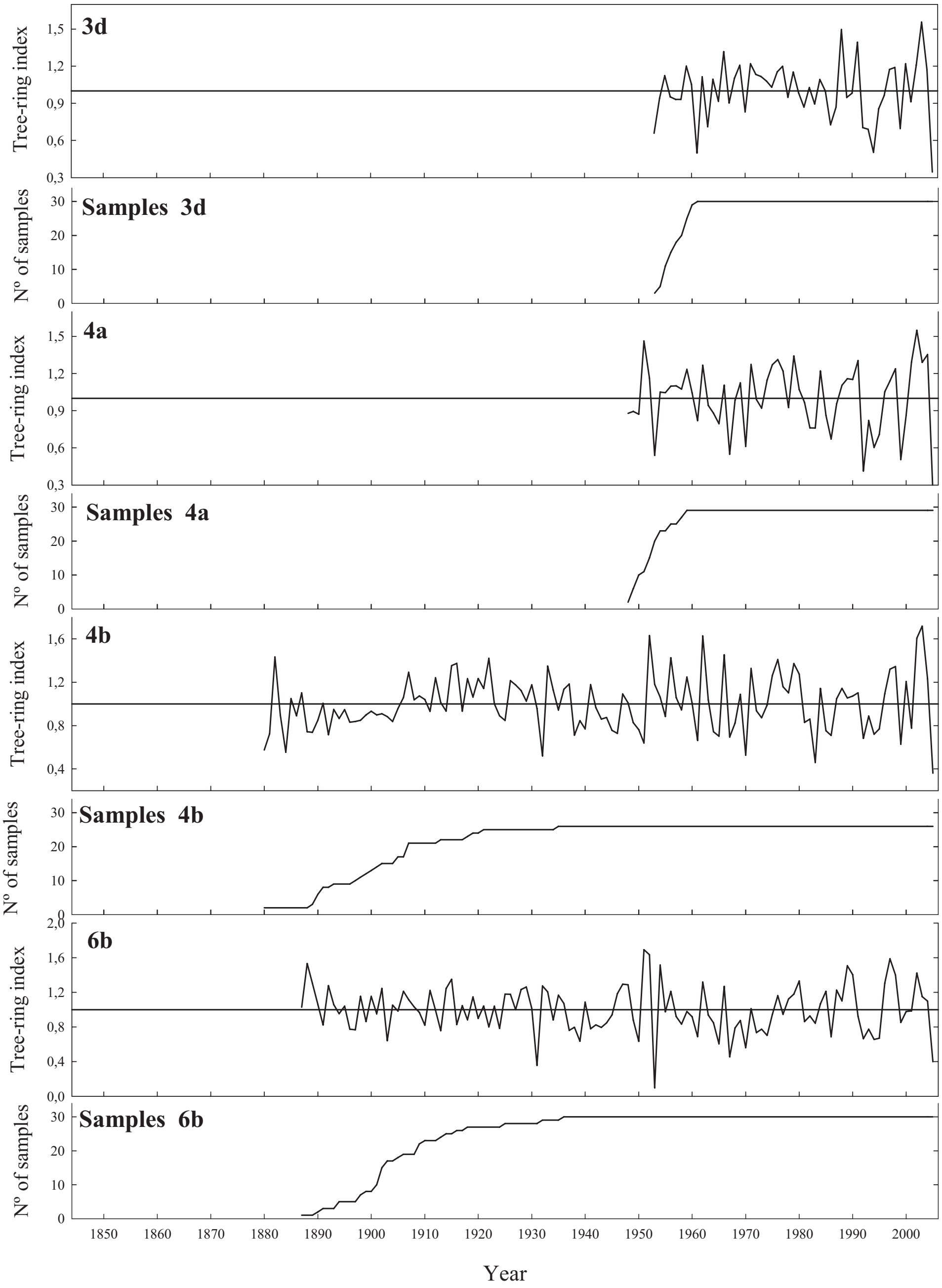


Figure 3. Continued.

were the only ones among all the sites analysed that showed a negative correlation with autumn temperature prior to the growing season; in addition, temperature had a negative effect on growth in later springtime and early summer at both sampling sites (June and July). The impact of rainfall during the growing season was shared among all the sampling sites and, in this ecoregion, it varied from May to June (Site 2a) and from April to June (Site 2b) (Figs. 6, 2a, 2b).

The four chronologies belonging to the *Catalano-Aragonesa* ecoregion showed a clear association with rainfall in August prior to the growing season and during the growing season at all the sampling sites. Sites 3a and 3c also showed a positive association with winter rainfall previous to the growing season. Site 3a was placed at lower altitude (> 200 m.) than the other sites analysed and it was the only one that showed a positive association with temperature in winter (February). This site also had a different behaviour compared with the other sampling sites, showing a negative association with summer temperature (Figs. 6, 3a–3d).

Both chronologies belonging to the *Litoral-Mediterránea* ecoregion also showed a clear association with rainfall in springtime (April and May at Site 4b), in springtime and

Table III. Descriptive statistics of the nine *Pinus pinaster* chronologies in central Spain. SD: standard deviation; MS: mean sensibility; SNR: signal to noise ratio; EPS: expressed population signal; Var.: variance in first eigenvector; and Mean Corr.: mean correlation among trees.

	2a	2b	3a	3b	3c	3d	4a	4b	6b
Time span	1946–2005	1916–2005	1947–2005	1844–2005	1847–2005	1952–2005	1947–2005	1879–2005	1886–2005
Core number	30	27	30	26	29	30	29	26	30
Ring number	1635	2228	1487	3757	4128	1477	1579	2723	3043
Age range	44–60	68–90	42–59	131–156	124–158	45–54	48–59	72–127	72–120
SD	0.172	0.165	0.310	0.258	0.317	0.237	0.271	0.255	0.2764
MS	0.206	0.185	0.278	0.299	0.370	0.268	0.292	0.257	0.297
SNR	27.615	22.60	52.302	29.087	68.444	52.118	60.636	38.528	36.254
EPS	0.965	0.958	0.981	0.967	0.986	0.981	0.984	0.975	0.973
Var.	51.84	48.94	67.78	54.85	71.41	65.88	70.18	62.78	59.62
Mean corr.	0.488	0.466	0.635	0.528	0.702	0.642	0.684	0.606	0.573

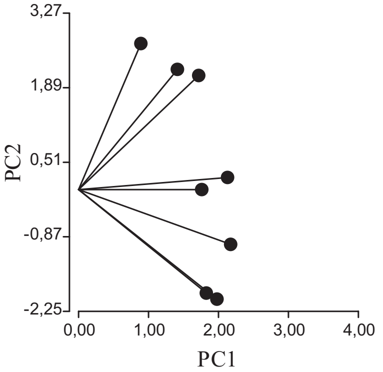


Figure 4. First against second eigenvector loading of the principal component analysis on all *Pinus pinaster* residual chronologies in central Spain for the common period 1952–2005.

summer in the growing season (March, May and August at Site 4a) and with August rainfall prior to the growing season at both sites. Site 4b also showed a positive association with temperature in winter, and high temperature during the growing season (May) may limit growth at Site 4a. Both sampling sites shared a similar variability explained by both variables, which varied between 63 and 64% (Figs. 6, 4a, 4b).

Finally, Site 6b (belonging to the *Manchega* ecoregion) showed a positive correlation not only with rainfall in May and December, but also with temperature in winter (Fig. 6, 6b).

3.3. Climate growth association over time: the Kalman filter technique

Results suggested a changing association between growth and climatic variables from non-significant to significant ($^{*}p < 0.05$) at six of the sampling sites. Table V show a summary of the Kalman filter analysis results that includes all the sampling sites and the climatic variables studied.

The change in association between growth and climatic conditions took place during the 80s. During this period, precipitation changed from non-significant to positively significant at four sampling sites. This change occurred in winter prior to the growing season (December and January, Site 3a) and at the beginning of the growing season (March, Site 4a or April and May, Sites 3c and 3d). Temperature also showed a changing association with growth at two sampling sites (4a and 6b): temperature became negatively significant on radial growth in springtime (May) at Site 4a. Finally, a positive change was found at Site 6b in relation to winter temperature (February). As an example, Figure 7 shows the changing association between radial growth and climatic variables at Site 4a.

Table IV. Pearson correlation coefficient between the first principal component of the principal component analysis and the residual chronologies of *Pinus pinaster* ($^{**}p < 0.01$ and $^{***}p < 0.001$).

Chronology	PC1
2a	0.65^{***}
2b	0.39^{**}
3a	0.60^{***}
3b	0.78^{***}
3c	0.85^{***}
3d	0.94^{***}
4a	0.92^{***}
4b	0.74^{***}
6b	0.76^{***}

4. DISCUSSION

*Pinus pinaste*r is a reliable species for dendrochronological studies, showing good correlation between trees growing at the same sampling site, high signal related to total noise and accurate statistical values that mean clear response to environmental factors. This species also shows accurate performance in studying the association between tree growth and global change showed by a changing relationship with climatic variables over time.

The statistics that characterised the chronologies suggest that the tree-ring series accurately reflects one or more causal factors (including climate), shown by the mean sensitivity values (MS) that are similar to the 0.16 to 0.34 values found in previous studies on *Pinus sylvestris* L.*,* *Pinus nigra* Arnold,

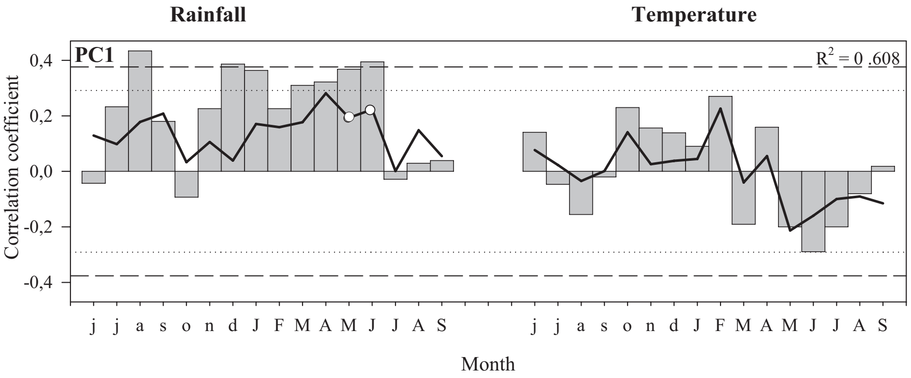


Figure 5. Correlation coefficients (bars) and bootstrapped response function (lines) that indicate the effect of regional climatic variables and growth of *Pinus pinaster* during the 1960–2005 period. The period analysed covers from June of the year prior to growth to September of the current growing year. Bars outside dashed lines show significant coefficients at $^{**}p < 0.01$. Bars outside dotted lines show significant coefficients at $^{*}p < 0.05$. White circles indicate months where the bootstrapped response function coefficients are significant at $p < 0.05$. R^2 values show the total variance explained by both variables. Lower case letters indicate months prior to the growing year. Upper case letters indicate the growing year months.

Pinus pinaster Ait. and *Pinus mugo* ssp. *uncinata* Turra. growing in Spain (Richter et al., 1991). Signal-to-noise ratio (SNR) values that vary from 22.6 to 66.44 suggest that the proportion of the explainable variation due to climate or other causal factors divided by the unexplainable variation or residual is sufficiently high for all the sampling sites. Expressed population signal (EPS), ranging from 0.958 to 0.986, is accurate enough (> 0.85) for these studies (Wigley et al., 1984); this suggests that these chronologies describe the infinite, hypothetical population of *Pinus pinaster* at each sampling site well enough. First eigenvector variance ranges from 48.94 to 70.18%, indicating good homogeneity within the same site. Summing up, the nine 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.

A common growth pattern among all series has been detected. PCA analysis suggests a clear strong common variance among all the sampling sites (explaining over 56% of variability) and a positive correlation ($^{**}p < 0.01$ and $^{***}p < 0.001$) with the PC1 axis. Although the sampling sites included trees of different ages and trees from different ecoregions, all the series can be considered to share a common variance related to causal factors. These results coincide with a previous study in Spain which suggested that pine species growing in the southern dendroecological section of the Iberian Peninsula (according to the division made by the authors), like eight of the chronologies in this study, could have a common growth response to environmental factors (Richter et al., 1991). PCA applied to analysing three pine species in Spain (*Pinus nigra* Arnold, *Pinus sylvestris* L. and *Pinus uncinata* Ram.) explained 32.5% of variability among the chronologies (Andreu et al., 2007). Our higher variability (56%) may be due to the fact that only one pine species was included in this study.

There are previous studies which consider PCA to be adequate for estimating a common growth variance among and within species (Andreu et al., 2007; Girardin and Tardif, 2005). In this study, the PCA analysis results obtained suggest a clear strong common variance among all the sampling sites, indicated by 56% of the growth variability explained by PC1. All sampling sites show a significant positive PC1 correlation, suggesting a sound common variance. The high correlation coefficient values (more than 0.6, except for Site 2b) emphasise the common growth pattern among all series. Another fact that explains the high variability shared by the sampling sites is that they were located about 450 km apart, a figure previously considered as too long a distance to guarantee good cross-dating among different pine chronologies (Richter and Eckstein, 1990).

High correlation on radial growth between individuals of *Abies alba* Mill. and *Picea abies* Karst. growing under different environmental conditions was also found in France, suggesting that tree-ring growth is not modified by local site characteristics (Lebourgeois, 2007). The total variance explained by average climatic variables and regional radial growth index of *Pinus pinaster* (60%) can be considered appropriate considering tree-rings rarely cover more than 60% of the variance registered in instrumental records, and 40 to 50% is quite a common level (Fritts H. 1991; Jones et al., 1998). Richter et al. (1991) found 68% of the total variance explained by precipitation and temperature in pine species growing in the Iberian Peninsula.

Rainfall is the dominant climatic variable that has a significant association with *Pinus pinaster* growth, as the stepwise correlation analysis indicated. Rainfall effect constitutes the significant climatic variable in both the regional and local analyses. This could be the reason why all the sites share a high common variance explained by PC1. Other factors, such as genetic provenance or specific plasticity based on physiological adaptations, can interact with precipitation. The importance of precipitation, independently of sampling site location, has also been reported for *Pinus sylvestris* L., *Pinus nigra* Arnold. and *Pinus pinea* L. growing in the Iberian Peninsula (Campelo et al., 2007; Richter, 1988). Rainfall during and prior to the

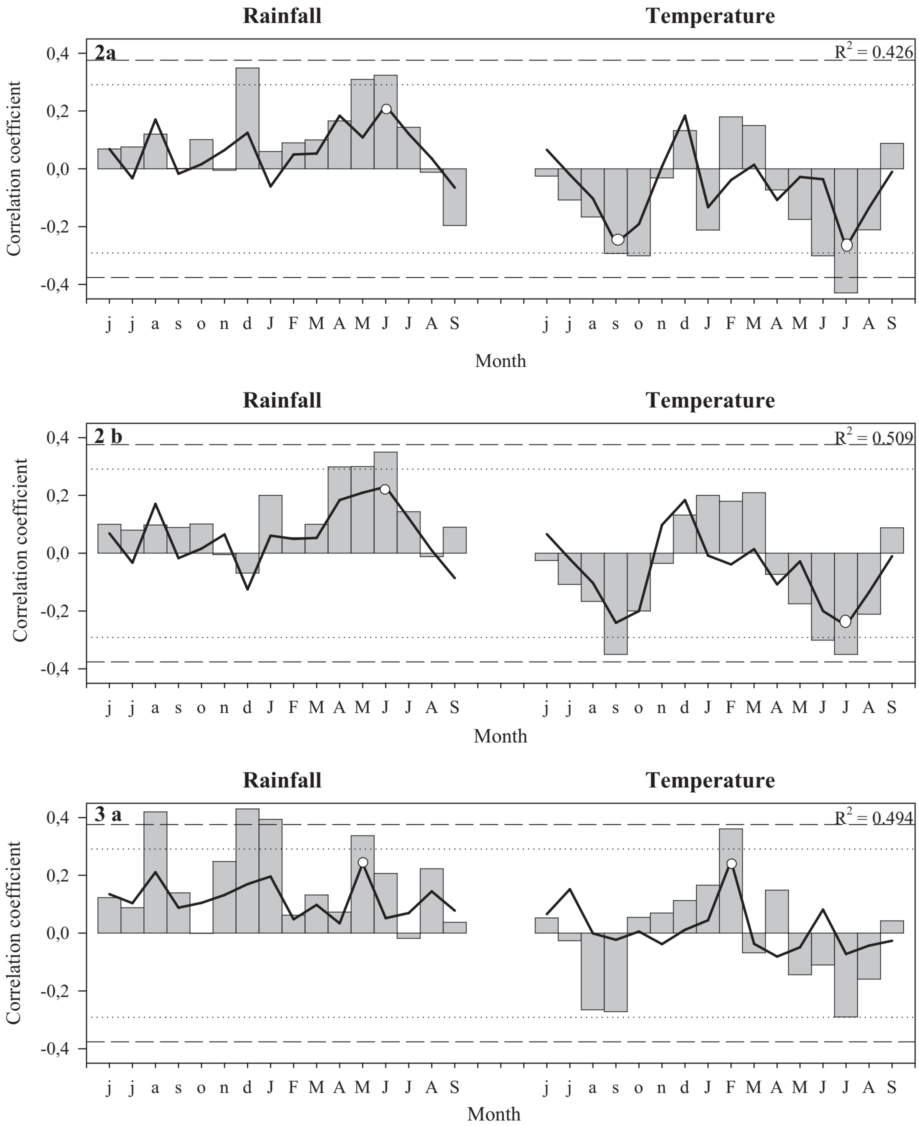


Figure 6. Correlation coefficients (bars) and bootstrapped response function (lines) that indicate the effect of local climatic variables and growth of *Pinus pinaster* at sites 2a, 2b, 3a, 3b, 3c, 3d, 4a, 4b and 6b during the 1960–2005 period. The period analysed covers from June of the year prior to growth to September of the current growing year. Bars outside the dashed lines show significant coefficient at $^{**}p < 0.01$. Bars outside the dotted lines show significant coefficients at $^{*}p < 0.05$. White circles indicate months where the bootstrapped response function coefficients are significant at $^{*}p < 0.05$. R^2 values show the total variance explained by both variables. Lower case letters indicate the months prior to the growing year. Upper case letters indicate the growing year months.

growing season had a positive effect on growth of four different pine species (*Pinus sylvestris, Pinus nigra, Pinus mugo* spp. *uncinata* and *Pinus pinaster*) when they were studied together (Richter et al., 1991). A positive effect of summer rainfall on three pine species (*Pinus sylvestris, Pinus nigra* and *Pinus uncinata*) has also been found in the Iberian Peninsula (Andreu et al., 2007).

Precipitation effect on radial or diameter tree growth, especially at young ages, has also been reported for other coniferous species in northern California, concluding that precipitation is the most important factor limiting growth for the six coniferous species analysed (Yeh and Wensel, 2000).

The effect of temperature on tree growth varied among sites. No effect was recorded at the sites placed at the highest positions (3b, 3c and 3d) and a negative effect was recorded at five sampling sites (2a, 2b, 3a, 3d and 4a). A positive temperature effect in winter prior to the growing season (Sites 3a, 4b and 6b) coincided with a previous study made on four pine species that pointed out the positive significant effect of winter temperature (Richter et al., 1991). The difference found out in this study is that temperature effect could not be simplified to a common response in all the sampling sites because local issues, as for example slope or aspect, could determine the association between this variable and radial growth.

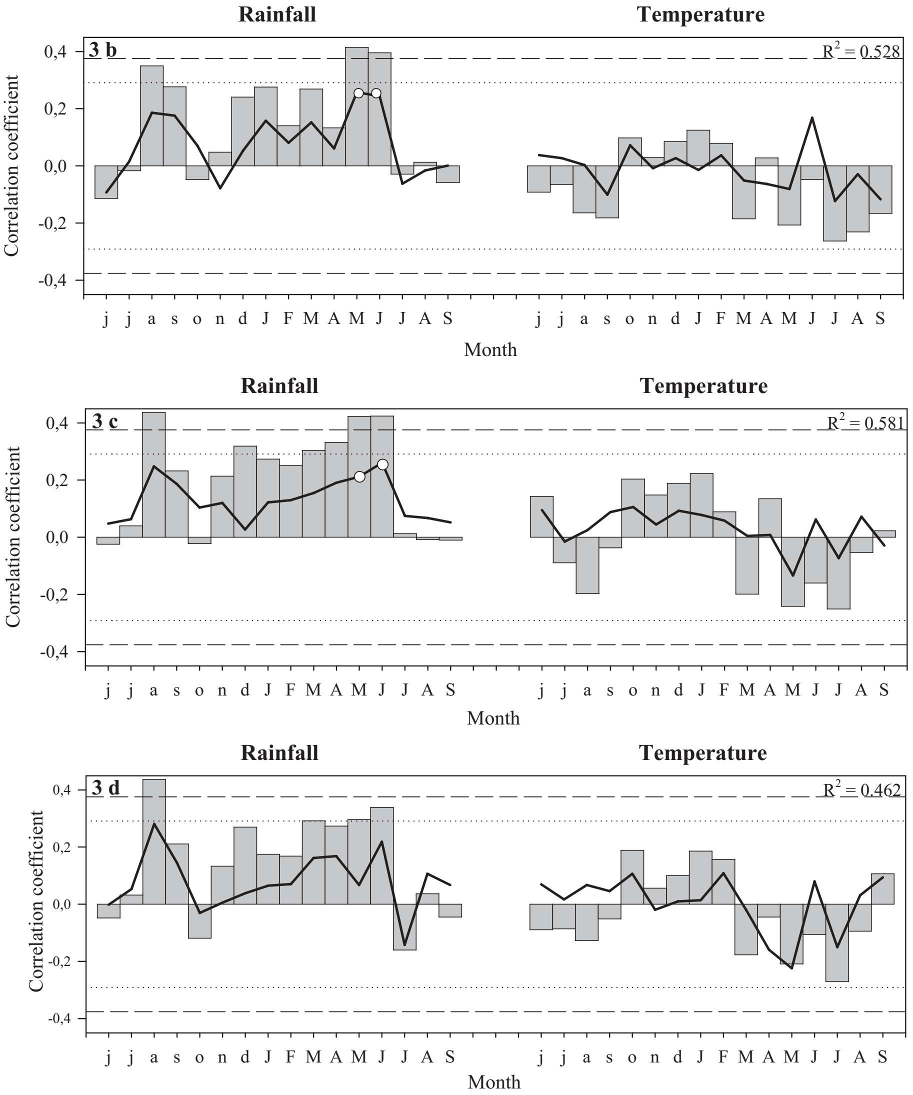


Figure 6. Continued.

Although Richter et al. (1991) did not find significant differences in growth patterns among different pine species using a PCA, they pointed out that the Iberian Peninsula could be divided in two main areas according to climate response (northern and southern). According to this division, all the sampling sites in this study belong to the southern area except for the *Duriense* ecoregion (northern area); the sampling sites in this ecoregion showed not only similarities with the other sampling sites (rainfall), but also differences, due to it is the only ecoregion that showed a negative association with temperature at the beginning of autumn prior to the growing season.

Pinus pinaster radial growth shows a strong association with precipitation and temperature. Temperature effect is less evident than precipitation. However, intercorrelation between precipitation, temperature and evapotranspiration can limit environmental moisture and, consequently, restrict tree growth. However, the predicted temperature trend until 2080 will probably not lead to an unsuitable environment for *Pinus pinaster* (Harrison et al., 2006). As *Pinus pinaster* is a pre-Mediterranean species that has suffered different environmental changes (from subtropical environments to Mediterranean ones) (DiCarlo, 1931), it can also deal with very atypical severe droughts, such as that of 1994 (Peñuelas et al., 2002), and it shows a better water-use efficiency than *Pinus sylvestris* (Martínez-Vilalta and Piñol, 2002). These facts indicate that *Pinus pinaster* might be well adapted to summer rainfall reductions.

A change in growth response in different pine species as a response to climatic conditions has been reported (Andreu et al., 2007). Results show a changing climate effect on growth and some variables that were non-significant 30 years ago have been stated to be significant since the 1980s. The climatic warming of the 1980–1995 period was characterised by intense droughts that produced severe damage in woody species (Peñuelas et al., 2001). Our findings indicate that some climatic variables (May temperature and April–May

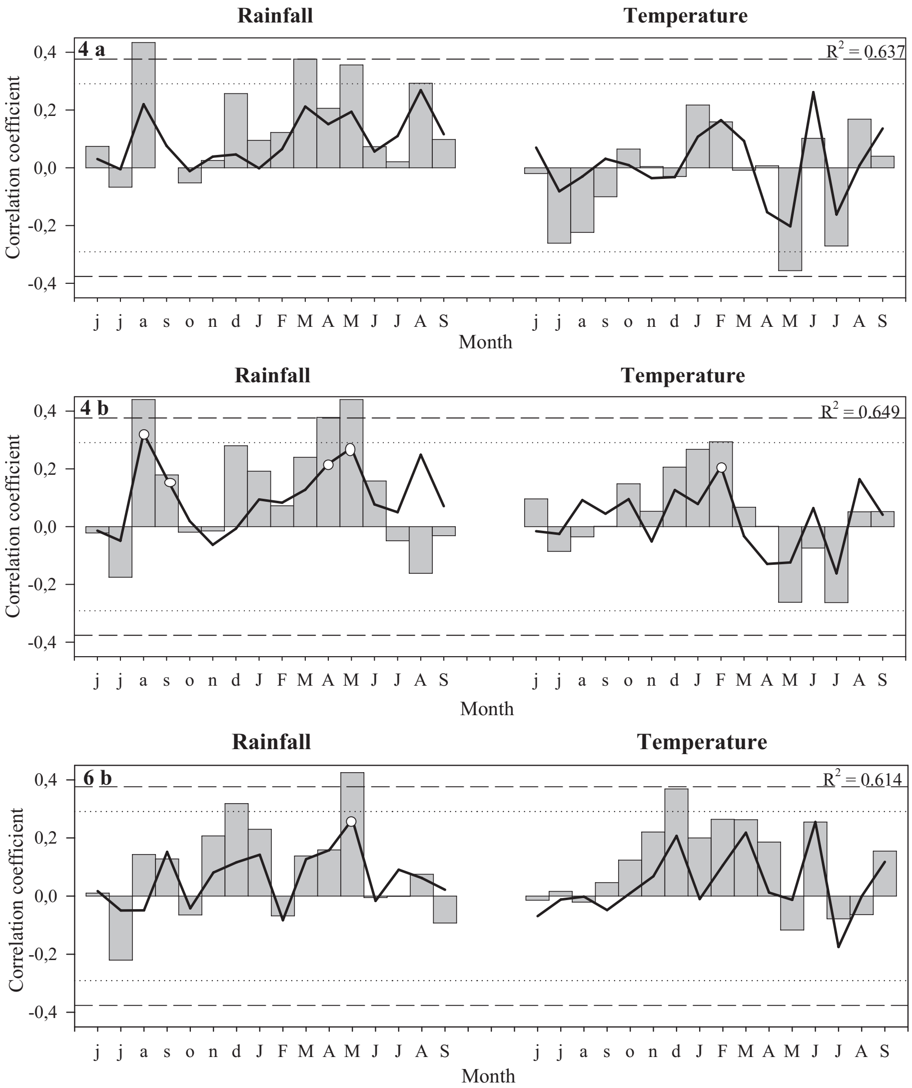


Figure 6. Continued.

Table V. Kalman filter analysis results between *Pinus pinaster* radial growth and monthly climate variables (mean temperature and rainfall) at all the sampling sites.

Site	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
2a				T	T		*r*					*r*+	*r* T			
2b				T							*r*	*r*	*r* T	T		
3a			*r*				*r*+	*r*+	*t*			*r*		T		
3b			*r*									*r*	*r*			
3c			*r*+				*r*			*r*	*r*+	*r*+	*r*			
3d			*r*+							*r*	*r*+	*r*+	*r*			
4a			*r*							*r*+		*r* T+			*r*	
4b			*r*						*t*		*r*	*r*				
6b							*r t*		*t*+	*t*		*rt*				

Letters indicate the months where the association was statistically significant ($^*p < 0.05$): lower-case *r* means monthly rainfall, and the letter *t* means mean monthly temperature. Capital letters refer to a negative association with climate variables. Letters with (+) symbol indicate the months when the association changed over time.

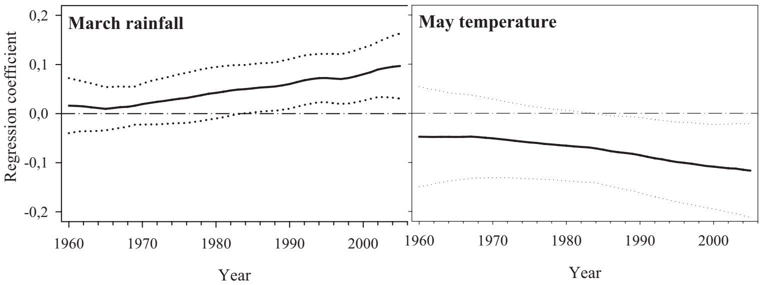


Figure 7. Kalman filter analysis results for the time-dependent relationships between radial growth and climatic variables (March rainfall and May temperature) at Site 4a. The solid lines show the regression coefficient and the dotted lines are the confidence intervals at a 95% confident level.

rainfall) have changed from non-significant to significant in the last 30 years (from 1980 until now). This coincides with previously-reported phenological changes in plant life (related to temperature) in the Mediterranean region. In addition, these changes started during the 80s, which could mean a changing association with climatic factors (Peñuelas et al., 2001; 2002).

Summing up, *Pinus pinaster* radial growth is strongly associated with water supply, which means that it could be an excellent tool for reconstructing past weather conditions in the Iberian Peninsula, especially the temporal fluctuations of rainfall. On the other hand, a changing association between growth and climatic variables has shown that this species could be a good candidate as accurate tool for studying global change effect on tree growth.

Acknowledgements: The authors thank Cristotal Ordóñez for field assistance, María José Fernández Nieto for providing meteorological data and Dr. Vicente Rozas, Dr. Estetan Jobbágy and Dr. Mariano Morales; the editor and two anonymous referees for their useful comments. Thanks are also extended to Etienne Cartuyvels, Cinnamon Nolan and María Laura Cangiano for improving the French and English versions. This study was supported by Research projects from the Regional *Castilla y León* government (Spain) (Project Code VA096A05), the Spanish Ministry of Science and Education (Project Code AGL2007-65795-C02-01) and by the Alβan Programme, the European Union Programme of High Level Scholarships for Latin America (Scholarship No. E05D049920AR).

REFERENCES

Alía M., Martín S., De Miguel J., Galera R., Agúndez D., Gordo J., Catalán G., Gil L., 1996. Regiones de procedencia *Pinus pinaster* Aiton., Dirección general de Conservación de la Naturaleza, Madrid, 75 p.

Andreu L., Gutiérrez E., Macias M., Ribas M., Bosch O., and Camarero J., 2007. Climate increases regional tree-growth variability in Iberian pine forest. Glob. Chang. Biol. 13: 804–815.

Bakkenes M., Alkemade J.R.M., Ihle F., Leemansand R., and Latour J.B., 2002. Assessing effects of forecasted climate change on the diversity and distribution of European higher plants for 2050. Glob. Chang. Biol. 8: 390–407.

Blanco E., Casado M., Costa M., Escribano R., Gracía Antón M., Génova M., Gómez Manzaneque G., Gómez Manzanaque F., Moreno J., Morla C., Regato P., and Sainz Ollero H., 1997. Los bosques ibéricos, Editorial Planeta, Barcelona, España, 572 p.

Briffa K.R., 1995. Interpreting high-resolution proxy climate data-the example of dendroclimatology. In: von Storch H. and Navarra A. (Eds.), Analysis of climate data variability, application of statistical techniques, New York, Springer, pp. 77–94.

Campelo F., Nabais C., Freitas H., and Gutiérrez E., 2006. Climatic significance of tree-ring width and intra-annual density fluctuations in *Pinus pinea* from a dry Mediterranean area in Portugal. Ann. For. Sci. 64: 229–238.

Cook E.R. and Holmes R.L., 1984. Program ARSTAN users manual. Lab. Tree Ring. Res. Univ. of Arizona, Tucson, Arizona, EUA.

Cook E.R. and Kairiuskstis L.A., 1990. Methods of Dendrochronology, Applications in the Environmental Sciences. Kluwer Academic Publishers, 393 p.

D'Arrigo R.D. and Jacoby G.C., 1992. A tree-ring reconstruction of New Mexico winter precipitation and its relation to El Niño/Southern Oscillation events. In: Diaz H.F. and Markgraf V. (Eds.). El Niño. Historical and paleoclimatic aspects of the Southern Oscillation. Cambridge, University Press, pp. 243–258.

DGCN, 1998. Segundo Inventario Forestal Nacional 1986–1996. Ed. Ministerio de Medio Ambiente, España.

DGCN, 2002. Plan Forestal Español. Ed. Ministerio de Medio Ambiente, España.

Di Castri F., 1981. Mediterranean-type shrublands of the world. In: di Castri F., Goodall D., and Specht R.L. (Eds.), Mediterranean-type shrub lands, Elsevier Scientific Publishing Amsterdam, The Netherlands, 643 p.

Di Rienzo J., Balzarini M., Casanoves F., Gonzalez L., Tablada E., and Robledo C., 2002. Infostat Software Estadístico versión 2. Grupo infoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Elena-Roselló R., 1997. Clasificación biogeoclimática de España Peninsular y Balear Madrid: Ministerio de Agricultura, Pesca y Alimentación, Madrid, 100 p.

Esteban-Parra M., Rodrigo F., and Castro Diéz Y., 1998. Spatial and temporal patterns of precipitation in Spain for the period 1880–1992. Int. J. Climatol. 18: 1557–1574.

Fritts H., 1976. Tree Ring and Climate, Academic Press Inc., London, 567 p.

Fritts H., 1991. Reconstructing large-scale climatic patterns from tree-ring data, The University of Arizona Press, Tucson, USA, 286 p.

Fritts H.C., 1998. Factors preconditioning growth with Kalman filter: an empirical model of the tree ring response to monthly variations in climate. Laboratory of tree-ring research, University of Arizona, Tucson, USA.

Fritts H.C., 1999. PRECON version 5.17, http://www.arizona.edu/webhome/hal/dlprecon.html.

Fritts H.C. and Swetnam T., 1989. Dendroecology: a tool for evaluating variations in past and present forest environments. Adv. Ecol. Res. 19: 111–188.

Girardin M. and Tardif J., 2005. Sensitivity of tree growth to the atmospheric vertical profile in the Boreal Plains of Manitoba, Canada. Can. J. For. Res. 35: 48–64.

Grissino-Mayer H.D., 2001. Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. Tree-Ring Res. 57: 205–221.

Harrison P., Berry P., Butt N., and New M., 2006. Modelling climate change impacts on species' distributions at the European scale: implications for conservation policy. Environ. Sci. Policy 9: 116–128.

Holmes R.L., 1983. Computer-assisted quality control in tree-ring dating and measurement. Tree-ring Bull. 43: 69–78

Holmes R.L., 2001. Dendrochronology Program Library. Available from the Laboratory of Tree Ring Research, University of Arizona, Tucson, USA.

Hughes M., 2002. Dendrochronology in climatology – the state of the art. Dendrochronologia 20: 95–116.

IPCC, 2007. Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jones P.D., Jónsson T., and Wheeler D., 1997. Extension to the North Atlantic Oscillation using early instrumental pressure observations from Gibraltar and South-West Iceland. Int. J. Climatol. 17: 1433–1450.

Jones P.D., Briffa K.R., Barnett T.P., and Tett S.F.B., 1998. High-resolution palaeoclimatic records for the last millennium: interpretation, integration and comparison with General Circulation Model control-run temperatures. Holocene 8: 455–471.

Lebourgeois F., 2007. Climatic signal in annual growth variation of silver fir (*Abies alba* Mill.) and spruce (Picea abies Karst.) from the French Permanent Plot Network (RENECOFOR). Ann. For. Sci. 64: 333–243.

Martínez-Vilalta J. and Piñol J., 2002. Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. For. Ecol. Manage.161: 247–256.

Nicolas A. and Gandullo J., 1967. Ecología de los pinares españoles. 1, *Pinus pinaster* Ait. Ministerio de Agricultura, Madrid, España, 311 p.

Parry M., 2000. Assessment of potential effects and adaptations for Climate change in Europe: The Europa ACACIA Projet. Jackson Environments Institute, University of East Anglia, Norwich, UK.

Peñuelas J., Lloret F., and Montoya R., 2001. Severe drought effects on Mediterranean Woody Flora in Spain. For. Sci. 47: 214–218.

Peñuelas J., Fillela I., and Comas P., 2002 Change plant and animal life cycles from 1952 to 2000 in the Mediterranean region. Glob. Chang. Biol. 8: 531–544

Regent Instrument Inc., 2002. Windendro^{TM} v. 2002a, Québec, Qc.

Richter K.,1988. Dendrochronologische und dendroklimatologische Untersurchungen an Kiefern (*Pinus* sp.) in Spanien. Diss., Universität Hamburg, 296 p.

Richter K. and Eckstein D., 1990. A proxi summer rainfall record for southeast Spain derived from living and historical pine trees. Dendrochronologia 8: 67–82.

Richter K., Eckstein D. and Holmes R.L., 1991. The dendrochronological signal of pine trees (*Pinus* spp.) in Spain. Tree-Ring Bull. 51: 1–13.

Rodrigo F., Esteban-Parra M., Pozo-Vázquez D., and Castro-Diéz Y., 2000. Rainfall variability in southern Spain on decadal to centennial time scales. Int. J. Climatol. 20: 221–732.

Schweingruber F.H., 1993. Trees and wood in Dendrochronology, Springer series in Wood Science, Springer-Verlag, 474 p.

Schweingruber F.H., 1996. Tree rings and environment: Dendroecology, Paul Haupt Publisher, Berne, Sttutgart, Vienna, 602 p.

Sokal R.R. and Rohlf F.J., 1995. Biometry: the principles and practice of statistics in biological research, 3rd ed., WH Freeman and Co., New York, USA, 358 p.

Stokes M. and Smiley T., 1968. An Introduction to Tree-Ring Dating, University of Arizona Press, Tucson, USA, 120 p.

Tessier L., Guibal F., and Schweingruber F., 1997. Research strategies in dendroecology and dendroclimatology in mountain environments. Clim. Chang. 36: 499–517.

Wigley T.M.L., Briffa K.R., and Jones P.D., 1984. On the average value of correlated time series, with applications in dendroclimatology and hydrometeorology. J. Appl. Meteorol. Climatol. 23: 201–213.

Yen H. and Wensel L., 2000. The relationship between tree diameter growth and climate for coniferous species in northern California. Can. J. For. Res. 30: 1463–1471.

II

This article was accepted to be published In Proceeding of the Dendrosymposium 2007. May 3rd – 6th 2007, Riga, Latvia. Scientific Technical Report STR08/05. Edited by Didzis E., Brumelis G., Gärtner H., Helle G., Schleser G. Vol.6 (2007) Bogino S., Bravo F. SOI and NAO impacts on Pinus pinaster Ait. growth in Spanish forests, pages 21–26, and is reprinted with kind permission of the editors.

SOI and NAO impacts on *Pinus pinaster* Ait. growth in Spanish forests

RESUMEN

Se analizó el impacto de los índices atmosféricos (NAO y SOI) sobre el crecimiento radial de pino mediterráneo (*Pinus pinaster* Ait.) en los bosques de España usando técnicas dendroclimatológicas. Se estudió la respuesta del crecimiento a los índices atmosféricos de manera dependiente e independiente del tiempo aplicando el análisis de correlación, la función respuesta del remuestreo *bootstrapped* y el filtro de Kalman. En el área de distribución natural de la especie en el este de España se construyeron cuatro cronologías. Se observó una señal común de crecimiento lo que sugiere que los árboles que crecen en el mismo sitio tienen una respuesta similar a los factores ambientales. La varianza total explicada por los índices atmosféricos varió entre 9,56 y 37,56% en el período analizado de 1950-2005. Los valores estadísticos que caracterizan las cronologías sugieren una fuerte asociación entre el crecimiento del pino mediterráneo y los factores ambientales. Los resultados mostraron una asociación positiva entre los sitios de muestreo y el índice SOI, sin embargo, esta asociación no es significativa excepto en sólo un sitio de muestreo. Por otra parte, el índice NAO en el invierno tiene un efecto negativo sobre el crecimiento radial en dos sitios de muestreo. El análisis a través del tiempo mostró que sólo el índice SOI cambió en los últimos 50 años. A pesar de que los índices atmosféricos explican menos la variabilidad del crecimiento radial de esta especie que las variables climáticas, temperatura y precipitación, su efecto se puede registrar en los anillos de crecimiento y es vulnerable de sufrir cambios a través del tiempo.

SOI and NAO impacts on *Pinus pinaster* Ait. growth in Spanish forests

S. Bogino[1,2] & F. Bravo[1]

[1] *Departamento de Producción Vegetal y Recursos Forestales. Universidad de Valladolid, Avda. de Madrid 44. 34004 Palencia. Spain. TE. 34 979108427.*

[2] *Departamento de Ciencias Agropecuarias. Facultad de Ingeniería y Ciencias Económico-Sociales. Universidad Nacional de San Luis. Argentina. Email: sbogino@fices.unsl.edu.ar*

Introduction

Global change provides an extraordinary research opportunity and challenges for dendroclimatologists and other scientists who investigate the natural variability in the Earth's system (Hughes 2002). Climate has been used as a source of explanation for changes in the size and state of the tree-ring and it should be used to predict future tree-ring growth (Hughes 2002). The North Atlantic Oscillation (NAO) is traditionally defined as the normalized pressure difference between the Azores and Iceland. The NAO pattern is most pronounced both, in intensity and area coverage, during the winter. This phenomena is considered to be the most important source of climate variability in Europe, northern Africa and eastern North America; affecting temperature, precipitation and atmospheric circulation (Hurrell 1995, Hurrel & van Loon 1997). The Southern Oscillation Index (SOI) refers to the pressure variation between Darwin (Australia) and Tahiti. This pressure variation defines the cyclic warming and cooling of the equatorial Pacific Ocean, commonly known as El Niño phenomena (Bjerknes 1966). The impact of the SOI is felt mainly in the Pacific, however, its effect seems to influence climatic variability on a global scale (Trenberth et al. 1998).

The NAO and SOI should be considered as the major sources of the inter-annual variability of weather and climate around the world (Hurrell 1995, Hurrell & van Loon 1997). Over the last five centuries the connection between the mean winter precipitation over the Mediterranean and the NAO has turned out to be stable, with highly negative correlations throughout the period (Cook et al. 2001). *Pinus pinaster* Ait. occurs naturally in the western Mediterranean Basin, in the northern rim (France, Italy, Portugal and Spain) and in the southern rim (Algeria, Morocco and Tunisia). It is a characteristic species of the Mediterranean forests and its main distribution area is across the Iberian Peninsula where it covers about 2.4 million hectares (Blanco et al. 1997). It is adapted to different environments and, consequently, shows a wide ecological variety of adaptations: it survives under high or low temperatures, under regular or variable rainfall as well as under sever droughts; it is also adapted to the extremely cold winter in the centre of the peninsula and to the mild temperature next to the Atlantic ocean coast (Blanco et al. 1997).

There are no previous studies made on the impact of the SOI and NAO indexes on conifers growing in the Iberian Peninsula. Because of many scientists arguing about both indexes global impact on the earth's surface, it could be an excellent opportunity to analyse the relationship between these indexes and the growth of woody species. The aim of this study was to analyze the relation between the *Pinus pinaster* 's tree-ring width and the NAO and

SOI atmospheric indexes in Eastern Spain. This objective was addressed analyzing sixty trees cored at four different sites. Correlation analysis, bootstrapped response function and Kalman filter analysis were applied to study both, time-independent and time-independent growth responses to atmospheric indexes.

Material and Methods

Study sites and laboratory methods

Four sampling sites were selected In Central Spain. The sites were located between 920 and 1,437 m a.s.l. (Fig.1, Tab. 1).

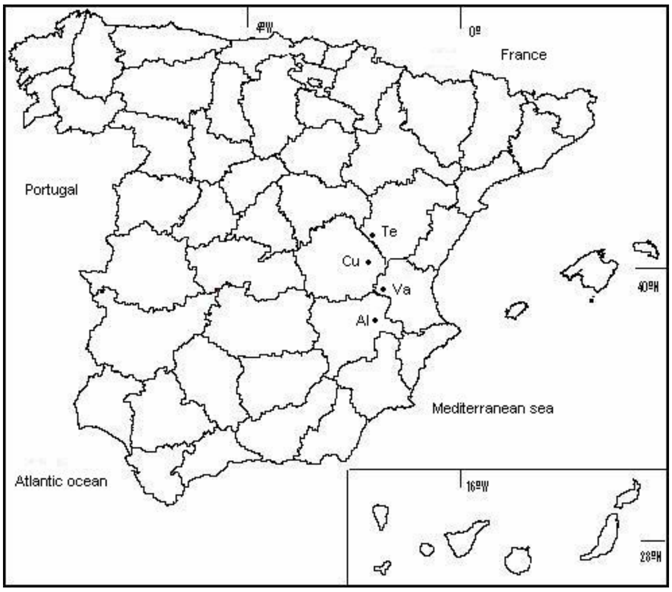


Figure 1: Geographical location of P. pinaster sampling sites in the Iberian Peninsula. The round points indicate the sampling sites. Sites codes: Te: Teruel; Cu: Cuenca; Va: Valencia; Al: Albacete.

The climate of the area is Mediterranean with sever droughts during the summer and precipitation from autumn to spring. Mediterranean Maritime pine grows on permeable soils, generally rich in organic matter, which have developed on calcareous or siliceous substrates. At each sampling site, in the summer of 2006, from fifteen dominant and co-dominant trees, two cores were extracted at a hight of 1.30 meter. Cores were polished and subsequently dated under a binocular microscope following standard dendrochronological techniques (Stokes & Smiley 1968). Sections were scanned at high resolution (2,000 dpi) with an Epson Expression 1640 XL scanner with a 0.01 mm accuracy. Tree-rings were measured using WinDENDRO© (Regent Instruments).

Statistical analysis

The NAO and SOI values were obtained from www.cru.uea.ac.uk/cru/data/nao.htm and www.cru.uea.ac.uk/cru/data/soi.htm (Jones et al. 1997). The COFECHA program 6.06P version (Grissino-Mayer 2001 available at: www.ltrr.arizona.edu) was applied to assess the data accuracy. This program calculates the correlation indices between the ring width series and also identifies errors such as missing or false rings. To eliminate the growth biological tendency and to minimise growth variation which was not present in all trees (Fritts 1976), the ARSTAN program, 2.07 version (Cook & Holmes 1984 available at: www.ltrr.arizona.edu) was used. To obtain a master chronology at each study site, the standardised series were averaged. These temporal series or master chronologies expressed the annual variations in radial growth of *P. pinaster* at each sampling place. The quality of the chronologies was evaluated using the mean sensitivity (MS) (Schweingruber 1996), the signal-to-noise ratio (SNR) (Fritts & Swetnam 1989) and the expressed population signal (EPS) (Wigley et al. 1984). A chronology is considered to be confident with a higher than 0.85 EPS value. The common growth signal between residual chronologies was analysed using the Pearson correlation coefficient (Sokal & Rohlf 1995).

To determine the climatic variables that control the growth of *Pinus pinaster*, atmospheric indexes were compared with residual chronologies from June previous to the growing season to September of the current growth year during the period 1950-2005. The PRECON program version 5.17 (Fritts 1999 available at: www.ltrr.arizona.edu) was used. This is a statistical model for analysing the tree-ring response to variations in climate using a stepwise multiple regression analysis. The coefficients are considered significant at a 95% level of confidence. The program also includes a bootstrapped response function to improve the statistical significance of the regression coefficient (*p* <0.05). In this analysis 999 interactions were made. To analyse the time dependent relationship between these atmospheric indexes and radial growth, Kalman filter analysis was applied (Visser & Molenar 1988).

Results

An evaluation of climate atmospheric indexes impact on radial growth of Mediterranean Maritime pines has been carried out. This evaluation was based on a dendrochronological analysis of dominant and co-dominant trees in four stands in Eastern Spain.

The descriptive statistic of all the chronologies showed that the mean sensitivity varied from 0.2571 to 0.3779, and the standard deviation varied from 0.2555 to 0.3179, according to the sampling site. The SNR fluctuated from 29.087 to 68.444, and the EPS values varied from 0.967 to 0.986. The total period covered by the chronologies varied from 120 in the shortest chronologies, to 162 years in the longest ones (Tab. 1).

Table 1: Coordinates, altitude, basal area (BA) and descriptive statistic of the four Pinus pinaster chronologies in Eastern Spain. SD: standard deviation; MS: mean sensibility; SNR: Signal to noise ratio; EPS: Expressed population signal. Te: Teruel; Cu: Cuenca; Va: Valencia; Al: Albacete.

	Te	**Cu**	**Va**	**Al**
UTM_X	639753	638858	648053	645583
UTM_Y	4464496	4467569	4411314	4411593
Altitud (m)	1437	1364	970	1090
BA($m^2.ha^{-1}$)	40.17	45.73	36.66	34.74
Time span	1844-2005	1847-2005	1879-2005	1886-2005
Core number	26	29	26	30
Ring number	3757	4128	2723	3043
Age range	124-162	124-158	72-127	72-120
SD	0.2589	0.3179	0.2555	0.2764
MS	0.2992	0.3708	0.2571	0.2978
SNR	29.087	68.444	38.528	36.254
EPS	0.967	0.986	0.975	0.973
Variance in first eigenvector	54.85	71.41	62.78	59.62
Mean correlation among trees	0.528	0.702	0.606	0.573

The four chronologies showed high SNR (over 29.087) and EPS (over 0.967), and the percentage of the variance accounted for the first eigenvector (over 54.85) reflected a strong common signal related to climatic-environmental factors. The Pearson correlation coefficient between all residual chronologies varied from 0.37 to 0.76 in the 1887-2005 common growth period (DF = 111 and *p**< 0.05). The association between radial growth and monthly climatic atmospheric indexes is shown in figure 2. The total variance explained by atmospheric indexes varied from 8.95 to 37.46%.

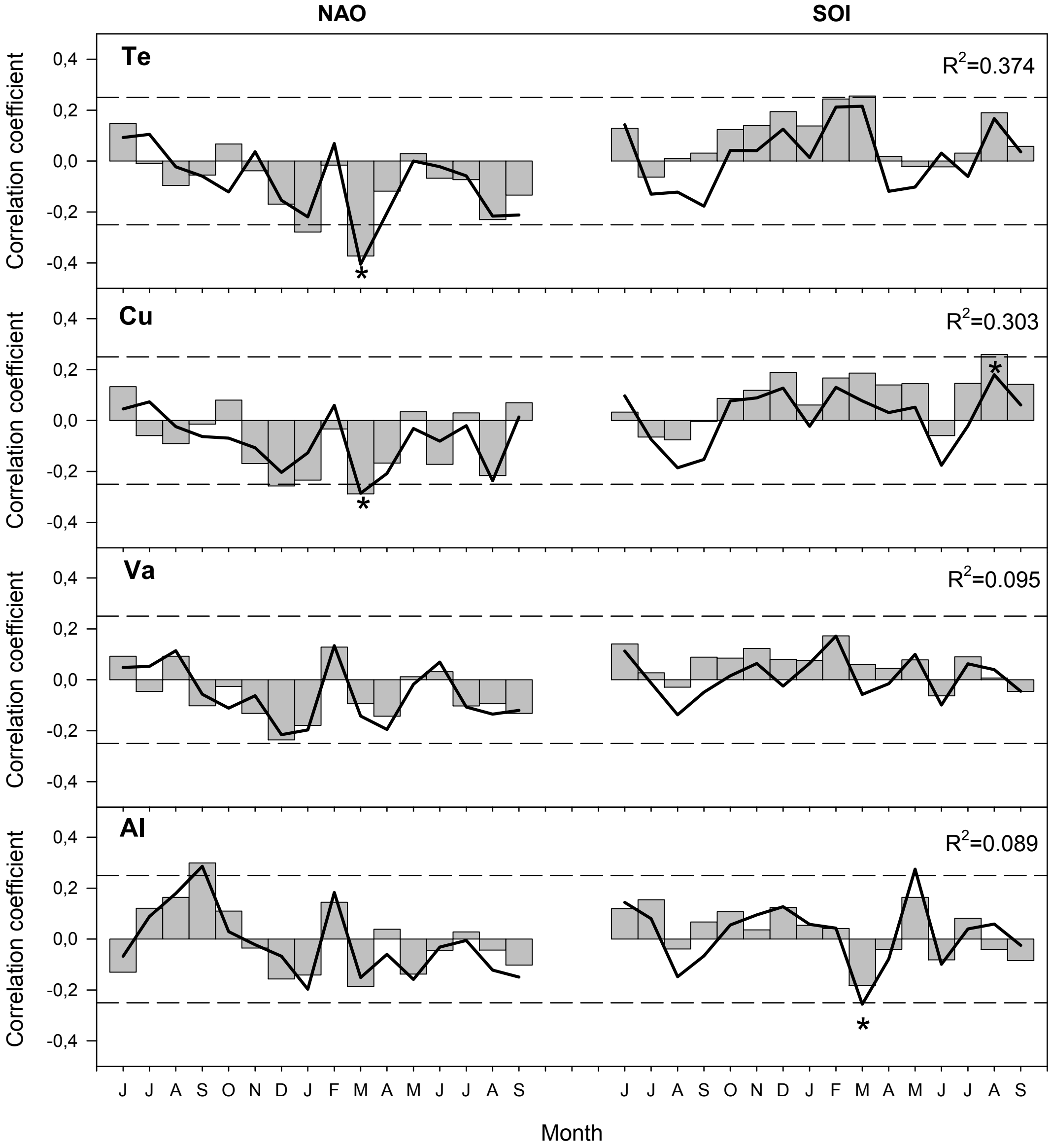


Figure 2: Regression coefficients (bars) and bootstrapped response function (lines) which relate the effect of climatic atmospheric indexes and growth of Pinus pinaster during the 1950-2005 period. The analysed period is from June to the previous growing season to September of the current growing season. Bars higher than the dashed lines show significant coefficient at the 0.05 level. Asterisks point the months where the bootstrapped response function coefficients are significant at the 0.05 level. R^2 *values show the total variance explained by both indexes. Sites codes: Te: Teruel; Cu: Cuenca; Va: Valencia; Al: Albacete*

The total variance explained by the NAO and SOI indexes is higher in the sites at higher positions (chronologies Te and Cu). In these places there is a significant negative association between the NAO index and growth during January and March (site Te) and December and March (site Cu), but only March is significant in the bootstrapped analysis. Only one place showed a positive association with NAO values during September prior to the growing season (Site Al), but this association was not significant in the bootstrapped analysis. The association with the SOI was positive in all the analysed sites, but it was only significant, in the correlation coefficient and the in the bootstrapped response function, in place Cu. Only site Al showed a negative association with the SOI, shown by the bootstrap coefficient during March previous to the growing season.

The Kalman filter showed that no place showed a changing association through time with NAO index. Only place Te showed a changing association with SOI, statistically significant in February, from 1982 to 1987. This significant association was coincident with the strongest El Niño phenomena recorded during the last century.

Discussion

It is difficult to find a simple linear correlation between radial growth and atmospheric indexes because their global effects and their impact on regional climatic variables are not yet completely understood.

In these results, the total variance explained by NAO and SOI indexes suggested that the signal is weak if it is compared with regional climatic variables. However, the negative correlation with NAO during winter in two sites, and the changing effect of SOI index through time in one site, offer new information about the association between atmospheric indexes and coniferous species growing in the Iberian Peninsula.

Although atmospheric indexes explain less variability than other regional climatic variables, these results emphasis that these indexes effects could be recorded on tree-ring and they could have a sensible effect in growth of woody species, even if their action centres are located too far away from the analysed sites.

Previous studies have determined an opposite relation between winter NAO index and precipitation on the Iberian Peninsula (Esteban Parra et al.1998) and in this study two sites showed a negative winter correlation with the NAO index, consequently, these results suggest that these negative relation between NAO and growth could be associated with a moisture availability that could affect growth. Also, NAO effect is related to altitudinal position: the highest sites showed a significant relation with NAO during winter but there was no association with NAO values at the lowest analysed sites.

The association between growth and the NAO index was different from *Pinus sylvestris* across Northern Fennoscandia where this species had a positive correlation between early winter NAO indexes previous to the growing season and late spring NAO (Macias et al. 2004). In our study, only one place showed a positive association with the NAO during the autumn previous to the growing season. The positive correlation these authors found in Fennoscandia between the NAO winter index and growth does not exist in Spanish Mediterranean Maritime pine forests at any sampling site. This difference could be explained by the fact that the effect of the NAO index on Northern Europe is opposite to the effect in Southern (Hurrel 1995).

In Spain, the relation between the SOI and plant growth has only been previously analysed on annual crops (Gimeno et al. 2002). As far as we know, there is no study focused on forest growth related to the SOI in our region. According to Trenberth et al. (1998) the SOI index mainly affects the Pacific area, but they consider that its effect might also influence climatic variability on a global scale. In this study a positive association was found between the index named above and growth in August in one of the highest sites, and negative in March in one of the lowest altitudinal position sites. Unfortunately, previous studies on the SOI effect in Spain are contradictory, as a consequence of that, future studies have to be made in order to understand better the opposite effect, according to the site and the changing impact through time. Modelling the impact of climate change on the distribution of species on a European scale under future climatic scenarios, Spain's environment will become unsuitable for *Pinus pinaster* by 2080 (Harrison et al. 2006). This would be coherent if growth were associated with precipitation, but the comprehensive general circulation models used for future climate projections leave us with an indeterminate picture of ENSO's future. Some observers predict more ENSO activity, others less, with the highly uncertain forecast consensus indicating little change (Cane 2005). Considering that this index shows a peculiar association with growth, which changes through time, future studies will have to be carried out.

Results can serve both, to understand climate/forest growth associations, and to determine which climatic variables can be useful for improving empirical models in order to help forest managers to adopt decisions in the future within the context of an extremely unpredictable climatic scenario.

Acknowledgements

The authors thank Cristobal Ordóñez for field assistance.This study was supported by the Research project from Junta de Castilla y León (Spain) Code: AGL 2004-07094-CO2- and by the Programme Alβan, the European Union Programme of High Level Scholarships for Latin America, scholarship # E05D049920AR.

References

Bjerknes, J. (1966): A possible response of the atmospheric Hadley circulation to equatorial anomalies of ocean temperature. *Tellus 18:* 820–829.

Blanco, E., Casado, M., Costa, M., Escribano, R., Gracía Antón, M., Génova, M., Gómez Manzaneque, G., Gómez Manzanaque, F., Moreno, J., Morla, C., Regato, P., Sainz Ollero, H. (1997): Los bosques ibéricos. Editorial Planeta.

Cane, M. (2005): The evolution of El Niño, past and future. *Earth and Planetary Science Letters 230*: 227-240.

Cook, E.R., Holmes, R.L. (1984): Program ARSTAN users manual. Lab. Tree Ring. Res. Univ. of Arizona, Tucson, Arizona, EUA.

Cook, E.R., Glitzenstein, J.S., Krusic, P.J., Hracombe, P.A. (2001): Identifying functional groups of trees in west Gulf Coast forests USA: a tree-ring approach. *Ecological applications 11*: 883-903.

Esteban-Parra, M., Rodrigo, F., Castro Diéz, Y. (1998): Spatial and temporal patterns of precipitation in Spain for the period 1880-1992. *Int. Journal of Climatology 18*: 1557- 1574.

Fritts, H.C. (1976): Tree Ring and Climate, Academic Press Inc, London.

Fritts, H.C. (1999): PRECON vers. 5.17, http://www.arizona.edu/webhome/hal/dlprecon.html

Fritts, H.C., Swetnam, T. (1989): Dendroecology: a tool for evaluating variations in past and present forest environments. *Advances in Ecological Research 19:* 111-188.

Gimeno, L., Ribera, P., Iglesias, R., de la Torre, L., García, R., Hernández, E. (2002): Identification of empirical relationships between indices of ENSO and NAO and agricultural yields in Spain. *Climate research 21*: 165-172.

Grissino-Mayer, H.D. (2001): Assessing crossdating accuracy: a manual and tutorial for the computer program COFECHA. *Tree-Ring Res. 57*: 205–221.

Harrison, P., Berry, P., Butt, N., New, M. (2006): Modelling climate change impacts on species' distributions at the European scale: implications for conservation policy. *Environmental Science & Policy 9:* 116-128.

Hughes, M. (2002): Dendrochronology in climatology – the state of the art. *Dendrochronologia 20:* 95-116.

Hurrell, J.W. (1995): Decadal trends in the North Atlantic Oscillation and relationships to regional temperature and precipitation. *Science 269:* 676-679.

Hurrel, J., Van Loon, H. (1997): Decadal variations in climate associated with the North Atlantic Oscillation. *Clim. Change 36:* 301-326.

Jones, P.D., Jonsson, T., Wheeler, D. (1997): Extension to the North Atlantic Oscillation using early instrumental pressure observations from Gibraltar and South-West Iceland. *Int. J. Climatol. 17:* 1433-1450.

Macias, M., Timonen, M., Kirchhefer, A., Lindholm, M., Eronen, M., Gutiérrez, E. (2004): Growth variability of Scots pine (*Pinus sylvestris* L.) along a west-east gradient across northern Fennoscandia: a dendroclimatic approach. *Arct. Antart. Alpine Res. 36*: 565-574.

Schweingruber, F.H. (1996): Tree rings and environment: Dendroecology, Haupt, Berne.

Sokal, R.R., Rohlf, F.J. (1995): Biometry: the principles and practice of statistics in biological research, 3rd edition, WH Freeman and Co., New York, UEA.

Stokes, M.A., Smiley, T.L. (1968): An Introduction to Tree-Ring Dating, University of Arizona Press, Tucson, UEA.

Trenberth, K., Branstator, G., Karoly, D., Kumar, A., Lau, N., Ropelewski, C. (1998): Progress during TOGA in understanding and modelling global teleconnections associated with tropical sea surface temperatures. *Journal of Geophysical Research 103*: 14291–14324.

Visser, H., Molenaar, J. (1988): Kalman filter analysis in dendroclimatology. *Biometrics 44:* 929-940.

Wigley, T.M.L., Briffa, K.R., Jones, P.D. (1984): On the average value of correlated time series, with applications in dendroclimatology and hydrometeorology. *J. Clim. Appl. Meteorol. 23:* 201-213

III

This article was submitted in Journal of Vegetation Sciences. Bogino S., Fernández Nieto M, Bravo F. Drought index and radial growth of Pinus sylvestris L. in its southern and western distribution threshold. Journal of Vegetation Sciences.

Drought and climate effect on radial growth of *Pinus sylvestris* L. in its southern and western distribution threshold

RESUMEN

Se estudió la relación entre el ancho de los anillos de crecimiento de *Pinus sylvestris* y las variables climáticas (precipitación y temperatura), el índice de sequía relativa y el efecto de los pulsos e interpulsos de la disponibilidad de agua. Estos estudios se realizaron en el extremo sudoeste de la distribución mundial de esta especie en la Península Ibérica y usando técnicas dendrocronológicas. Se construyeron seis cronologías de ancho de anillo. La relación entre el ancho de los anillos de crecimiento con el clima se determinó usando el coeficiente de correlación de Pearson y la función respuesta del remuestreo *bootstrapped.* Se aplicaron el índice de sequía relativa y el análisis de series temporales para determinar el efecto de la aridez y la periodicidad en el crecimiento radial. El ancho de los anillos de crecimiento está positivamente correlacionado con la lluvia de la estación de crecimiento en todos los sitios. El efecto de la temperatura media varió de acuerdo con el sitio. También se encontró una respuesta climática de acuerdo con la edad: los árboles jóvenes tuvieron una mayor variabilidad explicada por el clima que los árboles más longevos. Entre los ejemplares adultos también hubo correlación entre añillos de crecimiento sucesivos. La relación significativa entre el crecimiento radial y el índice de sequía sugiere que la sequía es un factor de control que afecta el crecimiento radial de esta especie en el límite sudoeste de su distribución mundial. La periodicidad relacionada con el efecto de los pulsos e interpulsos de disponibilidad de agua es de dos a dos y medio años lo que sugiere un fuerte impacto de la variación interanual de la precipitación sobre el crecimiento radial.

Drought and climate effect on radial growth of *Pinus sylvestris* L. in its southern and western distribution threshold

Bogino, Stella M.[1,2*]; Fernández Nieto, María J.[3,4] & Bravo, Felipe[1,5]

[1] Joint Unit UVa-INIA 'Sustainble Forest Management'. Departamento de Producción Vegetal y Recursos Forestales, Universidad de Valladolid, Avda. de Madrid 44, 34004 Palencia, Spain; [2] Departamento de Ciencias Agropecuarias. Facultad de Ingeniería y Ciencias Económico-Sociales, Universidad Nacional de San Luis, Avda. 25 de Mayo 384, 5730 Villa Mercedes, San Luis, Argentina; [3] Joint Unit UVa-INIA 'Sustainable Forest Management'. Departamento de Física Aplicada, Universidad de Valladolid, Avda. de Madrid 44, 34004 Palencia, Spain; [4] E-mail: mariajose.fn@ono.com; [5]E-mail: fbravo@pvs.uva.es

* Corresponding author; Fax: +34 979108302; E-mail sbogino@fices.unsl.edu.ar

Abstract

Question: How climate affects radial growth of *Pinus sylvestris* growing in its limit worldwide distribution area?

Location: Mediterranean environments in the Iberian Peninsula.

Methods: Dendrochronological techniques were applied to build six tree-ring width chronologies of *P. sylvestris*. Tree-growth association with climate was analysed using correlation coefficient and bootstrapped response functions. Drought index (DRI) and a time series analysis were performed to detect aridity effect, autocorrelation between successive tree ring and periodicity in radial growth related to pulse and interpulse water events.

Results: Tree-ring width was positively correlated with rainfall in the growing season at all sites. Mean temperature effect varied according to site. A climatic response according to age was found: young stands had higher variability explained by climatic variables than old stands. Correlation between successive years was also detected in old stands. The significant association between radial growth and DRI suggests that drought is a commanding factor affecting radial growth of *P. sylvestris.* The periodicity related to pulse and interpulse events was two or two and a half years, suggesting a strong impact of interannual precipitation variation on radial growth.

Conclusions: Precipitation is a commanding factor on radial growth of Pinus silvestris whereas temperature effect changes according to the site. Cambial age determine the climate growth association. Drought events may be used to estimate both radial growth and growth periodicity.

Keywords: Dendroclimatology; Scots pine; tree ring; pulse and interpulse.

Abbreviations: DRI = Drought index; MS = Mean sensitivity; SNR = signal-to-noise ratio; EPS = Expressed population signal; ACF = Autocorrelation function

Introduction

Growth-climate relationships are crucial to understanding and modeling the carbon sequestration process in forests and to developing forestry strategies to mitigate climate change impact. 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). Extreme sites, such as the upper tree line or dry sites, have been the most valuable for dendroclimatological studies because the number of important influencing factors is reduced and the possibilities for interpreting growth patterns are increased (Fritts 1976).

In arid and semi-arid ecosystems, where water is a limiting resource, water availability experiences two phases: “pulse”, when the resource is available, and “interpulse”, when water availability is too low for plant use (Noy-Meir 1973; Goldberg and Novoplansky 1997). Mediterranean environments in the Iberian Peninsula, which are water limited ecosystems, are characterised by summer droughts and high interannual variability of precipitation and temperature. In summer months, when temperature is favourable for growth, moisture is limiting; whereas in winter, when moisture is available, low temperature limits further growth (Mooney and Dunn 1970).

Scots Pine (*Pinus sylvestris* L.) is the pine species with the most extensive ecological area in the world (Blanco et al. 1997) and represents one of the most important dendroecological species in Europe (Schweingruber 1996). *P sylvestris* is one of the most important tree species in Spain due to both the area covered (1 280 000 ha) and the diverse functions that the woodlands have (Montero et al. 2008). Forests of *P. sylvestris* in Spain are the southern and western distribution threshold worldwide of that species (Barbéro et al. 1998) and 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).

The forecast of climate change impact on the diversity and distribution of European higher plants for the year 2050 pointed out that Spain could be one of the most vulnerable areas of species lost mainly due to climatic variability (Bakkenes et al. 2002). Previous studies in the Iberian Peninsula concluded that, under severe dry phenomena, woody species showed that shrubs evolved under Mediterranean climate conditions, were more damaged by the drought than the earlier evolved pre-

Mediterranean genera, mostly trees (Peñuelas et al. 2001), and among all pine species, *P. sylvestris* had the highest mortality rate under severe droughts in the 1990s (Martínez-Vilalta and Piñol 2002).

On the other hand, ecophysiological changes in trees relate to age (Bond 2000; Bond & Franklin 2002) also emphasises the importance of considering tree age in climate-growth responses analysis. Previous studies show contradictory results between tree age and growth-climate response, according to the species analysed: for example, no differences were found between young and old trees in *Pinus aristata* Engelm.(Fritts 1976) and *Larix lyalii* Parl. (Colenutt & Luckman 1991), but a higher climate effect was recorded on old trees than on young ones of *Larix decidua* and *Pinus cembra* in Italian Alps (Carrer & Urbinati 2004) and *Abies lasiocarpa* (Hook.) Nutt. in North America (Peterson & Peterson 1994). Previous dendroclimatological studies of *P. sylvestris* in the Iberian Peninsula concluded that this species is vulnerable to rainfall variability in the growing season (Guttierrez 1990; Richter et al. 1991; Fernández et al. 1996) but the authors did not set up the age effect on climate response.

Despite the fact that predictions of climate models still have uncertainties, one result appears clear: future climate may be characterised by greater extremes and, perhaps, more erratic fluctuations, with potentially strong effects on interannual to intraseasonal rainfall variability (Schiwinning et al. 2004). Consequently, it is essential to understand how different species may be impacted by temporal variations in water supply (Easterling et al. 2000). Furthermore, in arid and semiarid environments, it is critically important to interpret short-term responses of individuals and populations to precipitation (Chesson et al. 2004). Different responses of species to changes in intra-season recharge have been reported for grass and shrub (Jobbágy & Salas 2000; Oesterheld et al. 2001) and for annual species which grow in Mediterranean environments (Sher et al. 2004) but, to our knowledge, there are no previous studies on woody species related to the pulse-interpulse theory in Mediterranean environments in the Iberian Peninsula.

Due to in Mediterranean environments in the Iberian Peninsula, *P. sylvestris* grows under water limited conditions, dendroclimatological studies may provide valuable information about the association with climatic variables depending on the cambial age, the drought effect and the interannual water availability effect (pulse and interpulse) on radial growth.

The following questions were addressed: Which climatic variables are significant in radial growth of *Pinus sylvestris*? Does the cambial age affect the climate response? May be predicted radial growth according to drought events? Is it possible to predict growth periodicity relate to “pulse” and “interpulse” water events?

Material and methods

Study sites, field work and laboratory methods

Six sampling sites were selected in the distribution area of *Pinus sylvestris* in the Iberian Peninsula (Fig.1; Table 1). Three sites had individuals older than 100 years old and three sites had individuals younger than 100 years old. In the summer of 2006, two cores were extracted at 1.30 m above ground level from fifteen dominant and co-dominant trees at each sampling site. Cores were glued onto channelled wood, dried for two weeks and polished with progressively finer grade sandpaper. Tree rings were dated to establish correctly the calendar year in which a tree ring was formed. Dating was achieved using a binocular microscope following standard dendrochronological techniques (Stokes & Smiley 1968; Fritts 1976; Cook & Kairiukstis 1990). The transverse section cores were scanned at high resolution (2 000 dpi) with an Epson Expression 1640 XL scanner at 0.01-mm accuracy, and rings were measured using

WinDENDRO software (Regent Instrument Inc. 2002). 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's 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.

Climatic data

Monthly precipitation and mean monthly temperature, provided by the *Agencia Estatal de Meteorología* (National Meteorological Agency, Spain), were selected to assess the climate-growth relationship. Recorded meteorological data that varied from 61 to 71 yr pertain to four meteorological stations placed at less than 30 km of the sampling sites: *Miranda del Ebro (Burgos), Villafría (Burgos), Observatorio (Soria)* and *Aldea del Rey Niño (Avila)* (Table 2). The climate-diagrams of the Meteorological Stations are provided in Fig. 2. The HOM component (Homogeneity of Meteorological Data) of the Directory Program Library for Dendrochronology (Holmes 1983; available at www.ltrr.arizona.edu) was used to determine the homogeneity of the climatic variables.

Growth-climate relationship analysis

To eliminate biological trends in tree-ring series and to minimize growth variations that are not shared by most trees (Fritts 1976), the v6.05P ARSTAN program (Cook & Holmes 1984; Holmes 2001; available at www.ltrr.arizona.edu) was used. Standardisation 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, the standardised series were averaged. These temporal series or master chronologies expressed the annual variations in *P.sylvestris* radial growth at the population level in each sampling site.

Chronology quality was evaluated using mean sensitivity (MS), is the degree to which a tree reacts to environmental factors or the measure of the year-to-year variability (Schweingruber 1996); signal-to-noise ratio (SNR), the proportion of the variability explained by climate or other causal factors divided by the residual or unexplained variability (Fritts & Swetnam 1989); and expressed population signal (EPS), which describes how a finite sample estimates the hypothetical infinite population (Wigley et al. 1984; Briffa 1995). A chronology was considered useful when it had an EPS value of higher than 0.85.

To determine the climatic variables that control *P. sylvestris* radial growth, mean monthly temperature and monthly rainfall were compared to the local chronologies at each sampling site. The local chronologies were compared with the meteorological station closest to the analysed site. The period explored was from the previous June to September of the current growth year. The v 5.17 PRECON program (Fritts 1999; available at www.ltrr.arizona.edu) was used to compute the response functions of tree growth to climate by means of a multiple stepwise regression. Coefficients were considered significant at *$p<0.05$ and **$p<0.01$. A bootstrapped analysis was also applied to improve the robustness of the correlation coefficients. In this analysis, 1000 bootstrap interactions were made (Mooney and Duval 1993).

Drought index effect and pulse and interpulse analysis

The drought index (DRI) of Thornthwaite (1948) was applied to detect pulse-interpulse effect on radial growth of *Pinus sylvestris*. The DRI at each sampling site was calculated from the months that at the response function analysis showed a significant association between precipitation and radial growth (May and June at *Miñon*, *Oña* and *Arauzo de Miel* sites; June and July at *El Espinal* site and July and August at *Molino Piqueras* and *Amogable* sites).

Monthly DRI was calculated by the formula (1):

$$\text{(1)} \qquad DRI = P - PET$$

Where DRI is the drought index, P = monthly precipitation and PET = the potential evapotranspiration estimated from the monthly mean temperature and the geographical position of the meteorological station (Thornthwaite 1948).The Pearson's correlation coefficient was applied between DRI and tree ring width at each sampling site (Sokal and Rohlf 1995).

A time series analysis was performed to detect both autocorrelation between consecutive tree-ring index and periodicity in the radial growth fluctuation due to pulse and interpulse frequency (Box and Jenkins 1976). Tree-ring index autocorrelation of the six sampling sites was estimated using the autocorrelation function (ACF). The ACF provided information about the correlation between a specific year and the previous one. Coefficients statistically significant were those higher than the lines at a confidence level of 95%.The lag number changed according to the sampling site due to the fact that the series length was different. All lags were shorter than 25% of the total tree-ring series at each sampling site. Periodograms of chronologies were also calculated. The occurrence and periodicity of cycles was determined using the inverse of the peak of highest intensity which results from the spectral density due to the frequency. In order to eliminate the high peak in the lower frequency that may hide other cycles, the original series were transformed in a first order integrated series using a first difference transformation (Box and Jenkins 1976). The statistical analysis was performed using Infostat V.2 (Di Rienzo et al. 2002).

Results

The six chronologies had high SNR, EPS and percentage of the variance accounted for the first eigenvector. The descriptive statistics showed that mean sensitivity varied from

0.15 to 0.26 and standard deviation varied from 0.18 to 0.35, depending on the sampling site. SNR varied from 10.67 to 24.74 and EPS values varied from 0.91 to 0.96. All chronologies analysed had high SNR (values always over 10) and EPS (always over 0.91), while the variance accounted by the first eigenvector was over 35 % (Table 3). The association between radial growth and climatic variables (mean monthly temperature and monthly precipitation) at the *Miñón* and *Oña* sites showed that rainfall in the growing season (May and June) affects growth of this species positively. On the other hand, temperature showed a positive correlation in February and a negative correlation in October prior to the growing season (Fig.3). The association between radial growth and climatic variables at the *El Espinar* site showed that rainfall during the growing season affects growth of this species positively (June and July). High temperatures during the growing season limited growth (Fig. 3). At the *Molino Piqueras* site, a positive association with rainfall during the growing season was found (July and August); temperature showed a significant negative relationship with growth in September, and positive in February and April prior to the growing season (Fig.3). The *Amogable* site also showed a positive association with rainfall in the growing season (July) and with mean temperature in February. Temperature limited growth in October prior to the growing season (Fig.3). Finally, rainfall in May and June had a significant effect on growth at the *Arauzo de Miel* site (Fig. 3). The total variance explained by both variables ranged from 31.2% to 60.2%, according to the sampling site and the tree age (Fig.3). The *Amogable* site showed a higher variance than the other sites analysed, but the period studied was shorter than at the other sites [from 1960 to 2005 (Fig.3)]. The Pearson's correlation coefficient between tree-ring index and DRI showed a significant association in all sampling sites [* $p < 0.05$; **$p < 0.01$;***$p < 0.001$ (Table 4)] as an example two chronologies from an old stand (*Miñon* site) and a young stand

(*Molino Piqueras* site), showing that low tree-ring width coincides with negative DRI values, are presented in Fig. 4.

The ACF of the six chronologies performed to detect autocorrelation between successive tree-ring growth values suggested that autocorrelation coefficients in young stands were not significant (*Oña*, *Molino Piqueras* and *Amogable* sites). However, this association changed in old stands, where a significant association between current year tree growth with that of one, two or three years before was detected [*Miñon*, *El Espinar* and *Arauzo de Miel* sites (Fig. 5)].

The time series analysis used to detect periodicity in fluctuation in tree-ring growth relating to pulse and interpulse water availability events suggested that the strong intensity variation in precipitation between years determined a clear variation in tree growth showed by a periodicity of two or two and a half years (except for the *El Espinar* site) (Table 5 and Fig.6).

Discussion

The relationship between climate variables and radial growth has been identified in Scots pine stands in Spain using dendrochronological methods. *Pinus sylvestris* is a useful species in dendrochronological studies showing accurate statistical values that mean clear response to environmental causal factors. The six chronologies have high Mean sensitivity (MS), signal to noise ration (SNR), Expressed population signal (EPS) and percentage of the variance accounted for the first eigenvector, suggesting a strong common signal to related-climatic environmental factors. The mean sensitivity values are comparable with the 0.16 to 0.34 ones found for four different pine species growing in the Iberian Peninsula (Richter et al. 1991) and on two chronologies of *P. sylvestris* located on the Central Mountains of Spain (MS from 0.13 to 0.20; Fernández et al. 1996).

Expressed population signal (EPS) values were higher than 0.85 suggesting that these chronologies describe the infinite, hypothetical population of *P.sylvestris* at each sampling site well enough (Wigley et al. 1984). Signal-to-noise ratio (SNR) values suggest that the proportion of explainable variation due to climate or other causal factors divided by the unexplainable variation or residual is high enough in all the sampling sites. The first eigenvector variance indicated good homogeneity inside the same site which is comparable with the 35 to 64% values found in four pines species studied in Spain (Richter et al. 1991).

The association between growth and climate shows that rainfall in the growing season has a positive significant effect on radial growth among all sites, no matter what their altitudinal location, geographical position or cambial age are. Differences in rainfall effect within the growing season were found: at sites located at the lowest altitudinal position (*Miñon, Oña* and *Arauzo de Miel*), this effect happened in spring (May and June); in contrast, with sites placed at a higher altitudinal position, the association occurred in late spring and early summer [June and July (*El Espinar* site)] and summer [July and August (*Molino Piqueras* and *Amogable* sites)]. This different association may be due to temperature. Sites located at the lowest altitudinal positions are warmer; consequently, the growing season starts early, and the spring rainfall is a driving factor that affects growth. However, sites located at the highest altitudinal position are colder, the growing season starts later and growth depends on summer rainfall. These results emphasised how important the within-season precipitation dynamic is; it can be equal to or more important than the seasonal or annual total for plant growth (Fay et al. 2000; Knapp et al. 2002).

These results also complement previous studies that emphasised the essential effect of precipitation on radial growth of different *Pinus s*pecies growing in the Iberian

Peninsula under Mediterranean climatic conditions as, for example, for *Pinus nigra* Arnold, *Pinus halepensis* Mill. and *Pinus pinaster* Ait. (Génova 1994; Fernández et al. 1996; Raventós et al. 2001; Martín-Benito et al. 2008; Bogino & Bravo 2008).

Previous studies in Spain suggest both the decrease in rainfall and the increase in mean temperature have to be considered as the most limiting factors that affect growth of the Scots pine (Gutierrez 1989) and previous studies on four pine species, including *P. sylvestris*, analysed together also emphasised the positive impact of winter temperatures (December and February) on radial growth (Richter et al., 1991). Additionally, one study in the Central Pyrenees for the 1952 – 1993 period also pointed out the positive impact of temperatures (November and May) on radial growth of *P. sylvestris* (Tardif et al. 2002). In this study, the importance of rainfall was found through all the analysed sites, but mean temperature effect changed according to the site: from positive in winter prior to the growing season (*Miñón, Oña, Molino Piqueras* and *Amogable* sites) to negative both in fall prior to the growing season (*Miñón, Oña, Molino Piqueras* and *Amogable* sites) and in summer in the growing season (*El Espinar* and *Amogable* sites).

Consequently, it is inferred that the local growth pattern of this species is the result of a changing association with mean temperature according to the site analysed. Previous studies suggest that species growing in temperate areas (Tessier et al. 1994; Dittmar et al. 2003; Pederson et al. 2004) could not have a common climatic response and *P. sylvestris* in Spain has a variable response to climatic variables depending on the sampling site (Gutierrez 1989).

Age-dependent climate response showed that young stands have higher variability explained by climatic variables in the response function analysis (from 53 to 69%) and are not vulnerable to previous growth; in contrast, old stands have a lower percentage of the variability explained by climatic variables (from 31 to 39%) but are vulnerable to previous growth. Ecophisiological changes relate to tree age emphasised a reduction in photosynthesis and in stomatal conductance, changes in leaf structures and in canopy structure (Bond 2002, Bond & Franklin 2002) that may imply a variable association between radial growth and climate according to the age. *Pinus ponderosa* Douglas ex. C. Lawson in Oregon showed that when trees get older the water storage capacity in stems increases which provided a buffer against short-term water stress (Anthoni et al. 2002) and may imply, as in our results, a lower vulnerability to climatic conditions in old trees. On the other hand, the reaction of a tree to a drought period is a lower needle amount as a result of increased needle loss (Rebertez & Dobbertin 2004), which may involve a longer recovery period for old trees than young ones and consequently, a correlation between subsequent tree ring.

The significant association between radial growth and DRI in the growing season suggests that this index is an accurate tool to predict restrictions in *P. sylvestris* radial growth. The DRI accuracy to estimate growth restrictions and mortality of *P.sylvestris* had been applied in Switzerland with similar correlation coefficient to our sampling sites (Bigler et al. 2006).

The chance to estimate the impact of drought events on growth of *P.sylvestris* becomes important considering that previous studies in Spain concluded that among all pine species, *P. sylvestris* had the highest mortality rate under severe droughts (Martínez-Vilalta and Piñol 2002) and in Switzerland drought processes are considered as a major factor of dead increase of *P. sylvestris* (Eilmman et al. 2006). Considering a higher precipitation variability in the future and that the mean annual temperature has increased 1.6ºC in the last century in the Iberian Peninsula (IPCC 2007) the applications of these results in models to predict future growth may be essential to corroborate the forecast of climate change that suggest that Spain could be one of the most vulnerable areas of species lost mainly due to climatic variability (Bakkenes et al. 2002).

When growth periodicity is analysed to determine “pulse” and “interpulse” periods in *P. sylvestris* growth, it is clear that the strong precipitation variability and the significant effect that this climatic variable has on tree-ring growth result in a strong variability between successive years that is reflected as periods of two or two and a half years in the time series analysis. Even though, depending on the sampling site, other cycle periods were detected, two-year periodicity is stronger than other long-period cycles.

Conclusions

Pinus sylvestris shows a relationship that changes depending on the climatic variable analysed: rainfall in the growing season is the driving climatic variable that controls growth in all the sites analysed, while the association with temperature changes according to the site and could be positive or negative. Results that relate cambial age and climate suggest the importance of considering this variable when the growth climate association is analysed. Drought is a commanding factor affecting growth and should be considered in models that forecast the climate change impact in Mediterranean environments.

Acknowledgements

The authors thank Cristotal Ordóñez for field assistance. Thanks are also extended to Dr. Mariano Morales, Esteban Jobbagy and Javier Gyenge for their useful comments on the manuscript, to for improving the English version and to the *Agencia Estatal de Meteorología* for providing meteorological data. This study was supported by Research projects from the Regional *Castilla y León* government (Spain) (Project Code VA096A05), the Spanish Ministry of Science and Education (Project Code AGL2007-

65795-C01-01) and by the Alβan Programme, the European Union Programme of High Level Scholarships for Latin America (Scholarship No. E05D049920AR).

References

Andreu, L., Gutiérrez, E., Macias, M., Ribas, M., Bosch, O. & Camarero, J. 2007. Climate increases regional tree-growth variability in Iberian pine forest. *Global Change Biology* 13, 804-815.

Bakkenes, M., Alkemade, J.R.M., Ihle, F., Leemansand, R. & Latour, J.B. 2002. Assessing effects of forecasted climate change on the diversity and distribution of European higher plants for 2050. *Global Change Biology* 8, 390-407.

Barbéro, M., Loisel, R.& Quezel, P. 1998. Pines of the Mediterranean Basin. In: Richardson D.M. (ed.). *Ecology and Biogeography of Pinus,* pp. 153-170. Cambridge University Press, Cambridge, UK.

Bigler, C., Ulrich Bräker, O., Bugmann, H., Dobbertin, M. & Rigling, A. 2006. Drought as an Inciting Mortality Factor in Scots Pine Stands of the Valais, Switzerland. *Ecosystems* 9, 330-343.

Blanco, E., Casado, M., Costa, M., Escribano, R., García Antón, M., Génova, M., Gómez Manzaneque, G., Gómez Manzanaque, F., Moreno, J., Morla, C., Regato, P. & Sainz Ollero, H. 1997. *Los bosques ibéricos*. Editorial Planeta, Barcelona, España.

Box, G.E.P. & Jenkins, G.M. 1976. *Time Series Analysis, Forecasting and Control, Revised edn*. Holden-Day.San Francisco.CA.

Bogino, S. & Bravo, F. 2008. Growth response of *Pinus pinaster* Ait. to climatic variables in central Spanish forests. *Annals of Forest Science* 65, 506-518.

Bond, B. 2000. Age-related changes in photosynthesis of woody plants. *Trends in plant science* 5, 349-353.

Bond, B. & Franklin, J. 2002. Aging in Pacific Northwest forests: a selection of recent research. *Tree Physiology* 22, 73-76.

Briffa, K.R. 1995. Interpreting high-resolution proxy climate data-the example of dendroclimatology. In: von Storch, H. & Navarra, A. (eds.) *Analysis of climate data variability, application of statistical techniques*, pp.77–94. Berlin, Springer Verlag.

Carrer, M.& Urbinatti, C. 2004. Age-dependent tree-ring growth responses to climate in *Larix decidua* and *Pinus Cembra. Ecology* 85, 730-740.

Catalán, G. 1991. *Las Regiones de Procedencia de Pinus sylvestris L. y Pinus nigra Arn. Subsp. Salzmannii (Dunal) Franco en España.* ICONA, Madrid.

Chesson, P., Gebauer, R., Schwinning, S., Huntly, N., Wiegand, K., Ernest, M., Sher, A., Novoplansky, A. & Weltzin, J. 2004. Resource pulses, species interactions and diversity maintenance in arid and semi-arid environments. *Oecologia* 141, 236-253.

Colenutt, M. & Luckman, B. 1991.The dendrochronological characteristics of alpine larch. *Canadian Journal of Forest Research* 25, 777-789.

Cook, E.R. & Holmes, R.L. 1984. *Program ARSTAN users manual.* Lab. Tree Ring. Res. Univ. of Arizona, Tucson, Arizona, EUA.

Cook, E.R. & Kairiukstis, L.A. 1990. *Methods of Dendrochronology: applications in the environmental sciences*. Kluwer, Dordrecht.

Di Rienzo, J., Balzarini, M., Casanoves, F., González, L., Tablada, E. & Robledo, C. 2002. *Infostat Software Estadístico versión 2.* Grupo infoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Dittmar, C., Zech, W. & Elling, W. 2003. Growth variations of common beech (*Fagus sylvatica* L.) under different climatic and environmental conditions in Europe-a dendroecological study. *Forest Ecology and Management* 173, 63-78.

Easterling, D.R., Meehl, G.A., Parmesan, C., Changnon, S.A., Karl, T.R. & Mearns, L.O. 2000. Climate extremes: observation, modelling, and impact. *Science* 289, 2068-2074.

Fay, P.A., Carlisle, J.D., Knapp, A.K., Blair, J.M. & Collins, S.L. 2000. Altering rainfall timing and quantity in a mesic grassland ecosystem: design and performance of rainfall manipulation shelters. *Ecosystems* 3, 308-319.

Fernández, A., Génova, M., Creus, J. & Gutiérrez, E. 1996. Dendroclimatological investigation covering the last 300 years in Central Spain. Dean, J. Meko, D. & Swetnam, T. (eds) *Tree Rings, Environments and Humanity*, pp 181-190. Radiocarbon, U. of Arizona; Tucson, USA:

Fritts, H.C. 1976. *Tree Ring and Climate*, Academic Press Inc, London.

Fritts, H.C. 1999. PRECON version 5.17, http://www.arizona.edu/webhome/hal/dlprecon.html.

Fritts, H.C. & Swetnam, T. 1989. Dendroecology: a tool for evaluating variations in past and present forest environments. *Advances in Ecological Research*19, 111-188.

Génova, M. 1994. *Dendroecología de Pinus nigra Arnold subsp. salzmannii (Dunal) Franco y Pinus sylvestris L. en el Sistema Central y en la serranía de Cuenca (España).* Tesis doctoral. Departamento de Biología. Universidad Autónoma de Madrid.

Goldberg, D. & Novoplansky, A. 1997. On the relative importance of competition in unproductive environments. *Journal of Ecology* 85, 409-418.

Grissino-Mayer, H.D. 2001. Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. *Tree-Ring Research* 57, 205-221.

Gutierrez, E. 1989. Dendroclimatological study of *Pinus sylvestris* L. in southern Catalonia (Spain). *Tree-ring Bulletin* 49, 1-9.

Gutiérrez, E. 1990. Dendroecología de *Pinus sylvestris* L.en Catalunia. *Orsis* 5, 23-41.

Holmes, R.L. 1983. Computer-assisted quality control in tree-ring dating and measurement. *Tree-ring Bulletin* 43, 69-78.

Holmes, R.L. 2001. *Dendrochronology Program Library.* Available from the Laboratory of Tree-ring Research, University of Arizona, Tucson, USA.

IPCC. 2007. *Fourth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jobbágy, E. & Sala, O. 2000. Controls of grass and shrub aboveground production in the Patagonian steppe. *Ecological Applications* 10, 541-549.

Knapp, A.K., Fay, P.A., Blair, J.M., Collins, S.L., Smith, M.D., Carlisle, J.D., Harper, C.W., Danner, B.T., Lett, M.S. & Mc Carron, J.K. 2002. Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science* 298, 2202-2205.

Martín Benito, D., Cherubini, P., del Río, M. & Cañellas, I. 2008. Growth response to climate and drought in *Pinus nigra* Arn. trees of different crown classes. *Trees* 22, 263-273.

Martínez-Vilalta, J. & Piñol, J. 2002. Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. *Forest Ecology and Management* 161, 247-256.

Montero, G., del Río, M., Roig, S. & Rojo, A. 2008. Silvicultura de *Pinus sylvestris* L. In: Serrada, R., Montero, G. & Reque, J. (eds.) *Compendio de Selvicultura Aplicada en España*, pp. 503-534. INIA, Ministerio de Educación y Ciencia, España.

Mooney, H. & Dunn, L. 1970. Convergent Evolution of Mediterranean-Climate evergreen sclerophyll shrubs. *Evolution* 2, 292-303.

Mooney, C.Z. & Duval, R.D. 1993. *Bootstrapping: a nonparametric approach to statistical inference.* Sage University Paper series on Quantitative Applications in the Social Sciences, 07-095. Sage, Newbury Park.

Noy-Meir, I. 1973. Desert ecosystems: environment and producers. *Annual Reviews in Ecology and Systematics* 4, 25-41.

Oesterheld, M., Loreti, J., Semmartin, M. & Sala, O. 2001 Inter-annual variation in primary production of a semi-arid grassland related to previous-year production. *Journal of Vegetation Science* 12, 137-141.

Pederson, N., Cook, E., Jacoby, G., Peteet, D. & Griffin, K. 2004. The influence of winter temperature on the annual radial growth of six northern range margin tree species. *Dendrochronologia* 22, 7-29.

Peterson, D. W & Peterson D.L. 1994. Effects of climate on radial growth of subalpine conifers in the North Cascade Mountains. *Canadian Journal of Forest Research* 24, 1921-1932.

Peñuelas, J., Lloret, F. & Montoya, R. 2001. Severe drought effects on Mediterranean Woody Flora in Spain. *Forest Science* 47, 214-218.

Raventós, J., De Luís, M., Gras, M., Cufar, K., González-Hidalgo, J., Bonet, A. & Sánchez, J. 2001. Growth of *Pinus pinea* and *Pinus halepensis* as affected by dryness, marine spray and land use changes in a Mediterranean semiarid ecosystem. *Dendrochronologia* 19, 211-220.

Rebetez, M. & Dobbertin, M. 2004. Climate change may already threaten Scots pine stands in the Swiss Alps. *Theoretical and Applied Climatology*. 79, 1-9.

Regent Instrument Inc. 2002. Windendro ™ v.2002a. Québec, Qc.

Richter, K., Eckstein, D. & Holmes, R.L. 1991. The dendrochronological signal of pine trees (*Pinus* spp.) in Spain. *Tree-Ring Bulletin* 51, 1-13.

Schweingruber, F. 1996. *Tree rings and environment: Dendroecology*. Haupt, Berne.

Schwinning, S., Sala, O., Loik, M. & Ehleringer, J. 2004. Thresholds, memory, and seasonality: understanding pulse dynamics in arid/semi-arid ecosystems. *Oecologia* 141, 191-193.

Sher, A., Goldberg, D. & Novoplansky, A. 2004. The effect of mean and variance in resource supply on survival of annuals from Mediterranean and desert environments. *Oecologia* 141, 353-362.

Sokal, R.R. & Rohlf, F.J. 1995. *Biometry: the principles and practice of statistics in biological research.* 3rd ed. WH Freeman and Co., New York, USA.

Stokes, M. & Smiley, T.1968. *An Introduction to Tree-Ring Dating*. University of Arizona Press, Tucson, UEA.

Tardif, J., Camarero, J., Ribas, M. & Gutiérrez, E. 2002. Spatiotemporal variability in tree growth in the central Pyrenees: climatic and site influences. *Ecological Monographs* 73, 241-257.

Tessier, L., Nola, P. & Serre-Bachet, F. 1994. Deciduous *Quercus* in the Mediterranean region: tree ring/climate relationship. *New Phytologist*. 126, 355-357.

Tessier, L., Guibal, F. & Schweingruber, F. 1997. Research strategies in dendroecology and dendroclimatology in mountain environments. *Climatic Change* 36, 499-517.

Thornthwaite, CW. 1948. An approach toward a rational classification of climate. Geographical Review 38, 55-94.

Wigley, T.M.L., Briffa, K.R. & Jones, P.D. 1984. On the average value of correlated time series, with applications in dendroclimatology and hydrometeorology. *Journal of Applied Meteorology* 23, 201-213.

Table 1. Geographical position of six sampling sites of *Pinus sylvestris* in the Iberian Peninsula.

Site name	Code	Latitude N	Longitude W	Altitude (m.a.s.l.)
Miñón	MIN	42º 54' 46”	03º 21' 27”	860
Oña	OÑA	42º58' 22”	03º 18' 12”	760
El Espinar	ESP	40º 38' 58”	04º 12' 07”	1426
Molino Piqueras	MOL	42º 04' 36”	02º 30' 18”	1676
El Amogable	AMO	41º 50' 44”	02º 55' 48”	1134
Arauzo de Miel	ARA	41º 53' 04”	03º 21' 32”	1081

Table 2. Meteorological data used in this study. Rainfall: Annual precipitation; Temp.: Mean annual temperature; Site: Code of the sampling sites related to the meteorological station; Period: Time with data available (*Agencia Estatal de Meteorología*, Spain).

Meterological station	Latitude	Longitude	Altitude (m)	Rainfall (mm)	Temp.(ºC)	Site	Period
Miranda del Ebro (Burgos)	42º 40' 42”	02º 57' 20”	520	529.97	12.08	MIN - OÑA	1936-2005
Villafría (Burgos)	42º 21' 22”	03º 37' 57”	890	564.67	10.15	ARA	1943-2005
Observatorio (Soria)	41º 46' 00”	02º 28' 00”	1082	529.85	10.59	MOL - AMO	1944-2005
Aldea del rey Niño (Avila)	41º 34' 35	04º 42' 02"	1160	522.24	9.17	ESP	1935-2005

Table 3. Descriptive statistic of the six chronologies of *Pinus sylvestris* in Spain. SD: standard deviation; MS: mean sensitivity; SNR: Signal to noise ratio; EPS: Expressed population signals. Var.: Variance in first eigenvector and Mean. Corr.: Mean correlation among trees.

	MIN	OÑA	ESP	MOL	AMO	ARA
Time span	1867-2005	1932-2005	1811-2005	1945-2005	1949-2005	1846-2005
Core number	29	28	29	30	30	24
Ring number	2956	1845	4803	1528	1538	4490
Age range	81-140	55-74	99-195	40-61	46-57	127-160
SD	0.28	0.18	0.24	0.35	0.19	0.30
MS	0.26	0.20	0.19	0.25	0.15	0.18
SNR	18.82	20.13	19.89	24.74	15.30	10.67
EPS	0.95	0.95	0.95	0.96	0.93	0.91
Var.	43.17	46.41	45.95	54.99	38.23	35.88
Cor.	0.40	0.43	0.43	0.51	0.34	0.30

Table 4. Pearson’s correlation coefficient between tree-ring index of *Pinus sylvestris* and DRI (May and June at *Miñon*, *Oña* and *Arauzo de Miel* sites; June and July at *El Espinal* site and July and August at *Molino Piqueras* and *Amogable* sites) (* $p < 0.05$; **$p < 0.01$;***$p < 0.001$).

	Period	**Correlation**
MIN	1936-2005	0.47***
OÑA	1936-2005	0.31**
ESP	1944-2005	0.31*
MOL	1944-2005	0.56***
AMO	1935-2005	0.46**
ARA	1935-2005	0.43***

Table 5. Periodicity of the six chronologies of *Pinus sylvestris* in central Spain. The period analysed and the intensity of the frequency are also included.

Site	**Analysed Period**	**Periodicity**	**Intensity**
Miñón	1867 – 2005	2.57	758 535
Oña	1932 – 2005	2.55	408 236
El Espinal	1811 – 2005	5.73	973 318
Molino Piqueras	1945 – 2005	2.44	787 462
Amogable	1949 – 2005	2.35	404 511
Arauzo de Miel	1846 – 2005	2.34	841 498

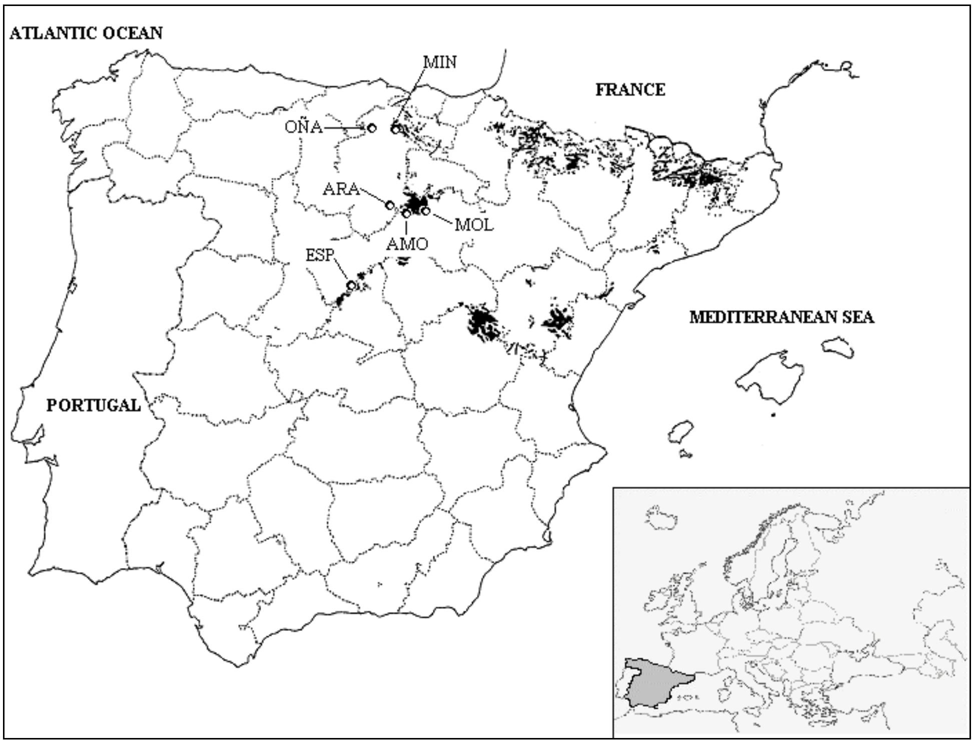
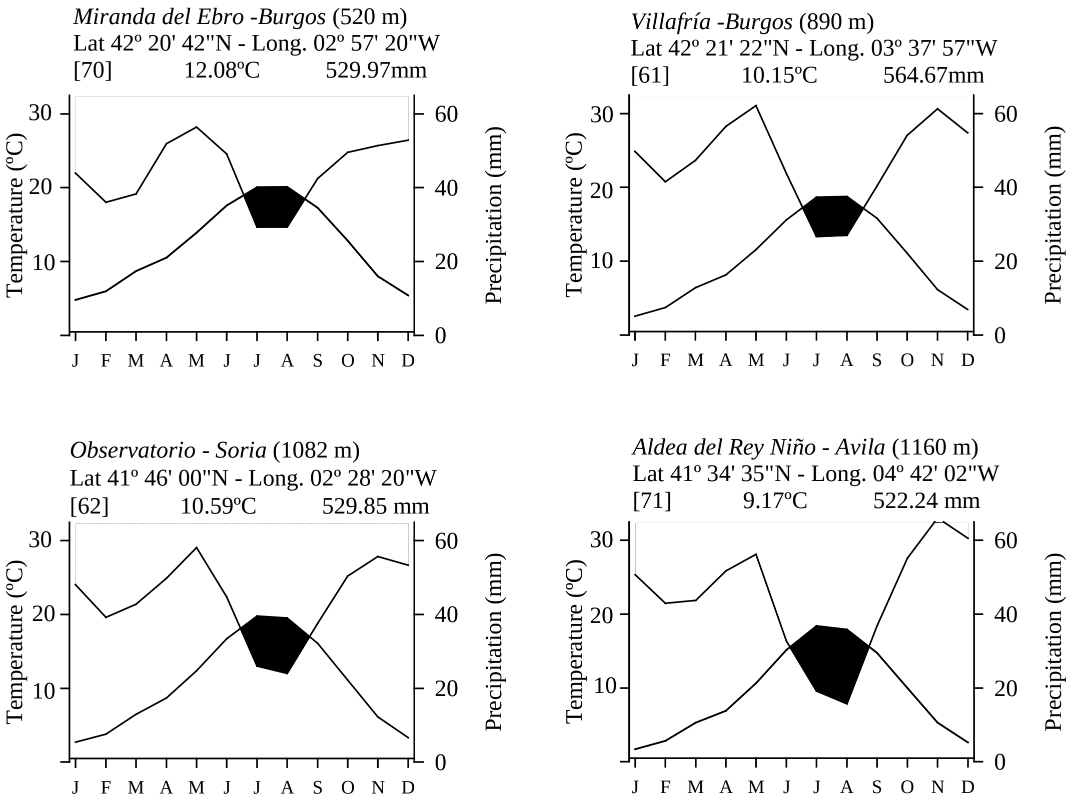


Fig.1. Sampling sites set up across the natural distribution area of *Pinus sylvestris* woodlands in Spain [Shaded area (Catalán 1991)]. Site codes: MIN: *Miñón*; OÑA: *Oña*; ESP: *El Espinar*; MOL: *Molinos Piqueras*; AMO: *Amogable*; ARA: *Arauzo*.



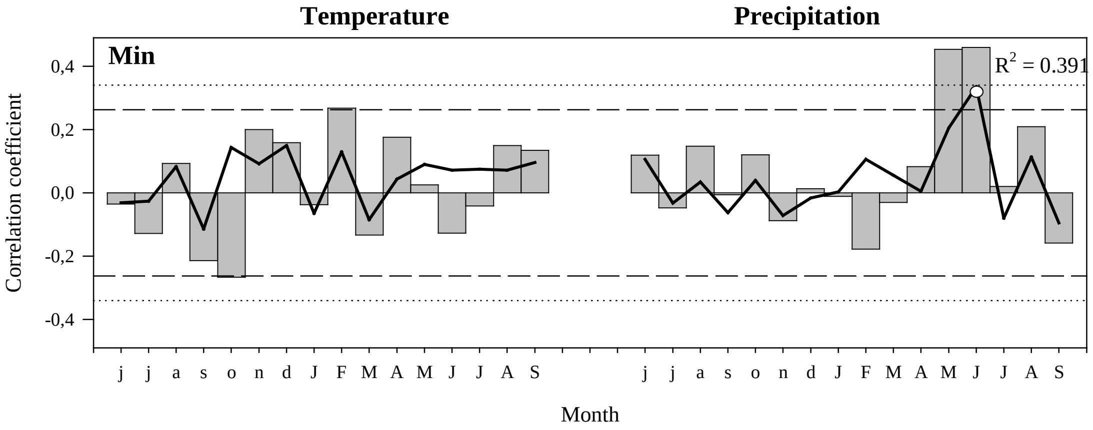
Miranda del Ebro -Burgos (520 m)
Lat 42º 20' 42''N - Long. 02º 57' 20''W
[70] 12.08ºC 529.97mm

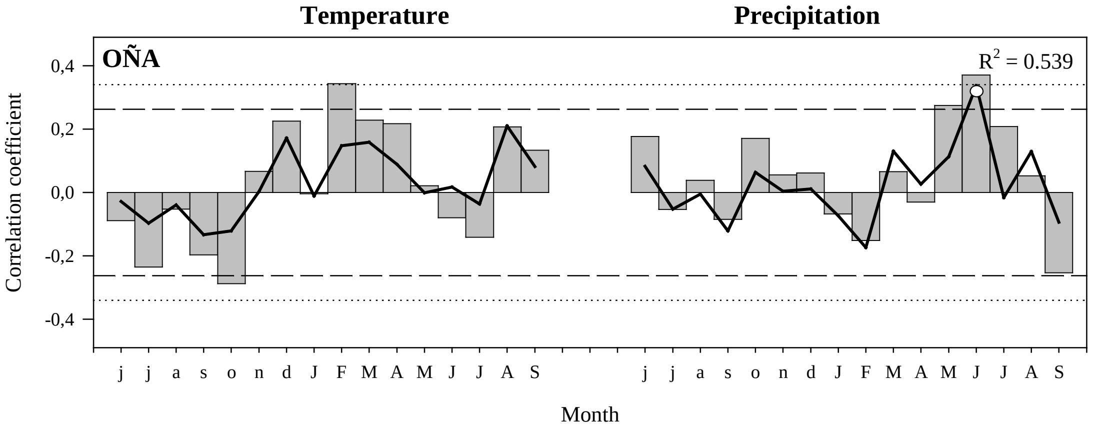
Villafría -Burgos (890 m)
Lat 42º 21' 22''N - Long. 03º 37' 57''W
[61] 10.15ºC 564.67mm

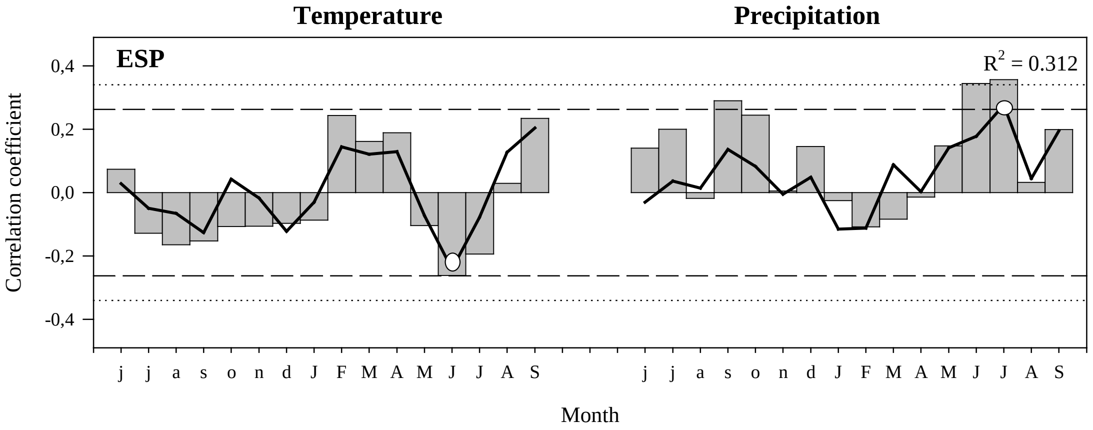
Observatorio - Soria (1082 m)
Lat 41º 46' 00''N - Long. 02º 28' 20''W
[62] 10.59ºC 529.85 mm

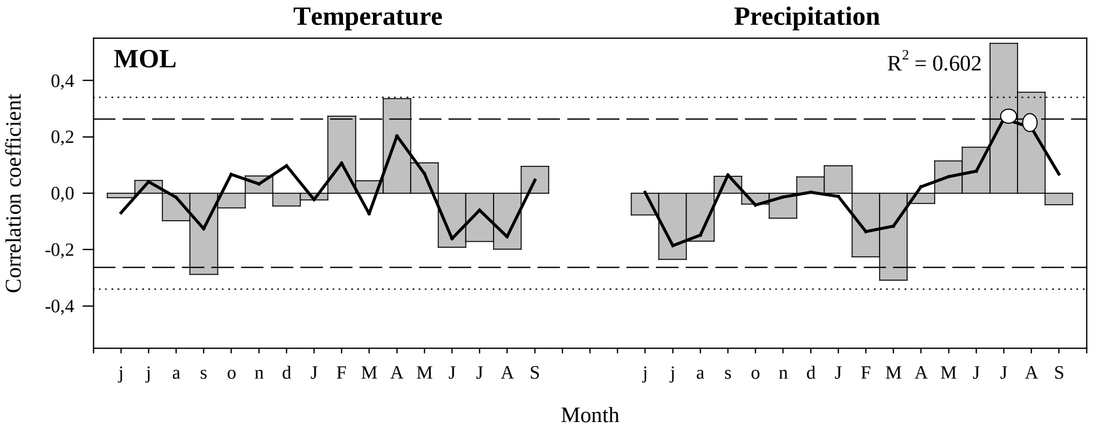
Aldea del Rey Niño - Avila (1160 m)
Lat 41º 34' 35''N - Long. 04º 42' 02''W
[71] 9.17ºC 522.24 mm

Fig. 2. Climate diagrams of *Miranda del Ebro (Burgos), Villafría (Burgos), Observatorio (Soria)* and *Aldea del Rey Niño (Avila)* meteorological stations.


Temperature
Precipitation
Min
R^2 = 0.391
Correlation coefficient
0,4
0,2
0,0
-0,2
-0,4
j j a s o n d J F M A M J J A S
j j a s o n d J F M A M J J A S
Month


Temperature
Precipitation
OÑA
R^2 = 0.539
Correlation coefficient
0,4
0,2
0,0
-0,2
-0,4
j j a s o n d J F M A M J J A S
j j a s o n d J F M A M J J A S
Month


Temperature
Precipitation
ESP
R^2 = 0.312
Correlation coefficient
0,4
0,2
0,0
-0,2
-0,4
j j a s o n d J F M A M J J A S
j j a s o n d J F M A M J J A S
Month


Temperature
Precipitation
MOL
R^2 = 0.602
Correlation coefficient
0,4
0,2
0,0
-0,2
-0,4
j j a s o n d J F M A M J J A S
j j a s o n d J F M A M J J A S
Month

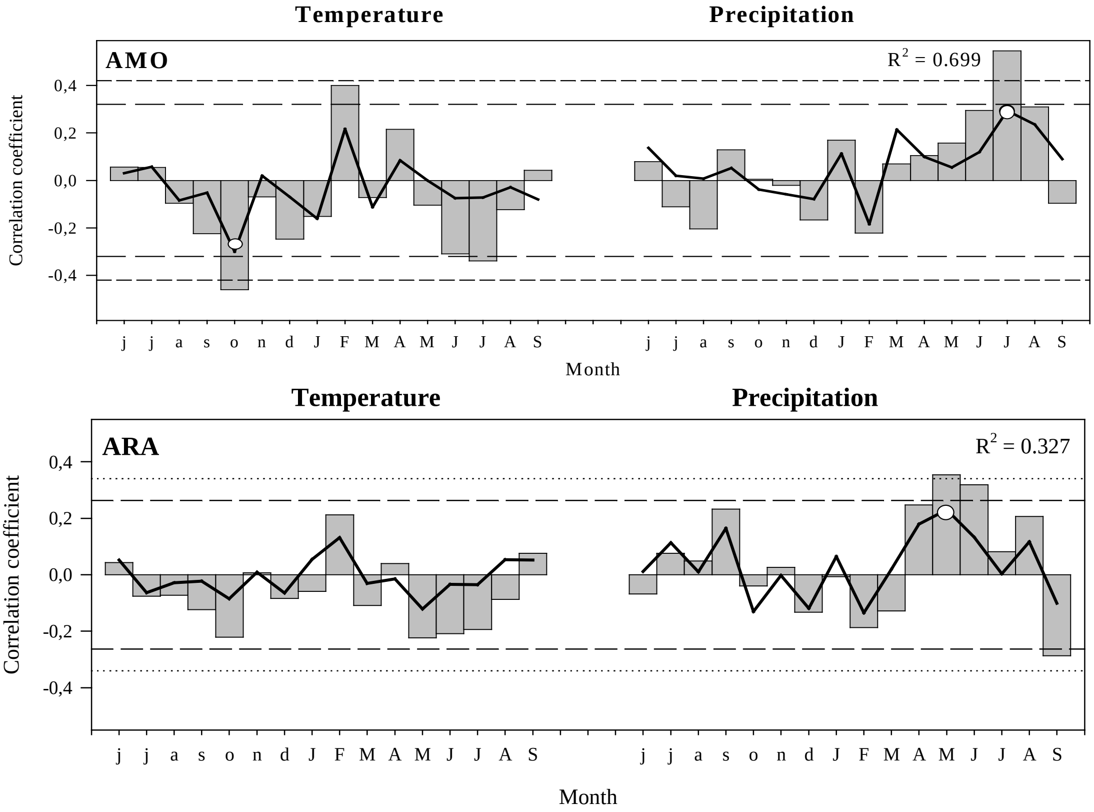


Fig. 3. Correlation coefficients (bars) and bootstrapped response function (lines) that relate the effect of regional climatic variables (mean monthly temperature - monthly precipitation) and growth of *Pinus sylvestris* during the 1950-2005 period at *Miñon, Oña; El Espinar, Molino Piqueras and Arauzo de Miel* sites. *Amogable* site was analysed from 1960-2005. The analysed period is from June of the previous growing season to September of the current growing season. Bars higher than the dashed lines show a significant coefficient at $p < 0.05$. Bars higher than the dotted lines show a significant coefficient at $p < 0.01$. White circles indicate the months where the bootstrapped response function coefficients are significant at $p < 0.05$. R^2 values show the total variance explained by both variables. Lower case letters indicate the months prior to the growing season. Upper case letters indicate the growing season months.

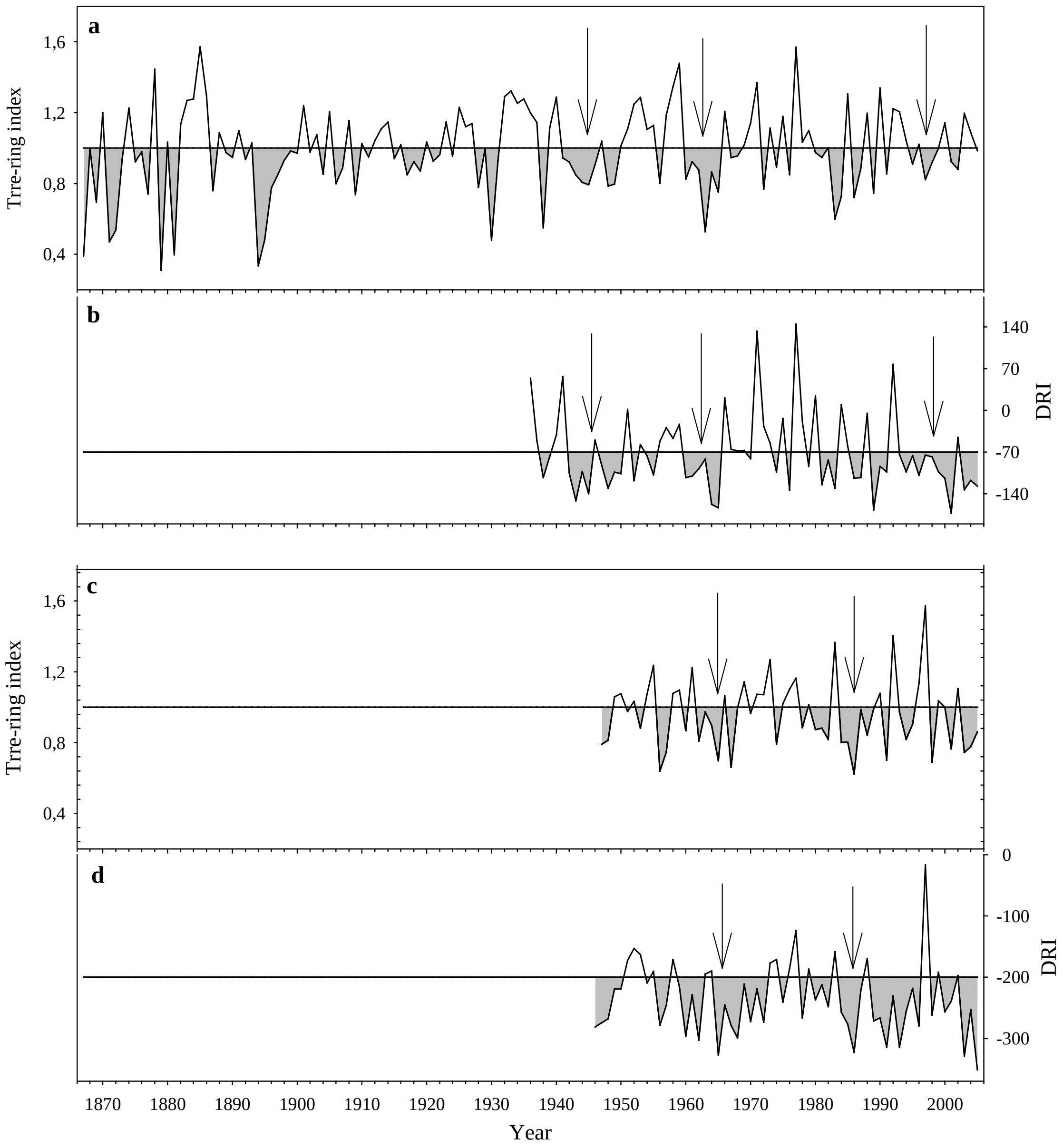


Fig. 4. Radial growth index of *Pinus sylvestris* at the *Miñon* site (a); May and June DRI from the *Miranda del Ebro* meteorological station (b) and radial growth index at the *Molino Piqueras* site (c); and July and August DRI from *Observatorio, Soria* meterological station (d). Arrows indicates low tree-ring growth that coincides with low DRI values.

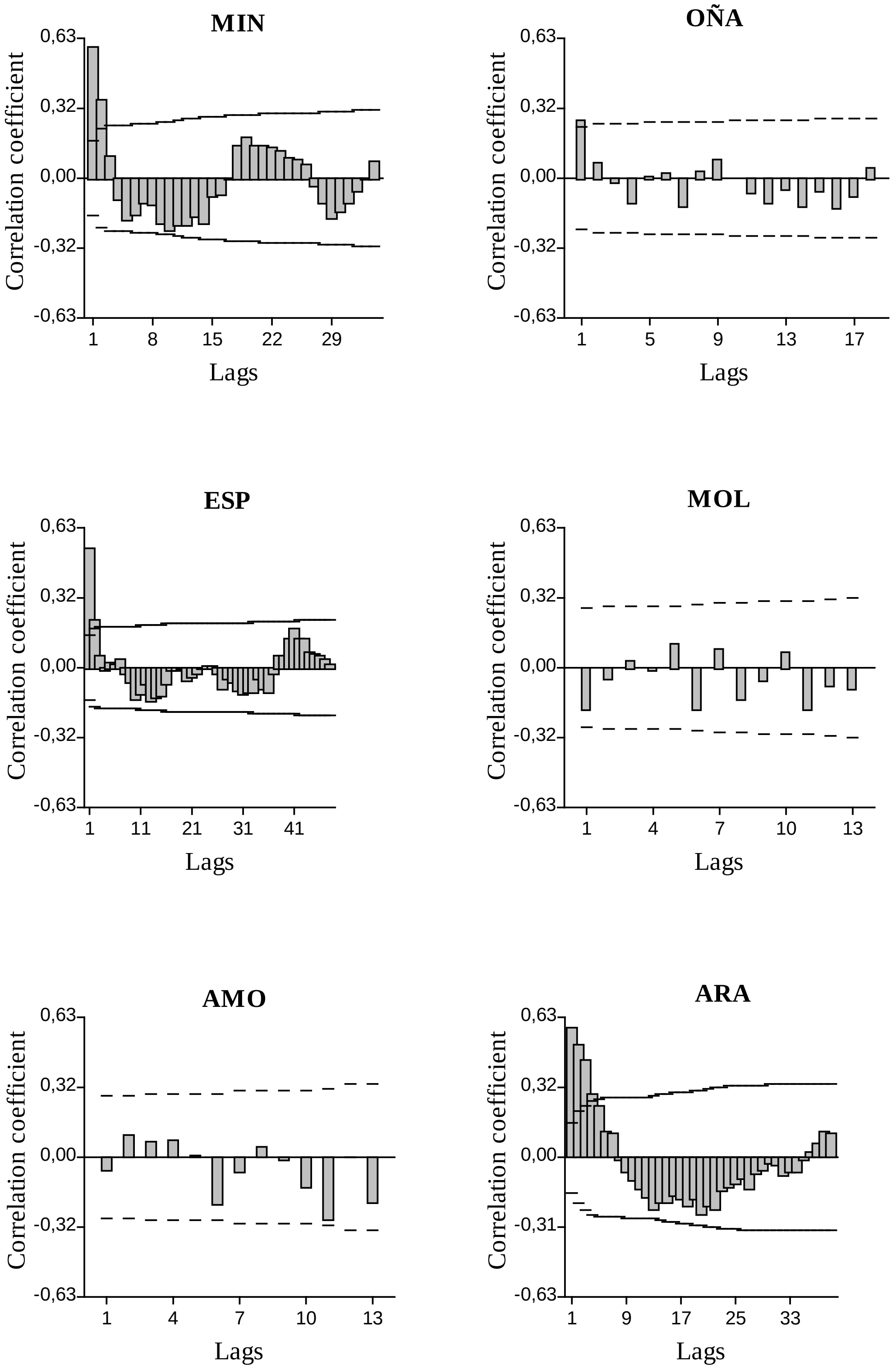


Fig. 5. Autocorrelation function between tree-ring growth at the six sampling sites of *Pinus sylvestris* in the Iberian Peninsula. Bars higher than the horizontal lines showed autocorrelation values statistically significant at a 95% confidence level.

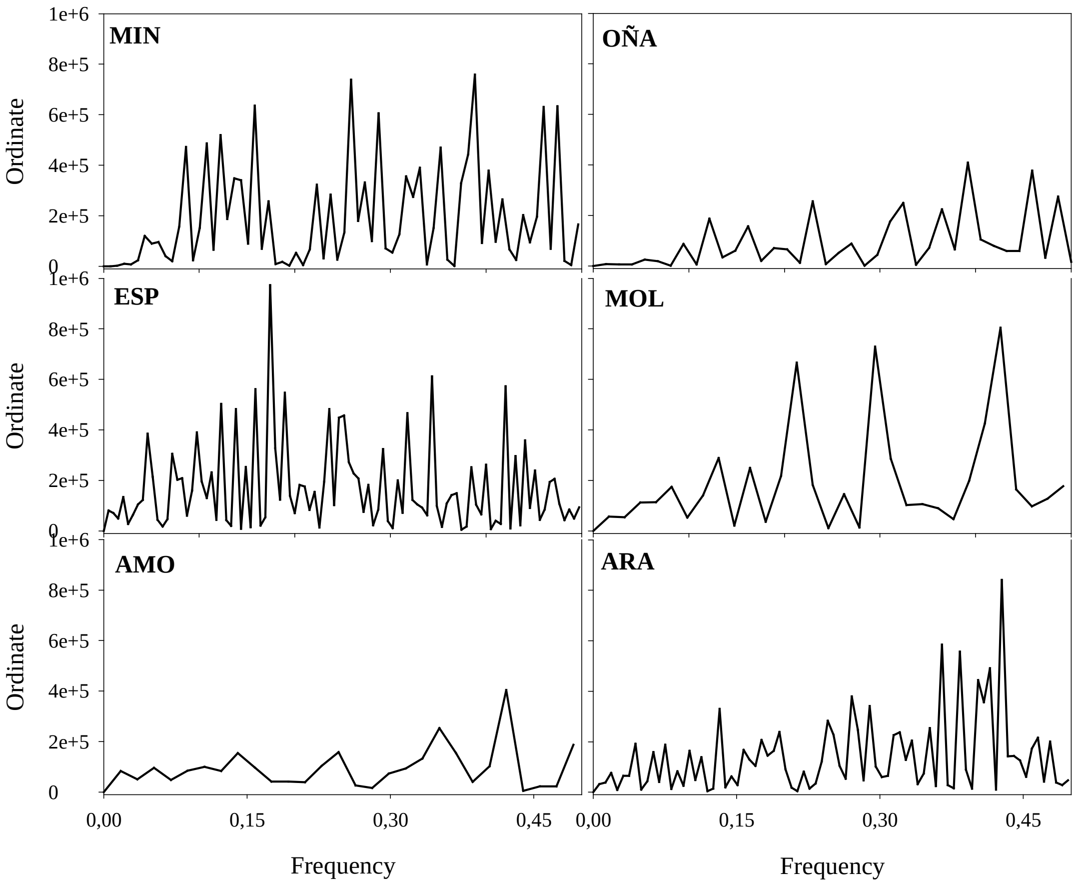


Fig. 6. Periodogram of the radial growth index fluctuation of *Pinus sylvestris* at six sampling sites in the Iberian Peninsula.

IV

This article was submitted in Canadian Journal of Forest Research. Bogino S., Bravo F. Climate and intra-annual density fluctuations in Pinus pinaster in Spanish woodlands. Canadian Journal of Forest Research.

Climate and intra-annual density fluctuations in *Pinus pinaster* in Spanish woodlands

RESUMEN

Las características anatómicas de fluctuaciones intra anuales de densidad (FIAD) de los anillos de crecimiento, los diferentes tipos de FIADs de acuerdo con la edad, los cambios en la densidad en el último siglo y de cómo esos cambios de densidad están asociados a las variables climáticas, se analizaron en *Pinus pinaster* Ait. en España.

Se seleccionaron cinco sitios de muestreo en el área natural de distribución de *P. pinaster* en la Península Ibérica. Se extrajeron dos muestras de barreno a 1,30 metros del nivel del suelo de quince árboles dominantes y co-dominantes en cada sitio de muestreo. Las muestras se prepararon de acuerdo con técnicas dendrocronológicas y luego se dataron y se estabilizaron las FIADs. Para el análisis estadístico se aplicó el análisis de la varianza (ANAVA), el coeficiente de correlación de Pearson y la función logística. Los resultados mostraron: 1) Nueve características anatómicas diferentes de FIADs; 2) Un incremento en al frecuencia de FIADs desde el año 1940 hasta el presente; 3) Que la frecuencia de FIADs fue más alta en los árboles jóvenes que en los viejos; 4) Se determinó una correlación negativa entre el crecimiento radial y los cambios de densidad y 5) Las fluctuaciones de la densidad se podrían predecir con el uso de una función logística que tiene a la lluvia mensual y a la temperatura media mensual como variables independientes. Los estudios de cambios intra-anuales o anomalías en el crecimiento radial podrían ser muy útiles para aplicaciones ecológicas y climatológicas bajo condiciones de cambio climático

Climate and intra-annual density fluctuations in *Pinus pinaster* in Spanish woodlands

Stella Bogino[1,2], Felipe Bravo[1]

[1] Joint Unit UVa-INIA ‘Sustainble Forest Management’. Departamento de Producción Vegetal y Recursos Forestales, Universidad de Valladolid, Avda. de Madrid 44, 34004 Palencia, Spain.

[2] Departamento de Ciencias Agropecuarias. Facultad de Ingeniería y Ciencias Económico-Sociales. Universidad Nacional de San Luis, Avda. 25 de mayo 384, 5730 Villa Mercedes, San Luis, Argentina. sbogino@fices.unsl.edu.ar

Abstract

The anatomical characteristics of intra-annual density fluctuations (IADFs), the differences in IADFs according to cambial age, changes in IADFs in the last century and how IADFs are related to radial growth and climate were analyzed in *Pinus pinaster* Ait. stands in Spain. Five sampling sites were selected throughout the natural distribution area of *Pinus pinaster* in the Iberian Peninsula. Two cores were extracted, at 1.30 m above ground, from fifteen dominant and co-dominant trees at each sampling site. The cores were prepared according to dendrochronological techniques and were then dated and the stabilized IADFs were determined. The data were analyzed by ANOVA, Pearson's correlation and the logistic function. Results showed: 1) Nine different anatomical characteristics in IADFs; 2) An increase in frequency of IADFs from the 1940s to the present; 3) That the mean frequency of IADFs was higher in younger than older trees; 4) A negative correlation between radial growth and IADFs and 5) That density fluctuations may be predicted by use of a logistic model, with monthly rainfall and temperature as independent variables. Studies of intra-annual features or anomalies in radial growth may be useful for ecological and climatological applications under changing scenarios worldwide.

Introduction

Intra-annual density fluctuations (IADFs), which include false rings, growth bands, double rings and multiple rings, are anomalies in ring growth that are formed by latewood-like cells within the earlywood, or earlywood-like cells within the latewood (Fritts 1976). Cambial activity may cease at very low temperatures, as for example, in winter in temperate regions, and also during periods of drought in hot, dry summers (Larcher 1995). Dry conditions may result in tracheids of smaller radius, before the regular latewood formation has started. When more favourable growing conditions return (rainfall), the subsequently formed cells are again larger with thinner walls (Fritts 1976). Small tracheids are formed earlier for the same reason as latewood is formed, but in IADFs this is followed by a reversal to earlywood production (Zahner 1963). The boundary between the earlywood cells that form the false ring exhibits a more gradual increase in cell diameter and decrease in cell wall thickness than the abrupt change in cell diameter associated with a true ring boundary (Fritts 1976).

When IADFs are properly identified, intra-annual structures from trees growing in xeric environments may be used to develop records of growing seasons characterized by early drought and followed by moist conditions (Villalba and Veblen 1996). Species growing under a Mediterranean climate, which is characterized by summer drought and high inter-annual variability in precipitation and temperature, commonly show special anatomical characteristics in tree rings (Schweingruber 1993). Consequently, the importance of incorporating intra-annual features or anomalies in radial growth may be useful for ecological and climatological interpretation (Tessier et al. 1997).

Climate shifts have already taken place in the Iberian Peninsula, as exemplified by the reduction of rainy days on the southern coast of Spain and in the Pyrenees in the last century [by 50 and 30% respectively (Esteban-Parra et al. 1998; Rodrigo et al. 2000)]. On the other hand, forecasts predict more frequent drought events due to a decrease in the number of rainfall events in summer (IPCC 2007).

The Mediterranean Maritime pine (*Pinus pinaster* Ait.) is a characteristic species in Mediterranean forests, with the main populations located in the Iberian Peninsula (Blanco et al. 1997). This species inhabits a wide range of environments (Nicolas and Gandullo 1967), and is able to survive high or low temperatures, regular or variable rainfall as well as severe droughts. It is adapted to the extremely cold winters of the continental climate in central Spain and to the mild, temperate climate of the Atlantic coast in the western Iberian Peninsula (Alía et al. 1996; Blanco et al. 1997).

In Spain, the Mediterranean Maritime pine grows in both natural (1 million ha) and planted (0.6 million ha) pure and mixed woodlands (DGCN 1998; 2002), in areas where the climate is characterized by irregular rainfall between and within years, and high temperatures in summer. The soils are sandy and rocky and very well drained (Blanco et al. 1997). The relationship between the trigger factors and false ring formation will serve as good proxy data for reconstructing droughts, flooding, frosts, and insect outbreaks (Copenheaver et al. 2006). As conifers growing in dry environments tend to produce false rings, and as dendroclimatological studies concluded that *Pinus pinaster* is very susceptible to rainfall during the growing season [especially in spring and early summer (Bogino and Bravo 2008)], the study of IADFs in *Pinus pinaster* may be a useful tool for enhancing dendroclimatological studies based on tree ring width fluctuations, and for reconstructing past intra-annual climate events.

The objectives of the present study were a) to identify the different types of IADFs in *Pinus pinaster;* b) to specify the differences in IADFs according to cambial age; c) to estimate changes in frequency of IADFs in the last century; d) to determine the relationship between IADFs and radial growth and e) to elaborate a model to predict the probability of IADF occurrence.

Materials and Methods

Five sampling sites (Table 1) were selected in the distribution area of *Pinus pinaster* in the Iberian Peninsula (Fig.1). Site index was determined by use of the site index curves proposed by Bravo Oviedo et al. (2004).

During the summer of 2006, two cores were extracted, at 1.30 m above ground level, from fifteen dominant and co-dominant trees at each sampling site. As growth of tapped trees may be affected by resin extraction, such trees were avoided. Cores were glued on to channelled wood, dried for two weeks and polished with progressively thinner sandpaper. To establish correctly the calendar year in which a tree ring was formed, tree rings were dated by use of a binocular microscope Nikon SMZ1000 (20X), according to standard dendrochronological techniques (Stokes and Smiley 1968; Fritts 1976; Cook and Kairiukstis 1990). The cores in transverse sections were scanned at high resolution (2.000 dpi) with an Epson Expression 1640 XL scanner (0.01-mm accuracy), and rings were measured by use of the WinDENDRO programme (Regent Instrument Inc. 2002). The v6.06P COFECHA programme (Holmes 2001; Grissino-Mayer 2001; available at www.ltrr.arizona.edu) was applied to assess measurement and dating accuracy. COFECHA allows calculation of the Pearson correlation indices for the indexed tree ring series and a master reference chronology in a series of consecutive, partially overlapping segments of a length specified by the user. Once the cores were dated accurately, they were re-examined in a binocular microscope Nikon SMZ1000 (20X). Differentiation between a true tree-ring and an IADF was established by the clear boundary in the true annual ring and the progressive and gradual transition in cell size and wall thickness in the IADFs [Masiokas and Villalba 2004 (Fig. 2)]. Photographs were obtained with a digital camera Leica S8APO with a binocular microscope Leica DFC290. Images were constructed with Adobe Photoshop® CS3 Extended version 10.0 (Adobe® Systems Inc. 2007) from 16 bits to 8 bits; only brightness, contrast and colour were modified. As the number of samples changed over time, the relative frequency was calculated with the following formula:

[1] $F = n/N$

where F is the number of IADFs per year; n the number of trees that formed the IADF and N the total number of trees analyzed. As the number of samples changed over time, the bias in the frequency was assessed by calculating the stabilized IADF frequency (f), according to the formula of Osborn et al (1997):

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

The Pearson's correlation between stabilized IADFs was applied to detect common patterns in IADFs at different sites. ANOVA and Fisher's test were used to detect significant differences in stabilized IADF between sites and over time. To determine the variability in IADFs over time, sampling sites of more than 100 years old were subdivided into two periods (between 1886 and 1939 and between 1940 and 2005). The Pearson's correlation between the frequency of IADFs and the residual tree-ring series (data from Bogino and Bravo 2008) was also calculated to analyse the relationship between IADFs and radial growth (Sokal and Rohlf 1995). Infostat V.2 software (Di Rienzo et al. 2002) was used to statistical analysis.

The nonlinear logistic equation form was chosen to model the probability of occurrence of IADFs in *Pinus pinaster* rings:

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

where P is the probability of IADFs and $Z = b_0+b_1(x_1)+b_2(x_2)+\ldots\ldots+b_k(x_k) + \varepsilon$ where $x_{1;}\, x_2 \ldots\ldots x_k$ are the climatic variables and $b_{0;}\, b_{1;}\, b_2 \ldots\ldots b_k$ are unknown parameters of the model and ε is a normal random error $N(0,\sigma^2)$.

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. 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 area under the receiver operating characteristic (ROC) curve and the expected behavior - as indicated by the signs of the parameters estimates.

The area under the ROC curve can be considered as an estimator of accuracy. This curve, which is widely used in health sciences and but not in forestry, 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). PROC LOGISTIC of SAS 9.1 (SAS Institute Inc. 2004) was used to fit the model.

Monthly rainfall and mean monthly temperatures at sites Cu1, Cu2 and Cu3 (recorded at the *Yemeda* meteorological station), and rainfall and mean temperature at sites Te1 and Te2 (recorded respectively at the *Cella* and *Pantano de la Toba* meteorological stations) (*Agencia Estatal de Meteorología,* Spain) were used to assess the relationships between IADFs and climatic variables (Table 2).

Results

Pinus pinaster is characterized by different anatomical features. A total of 11 930 tree rings were analyzed from trees from the five sampling sites and a total of 1 038 IADFs were detected in the trees analyzed. Even though the species showed different anatomical IADFs they were rather easy to identify by correct preparation of the samples. The data summarising the results of the five sampling sites are shown in Table 3.

The IADFs were identified as: multiple rings (Fig. 2a-b); double rings (Fig. 2c); latewood at the beginning of the growing season (Fig. 2d); earlywood between latewood (Fig. 2e); latewood between earlywood in the middle of the tree ring (Fig. 2f); close to the latewood (Fig. 2g); density fluctuations in latewood (Fig. 2h), and latewood between earlywood (Fig. 2i).

Stabilized IADF frequency in relation to calendar year and samples analyzed at the five sampling sites are shown in Fig. 3. Samples showed an increase in IADFs from the

1940s to the present, except in sample Te1. The absence of IADFs at this site may be attributed to the extreme thinness of the tree rings (less than 0.3mm, Fig. 4A), which made it impossible to detect IADFs. The different anatomical features of one tree at three different sampling sites are shown in Fig. 4 (two sites older and one younger than 100 years, Fig. 4A, C and B, respectively). There were IADFs at all sampling sites in 1961 and 1983, with a stabilised frequency higher than 3. A low frequency of IADFs was recorded in the 1970s at all sampling sites.

The Pearson's correlation between stabilized IADFs was positive and significant for all comparisons between sampling sites [*$p<0.05$ and ***$p<0.001$ (Table 4)].

The results of the ANOVA and Fisher's test performed to detect significant differences between IADFs at different sampling sites (from the common growth period 1953-2005) showed that younger sampling sites have higher IADFs than older sampling sites (Table 5). However, Fisher's test does not enable complete separation of young and old stands. The higher mean IADFs at sites Te2 and Cu3 also coincided with the highest site index, but as these sites are also the youngest in terms of cambial age, it was not possible to ensure that the higher IADFs are related to site index.

Significant differences were found in the last 120 years in the stabilized IADFs. Results showed statistically significant differences between both periods in samples Cu2 and Cu1 and no differences in sample Te1 (Table 6).

The Pearson's correlation coefficients showed a negative significant correlation (*$p<0.05$; ***$p<0.001$) between tree-ring growth and stabilised IADF at all sampling sites (Table 7). The results suggest that IADFs restrict growth at all sampling sites.

The logistic function used to predict the possibility of the occurrence of IADF estimated that 18 monthly climatic variables out of 24 had a significant effect on predicting future IADFs (Table 8). Drought events in May and July had a positive impact on IADF while wet periods in April and June also promoted IADF. Such pulses in precipitation (rainy months follow by dry months) are typical of Mediterranean areas. Increases in temperatures also had a generally positive impact on IADFs. The value of the area under ROC curve (0.84) shows that the accuracy of model is good enough to use it to predict occurrence of IADFs (Fig. 5).

However, statistical significance must be accompanied by biological significance and consistency of parameter signs and values. In the IADF logistic model developed for *Pinus pinaster*, low precipitation in January, March, May and July had a negative impact on IADF, and high precipitation in April, June and August had a positive effect. Temperature had a positive impact on IADF, except in August and October.

Discussion

The impact of climate on intra-annual density fluctuations (IADFs) in *Pinus pinaster* in Central Spain was studied. *Pinus pinaster* showed nine different anatomical structures that confirm the tendency of Mediterranean species, and this species in particular, to develop special anatomical structures (Schweingruber 1993). Even though IADFs, which occur in all series at all sampling sites, may previously have limited dendroclimatological studies in *Pinus pinaster* (Schweingruber 1993), when the correct date is obtained, the significant association between IADFs and radial growth or climatic variables provides a useful proxy for complementing and enhancing dendroclimatological data (Bogino and Bravo 2008).

Different categories of IADFs that were found in the present study were also observed in *Pinus pinea* L.from a dry Mediterranean area in Portugal in a study in which Campelo et al. (2006) determined four different anatomical categories of IADFs, which suggest different anatomical characteristics in IADFs in Mediterranean pine species.

A higher tendency of young trees to develop more IADFs was also found in *Pinus banksiana* Lamb., *Pinus radiata* D.Don; *Pinus edulis* Engelm.and *Pinus ponderosa* Douglas ex. C. Lawson growing in EUA, and in *Pinus sylvestris* L. in semiarid low forests on the border of the central Alps and central Siberia (Schulman 1938, Rigling et al. 2001; Copenheaver et al. 2006). In the southern Patagonian Andes under a cold temperate climate (300 mm year^{-1} rainfall and mean temperature of 13.2 ºC and 0.8ºC in January and July, respectively), *Nothofagus pumilio* (Poepp. et Endl.) Krasser showed a variation in IADFs in the last sixty years presumably related to both a long-term warming trend and a significant decrease in precipitation (Masiokas and Villalba 2004). The highly significant difference in IADF frequency between the beginning and the last part of the last century found in *Pinus pinaster* in this study may be explained by the increase in drought events in the last fifty years in the Iberian Peninsula (IPCC 2007). Previous studies that relate IADFs to growth of *Pinus sylvestris* in Switzerland determined a positive association between tree-ring growth and IADFs (Rigling et al. 2001); these results contrast with the present results in which a significant negative relationship between radial growth and IADFs was found, suggesting that the environmental variables that produce IADFs also limit growth.

An increase in mean temperature in winter and spring leads to a higher probability of IADFs, while such an increase in May, or a reduction in precipitation in April, has a negative impact on IADFs (Table 8). These results are consistent with those of previous studies in *Pinus nigra* Arnold in Austria, where precipitation in May was the determining factor in false tree-ring formation (Wimmer et al. 2000), and in *Pinus pinea* in Portugal where early drought events were found to determine IADFs (Campelo et al. 2006). The precipitation pulses that determine IADFs in the present study were also found in *Astrocedrus chilensis* (D. Don) Florin et. Bout., a coniferous species growing in the forest-steppe ecotone in the Argentinean Patagonia, where IADFs are associated with extremely dry springs followed by wet summers (Villalba and Veblen 1996). The positive impact of precipitation in April and June in the present study coincide with the positive effect of moist-cool conditions in the middle of the growing season in *Pinus sylvestris* in dry areas in Valais (Switzerland), whereas in moderately dry sites, there must be an additional warm period in early summer in order to initiate IADFs (Rigling et al. 2001; 2002). In Mediterranean areas, double rings of *Quercus ilex* L. were triggered by positive effect of precipitation and negative effect of temperature in August (Campelo et al. 2007), which is consistent with the present results that showed both variables to have the same effect on IADFs on *Pinus pinaster*.

Latewood is formed from carbohydrates produced during photosynthesis which is the result of water stress and temperature (Kozlowski et al. 1991). IADFs of *Pinus pinaster* growing in Tuscani Italy had a higher 13C/12C isotope ratio in latewood than earlywood, which suggests a better water use efficiency (De Micco et al. 2007). The present results emphasised the impact of drought events on IADFs (significant effect of rainfall in the growing season) and suggest a physiological response of *Pinus pinaster* to unfavourable climatic conditions and the development of anatomical structures that lead to better water use efficiency. Isotope analysis in the Iberian Peninsula, showed that *Pinus pinaster* makes more efficient use of water under severe drought events than *Pinus sylvestris* and *Pinus nigra* (Martinez Vilalta and Piñol 2002).

The present results contrast with those of a previous study on *Pinus banksiana* growing in the United States, where IADFs are not related to climatic factors (Copenheaver et al. 2006). The effect of climate on IADFs of *Pinus pinaster* is determined by both the significant correlation between stabilised IADFs at all sampling sites -suggesting the impact of the driving factor (climate)- and the accurate results of the logistic function.

Climate change scenarios developed by IPCC (2007) show that irregularity in intra-annual rainfall and trends of increasing temperature should be expected during the next decades. According to the present results, this predicted future climatic situation will lead to a higher occurrence of IADFs.

In the present study, the frequency of stabilized IADFs in *Pinus pinaster* was higher in more productive sites (higher site index), thus demonstrating the importance of the incorporation of intra-annual features in dendroecological and dendroclimatological studies, which is highly recommended because it enables differentiation of site types (Rigling et al. 2001; 2002). Extensive studies are needed to provide further insight in this finding.

In conclusion, the present study provides information about the different anatomical characteristics of IADFs in *Pinus pinaster*, which is a useful tool in the application of dendrochronological techniques to date samples. IADFs are determined by cambial age and have increased in frequency in the last sixty years. Finally, the probability model used showed that rainfall pulses in late winter and spring and higher temperatures will lead to a more frequent occurrence of intra-annual density fluctuations in *Pinus pinaster* trees growing under Mediterranean climate conditions.

Acknowledgements

The authors thank Cristotal Ordóñez for field assistance and María José Fernández Nieto for providing meteorological data. Thanks are also extended to Dr. Mariano Morales for their useful comments on the manuscript; Iñaki Extebeste Larrañaga for whose valuable contribution helped us with the development of Figures 2 and 4 and to Christine Francis and María Laura Cangiano for improving the English version. This study was supported by the Spanish Ministry of Science and Education (Project Code AGL2007-65795-C02-01) and by the Alβan Programme, the European Union

Programme of High Level Scholarships for Latin America (Scholarship No. E05D049920AR).

Referentes

Adobe® Systems Inc. 2007. Adobe Photoshop CS3 Standard beta, Version 10.0 user guide San José, Calif, EUA.

Alía, M., Martín, S., De Miguel, J., Galera, R., Agúndez, D., Gordo, J., Catalán, G., and Gil, L. 1996. Regiones de procedencia *Pinus pinaster* Aiton. INIA-CIFOR, Madrid.

Blanco, E., Casado, M., Costa, M., Escribano, R., Gracía Antón, M., Génova, M., Gómez Manzaneque, G., Gómez Manzanaque, F., Moreno, J., Morla, C., Regato, P., and Sainz Ollero, H. 1997. Los bosques ibéricos, Editorial Planeta, Barcelona, España.

Bogino, S., and Bravo, F. 2008. Growth response of *Pinus pinaster* Ait. to climatic variables in central Spanish forests. Ann. For. Sci. In press.

Bravo-Oviedo, A., Del Río, M., and Montero, G. 2004. Site index curves and growth model for Mediterranean maritime pine (*Pinus pinaster* Ait.) in Spain. For. Ecol. Manage. **201**:187-197.

Campelo, F., Nabais, C., Freitas, H., and Gutiérrez, E. 2006. Climatic significance of tree-ring width and intra-annual density fluctuations in *Pinus pinea* from a dry Mediterranean area in Portugal. Ann. For. Sci. **64**: 229-238.

Campelo, F., Gutiérrez, E., Ribas, C., Nabais, C., and Freitas, H. 2007. Relationship between climate and double rings in *Quercus ilex* from northeast Spain. Can. J. For. Res. **37**: 1915-1923.

Cook, E.R., and Kairiukstis, L.A. 1990. Methods of Dendrochronology: applications in the environmental sciences. Kluwer, Dordrecht.

Copenheaver, C., Pokorski, E., Currie, J., and Abrams, M. 2006. Causation of false ring formation in *Pinus banksiana*: A comparison of age, canopy class, climate and growth rate. For. Ecol. Manage. **236**: 348-355.

De Micco, V., Saurer, M., Aronne, G., Tognetti, R. and Cherubini, P. 2007. Variations of wood anatomy and δ13C within-tree rings of coastal *Pinus pinaster* showing intra-annual density fluctuations IAWA J. **28** (1): 61–74.

DGCN 1998. Segundo Inventario Forestal Nacional 1986-1996. Ed. Ministerio de Medio Ambiente, España.

DGCN 2002. Plan Forestal Español. Ed. Ministerio de Medio Ambiente, España.

Di Rienzo, J., Balzarini, M., Casanoves, F., González, L., Tablada, E., and Robledo, C. 2002. Infostat Software Estadístico versión 2. Grupo infoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Esteban-Parra, M., Rodrigo, F., and Castro Diéz, Y. 1998. Spatial and temporal patterns of precipitation in Spain for the period 1880-1992. Int. J. Climatol. **18**: 1557-1574.

Fritts, H. 1976. Tree Ring and Climate, Academic Press Inc, London.

Grissino-Mayer, H.D. 2001. Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. Tree-Ring Res. **57**: 205–221.

Hair, J. E., Anderson, R.E., Tatham, R. L., and Black, W.C. 1998. Multivariate data analysis. 5th ed. Prentice Hall. Upper Saddle River, NJ, USA.

Holmes, R.L. 2001. Dendrochronology Program Library. Available from the Laboratory of Tree Ring Research, University of Arizona, Tucson, USA.

IPCC 2007. Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Kozlowski, T.T., Kramer, P.J., and Pallardy, S.G. 1991. The physiological ecology of woody plants, Academic Press, New York, EUA.

Larcher, W. 1995. Physiological plant ecology. 3rd ed. Springer-Verlag Berlin.

Martínez-Vilalta, J., and Piñol, J. 2002. Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. For. Ecol. Manage.**161**: 247-256.

Masiokas, M., and Villalba, R. 2004. Climatic significance of intra-annual bands in the wood of *Nothofagus pumilio* in southern Patagonia. Trees **18**: 698-704.

Nicolas, A.; and Gandullo, J. 1967. Ecología de los pinares españoles. 1, *Pinus pinaster* Ait. Ministerio de Agricultura, Madrid, España.

Osborn, T.J., Briffa, K.R. and Jones, P.D. 1997. Adjusting variance for sample-size in tree-ring chronologies and other regional mean time series. Dendrochronologia **15**: 1–10.

Regent Instrument Inc. 2002. Windendro ^{TM} v.2002a. Québec, Qc.

Rigling, A., Waldner, P., Forster, T., Bräker, O., and Pouttu, A. 2001. Ecological interpretation of tree-ring width and intraannual density fluctuations in *Pinus sylvestris* on dry sites in the central Alps and Siberia. Can.J.For.Res. **31**(1):18-31.

Rigling, A., Bräker, O., Schneiter, G., and Schweingruber, F. 2002. Intra-annual tree-ring parameters indicating differences in drought stress of *Pinus sylvestris* forests within the Erico-Pinion in the Valais (Switzerland). Plant ecology **163**:105-121.

Rodrigo, F., Esteban-Parra, M., Pozo-Vázquez, D., and Castro-Diéz, Y. 2000. Rainfall variability in southern Spain on decadal to centennial time scales. Int. J. Climatol. **20**: 221-732.

SAS Institute Inc. 2004. SAS/STAT versión 9.1, User’s Guide. Cary, NC, USA.

Schulman, E. 1938. Classification of false annual rings in Monterey pine, Tree-ring Bull. **4**: 4–7.

Schweingruber, F.H. 1993. Trees and wood in Dendrochronology, Springer series in Wood Science, Springer-Verlag.

Sokal, R.R., and Rohlf, F.J. 1995. Biometry: the principles and practice of statistics in biological research, 3rd edition, WH Freeman and Co., New York, UEA.

Stokes, M., and Smiley T. 1968. An Introduction to Tree-Ring Dating, University of Arizona Press, Tucson, UEA.

Tessier, L., Guibal, F., and Schweingruber, F. 1997. Research strategies in dendroecology and dendroclimatology in mountain environments. Clim. Change **36**: 499-517.

Villalba, R., and Veblen, T. 1996. A tree-ring record of dry spring-wet summer events in the forest-steppe ecotone, northern Patagonia, Argentina. *In* Proceeding of International Conference: Tree rings, environment and humanity, 17-21 May 1994, Tucson, Arizona. *Edited by* Dean, JS, Meko, DM, Swetnam, TW. Department of Geosciences, University of Arizona, Tucson, EUA. Pp. 107–116.

Wimmer, R., Strumia, G., and Holawe, F. 2000. Use of false ring s in Austrian pine to reconstruct early growing season precipitation. Can.J.For.Res. **30**:1691-1697.

Zahner, R, 1963. Internal moisture stress and wood formation in conifers. For. Prod. J. **13**: 240-247.

Table 1. Geographical, altitudinal position and site index of five sampling sites of *Pinus pinaster*.

Sampling	**Site code**	**Latitude N**	**Longitude W**	**Altitude (m)**	**SI**
P16106	Cu2	39º 50' 17”	01º 16' 11”	970	9.67
P16008	Cu3	39º 48' 56”	01º 15' 36”	920	20.22
P16208	Cu1	39º 50' 28”	01º 17' 54”	1090	9.38
P44005	Te1	40º 20' 47”	01º 21' 54”	1364	7.11
P44204	Te2	40º 20' 01”	01º 21' 26”	1232	15.67

Table 2. Data from meteorological stations (*Agencia Estatal de Meteorología*, Spain).

Met. Station	**Altitude**	**Latitude (N)**	**Longitude (W)**	**Met. Var.**	**Period**	**Rainfall (mm)**	**Temp.(ºC)**
Yemeda	868	39 45' 40"	01 43' 17"	pp-tt	1950-2000	405.13 (±138.74)	12.41 (±0.87)
Cella	1023	40 27' 20"	01 17' 27"	pp	1939-2006	370.81 (±110.41)	12.01 (±0.52)
Pantano	1154	40 13' 19"	01 55' 33"	tt	1944-2005	764.68 (±253.12)	10.22 (±0.71)

Met. Var.: Climate variable: (pp: annual precipitation and tt: mean monthly temperature); Period: Time with data available; Rainfall: Annual precipitation; Temp.: Mean monthly temperature.

383 Table 3. Descriptive statistic of the IADFs in *Pinus pinaster* in the Iberian Peninsula.

	Cu2	**Cu1**	**Cu3**	**Te2**	**Te1**
Number of cores	27	25	27	25	25
Period	1877-2005	1886-2005	1948-2005	1953-2005	1846-2005
Cores with IADF (%)	100	100	100	100	100
Ring in total	2626	2637	1471	1328	3848
Ring with IADFs	209	182	278	209	160
Percentage of IADF	7.95	6.90	18.89	15.73	4.15
Mean stabilised IADF	0.3266	0.3067	0.9332	0.7617	0.1935

Table 4. Pearson's correlation coefficients for the stabilized IADFs for all comparisons between sampling sites.

	Te1	**Te2**	**Cu3**	**Cu2**
Te2	0,55***			
Cu3	0,38***	0,43***		
Cu2	0,27***	0,41***	0,63***	
Cu1	0,42***	0,44***	0,31*	0,64***

(*$p<0.05$ and ***$p<0.001$)

Table 5. Results of ANOVA and Fisher's test of the stabilized IADFs for the different sampling sites (from the common growth period 1953-2005).

ANOVA (SS type III)

Variance factor	SS	DF	MS	F	p-value
Model	19.81	4	4.95	3.62	0.0069
Site	19.81	4	4.95	3.62	0.0069
Error	356.04	260	1.37		
Total	375.86	264			

LSD Fisher Test; Alfa = 0.05 DMS = 0.44763

Error: 1.3694 df: 260

Site	Mean	n			
Te1	0.19	53	A		
Cu2	0.50	53	A	B	
Cu1	0.58	53	A	B	C
Te2	0.76	53		B	C
Cu3	1.01	53			C

*Different letters point significant differences (*p<= 0.05)*

Table 6.Results of ANOVA and Fisher's test of the stabilized IADFs for the periods between 1886 and 1939 (sites indicated with letter *a*) and between 1940 and 2005 (sites indicated with letter *b*) in sampling sites Cu2, Cu1 and Te1.

ANOVA (SS type III)					
Variance factor	SS	DF	MS	F	p-value
Model	14.69	5	2.94	4.89	0.0002
Site	14.69	5	2.94	4.89	0.0002
Error	212.80	354	0.60		
Total	227.49	359			

LSD Fisher Test; Alfa = 0.05 DMS = 0.44763				
Error: 1.3694 df: 260				
Site	Mean	n		
Cu1_a	0.05	54	A	
Te1_a	0.10	54	A	
Cu2_a	0.12	54	A	
Te2_b	0.22	66	A	
Cu1_b	0.54	66		B
Cu2_b	0.54	66		B

*Different letters point significant differences (*p<= 0.05)*

Table 7. Pearson’s correlation coefficients for the stabilized IADFs and the residual tree ring chronology for all comparisons between sampling sites.

	Crono-Te1	**Crono-Te2**	**Crono-Cu3**	**Crono-Cu2**	**Crono-Cu1**
Te1	-0.25***				
Te2		-0.28*			
Cu3			-0.39***		
Cu2				-0.30***	
Cu1					-0.31***

(*$p<0.05$ and ***$p<0.001$).

Table 8. Climatic variables with a significant effect on predicting future IADFs in *Pinus pinaster* under Mediterranean climate conditions.

Parameter	DF	Estimator	Standard error	Chi-square de Wald	Pr > ChiSq
Intercept	1	13.329	0.6212	46.040	0.0319
pp-January	1	-0.0131	0.00195	450.416	<.0001
pp_March	1	-0.0180	0.00235	584.210	<.0001
pp_April	1	0.00565	0.00223	64.318	0.0112
pp_May	1	-0.0441	0.00253	3.022.477	<.0001
pp-June	1	0.00697	0.00165	178.479	<.0001
pp_July	1	-0.00987	0.00241	167.630	<.0001
pp_August	1	0.0126	0.00168	562.980	<.0001
pp_September	1	0.0113	0.00145	601.814	<.0001
pp_October	1	0.0121	0.00190	404.570	<.0001
pp_November	1	0.00840	0.00125	450.177	<.0001
tt_January	1	0.0779	0.0289	72.743	0.0070
tt_March	1	0.1226	0.0386	101.007	0.0015
tt_April	1	0.1737	0.0433	161.020	<.0001
tt_May	1	0.2827	0.0322	768.682	<.0001
tt_June	1	0.2457	0.0356	477.218	<.0001
tt_August	1	-0.7385	0.0434	2.891.261	<.0001
tt_September	1	0.1823	0.0313	338.904	<.0001
tt_October	1	-0.1219	0.0347	123.406	0.0004

Parameter: climate variable (pp: monthly precipitation; tt: mean monthly temperature).

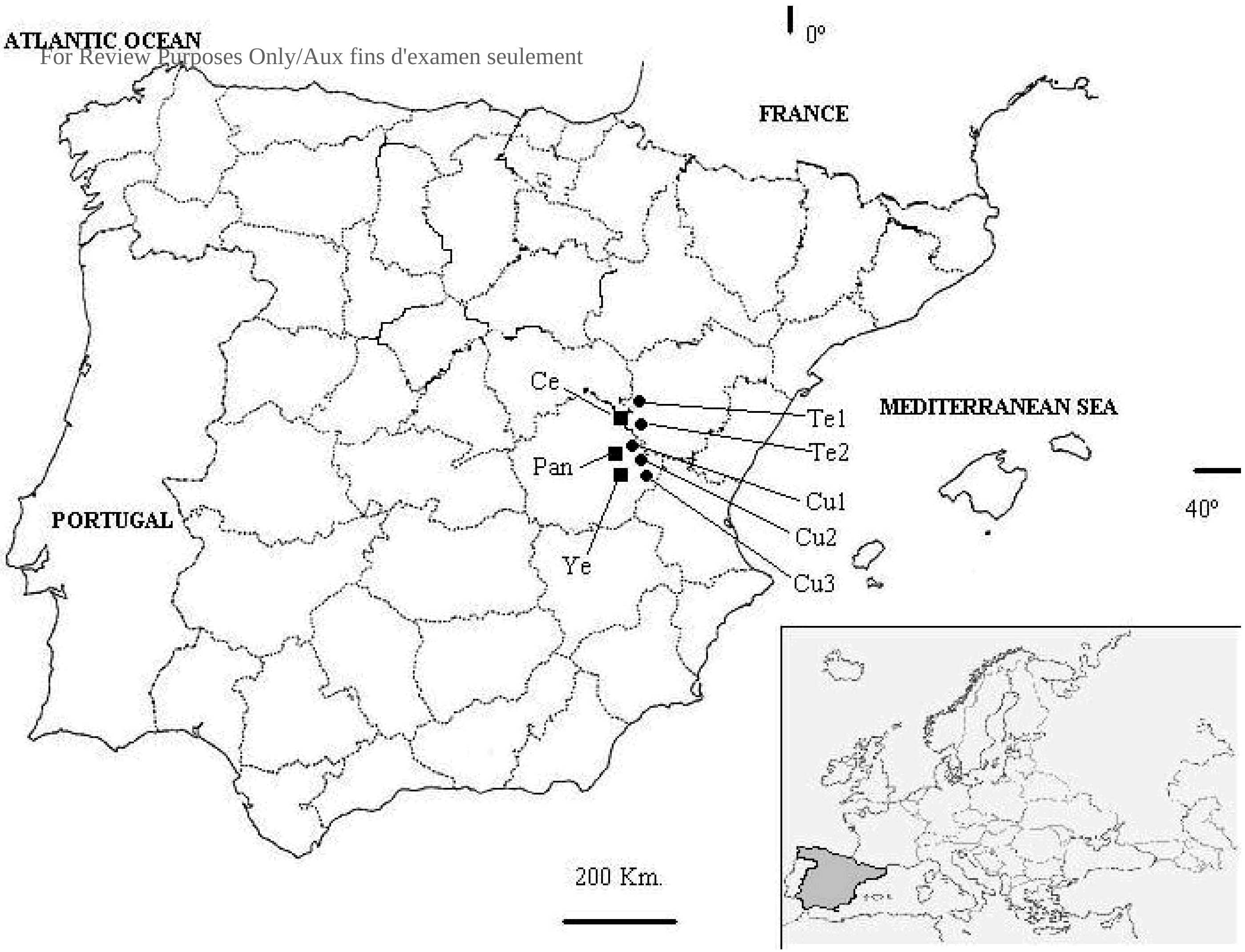
Fig. 1. Sampling sites established across the natural distribution area of *Pinus pinaster* in the Iberian Peninsula. Sampling sites:Te1, Te2, Cu1, Cu2 and Cu3. Meteorological stations: Ce: *Cella*, Pan: *Pantano de la Toba* and Ye: *Yemeda*.

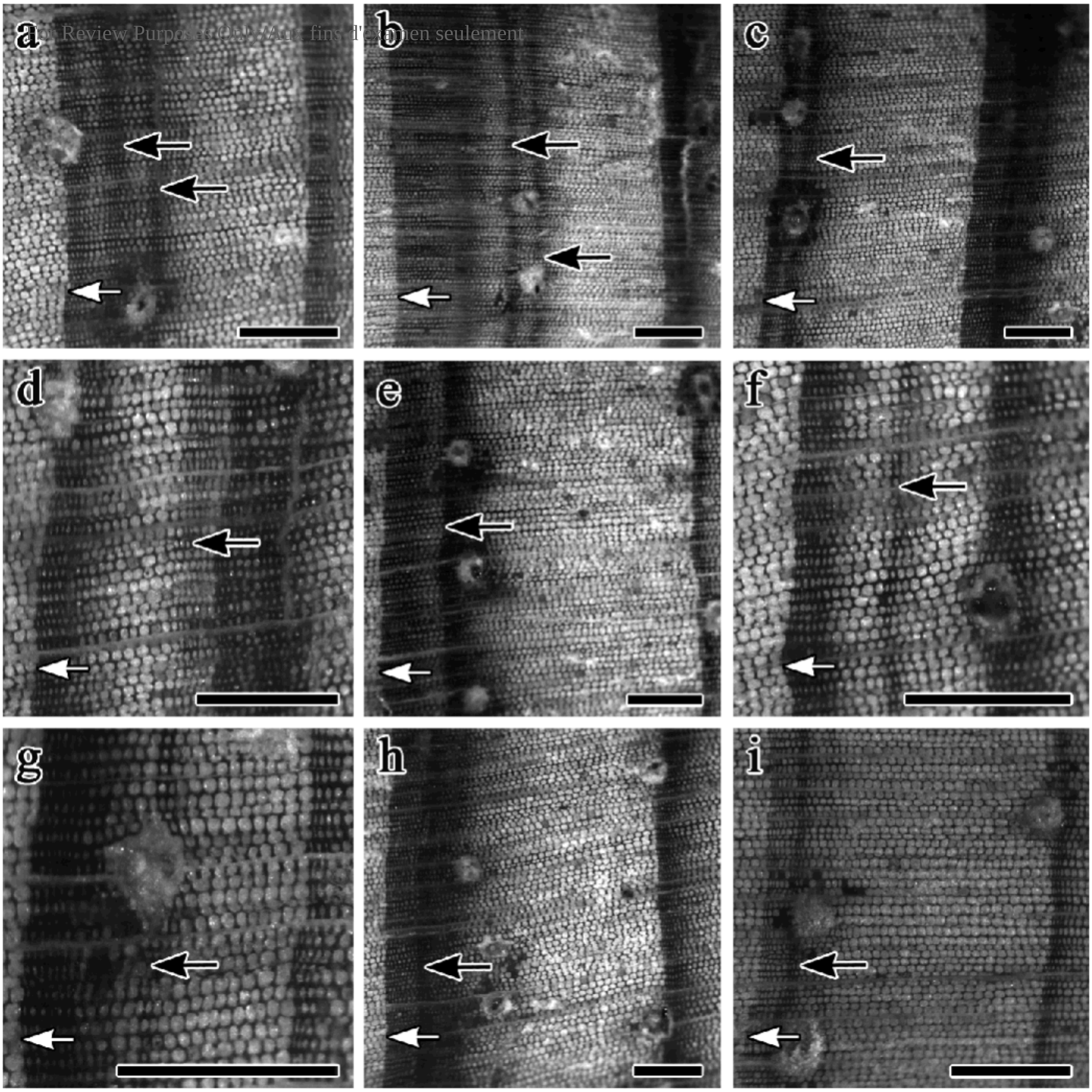
Fig. 2. Nine different IADFs in *Pinus pinaster* in the Iberian Peninsula. White arrows indicates the true tree ring boundary and black arrows the IADFs. The black scale bars point 0.5mm width.

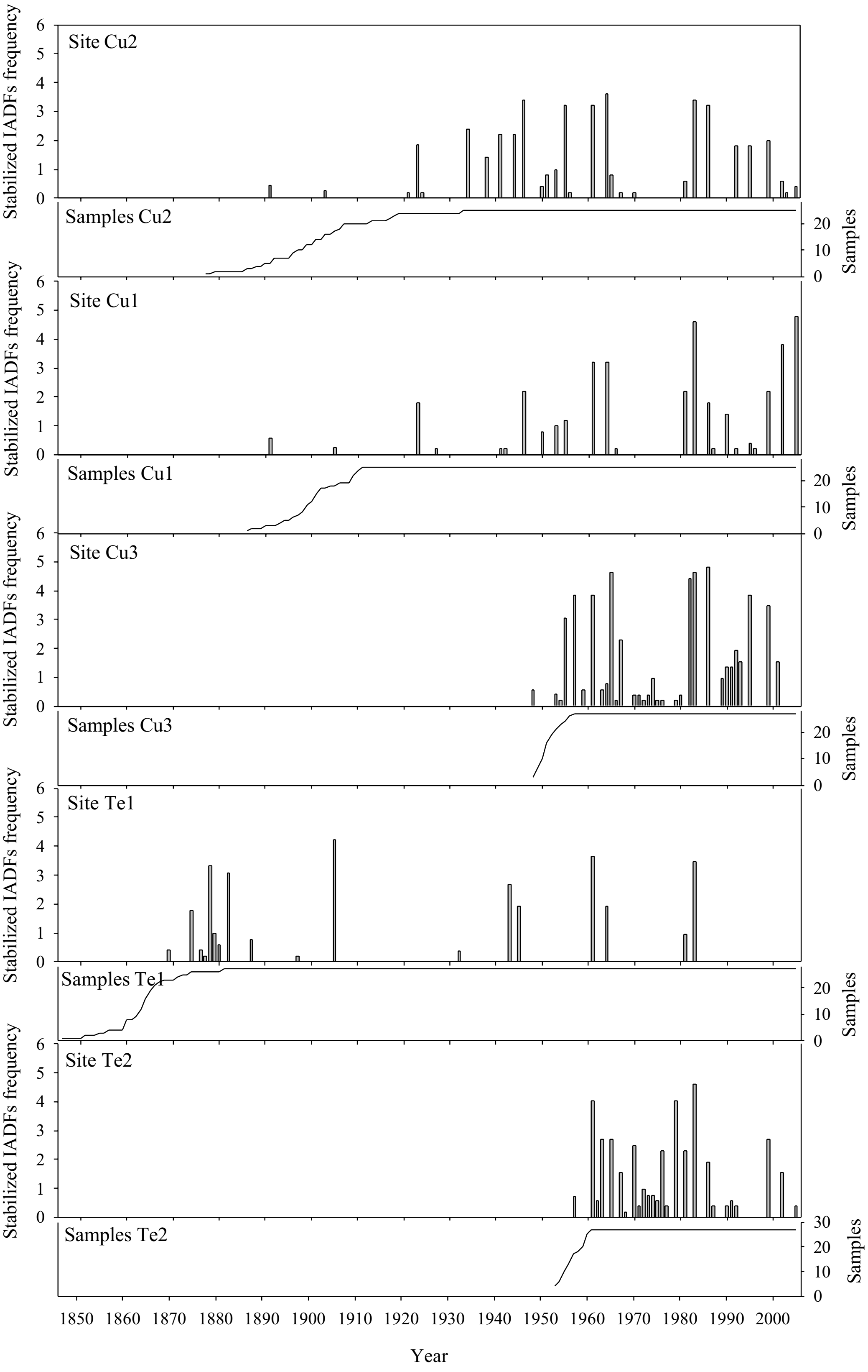
Fig. 3. Stabilized IADF of *Pinus pinaster* at five sampling sites in the Iberian Peninsula. The upper part of each figure shows the stabilized IADFs and the bottom part the number of samples analyzed.

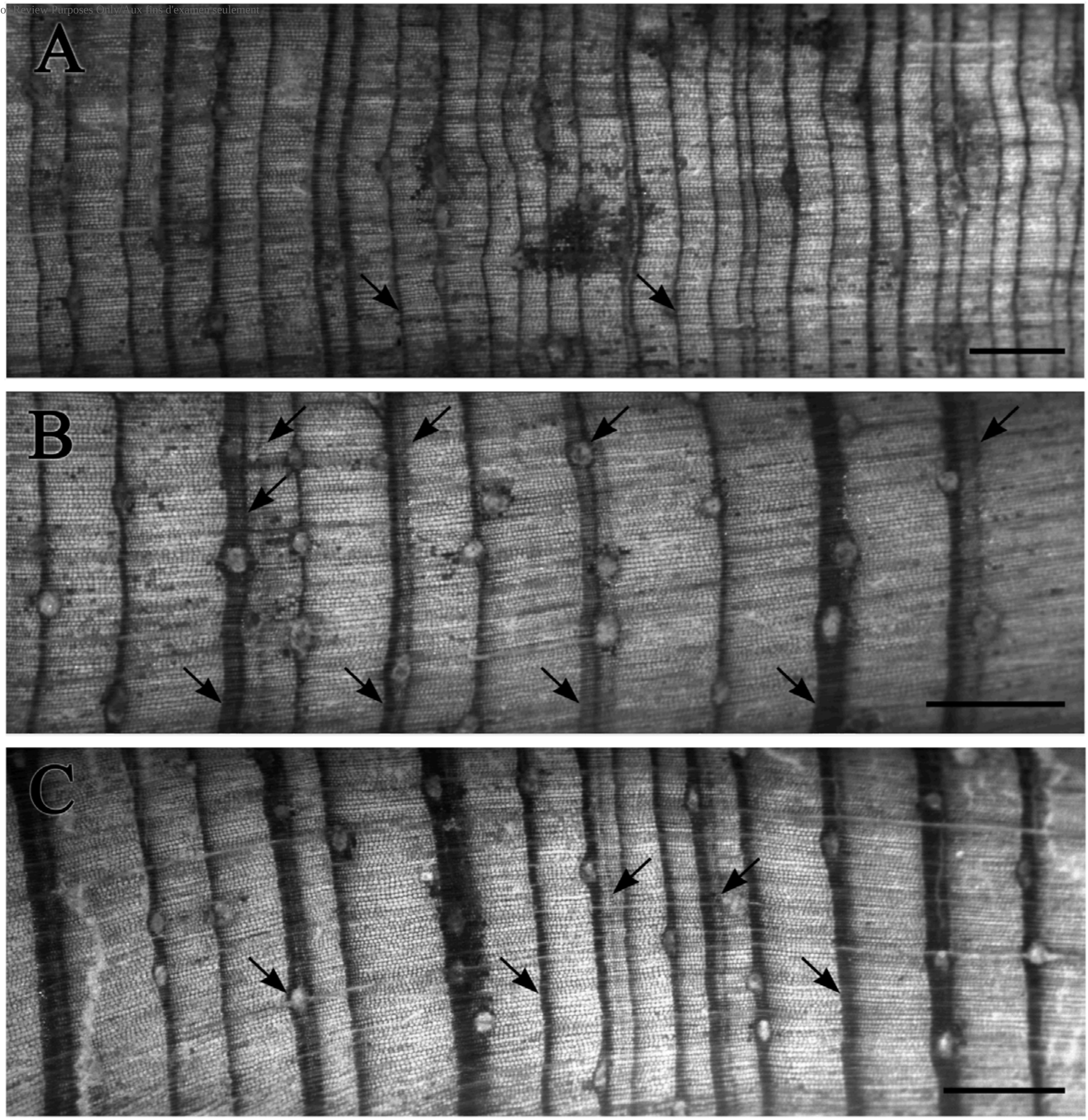
Fig.4. Different anatomical features of one tree at three different sampling sites (Sites Cu2, Cu1 and Te1). Arrow → indicates the true tree ring boundary and arrow ← , the IADFs. The black scale bars point 1mm width.

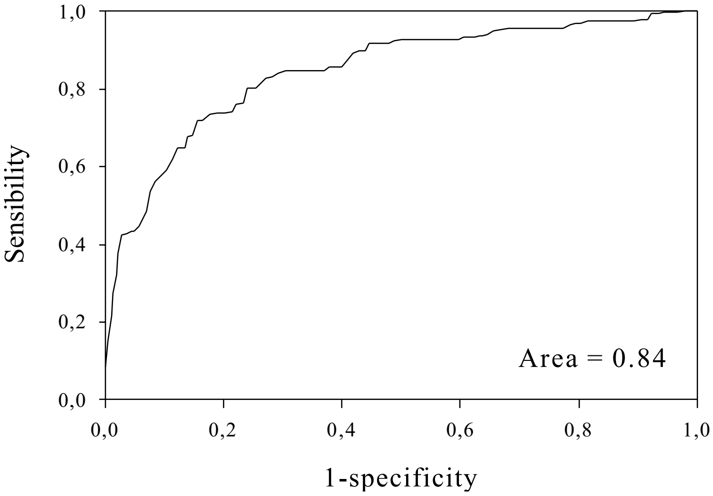
Fig. 5. ROC curve of the IADF model for *Pinus pinaster* in Central Spain.


ATLANTIC OCEAN
0º
FRANCE
PORTUGAL
MEDITERRANEAN SEA
40º
Ce
Te1
Te2
Pan
Cu1
Cu2
Cu3
Ye
200 Km.


a
b
c
d
e
f
g
h
i


Site Cu2
Samples Cu2
Site Cu1
Samples Cu1
Site Cu3
Samples Cu3
Site Te1
Samples Te1
Site Te2
Samples Te2
Stabilized IADFs frequency
Samples
0
1
2
3
4
5
6
10
20
30
1850
1860
1870
1880
1890
1900
1910
1920
1930
1940
1950
1960
1970
1980
1990
2000
Year


A
B
C


1,0
0,8
0,6
0,4
0,2
0,0
Sensibility
Area = 0.84
0,0
0,2
0,4
0,6
0,8
1,0
1-specificity

V

This article was submitted to Annals of Forest Sciences. Bogino S., Bravo F. Climate signals of stable carbon isotope ^{13}C in tree ring of Pinus pinaster and Pinus sylvesrtis in Mediterranean environments. Annals of Forest Sciences.

Stable carbon isotope ^{13}C in *Pinus pinaster* and *Pinus sylvestris* tree rings: climatic signals and water use efficiency in Mediterranean environments

RESUMEN

El objetivo de este capítulo es determinar las variables climáticas que controlan la variabilidad del isótopo carbono trece (δ^{13}C) en los anillos de crecimiento de *Pinus. pinaster* y *Pinus sylvestris.* Se seleccionaron cuatro árboles de dos sitios de muestreo, uno de *P.pinaster* y otro de *P. sylvestris*, emplazados en el centro de España. Se analizó material pulverizado de cada anillo de crecimiento para el período 1975-1999 con un espectrómetro. Se aplicó el coeficiente de correlación de Pearson entre todos los árboles de la misma especie y entre δ^{13}C y las cronologías residuales de ancho de anillo de crecimiento de un estudio previo con el objeto de determinar si existe relación entre δ^{13}C y el crecimiento radial. También se aplicó el coeficiente de correlación de Pearson entre δ^{13}C y las variables climáticas mensuales (máxima temperatura media mensual, precipitación mensual, humedad atmosférica mensual y horas de radiación solar) con el objeto de estimar qué variables climáticas son significativas sobre δ^{13}C. Las variables climáticas que correlacionaron con δ^{13}C se agruparon en modelos regresivos lineares. Los resultados muestran que: los árboles de *P. pinaster* tienen una correlación significativa entre todos los individuos (***$p<$ 0,001) mientras que el nivel de significación para *P. sylvestris* varió entre los individuos (*$p<0{,}05$, ** $p<0{,}01$,*** $p<0{,}001$). Sólo dos árboles de *P. sylvestris* no mostraron una correlación significativa. Se determinó una correlación negativa y significativa entre δ^{13}C y el crecimiento radial para el período 1975-1999 para las dos especies. *P. pinaster* mostró que la humedad desde invierno hasta el verano (enero a julio) y la lluvia en primavera (abril y mayo) tienen una correlación negativa y significativa con δ^{13}C, mientras que la temperatura media mensual de marzo y las horas diarias de radiación solar en enero, abril y mayo mostraron un efecto positivo sobre la δ^{13}C. En *P. sylvestris* la humedad en verano (julio) y en otoño (octubre) y la precipitación de octubre mostraron un efecto negativo sobre δ^{13}C, mientras que la temperatura media mensual de verano (junio y julio) y las horas de radiación solar de octubre mostraron un efecto positivo. Para *P. pinaster* la humedad relativa de enero a julio, la lluvia de enero a mayo, la temperatura máxima media mensual desde abril hasta julio y las horas de radiación solar de enero abril y mayo se seleccionaron para construir diferentes modelos regresivos lineares simples que relacionaron cada variable con δ^{13}C. Todas las variables mostraron una correlación significativa con δ^{13}C. Para *P. sylvestris* se seleccionaron la humedad relativa y la lluvia de julio y octubre, la temperatura máxima media mensual de junio y julio y las horas de radiación solar de octubre para construir los diferentes modelos de regresión simple. En conclusión, *P. pinaster* y *P. sylvestris* en los ambientes mediterráneos muestran una fuerte correlación con las variables climáticas lo que permite inferior su potencial como herramienta para el estudio del impacto del clima sobre los bosques mediterráneos.

Stable carbon isotopes ^{13}C in *Pinus pinaster* and *Pinus sylvestris* tree rings*:* climatic signals and water use efficiency in Mediterranean environments

Stella M. BOGINO[1,2*], Felipe BRAVO[1]

[1] Joint Unit UVa-INIA 'Sustainble Forest Management'. Departamento de Producción Vegetal y Recursos Forestales, Universidad de Valladolid, Avda. de Madrid 44, 34004 Palencia, Spain.

[2] Departamento de Ciencias Agropecuarias. Facultad de Ingeniería y Ciencias Económico-Sociales. Universidad Nacional de San Luis, Argentina. sbogino@fices.unsl.edu.ar

Stable carbon isotopes in *Pinus pinaster* and *Pinus sylvestris*

Dendrochronology /Maritime pine / Scots pine/ isotope

Dendrochronologie / pin maritime / pin sylvestre / isotope

Abstract

The isotopic composition of carbon stored in tree rings may represent a record of the variation in carbon thirteen ($\delta^{13}C$) as a result of physiological responses to environmental changes. The objective of the present study was to estimate the climatic variables that determine the values of $\delta^{13}C$ in tree rings of *Pinus pinaster* and *Pinus sylvestris* in central Spain. Pearson's correlation analysis was applied to $\delta^{13}C$ data for all trees of the same species, $\delta^{13}C$ and residual tree ring chronologies and $\delta^{13}C$ and monthly climatic variables. The climatic variables that were best correlated with $\delta^{13}C$ were grouped to construct simple linear regression models. The values of $\delta^{13}C$ data for all trees were significantly correlated in *P. pinaster* but results showed a variable degree of significance in *P. sylvestris*. $\delta^{13}C$ was significantly and negatively correlated with radial growth. In both species, $\delta^{13}C$ was negatively correlated with moisture and precipitation and positively with maximum temperature and hours of solar radiation. The linear regression models showed a significant correlation between climatic variables and $\delta^{13}C$. The values of $\delta^{13}C$ in *P. pinaster* and *P. sylvestris* in Mediterranean environments were strongly correlated with the climatic variables, which suggest that they may be of use for analysing tree behaviour under global change conditions.

Résumé

La composition isotopique du carbone fixé dans les cernes peut représenter un enregistrement de la variabilité du carbone treize ($\delta^{13}C$) qui est le résultat des réponses physiologiques aux changements environnementaux. L'objectif de ce travail était d'estimer les variables climatiques qui contrôlent le $\delta^{13}C$ dans les cernes de *P. pinaster* et de *P.*

sylvestris dans le centre de l’Espagne. Le coefficient de corrélation de Pearson a été appliqué entre le $\delta^{13}C$ de tous les arbres de la même espèce, entre le $\delta^{13}C$ et les chronologies résiduelles des cernes et entre le $\delta^{13}C$ et les variables climatiques mensuelles. Les variables climatiques qui présentaient la meilleure corrélation avec le $\delta^{13}C$ ont été regroupées pour élaborer des modèles de régression linéaire simple. Les résultats ont indiqué une corrélation significative entre les $\delta^{13}C$ de *P. pinaster*, tandis que les $\delta^{13}C$ des exemplaires de *P. sylvestris* ont montré un niveau de signification variable. Une corrélation négative significative a été déterminée entre le $\delta^{13}C$ et la croissance radiale. Les deux espèces en question ont présenté une corrélation négative entre le $\delta^{13}C$ et l’humidité et les précipitations, ainsi qu’une corrélation positive avec les températures maximales et les heures de rayonnement solaire. Les modèles de régression linéaire ont révélé une corrélation significative entre les variables climatiques et le $\delta^{13}C$. Les $\delta^{13}C$ de *P. pinaster* et de *P. sylvestris* dans des environnements méditerranéens ont présenté une forte corrélation avec les variables climatiques, ce qui pourrait suggérer que le $\delta^{13}C$ constitue un instrument adéquat pour analyser le comportement des arbres dans des conditions de changement global.

Introduction

The isotopic composition of carbon stored in the growth rings of trees may represent a record of variations in $^{13}C/^{12}C$ as a result of physiological responses to environmental changes (Francey and Farquhar, 1982). This ratio is expressed as $\delta^{13}C$, the proportional deviation of the $^{13}C/^{12}C$ ratio from the international accepted PDB carbonate standard (Craig, 1957). Since cellulose is not transferred between annual growth rings, intra and interannual seasonal events are recorded permanently in the $\delta^{13}C$ signal in tree rings (Tans et al., 1978). The value of $\delta^{13}C$ depends on stomatal conductance and the photosynthesis: when stomatal conductance predominates, relative humidity and soil moisture status are the determining factors, and when photosynthetic rate predominates, the main determining factors are solar radiation and temperature (McCarroll and Loaded, 2004).

Water use efficiency (WUE), the amount of carbon assimilated per unit leaf area per unit time at per unit cost of water (Ehleringer et al., 1993) and $\delta^{13}C$ are positively related to each other (Farquhar et al., 1989). The crucial interdependence between carbon and water relations of plants can be particularly well studied by the use of stable carbon isotopes. Determination of $\delta^{13}C$ was suggested as a sensitive method for long-term monitoring of physiological changes (Francey and Farquhar, 1982). Stomata tend to close under drought conditions as a mechanism for saving water, which leads to an improvement in the intrinsic WUE, the study of carbon isotope variability in tree rings over time may be applied to detect potential changes in WUE due to changing climate (Saurer et al., 2004). Previous studies on pines species in Northern America and on conifers species in northern Eurasia suggest an increasing trend in WUE in the last century (Saurer et al., 2004).

The Mediterranean climate is characterized by summer drought and high interannual variability in precipitation and temperature. Species growing in dry sites, such as Mediterranean sites, show annual changes in $\delta^{13}C$ in each tree ring as a result of the variable climatic conditions (temperature and precipitation) and therefore $\delta^{13}C$ may provide a strong indicator of both the severity of the climatic conditions and the changes in WUE (McCarroll and Loader, 2004).

Projections of the impact of climate change on the European distribution of higher plants in 2050 suggest that the Iberian Peninsula may be particularly vulnerable in terms of species loss (Bakkenes et al., 2002). Climate shifts have already taken place in the region, as exemplified by the reduction in rainy days by 50 and 30% over the southern coast of Spain and the Pyrenees, respectively, in the last century (Esteban-Parra et al., 1998; Rodrigo et al., 2000). On the other hand, the mean annual temperature has increased by about 1.6º C in the Iberian Peninsula over the last century (IPCC, 2007).

Maritime pine (*P. pinaster* Ait.) 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 (Nicolas and Gandullo, 1967), and 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).

Scots pine (*P. sylvestris* L.) is the most widely distributed species of pine in the world (Schweingruber, 1996) and in the Iberian Peninsula occupies 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).

In Spain, the Maritime pine and Scots pine occupy 1.6 and 1.21 million ha, respectively, as pure or mixed woodlands, and are two of the main species used in operational forestry (DGCN, 1998; 2002).

Previous studies in the Iberian Peninsula have determined the carbon and oxygen isotopes in tree rings of *Pinus nigra* Arnold, under Mediterranean climate conditions, and *P. sylvestris* and *Pinus uncinata* Miller growing in the northern region of the country (Treydte et al., 2007), but there is no information about stable carbon isotopes in tree rings of *P.*

pinaster and *P. sylvestris* growing under Mediterranean climate conditions. Given the high vulnerability of Iberian plant communities to climate change (Bakkenes et al., 2002) and the importance of *P. pinaster* and *P. sylvestris* forests in this region, stable carbon isotope ratios may provide very valuable information about the climatic variables that affect this variability and WUE as a result of globally changed conditions. These results may help us to explore how populations will behave in the dramatic climatic shifts expected in the future. The objectives of the study were: a) To estimate the climatic variables that determine the δ^{13}C signal in *P. pinaster* and *P. sylvestris* tree rings, and b) To analyse the variability in δ^{13}C in relation to water use efficiency.

Material and Methods

Four trees were selected from each of two samples (one of *P. pinaster* and one of *P. sylvestris*) from a previous dendroclimatological study (Bogino and Bravo, 2008, Bogino et al., 2008 Fig.1, Tab. I). Four trees were used as this is the number of samples that will yield acceptable average absolute δ^{13}C values (McCarroll and Pawellek, 1998).

Samples were obtained with an increment borer, at 1.30 m above ground level. Cores were glued on to channelled wood, dried for two weeks and polished with progressively thinner sandpaper. In order to determine the calendar year in which a tree ring was formed, tree rings were dated by use of a binocular microscope Nikon SMZ1000 (20X), according to standard dendrochronological techniques (Stokes and Smiley, 1968; Fritts, 1976; Cook and Kairiukstis, 1990). In a previous study, the cores in transverse sections were scanned and the v6.06P COFECHA programme (Holmes, 2001; Grissino-Mayer, 2001; available at www.ltrr.arizona.edu) was applied to assess measurement and dating accuracy (Bogino and Bravo 2008, Bogino et al., 2008). Absolute dating is essential for dendroclimatological studies, including isotope analysis, as it is impossible to compare climatic variables in one specific year with $\delta^{13}C$ isotope if the individual tree ring series are not dated correctly. The $\delta^{13}C$ isotope was determined on whole wood (Schleser et al., 1999a; Babour et al., 2001). Cellulose was not extracted from the wood as these two components were shown to yield highly correlated signals (Borella and Leuenberger, 1998) and even higher climatic signal can be detected in the untreated material (Loader et al., 2003). Powdered material was obtained for each tree ring with a Micromot 40E instrument with a 0.5 mm thick needle. The samples were obtained after identification of the previously dated tree ring by use of a binocular microscope. Each sample was analysed individually, the material was weighted, and for each tree ring, 0.2-0.3 mg of powered material was placed into tin capsules. The stable carbon isotopic was measured with an NA 2500 elemental analyser (CE Instruments, Rodano, Italy), with an isotope ratio mass spectrometer (Finningan MAT Delta plus, Bremen, Germany).

The isotopic composition ($\delta^{13}C$) of samples was determined with the formula,

$$[1] \qquad \delta^{13}C\ (‰) = [(^{13}C/^{12}C\ \text{sample}) / (^{13}C/^{12}C\ \text{PDB} - 1)] \times 10^3$$

where $\delta^{13}C$ (‰) is the proportional deviation from the international Peedee belemite (PDB) carbonate standard (Craig, 1957).

As a previous study showed a changing association between climatic variables and growth of *P. pinaster* (Bogino and Bravo, 2008) from the 1980s onwards, and phenological changes have been reported over the last 25 years (Peñuelas et al., 2002), the period analysed was between 1975 and 1999. The same period was considered for *P. sylvestris*.

Pearson's correlation analysis was applied to all trees of the same species to detect common patterns in $\delta^{13}C$, and between $\delta^{13}C$ and the residual tree ring chronologies of both species (data from Bogino and Bravo 2008, Bogino et al., 2008) to detect any significant associations between growth and isotope variability. Pearson's correlation analysis was also used for $\delta^{13}C$ and monthly climatic variables [monthly relative humidity (RH), monthly precipitation, monthly mean maximum temperature, and monthly hours of solar radiation] in order to estimate which environmental variables were statistically significant. The monthly climatic variables that were best correlated with $\delta^{13}C$ were grouped to construct simple linear regression models, as follows [$***p < 0.001$; (Sokal and Rohlf, 1995)],

[2] $y = a + bx + e$

where y is $\delta^{13}C$; x is the sum of climatic variables; a and $b$are unknown parameters of the model and e is a normal random error N(0,1).

Statistic analysis was carried out with the Infostat programme (Di Rienzo et al., 2002). Monthly precipitation, mean monthly temperature and mean monthly RH data from *Cuenca* and monthly hours of solar radiation data from *Molina de Aragón* (*Guadalajara)* meteorological stations for *P. pinaster* and data from *Soria* meteorological station for *P. sylvestris* by the *Agencia Estatal de Meteorología* [Spain (Tab. II)] were used to detect $\delta^{13}C$. Climate diagrams from the *Cuenca* and *Soria* meteorological stations are included (Fig. 2).

Results

The mean values characterizing each tree ring $\delta^{13}C$ in both species and in each year analysed showed that the highest and the lowest values of $\delta^{13}C$ (-22.62‰ and -25.87‰) corresponded to *P. pinaster,* whereas the corresponding values for *P. sylvestris* varied between -23.85‰ and -25.8‰ (Fig. 3).

The mean values characterizing the eight trees analysed are shown in Tab. III. The standard deviation for *P. sylvestris* was lower than 1‰ whereas *P. pinaster* was more variable throughout the 25 years, as indicated by a standard deviation of 1.41‰ in tree 48.

Pearson's correlation coefficient for trees of the same species showed a positive significant association in *P. pinaster* (***$p < 0.001$) and in *P. sylvestris* (*$p < 0.05$, **$p < 0.01$,***$p < 0.001$). In *P. sylvestris* only, trees 8 and 4 did not show any significant correlation (Tab. IV).

A negative significant correlation between $\delta^{13}C$ and radial growth was found for the 1975-1999 period. The Pearson's correlation coefficient for the residual tree ring chronologies and the mean $\delta^{13}C$ was r: -0.83 (***$p < 0.001$) for *P. pinaster* and r:-0.41 (*$p < 0.05$) for *P. sylvestris.*

Pearson's correlation coefficient for climatic variables and $\delta^{13}C$ in *P. pinaster* showed that moisture is a driving factor affecting the variability of $\delta^{13}C$ between winter and summer (January to July) (Fig. 4a). Rainfall also had a negative effect on $\delta^{13}C$ between winter and spring although the effect was only statistically significant in April and May (Fig. 4b). Monthly mean maximum temperature in March had a positive significant effect on $\delta^{13}C$ (Fig. 4c) and monthly hours of solar radiation in January, April and May (Fig. 4d).

Pearson’s correlation coefficient for climatic variables and δ^{13}C in *P. sylvestris* showed that moisture is a driving factor affecting δ^{13}C variability in summer (July) and in autumn (October) (Fig. 5a). Rainfall in October also had a negative effect on δ^{13}C (Fig. 5b). Monthly mean maximum temperature in summer (June and July) had a positive significant effect on δ^{13}C (Fig. 5c) as well as hours of solar radiation in October (Fig. 5d).

For *P. pinaster* RH from January to July, rainfall from January to May, maximum mean temperature from April to July and hours of solar radiation of January, April and May were selected to construct four different simple linear regression models that relate each variable and δ^{13}C (Fig. 6). For *P. sylvestris* RH in July and October months, rainfall in July and October, maximum mean temperature in June and July and hours of solar radiation in October were selected to construct different simple linear regression models (Fig. 7). All variables were significantly correlated with δ^{13}C in both species.

Discussion

The δ^{13}C is a useful tool that provides both reliable information about the climatic variables that affect the physiology of *P. pinaster* and *P. sylvestris* and WUE of these species growing under Mediterranean climatic conditions.

The confidence interval at 95% showed extremes values of δ^{13}C for trees in the same calendar year, which varied from –23.44‰ to –27.50‰ (year 1978) and from –22.83‰ to –26.45‰ (year 1994) in *P. pinaster* and *P. sylvestris*, respectively. This high variability between trees suggests an individual tree response in relation to fractionation [the ratio of carbon isotope ratios in reactant and products (Farquhar et al., 1989)]. The values of δ^{13}C data for all trees of *P. pinaster* were significantly correlated which suggests that this variability is driven by a strong environmental effect. The δ^{13}C values for individual *P.*

sylvestris trees were also significantly correlated but the coefficients were lower than in *P. pinaster* and two trees did not show any significant association. The mean $\delta^{13}C$ for trees ranged between 1.14‰ and 1.56‰, respectively in *P. pinaster* and *P. sylvestris*, and was lower than the variability of *P. sylvestris* in Finland, *Pinus edulis* Engelm. in the south-western EUA and *Fitzroya cupressoides* Johnst. in Patagonia as reported in previous studies (Leavitt and Long, 1984; Leavitt and Lara, 1994; McCarroll and Pawellek, 1998). The highly significant inverse correlation between $\delta^{13}C$ and tree ring width in *P. pinaster* and *P. sylvestris* emphasises the potential usefulness of this type of study for analysing the environmental factors that affect growth of *P. pinaster* and *P. sylvestris* under Mediterranean climatic conditions. The results are consistent with previous statements that suggest that species growing in variable environments such as Mediterranean environments show a changing ratio of $\delta^{13}C$ in each tree ring as a result of the variables climatic conditions (high temperature and low precipitation) and that $\delta^{13}C$ provides a strong indicator of the severity of these climate variables (McCarroll and Loader, 2004).

The $\delta^{13}C$ was significantly correlated with moisture, rainfall, maximum temperature and solar radiation, all of which are related to water availability and evapo-transpiration. The negative effect of moisture and rainfall prior to and throughout the growing season, and the positive effect of maximum temperature and solar radiation throughout the same period confirm the hypothesis that $\delta^{13}C$ in conifers worldwide is an indicator of drought stress in dry climates (Warren et al., 2001).

In *P. pinaster* the $\delta^{13}C$ accounted for rainfall from January to May and RH between January and July was 44% and 52%, respectively. On the other hand, rainfall and humidity in July and October on *P. sylvestris* accounted for 34% and 22% of the $\delta^{13}C$, respectively. In *P. sylvestris* growing in the Pyrenees mountains, October rainfall affects $\delta^{13}C$ whereas in

Pinus longaeva D.K.Bailey growing in White Mountains (California, USA) 46% of the δ^{13}C was related to drought indexes (Leavitt , 1994; Treydte et al., 2007). These results, together with the present results are consistent with a previous report that underlines the importance of water availability as a driving factor for isotope fluctuation in both arid and semiarid environments and in pine species (Warren et al., 2001).

Although temperature had a significant effect on drought (the higher the temperature, the greater the effect of drought), the results showed that each climatic variable analysed may have a significant effect on δ^{13}C in these Mediterranean environments. In the present study, maximum mean temperature was the only climatic variable that was more closely correlated with δ^{13}C in *P. sylvestris* than in *P. pinaster* (61% and 57%, respectively). This association was highly significant in both species and the significant effect varied according to the period analysed: summer temperature were significant for *P. sylvestris* (June and July) and spring temperatures (March) for *P. pinaster*. Correlations between δ^{13}C and summer temperatures have previously been reported in *Larix, Pinus* and *Picea* trees growing at high-latitude sites (59º–71ºN) (Saurer et al., 2004). Part of this signal may be due to the fact that hot summers are strongly correlated with high evaporation rates and thus, with δ^{13}C.

As the carbon isotopic is a measure of the balance between stomatal conductance and photosynthetic rate, when moisture is limiting, stomatal conductance predominates and the determining environmental factors are air RH and soil moisture, which are strongly correlated with RH and precipitation (Gagen et al., 2004). Stomatal conductance in pines species is controlled by soil moisture and RH (Leavitt, 1993; McCarroll and Pawellek, 2001). Although in this study RH and rainfall were clearly correlated with δ^{13}C, it is impossible to reject the idea that temperature also has a significant effect on δ^{13}C. A complex response of $\delta^{13}C$ to climate was also for *P. sylvestris* in Finland (McCarroll and Pawellek, 2001), even though the trees were growing in environments where temperature is a strongly growth-limiting factor. Considering that *P. sylvestris* in the Iberian Peninsula grows within the limit of the worldwide distribution, and Schleser et al. (1999b) reported that under these conditions, $\delta^{13}C$ tends to respond to one atmospheric parameter, these results are also consistent with those of McCarroll and Pawellek (2001) who concluded that the $\delta^{13}C$ response is complex.

The rate of photosynthesis depends on radiation and temperature (Beerling, 1994) and McCarroll and Pawellek (2001) reported a strong correlation between $\delta^{13}C$ and hours of summer sunshine, temperature, antecedent precipitation and RH according to the sampling site in a study of Scots pine in four sites in Finland. Even though the climatic conditions in the sites differ significantly from those in the present sites, summer temperature (June and July) and rainfall (July) have the same significant effect on $\delta^{13}C$.

It was previously concluded that the same species may have different isotopic responses in different environments (Sternberg and DeNiro, 1983; Leavitt and Long, 1984), and *P. pinaster* growing under maritime climatic conditions in Italy did not show any strong variation in $\delta^{13}C$ in different years, even thought there was a severe drought during the period analyzed (summer of 2001) and the authors commented on the limitation of the application of stable carbon analysis in assessing the severity of drought in environments characterized by seasonal aridity (De Micco et al., 2007). Even though we analysed the same species, the present results showed that *P. pinaster* is an accurate tool for studying climatic conditions, as shown by the significant correlation coefficients that underlined the recommendation made by Leavitt and Long (1986) that results for $\delta^{13}C$ in one species should not be extrapolated to other environments.

Even thought in same cases isotopes may be more closely correlated with climatic variables than tree ring width, use of one does not limit the use of the other, as for example in sub fossil chronologies of Scots pine in Finland the use of $\delta^{13}C$ would be limited for climatic reconstruction if not used with other estimates (McCarroll and Pawellek, 2001). Wood decay can affect the $\delta^{13}C$ and limit the use of carbon isotopes for the reconstruction of past climate conditions (Schleser et al., 1999a).

When WUE is analysed, the results suggest the importance of $\delta^{13}C$ studies for understanding the physiological changes in trees in relation to general changing environmental conditions. Phenological changes and the increased WUE in woody species in the Iberian Peninsula have been already reported (Peñuelas et al., 2002), and the significant correlation between RH and $\delta^{13}C$ in *P. pinaster* and *P. sylvestris* also corroborate these studies.

Even though environments in the Iberian Peninsula are suggested to be threatened under climatic scenarios (Bakkenes et al., 2004), both species showed a significant association with moisture, which indicates their capacity to cope with unfavourable climatic conditions. It is difficult to agree with Harrison et al. (2006) who suggested an unsuitable environment for these species from the year 2050 because the $\delta^{13}C$ studies revealed a clear adaptable capacity related to a better WUE.

In summary, *P. pinaster* and *Pinus sylvestris* growing in Mediterranean environments showed a significant correlation with climatic conditions which suggests that they are a very valuable tool for studying the effects of climate change. On the other hand, the potential use of both species for analysing physiological changes (WUE) in species due to changing environmental conditions emphasised the importance of this study for enhancing other tools used in dendroclimatological research.

Acknowledgements

The authors thank Cristotal Ordóñez for field assistance, María José Fernández Nieto for providing meteorological data and Christine Francis for improving the English version. Thanks are also extended to Drs. Jens Dyckmans and Reinhard Langel of the *Büsgen-Institut Kompetenzzentrum Stabile Isotope (KOSI) Georg-August Universität, Göttingen,* Germany, for their help with the isotopic analysis. This study was supported by Research projects from the Regional *Castilla y León* government (Spain) (Project Code VA096A05), the Spanish Ministry of Science and Education (Project Code AGL2007-65795-C02-01) and by the Alβan Programme, the European Union Programme of High Level Scholarships for Latin America (Scholarship No. E05D049920AR).

References

Alía M., Martín S., De Miguel J., Galera R., Agúndez D., Gordo J., Catalán G., and Gil L. 1996. Regiones de procedencia *Pinus pinaster* Aiton. INIA-CIFOR, Madrid.

Bakkenes M., Alkemade J.R.M., Ihle F., Leemansand R., and Latour J.B. 2002. Assessing effects of forecasted climate change on the diversity and distribution of European higher plants for 2050. Glob. Chang. Biol. 8 (4): 390-407.

Barbéro M., Loisel R., and Quezel P. 1998. Pines of the Mediterranean Basin. *Pinus.* Ecology and Biogeography of *Pinus.* D.M. Richardson (Ed.), Cambridge University Press, Cambridge, pp. 153-170.

Barbour M., Andrews J., and Farquhar, G. 2001. Correlations between oxygen isotope ratios of wood constituents of *Quercus* and *Pinus* samples from around the world. Australian J. of Plant Physiol. 28(5): 335-348.

Beerling D. 1994. Predicting leaf exchange and $\delta^{13}C$ responses to the past 30000 years of global environmental change. New Phytol. 128: 425-433.

Blanco E., Casado M., Costa M., Escribano R., Gracía Antón M., Génova M., Gómez Manzaneque G., Gómez Manzanaque F., Moreno J., Morla C., Regato P., and Sainz Ollero H. 1997. Los bosques ibéricos, Editorial Planeta, Barcelona, España.

Bogino S., and Bravo F. 2008. Growth response of *Pinus pinaster* Ait. to climatic variables in central Spanish forests. Ann. For. Sci. 65: 506-518.

Bogino S., Fernández Nieto M., and Bravo F. Drought index and radial growth of *Pinus sylvestris* L. in its southern and western distribution threshold. J. Arid Envir. Submitted.

Borella S., and Leuenberger M. 1998. Reducing uncertainties in d13C analysis of tree rings: pooling, milling and cellulose extraction. J. Geophys. Res. 103: 19519-19526.

Cook E.R., and Kairiukstis L.A. 1990. Methods of Dendrochronology: applications in the environmental sciences. Kluwer, Dordrecht.

Craig H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. Geochim. Cosmochim. Acta 12: 133-149.

De Micco V., Saurer M., Aronne G., Tognetti R., and Cherubini P. 2007. Variations of wood anatomy and $\delta^{13}C$ within-tree rings of coastal *Pinus pinaster* showing intra-annual density fluctuations IAWA J. 28 (1): 61-74.

DGCN 1998. Segundo Inventario Forestal Nacional 1986-1996. Ed. Ministerio de Medio Ambiente, España.

DGCN 2002. Plan Forestal Español. Ed. Ministerio de Medio Ambiente, España.

Di Rienzo J., Balzarini M., Casanoves F., Gonzalez L., Tablada E., and Robledo C. 2002. Infostat Software Estadístico versión 2. Grupo infoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Ehleringer J. R., Hall A. E., and Farquhar G. D. 1993. Stable Isotopes and Plant Carbon-Water Relations. Academic Press. San Diego, CA. 555 p.

Esteban-Parra M., Rodrigo F., and Castro Diéz Y. 1998. Spatial and temporal patterns of precipitation in Spain for the period 1880-1992. Int. J. Climatol. 18: 1557-1574.

Farquhar, G., Ehleringer J., and Hubick K. 1989. Carbon isotope discrimination and photosynthesis Annu. Rev. Plant Physiol. Plant Mol. Biol. 40: 503-537.

Francey R., and Farquhar G.1982. An explanation of $^{13}C/^{12}C$ variations in tree rings. Nature 297: 28-31.

Fritts H. 1976. Tree Ring and Climate, Academic Press Inc, London.

Gagen M., McCarroll D., and Edouard J. 2004. Latewood width, maximum density, and stable carbon isotope ratios of pine as climate indicators in a dry sub alpine environment, French Alps. Arctic, Antarctic, and Alpine Res. 36(2): 166-171.

Grissino-Mayer H.D. 2001. Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. Tree-Ring Res. 57: 205-221.

Harrison P., Berry P., Butt N., and New M. 2006. Modelling climate change impacts on species' distributions at the European scale: implications for conservation policy. Enviro. Sci. Polic. 9: 116-128.

Holmes RL 2001. Dendrochronology Program Library. Available from the Laboratory of Tree Ring Research, University of Arizona, Tucson, USA.

IPCC 2007. Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Leavitt S. 1993. Seasonal C^{13}/C^{12} changes in tree-ring species and site coherence, and a possible drought influence. Can. J. For. Res.23: 210-218.

Leavitt, S. 1994. Major wet interval in White Mountains medieval warm period evidenced in $\delta^{13}C$ of bristlecone pine tree rings. Clim. Change 26: 299-307.

Leavitt S., and Long A. 1984. Sampling strategy for stable carbon isotope analysis of tree rings in pine. Nature 311: 145-147.

Leavitt S., and Long A. 1986. Stable carbon isotope variability in tree foliage and wood. Ecology 67 (4): 1002-1010.

Leavitt, S., and Lara A., 1994. South American tree rings show declining $\delta^{13}C$ trend. Tellus 46: 152-157.

Loader N.J., Robertson I., and McCarroll D. 2003. Comparison of stable carbon isotope ratios in the whole wood cellulose and lignin of oak tree-rings. Palaeogeo., Palaeoclim., Palaeoecol. 196: 395-407.

Martínez-Vilalta J., and Piñol J. 2002. Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. For. Ecol. Manage.161: 247-256.

McCarroll D., and Pawellek F. 1998. Stable carbon isotope ratios of latewood cellulose in *Pinus sylvestris* from northern Finland: variability and signal-strength. The Holocene 8: 675-684.

McCarroll D., and Pawellek F. 2001. Stable carbon isotope ratios of *Pinus sylvestris* from northern Finland and the potential for extracting a climate signal from long Fennoscandian chronologies. The Holocene 11(5): 517-526.

McCarroll D., and Loaded N. 2004. Stable isotopes in tree rings. Quaternary Science Reviews 23: 771-801.

Nicolas A., and Gandullo J. 1967. Ecología de los pinares españoles. 1, *Pinus pinaster* Ait. Ministerio de Agricultura, Madrid, España.

Peñuelas J., Fillela I., and Comas P. 2002. Change plant and animal life cycles from 1952 to 2000 in the Mediterranean region. Glob. Chang. Biol. 8: 531-544.

Rodrigo F., Esteban-Parra M., Pozo-Vázquez D., and Castro-Diéz Y. 2000. Rainfall variability in southern Spain on decadal to centennial time scales. Int. J. Climatol. 20: 221-732.

Saurer M., Siegwolf R., and Schweingruber F. 2004. Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years Glob. Chang. Biol. 10: 2109-2120.

Schleser G.H., Frielingsdorf J., and Blair A. 1999a. Carbon isotope behaviour in wood and cellulose during artificial aging. Chem. Geol. 158: 121-130.

Schleser, G.H., Helle, G., Lücke, A. and Vos, H. 1999b. Isotope signals as climate proxies: the role of transfer functions in the study of terrestrial archives. Quat. Sci. Rev. 18: 927-943.

Schweingruber F. 1996. Tree rings and environment: Dendroecology. Haupt, Berne.

Sokal R.R., and Rohlf F.J. 1995. Biometry: the principles and practice of statistics in biological research, 3rd edition, WH Freeman and Co., New York, UEA.

Sternberg L., and De Niro J. 1983. Isotopic composition of cellulose from C_3, C_4 and CAM plants growing near one another. Science 220: 947-949.

Stokes M., and Smiley T. 1968. An Introduction to Tree-Ring Dating, University of Arizona Press, Tucson, UEA.

Tans P., DE Jong A., and Mook W. 1978. Chemical pre-treatment and radial flow of ^{14}C in tree rings. Nature 271: 234-235.

Treydte K., Frank, D. Esper, L. Andreu L.,Bednarz Z., Berninger F.,Boettger T., D'Alessandro C., Etien N., Filot M., Grabner M., Guillemin M., Gutierrez E., Haupt M., Helle G., Hilasvuori E., Jungner H., Kalela-Brundin M., Krapiec M., Leuenberger M., Loader N., Masson-Delmotte V., Pazdur A., Pawelczyk S., Pierre M., Planells O., Pukiene R., Reynolds-Henne C., Rinne K., Saracino A., Saurer M., Sonninen E., Stievenard M., Switsur V., Szczepanek M., Szychowska-Krapiec E., Todaro L., Waterhouse J., Weigl M., and Schleser G. 2007. Signal strength and climate calibration of a European tree ring isotope network. Geophys. Res. Let. 34, doi:10.1029/2007GL031106, L24302.

Warren C., McGrath J., and Adams M. 2001. Water availability and carbon isotope discrimination in conifers. Oecologia 127:476-486.

Table I. Geographical positions, altitude and time span of *P. pinaster* and *P. sylvestris* trees used in the isotope analysis.

	P.pinaster	***P.sylvestris***
Latitude N	39º 48' 56”	42º 04' 36”
Longitude W	01º 15' 36”	02º 30' 18”
Altitude (m)	920	1676
Time span (years)	1947-2005	1945-2005

Table II. Descriptive statistic for climatic variables recorded at the *Cuenca*, *Soria* and *Molina de Aragón* meteorological stations.

Variable	**N**	**Mean**	**S.D.**	**Min**	**Max**
Pp_Cuenca (mm)	25	500.29	119.44	266.2	700.6
Ttma_Cuenca (ºC)	25	18.83	0.687	17.71	20.13
Hu_Cuenca (%)	25	62.65	3.03	57.17	69.5
Ra_Molina (hours)	25	2046.92	127.3	1807.75	2288
Pp_Soria (mm)	25	505.77	102.92	370.4	859.6
Ttma_Soria (ºC)	25	16.81	0.689	15.55	18
Ra_Soria (hours)	25	2093.54	138.18	1833.92	2331.5
Hu_Soria (%)	25	67.68	3.38	61	73.83

Pp: Mean annual rainfall, Ttma: mean annual maximum temperature, Hu: mean annual relative humidity and Ra: mean monthly hours of solar radiation for the 1975-1999 period.

Table III. Descriptive statistics of the four *P. pinaster* and *P. sylvestris* individuals used in the isotope analysis in central Spain.

	n	**Mean**	**S.D.**	**Min.**	**Max.**
Pinus pinaster					
Tree 48	25	-24.52	1.41	-27.07	-22.2
Tree 44	25	-25.03	1.23	-27.1	-23.07
Tree 27	25	-23.89	0.96	-25.61	-22.07
Tree 53	25	-24.58	1.06	-26.05	-22.4
Pinus sylvestris					
Tree 23	25	-25.79	0.48	-26.65	-24.91
Tree 19	25	-24.23	0.78	-25.75	-22.85
Tree 8	25	-24.45	0.55	-25.51	-23.57
Tree 4	25	-24.6	0.58	-25.79	-23.2

n: number of tree ring analyzed; Mean: mean value of $\delta^{13}C$ for each tree analyzed; SD: Standard deviation; Min and Max: extreme values.

Table IV. Pearson's correlation coefficient for the values of $\delta^{13}C$ data for all trees in *P. pinaster* and *P. sylvestris.*

Pinus pinaster			
	Tree 48	Tree 44	Tree 27
Tree 44	0.82***		
Tree 27	0.8***	0.73***	
Tree 53	0.68***	0.7***	0.73***
Pinus sylvestris			
	Tree 23	Tree 9	Tree 8
Tree 19	0.59***		
Tree 8	0.41*	0.5**	
Tree 4	0.72***	0.78***	0.37

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

Figure 1. *P. pinaster* and *P. sylvestris* sampling sites in the Iberian Peninsula. The squares indicate the meteorological stations: So *Soria*, Mo *Molina de Aragón*, Cu *Cuenca*. The circles indicate the sampling sites: Syl *P. sylvestris*, Pin *P. pinaster.*

Figure 2 Climate-diagram for the *Cuenca* and *Soria* meteorological stations.

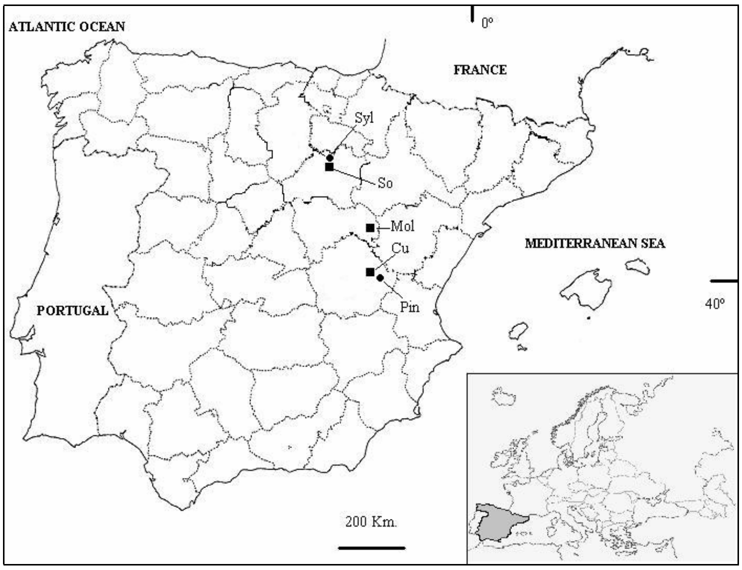
Figure 3 The mean values (thick lines) and confidence intervals at 95% (thin lines) for isotope analysis of Scots and Maritime pines in Spain.

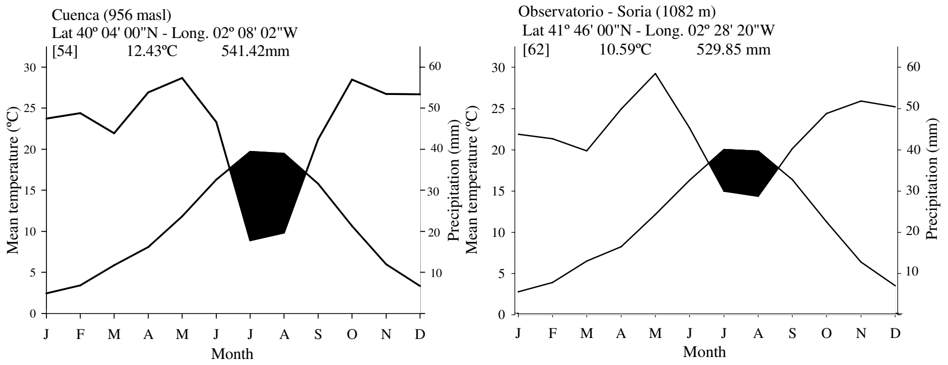
Figure 4. Pearson's correlation coefficients for $\delta^{13}C$ isotopes in *P. pinaster* and monthly RH (a), monthly rainfall (b), maximum mean monthly temperature (c) and monthly hours of solar radiation (d). Bars outside dashed lines show significant coefficients at $^{**}p < 0.01$. Bars outside dotted lines show significant coefficients at $^{*}p < 0.05$.

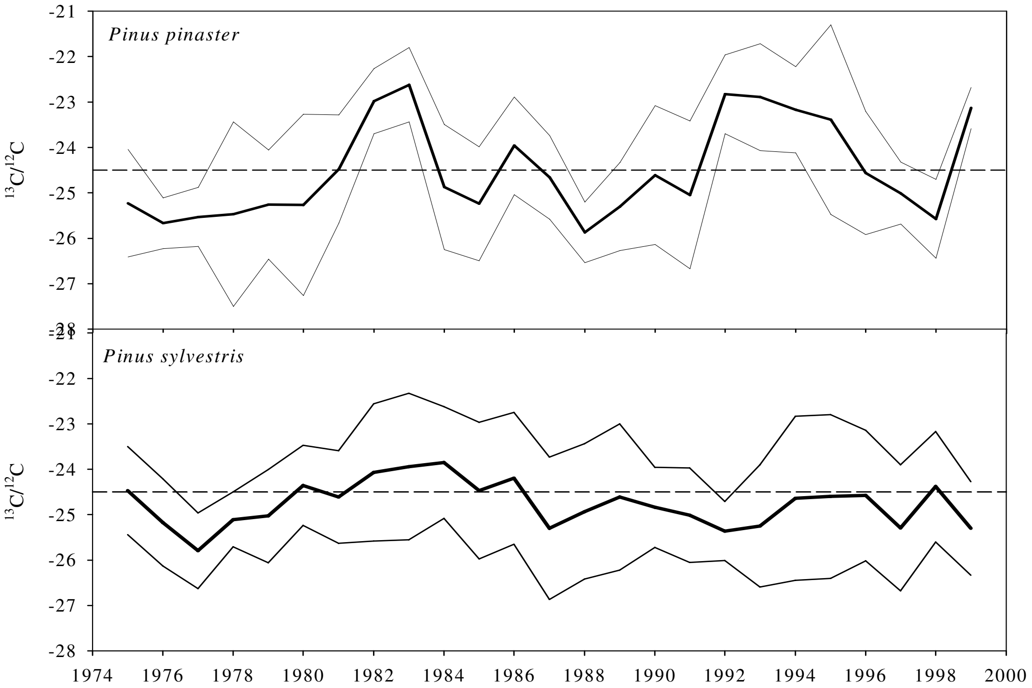
Figure 5. Pearson's correlation coefficients for $\delta^{13}C$ isotopes of *P. sylvestris* and monthly RH (a), monthly rainfall (b), maximum mean monthly temperature (c) and monthly hours of solar radiation (d). Bars outside dashed lines show significant coefficients at $^{**}p < 0.01$. Bars outside dotted lines show significant coefficients at $^{*}p < 0.05$.

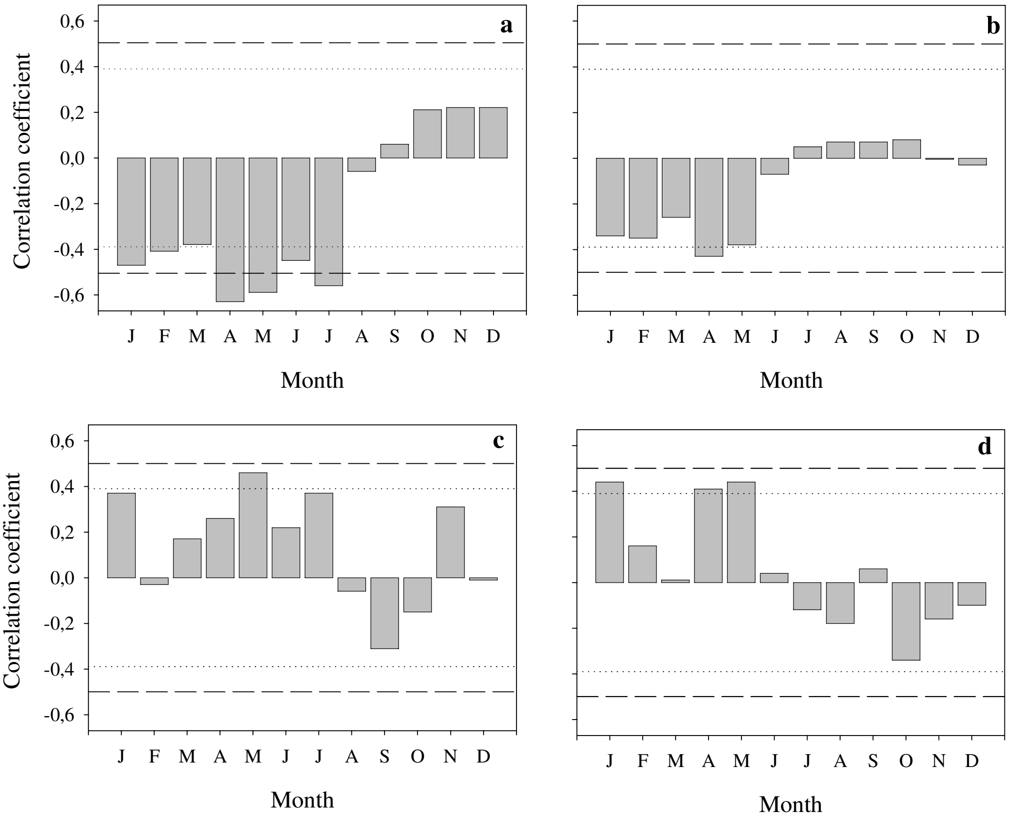
Figure 6. Linear regression models for $\delta^{13}C$ in *P. pinaster* and RH between January and July (a), rainfall between January and May (b), maximum mean temperature between April and July (c) and hours of solar radiation in January, April and May (d).

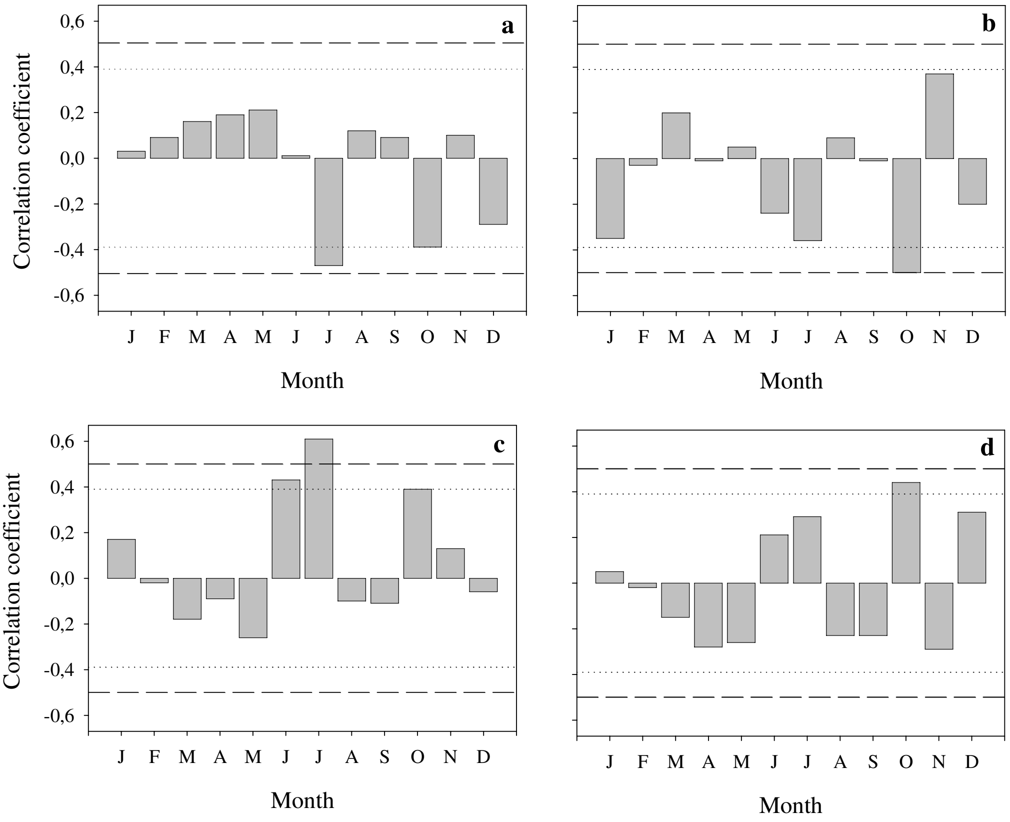
Figure 7. Linear regression between $\delta^{13}C$ in *P. sylvestris* and RH in July and October (a); rainfall in July and October (b) maximum mean temperature in June and July (c) and hours of solar radiation in October.

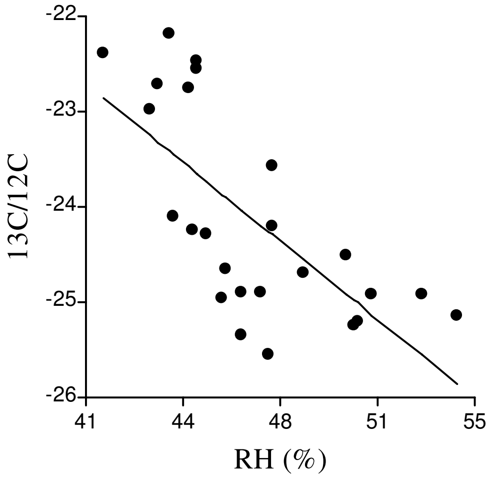

ATLANTIC OCEAN
0º
FRANCE
Syl
So
Mol
Cu
Pin
MEDITERRANEAN SEA
40º
PORTUGAL
200 Km.


Cuenca (956 masl)
Lat 40º 04' 00"N - Long. 02º 08' 02"W
[54] 12.43ºC 541.42mm
Mean temperature (ºC)
Precipitation (mm)
Month
J F M A M J J A S O N D
Observatorio - Soria (1082 m)
Lat 41º 46' 00"N - Long. 02º 28' 20"W
[62] 10.59ºC 529.85 mm
Mean temperature (ºC)
Precipitation (mm)
Month
J F M A M J J A S O N D

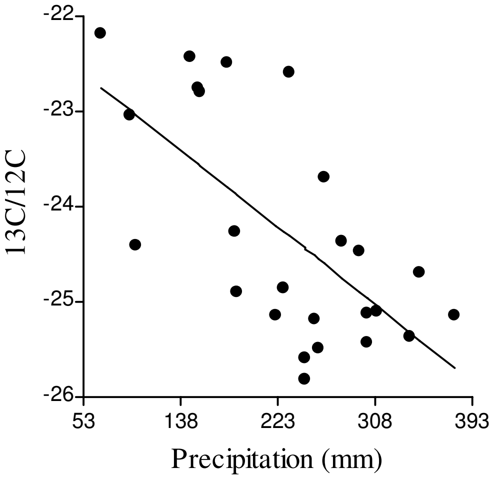

Pinus pinaster
Pinus sylvestris
$^{13}C/^{12}C$
$^{13}C/^{12}C$
-21
-22
-23
-24
-25
-26
-27
-28
1974
1976
1978
1980
1982
1984
1986
1988
1990
1992
1994
1996
1998
2000


a
b
c
d
Correlation coefficient
0,6
0,4
0,2
0,0
-0,2
-0,4
-0,6
J F M A M J J A S O N D
Month

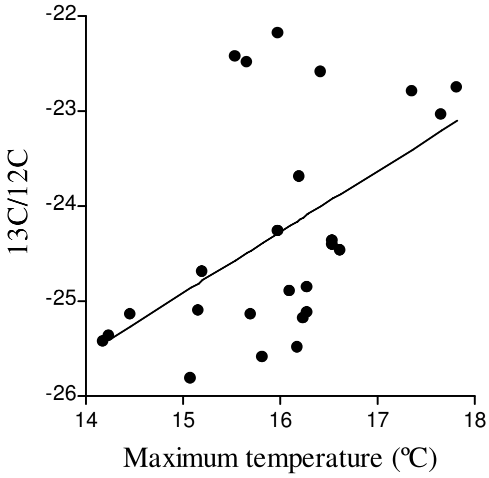

a
b
c
d
Correlation coefficient
0,6
0,4
0,2
0,0
-0,2
-0,4
-0,6
J F M A M J J A S O N D
Month



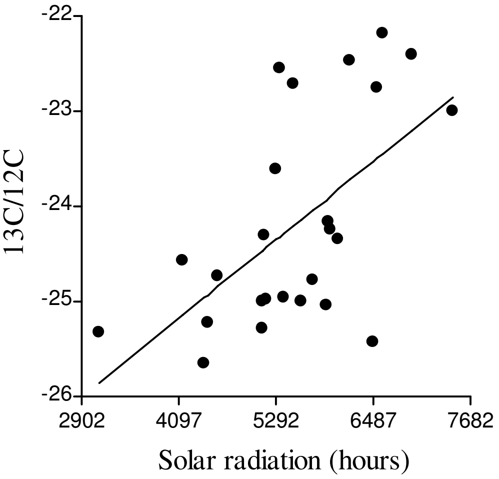
n = 25; $r^2 = 0.52$, $p < 0.0001$;
a = -14.25; b = -.22



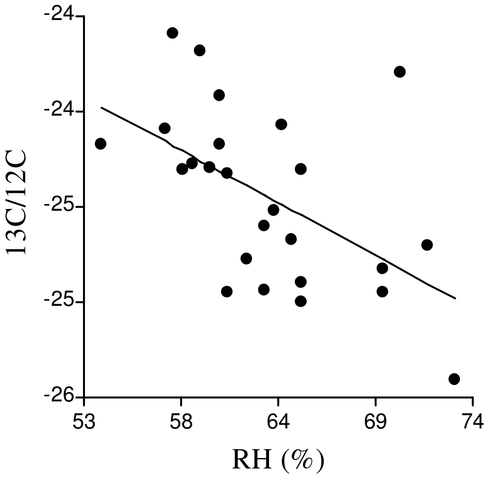
n = 25; $r^2 = 0.44$; $p = 0.0003$;
a = -22.54; b = -0.01



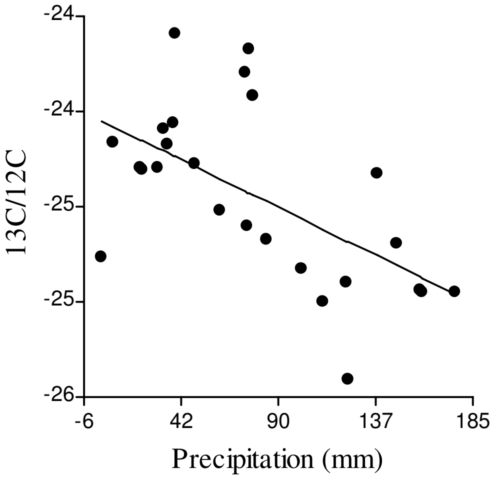
n = 25; $r^2 = 0.28$, $p = 0.0093$;
a = -34.14; b = 0.59



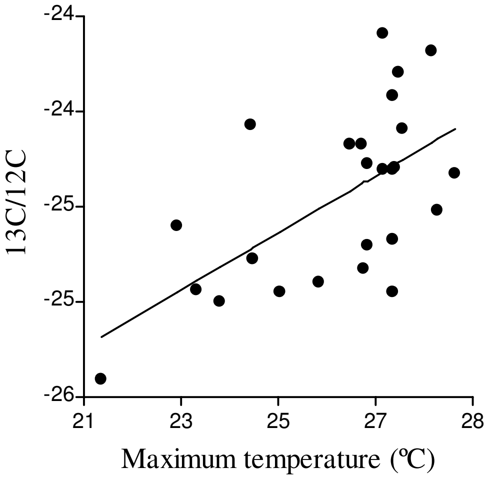
n = 25; $r^2 = 0.34$, $p = 0.0020$;
a = -28.07; b = $6.5.10^{-4}$



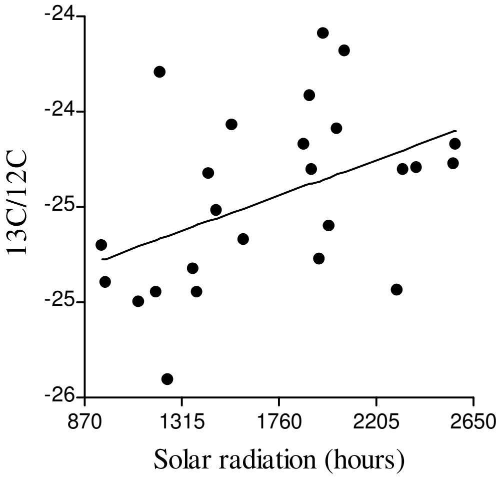
n = 25; $r^2 = 0.29$, $p = 0.0051$;
a = -21.27; b = -0.06



n = 25; $r^2 = 0.34$, $p = 0.002$;
a = -24.33; b = -0.01



n = 25; $r^2 = 0.37$; $p = 0.0013$;
a = -29.17; b = 0.17



n = 25; $r^2 = 0.20$; $p = 0.0262$;
a = -25.54; b = $4.5.10^{-4}$