Universidad de Huelva

Departamento de Ciencias Agroforestales

Influencia de factores ecológicos y selvícolas en el crecimiento diametral de la encina (*Quercus ilex* ssp. *Ballota* (Desf.) Samp.) en el suroeste de España

Memoria para optar al grado de doctor
presentada por:

Daniel Martín Pérez

Fecha de lectura: 22 de enero de 2016

Bajo la dirección de los doctores:

Francisco Javier Vázquez Piqué

Reyes Alejano Monge

Huelva, 2016

UNIVERSIDAD DE HUELVA
ESCUELA TÉCNICA SUPERIOR DE INGENIERÍA
Departamento de Ciencias Agroforestales

Influencia de factores ecológicos y selvícolas en el crecimiento diametral de la encina (*Quercus ilex* ssp. *ballota* (Desf.) Samp.) en el suroeste de España

Memoria presentada por Daniel Martín Pérez para optar al Grado de Doctor por la Universidad de Huelva

Daniel Martín Pérez
Directores:
Francisco Javier Vázquez Piqué
Reyes Alejano Monge

Huelva, Octubre de 2015

Dedicado a mis hijos,

Ángel y Nora.

Espero que me superen.

AGRADECIMIENTOS

En primer lugar, más que agradecer, les pido perdón a Olga y Ángel, por todo el tiempo que no he estado con ellos por dedicarme a la dichosa tesis, largas mañanas y tardes que me he exiliado encerrado dentro del cuarto del ordenador; o aquellas otras en que he tenido que exiliarles a ellos para quedarme yo tranquilo en casa, cavilando discusiones científicas rodeado de un mar de artículos que iban invadiendo poco a poco todas las habitaciones de la casa. Espero que de aquí en adelante pueda ir recuperando todo ese tiempo que les debo. Siguiendo con la familia, tengo que agradecer a mi madre, Elena, su apoyo para la realización de esta tesis, tanto personal como económico, puesto que podría decirse que ha sido también unas de las "entidades" que ha cofinanciado este trabajo, puesto que sin su ayuda hubiese sido difícil realizar algunas de las actividades que finalmente han permitido la presentación de esta tesis en su estado actual. Debo agradecer también a mi padre y a mis abuelos, que desgraciadamente, salvo abuelo Ángel, ya no se encuentran entre nosotros, por haberme animado a comenzar mis estudios, hace ya muchísimos años, tan lejos de mi tierra, y en una disciplina que en un principio les resultaba algo extraña. A todos ellos, por todo lo que me enseñaron, haciéndome tal y como soy, muchas gracias. También tengo que agradecer a mis suegros, Adolfo y Antonia, por acogerme de buen grado en su casa en aquel último periodo de trabajo de laboratorio, ya sin financiación, y por haber cuidado también de Ángel cuando tocaba algún viaje de congreso o durante mi estancia en Suiza, o en aquellos otros momentos de exilio forzado que ya he comentado.

Sin duda debo agradecer a mis directores de tesis, Javier Vázquez y Reyes Alejano, por haberme dado la oportunidad de realizar esta tesis, financiando la misma con sus respectivos proyectos, y dedicando toda la inconmensurable cantidad de tiempo necesaria para llevarla a buen puerto. De todas las temáticas disponibles para realizar una tesis doctoral que habían en el Departamento en aquel momento, no creo que el crecimiento de la encina fuera la más fea, pero sí de las más grandes por volumen de datos y complejidad, y de las que creo que más discusiones ha provocado entre unos y otros, hasta que al final parece que comenzamos todos a tener "algo de idea" de cómo se comporta la encina en cuanto a crecimiento; y digo esto con bastante humildad, porque de toda la complejidad que implica el conocimiento de las interrelaciones *existentes entre los diferentes factores que pueden afectar en campo al crecimiento de una especie como la encina, creo que es ya bastante exitoso defender esta tesis con los conocimientos que de ella se han obtenido. De Javier agradecer especialmente su guía en el tema de la estadística y de los modelos de crecimiento, que me evitó abrir los manuales de SAS más de lo saludablemente recomendable, aunque al final debo de reconocer que el uso de ese “programita” proporciona unas ventajas sustanciales. De Reyes agradecer sus buenas ideas. Lo de abordar el tema de los trade-offs fue un gran acierto, no solo porque es algo que no había hecho nadie hasta entonces en encina, sino debido también a que despejó algunas dudas sobre por qué una encina crecía inexplicablemente más que su vecina. Agradecimientos también a los demás profesores del Departamento de Ciencias Agroforestales que me han echado una mano en algunas de las cosillas de esta tesis, como Cristina Pérez-Carral con la metodología para calcular los modelos digitales del terreno, Raúl Tapias con el tema de los dendrómetros digitales, y Manuel Fernández con algunas cuestiones sobre la fisiología de la encina de las que “tomaba nota”. También agradecer a Juan Carlos Gutiérrez (Guti) y a Inmaculada Pulido por haberme introducido en el mundo de las Redes Neuronales y la Lógica Borrosa, aunque al final los modelos mixtos “ganaron” esta partida.*

Debo agradecer también a Guillermo Gea-Izquierdo, a Paolo Cherubini, y a todo el personal del WSL en Birmensdorf (Suiza), por haber hecho posible mi estancia de investigación para aprender dendrocronología en la encina (y por proximidad, en el pino piñonero), aunque si bien no tuve el tiempo suficiente para conseguir una serie sincronizada (no veáis lo difíciles que estaban esas rodajas de Calañas), el solo hecho de ver bajo la lupa y el microscopio los anillos de crecimiento y vasos xilemáticos de la encina me aclararon bastante las ideas respecto a los datos de los dendrómetros que había trabajado anteriormente.

Agradecimientos al Ministerio de Educación, por financiar el proyecto de investigación con el que me inicié en esta tesis con una beca (SUM2006-00026-00-00), y por la beca que obtuve posteriormente para la realización de mi estancia en el WSL; a la Junta de Andalucía, por financiar el proyecto de Excelencia en el que estuve contratado unos cuantos añitos (C03-192); y a la Universidad de Huelva, por ser la cuna de esta larga tesis que hoy concluye.

Por último, no podría dejar de agradecer a todos aquellos alumnos de la carrera de Ingeniería Técnica Forestal y Grado en Ingeniería Forestal, a los becarios y alumnos de doctorado, y a todo el personal investigador del Departamento de Ciencias Agroforestales, que han colaborado en la toma y procesado de los datos de esta tesis, que han sido muchos, y prefiero citarles en conjunto por si me olvido de los nombres en particular de algunos de ellos. Son todas esas personas que me echaron una mano, especialmente en campo, de forma desinteresada, prestando atención y aprendiendo, sin estar pendientes ni dar la lata sobre la hora a la que iban a llegar a casa para almorzar o echarse la siesta.

A todos ellos, gracias.

ÍNDICE

Resumen

La medición del crecimiento de los árboles proporciona información sobre su salud y el vigor de los bosques, los procesos fenológicos, los efectos del clima, su fisiología y estado hídrico, la competencia, la asignación de recursos dentro del árbol, y la influencia de las prácticas de gestión. La encina (*Quercus ilex* ssp. *ballota* (Desf.) Samp.) es la especie forestal más ampliamente distribuida en la Península Ibérica, siendo uno de los árboles más representativos de sus montes y dehesas.

En esta tesis se analiza el crecimiento diametral intraanual y las variaciones diarias del radio del tronco de encina, en 128 árboles con dendrómetros de banda, y en 9 árboles con dendrómetros electrónicos puntuales de alta resolución, situados en tres dehesas y en un monte mediterráneo mixto del suroeste de España. Este estudio abarca un periodo de nueve años y medio, desde mayo de 2003 hasta octubre de 2012, incluyendo años con contrastadas condiciones climáticas, especialmente en el régimen de precipitaciones. Además de los patrones de crecimiento diametral intraanual y de los ciclos diarios de variaciones en el radio, en este trabajo se ha analizado la influencia de las variables climáticas sobre el crecimiento, y los efectos del tamaño del árbol, los parámetros hidrológicos y la competencia sobre la variabilidad de crecimiento entre árboles. Asimismo, se ha analizado la existencia de *trade-offs* en la asignación de recursos entre el crecimiento diametral y la producción de bellota a nivel de árbol individual. Finalmente, se han evaluado los efectos de algunas prácticas tradicionales de tratamientos de suelo (laboreo, fertilización y siembra de leguminosas fijadoras de nitrógeno atmosférico) y podas sobre el crecimiento diametral.

Los resultados mostraron que el clima mediterráneo es la principal fuerza que dirige el crecimiento diametral de la encina, provocando un característico patrón intraanual con dos periodos principales de crecimiento. El primero tiene lugar durante el final del invierno y la primavera, y el segundo durante el final del verano y el otoño. Entre estos dos periodos principales de crecimiento se observaron dos periodos con bajas tasas de crecimiento, parada, o incluso contracciones del tronco, que sucedieron al final de la primavera y en el verano, cuando el estrés hídrico se incrementa, y al final del otoño y en el invierno, cuando las temperaturas disminuyen. Estos patrones de crecimiento intraanual variaron significativamente entre años debido a la alta variabilidad del clima mediterráneo, especialmente en relación a las precipitaciones. Las principales variables

climáticas que afectaron al crecimiento fueron aquellas relacionados con el estado hídrico del árbol. Las precipitaciones, la humedad del suelo y la humedad relativa estuvieron correlacionadas positivamente con el crecimiento, mientras que la evapotranspiración de referencia y la radiación solar tuvieron una influencia significativa y negativa en el crecimiento. Las temperaturas también estuvieron correlacionadas negativamente con el crecimiento, probablemente debido a su influencia negativa en relación con el aumento de la transpiración y de las pérdidas de agua, a pesar de que las bajas temperaturas disminuyeron las tasas de crecimiento al final del otoño y en el invierno.

Todos los árboles siguieron generalmente la misma señal climática y estuvieron altamente sincronizados, aunque también se detectó una gran variabilidad de las tasas de crecimiento entre árboles que no es explicada por el clima. El tamaño del árbol, representado por su circunferencia a la altura del pecho, y algunos índices de competencia, explicaron parte de esa variabilidad individual, indicando la existencia de competencia por los escasos recursos en las dehesas y montes mediterráneos de encina.

La producción de bellota tuvo un efecto significativo y negativo sobre el crecimiento diametral a nivel individual en la época en la que se produce el engorde de las bellotas, es decir, durante el final del verano y el otoño, pero solo durante años de alta producción. Estos resultados mostraron la existencia de un *trade-off* entre crecimiento diametral y reproducción en encina, que explica parte de la variabilidad individual en las tasas de crecimiento.

Los tratamientos de suelo realizados no influyeron significativamente en el crecimiento diametral, indicando en este sitio de estudio una baja respuesta de la encina a la competencia del matorral, compactación del suelo, infiltración de agua, y a la mejora en los nutrientes del suelo.

Los tratamientos intensos de poda tuvieron un efecto negativo significativo sobre el crecimiento diametral, especialmente en aquellos árboles localizados sobre peores suelos o sometidos a otros factores de estrés, por ejemplo la enfermedad de *la seca*, incrementando los efectos del estrés hídrico sobre el crecimiento diametral. Esta influencia negativa fue menos marcada en árboles con un buen estado sanitario y localizados sobre mejores suelos, a pesar de que las podas intensas disminuyeron ligeramente las tasas de crecimiento en primavera. Por lo tanto, las podas intensas podrían afectar al vigor y al estado vegetativo de los árboles en áreas donde su supervivencia ya se encontrase comprometida.

Abstract

Measurements of tree growth provide information about overall tree health and vigor of forests, phenological processes, effects of climate, physiology and water status, competition, resource allocation, and the influence of management practices. Holm oak (*Quercus ilex* ssp. *ballota* (Desf.) Samp.) is the most widespread species in the Iberian peninsula, being one of the most representative trees in forests and open woodland forests.

In this dissertation the intra-annual stem growth and the daily stem radius variations of holm oak were analysed in 128 trees with band dendrometers, and in 9 trees with high-resolution electronic point dendrometers, located in three open woodland forests and one mixed Mediterranean forest of SW Spain. The study comprised a period of nine and a half years, from May 2003 to October 2012, including years with contrasting climatic conditions, especially in precipitations. Apart from the intra-annual patterns of stem growth and the daily cycles of stem radius variations, the influence of climatic variables and the effects of tree size, hydrological parameters and competence on the variability of stem growth between trees have been assessed in this work. Moreover, the existence of *trade-offs* in the allocation of resources between stem growth and acorn production at individual tree level has been studied. Finally, the effects of traditional management practices as soil treatments (ploughing, fertilization and sowing with a N_2 fixation legume plant) and pruning on stem growth have been analyzed.

Results showed that the Mediterranean climate is the main driving force of the stem growth of holm oak, provoking a characteristic intra-annual pattern with two main periods of growth. The first one occurred during late winter and spring, and the second one during late summer and autumn. Between these two growth periods there were two periods of low growth rates or growth cessation, that occurred in late spring and summer, when water stress increase, and in late autumn and winter, when temperatures decreased. Intra-annual growth patterns varied significantly between years because the high variability of Mediterranean climate, especially in relation to precipitations. The main climate variables that affected stem growth were those related with tree water status. Precipitation, soil moisture and relative humidity were positively correlated to stem growth, but reference evapotranspiration and solar radiation had a significant negative effect on stem growth. Temperatures were negatively correlated to stem growth, probably because its negative influence in relation to increasing transpiration and water losses, despite low temperatures decreased growth rates in late autumn and winter.

All trees generally followed the same climatic signal and were highly synchronized, but there was also a high individual variability in growth rates that was not explained by climate. Tree size, represented by circumference at breast height, and some competition indexes explained part of that individual variability, indicating a competition for the scarce resources in open woodlands and Mediterranean forests of holm oak.

Acorn production had a significant negative effect on stem growth at individual level, during the time when acorns fattened, i.e., late summer and autumn, and occurred only in years of high acorn production or mast years. These results showed the existence of a *trade-off* between stem growth and reproduction in holm oak, that explained part of the individual variability in growth rates.

The soil treatments did not influence significantly the stem growth, indicating a scarce response of holm oak in our study site to shrubs competition, soil compaction and water infiltration, and soil nutrient improvement.

Intense pruning treatments had a significant negative effect on stem growth, especially in trees located on poor soils and under other stress factors, such as oak decline, increasing the effect of water stress on stem growth. This negative influence was significantly lesser in trees with good sanitary status located on better soils, despite intense pruning decreased slightly stem growth rates in spring. Therefore, intense pruning could affect the vigor and vegetative status of trees in areas where tree survival is already compromised.

1. Introducción

1. - INTRODUCCIÓN

1.1. - La encina: un árbol adaptado al ambiente mediterráneo

La encina o carrasca, *Quercus ilex* L., es un árbol corpulento y siempreverde de la familia Fagaceae que puede alcanzar hasta 25 m de altura, apareciendo como arbusto cerca de su límite altitudinal superior (Ruiz de la Torre 2006). La encina es uno de los árboles más característicos de los ecosistemas mediterráneos. Se localiza principalmente en el piso bioclimático Mesomediterráneo, aunque también habita en los pisos Termomediterráneo y Supramediterráneo (Rivas-Martínez 1987), pudiendo encontrarse en España desde el nivel del mar en regiones como Andalucía, Santander, etc., hasta los 2000 m en Sierra Nevada (Ruiz de la Torre 2006). Esta especie aparece en zonas con precipitacines superiores a los 350-400 mm anuales, siendo sustituida en zonas más secas por coníferas xerófilas o formaciones arbustivas (Rodà et al. 2009). Habita tanto en suelos calizos como silíceos, o incluso arenosos sueltos, rehuyendo los terrenos encharcados y tolerando mal los margosos o arcillosos excesivamente compactos; y falta en los suelos salinos o muy yesoso (Ruiz de la Torre 2006). La encina y el alcornoque (*Quercus suber* L.) son las principales especies de los bosques esclerófilos de la Península Ibérica, y representan el estado óptimo de sucesión vegetal de los ecosistemas donde se distribuyen (Ruiz de la Torre 2005). En España, la encina ocupa una superficie cercana a los 3 millones de hectáreas, de las cuales en torno al 75% se encuentran en terrenos de propiedad privada (Bravo et al. 2008). A escala global, esta especie ocupa en su área de distribución natural aproximadamente una superficie total de 6.5 millones de hectáreas (Quézel y Médail 2003, Fig. 1.1), apareciendo desde la Península Ibérica hasta Turquía por el norte, y de Marruecos a Túnez por el sur, desbordando la región mediterránea por el oeste de Francia y el norte de España (Ruiz de la Torre 2006).

En la Península Ibérica esta especie se encuentra representada por dos subespecies: *Quercus ilex* ssp. *ilex* L. y *Quercus ilex* ssp. *ballota* (Desf.) Samp., con marcadas diferencias anatómicas y ecofisiológicas (Corcuera et al. 2004, 2005). Estas dos subespecies iniciaron una temprana diferenciación genética, probablemente en respuesta a las contrastadas condiciones climáticas de sus áreas de distribución (Lumaret et al. 2002).

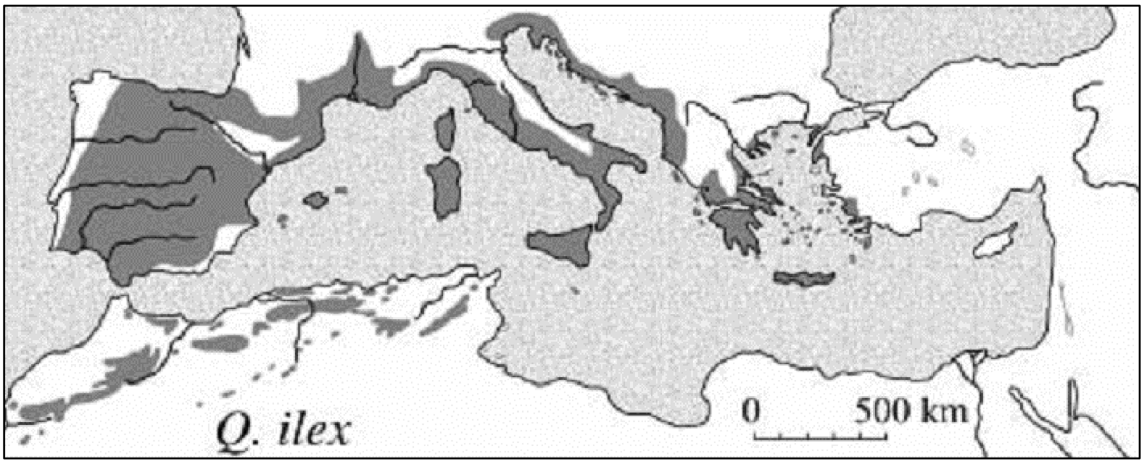

Fig. 1.1 Distribución natural de la encina según Quézel y Médail (2003). Tomado de Cavender-Bares et al. (2005)

En España, *Quercus ilex* ssp. *ilex* se encuentra restringida en el norte de la Península, de Galicia a Cataluña, en zonas litorales o con influencia oceánica, internándose hasta el norte de Burgos y Soria, y descendiendo por Levante hasta Valencia y Alicante, estando también representada en Baleares. Esporádicamente se encuentran también encinas de este taxón en las costas del sur de la Península (Ruiz de la Torre 2006). Por el contrario, *Quercus ilex* ssp. *ballota* es la subespecie con mayor distribución en la Península Ibérica, siendo junto con el alcornoque el árbol más representativo de las dehesas ibéricas (Cañellas et al. 2006), extendiéndose por todo el sur, centro y oeste de la Península, casi sin excepción (Ruiz de la Torre 2006).

1.2. - La encina en las dehesas ibéricas

Con el término *dehesa* (*montado* en Portugal) se define a aquellos sistemas agroforestales tradicionales de la Península Ibérica consistentes en un monte abierto con una densidad de arbolado que puede variar entre 20-100 pies/ha (San Miguel 1994; Serrada y San Miguel 2008), siendo frecuente una densidad media de entre 50-60 pies/ha (Fernández-Rebollo y Porras 1998). La baja densidad del arbolado, que implica una fracción de cabida cubierta en torno al 10-60% (Serrada y San Miguel 2008), facilita la entrada de luz hasta el suelo, permitiendo el desarrollo del sotobosque. El matorral de este estrato inferior, formado principalmente por vegetación pionera, es controlado continuamente por el manejo del ganado, los cultivos agrícolas en rotación, y mediante rozas, escardas y otras labores del suelo (Alejano et al. 2011) (Fig. 1.2). La producción

Fig. 1.2 Encina en una dehesa del Ándevalo Occidental (Huelva). Autor: Daniel Martín Pérez

principal del estrato inferior son los pastos, que en combinación con las bellotas producidas por el arbolado en las dehesas de *Quercus* spp., encina y alcornoque principalmente, permiten la cría de ganado porcino, vacuno, ovino e incluso caprino de calidad (San Miguel 1994; Eichhorn et al. 2006).

Las dehesas en España abarcan en la actualidad una superficie de entre 3.5 y 4 millones de hectáreas (Olea et al. 2005). Estos sistemas agroforestales se comenzaron a poner en práctica por el ser humano en la Península Ibérica hace 4500 años (Stevenson y Harrison 1992), hasta evolucionar en las dehesas actuales, que forman el sistema agroforestal dominante de España, y probablemente el más extendido de Europa (Eichhorn et al. 2006). Además de su importancia productiva, las dehesas son también ecosistemas con una alta biodiversidad, y constituyen unos paisajes únicos que sirven de refugio a muchas especies amenazadas, como el águila imperial ibérica (*Aquila adalberti*), el buitre negro (*Aegypius monachus*) o el lince ibérico (*Lynx pardinus*) (Carrete y Doñázar 2005).

1.3. - La importancia de los estudios del crecimiento de los árboles

El crecimiento de las especies forestales ha sido tradicionalmente estudiado por las Ciencias Forestales debido su importancia en relación a la producción de madera. Sin embargo, el estudio del crecimiento proporciona además un importante conocimiento en relación con la salud y el vigor de los ecosistemas (Nishimua et al. 2007; Martínez-Pastur et al. 2007), los efectos del clima sobre las especies (Fritts 1976; Gea-Izquierdo et al. 2011), procesos fisiológicos (Herzog et al. 1995; Zweifel et al. 2010), la competencia entre los árboles por los recursos (Gea-Izquierdo et al. 2009; Rodríguez-Calcerrada et al. 2011), la asignación de recursos a diferentes procesos dentro del propio árbol (Stearns 1989; Mund et al. 2010), o la respuesta de los árboles a la gestión de los montes (Cartan-Son et al. 1992; Gyenge et al. 2010).

Para el estudio del crecimiento de los árboles existen diferentes técnicas, cada una con sus ventajas e inconvenientes, por lo que deben seleccionarse dependiendo de los objetivos y el alcance de la investigación. En función de sus características, pueden distinguirse las siguientes técnicas o métodos para el estudio del crecimiento diametral:

1.- Estudios de crecimiento mediante dendrometría.

Consiste en la medición del árbol con técnicas de inventario forestal. Se pueden utilizar cintas métricas, forcípulas forestales, relascopios u otros dispositivos similares. El crecimiento se obtiene calculando las diferencias entre mediciones consecutivas. También pueden obtenerse y analizarse datos de crecimiento a partir de los datos de inventarios forestales. Estos métodos son muy poco fiables debido a la baja precisión de los dispositivos que se utilizan y al error que se produce al realizar las mediciones, por lo que no resultan adecuados para estudios de crecimiento diametral intraanual, o en el caso de especies de crecimiento lento, aunque sí pueden proporcionar datos de crecimiento con la precisión suficiente para intervalos de medición de un año o superiores. Ejemplo de estudios de crecimiento en los que se han utilizado estás técnicas son los realizados por Mayor y Rodà (1994) y Ogaya et al. (2003).

2.- Estudios dendrocronológicos.

Se fundamentan en el estudio de los anillos de crecimiento de la madera de los árboles, tanto su anchura como otras características histológicas, tales como fluctuaciones intraanuales de densidad, el tamaño y la densidad de las células conductoras (Fig. 1.3), la presencia de isótopos estables en la madera, etc. (Cook y Kairiukstis 1990).

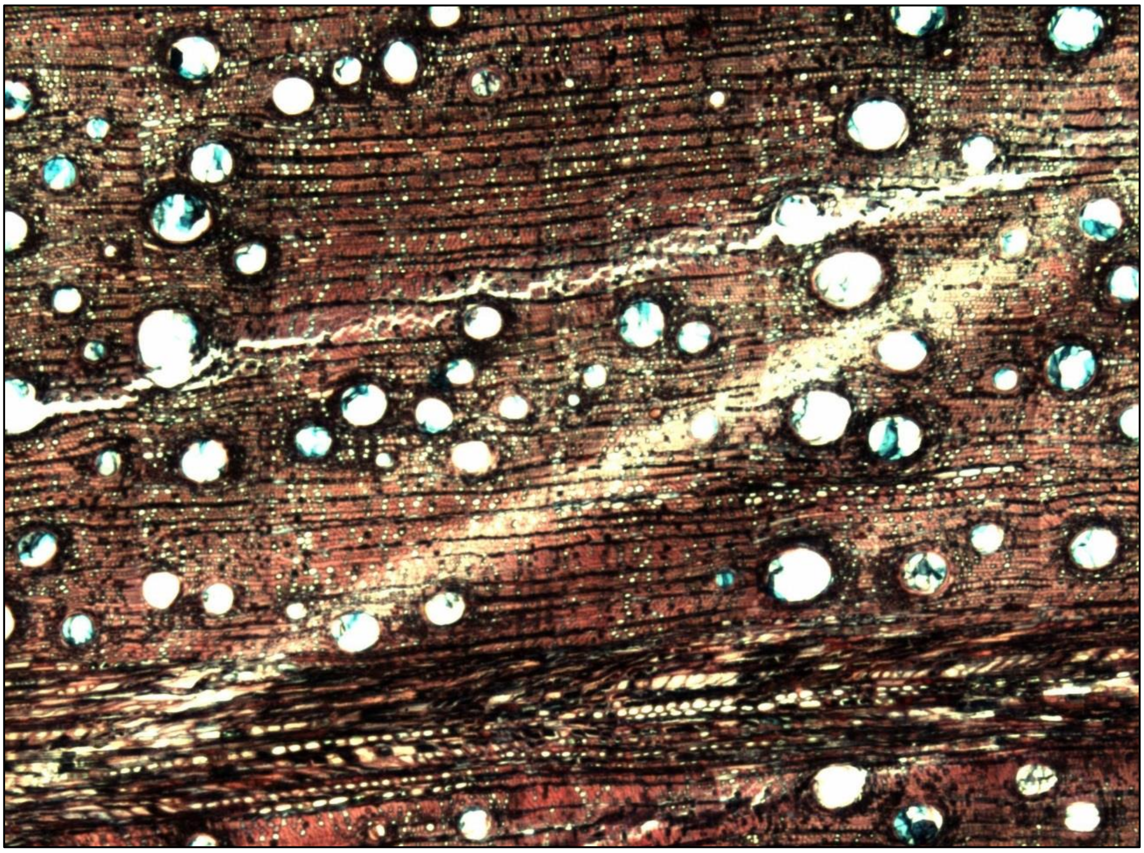

Fig. 1.3 Corte histológico de madera de encina. Autor: Daniel Martín Pérez

La dendrocronología tiene la ventaja de ser una de las escasas técnicas retrospectivas existentes para el estudio del crecimiento, ya que se analizan series temporales hacia el pasado, generalmente a partir del momento presente, pudiendo abarcar desde un periodo de algunos años hasta varios siglos (Speer 2010). Por tanto, los árboles vivos y la madera muerta constituyen en este caso un registro de las condiciones endógenas y ambientales que existían en el momento de su formación (Fritts 1976). Otra ventaja destacada de estas técnicas es que proporcionan valores exactos de crecimiento, al no encontrarse las mediciones influenciadas por el contenido de humedad del tronco de los árboles. Su principal desventaja es la baja resolución temporal en el análisis del crecimiento, normalmente anual, o estacional en el mejor de los casos. Otra desventaja es que muchas especies de árboles no forman anillos de crecimiento, o resulta muy difícil determinar los mismos. Finalmente, hay que señalar que los resultados obtenidos reflejan la respuesta de los árboles a unas condiciones y procesos ambientales del pasado, por lo que los mismos pueden resultar anacrónicos en el actual contexto de cambio climático (IPCC 2007), especialmente en el caso de los resultados obtenidos de series de crecimiento muy antiguas. Algunos ejemplos de estudios realizados con estas técnicas son Cherubini et al. (2003), Sarris et al. (2007) y Battipaglia et al. (2010).

3.- Análisis histológicos de la formación de madera.

Su objetivo es determinar con precisión los periodos de activación del cambium y la formación de xilema. En la actualidad se utilizan fundamentalmente dos técnicas: El *pinning* y el *microcoring*. El *pinning* (Wolter 1968) consiste en introducir una fina aguja a través de la corteza del árbol hasta el xilema exterior con el objetivo de provocar una herida en el cambium. Como consecuencia, la formación de madera se detiene junto al canal producido, y las células cercanas al mismo se modifican (Seo et al. 2007). El *microcoring* (Loris 1981) consiste en determinar directamente la formación de madera mediante la extracción de pequeñas muestras de la corteza y parte del xilema en intervalos cortos de tiempo (Makkinen et al. 2008). Ambas técnicas requieren la preparación de las muestras y el uso del microscopio para la observación de las células. La principal ventaja de estos métodos reside en que si se realizan correctamente se consigue detectar la activación del cambium y la expansión de las células (Rossi et al. 2006). La principal desventaja es que se tratan de unas técnicas muy difíciles de ejecutar y requieren un trabajo laborioso para la correcta preparación de las muestras, por lo que no se suelen aplicar a un gran número de árboles, ni durante periodos de estudio prolongados. Algunos estudios realizados con estas técnicas son los trabajos publicados por Schmitt et al. (2000), Rossi et al. (2006) y Makkinen et al. (2008).

4.- Mediciones con dendrómetros.

Los dendrómetros son dispositivos específicamente diseñados para la medición del crecimiento diametral de los árboles. Los dendrómetros se comenzaron a desarrollar a finales del siglo XIX (Böhmerle 1883; Friedrich 1890) y fueron mejorados a lo largo del siglo XX (p. ej. Friedrich 1905; MacDougal 1918; Fritts y Fritts 1955). Existen dos tipos principales: dendrómetros de banda de lectura manual y dendrómetros automáticos.

Los dendrómetros de banda de lectura manual consisten en una lámina fina y flexible, generalmente metálica, que se coloca alrededor del árbol, pasando su extremo a través de un collar y conectándola otra vez a si misma con un resorte que provoca una presión sobre el árbol. A medida que el árbol crece el muelle se expande y permite que la banda continúe extendiéndose, pudiendo medirse esa distancia de forma directa con la ayuda de una escala grabada sobre la banda o con otros instrumentos, como por ejemplo, calibres analógicos o digitales. Las mediciones se realizan en intervalos regulares de tiempo, siendo frecuente las mediciones semanales o mensuales. La principal ventaja de estos dispositivos es que son muy económicos, robustos y sencillos de instalar, por lo que se pueden utilizar para realizar estudios sobre un gran número de árboles, lo que es de vital importancia para aquellas especies con una alta variabilidad individual en el crecimiento. Otra ventaja destacada es que proporcionan una medición de todo el crecimiento en circunferencia de una sección trasversal del árbol, por lo que la misma no es influida por alteraciones locales del crecimiento en diferentes partes del tronco (Bormann y Kozlowski 1962). La precisión en la medición del crecimiento es significativamente superior a las mediciones realizadas con cintas métricas, forcípulas, etc., alcanzando valores de 0.01 mm en el caso de la utilización de calibres digitales. Sus mayores desventajas son la dificultad para distinguir entre crecimiento y contracción-expansión hídrica, por lo que no se recomienda su medición en días de lluvia, y el alto esfuerzo que es necesario emplear en el desplazamiento a campo y en las mediciones árbol por árbol, repetidas con mucha frecuencia. Algunos estudios de crecimiento en los cuales se han utilizados estos dispositivos son los realizados, por ejemplo, por Campelo et al. (2007), Miller et al. (2001) y Mund et al. (2010).

Los dendrómetros automáticos, dentro de los cuales también pueden incluirse los dendrógrafos, son dispositivos que proporcionan una medición continua de los cambios del tamaño del tronco de los árboles a una escala temporal de horas, minutos, e incluso segundos. Desde 1970 se han desarrollado dendrómetros automáticos electrónicos en los cuales las mediciones eran registradas en dataloggers, de forma similar a los modernos dendrómetros electrónicos existentes de la actualidad (Breitsprecher y Hughes 1975). Existen dos tipos fundamentales de dendrómetros electrónicos automáticos. Los dendrómetros electrónicos de banda, que registran cambios en el tamaño de la circunferencia de toda una sección transversal del árbol, y los dendrómetros electrónicos puntuales, que registran cambios en una zona determinada del tronco, proporcionando mediciones en relación a su radio. La principal ventaja de estos dispositivos es su alta resolución espacial y temporal, pudiendo registrar cambios de una magnitud cercana a la micra en intervalos de tiempo de hasta segundos. Esta alta resolución facilita la distinción entre crecimiento y contracción-expansión hídrica (Deslauriers et al. 2007), y permite además profundizar en el estudio de otros procesos fisiológicos relacionados con el estado hídrico del árbol, como la transpiración (Steppe et al. 2006), el flujo de savia (Herzog et al. 1995), la asimilación de carbono (Zweifel et al. 2010), etc. Su principal desventaja es que son dispositivos muy costosos de adquirir y mantener, por lo que su utilización suele estar limitada a estudios realizados sobre muy pocos árboles y durante periodos cortos de tiempo, que abarcan generalmente desde algunos meses hasta 2-3 años. Otra desventaja es que son dispositivos muy delicados y se deterioran con frecuencia debido a la acción de los factores meteorológicos, la fauna, etc., generando errores y huecos en las series de crecimiento. Algunos ejemplos de estudios de crecimiento en los que se han utilizados estos dispositivos son los trabajos realizados por Downes et al. (1999), Tardif et al. (2001), Deslauriers et al. (2007) y Wang et al. (2015).

1.3. – Antecedentes en el estudio del crecimiento diametral de la encina.

A pesar de su importancia ecológica y económica, la encina es una especie en la que tradicionalmente no se han realizado estudios exhaustivos sobre su crecimiento diametral y los factores ecológicos que lo rigen. Posiblemente este hecho sea debido a que se trata de una especie de crecimiento lento cuyo principal aprovechamiento es la bellota, estando restringidos sus aprovechamientos maderables principalmente a la obtención de las leñas provenientes de las podas, y a su beneficio en monte bajo (Bravo et al. 2008). La mayoría de los estudios científicos existentes sobre el crecimiento diametral de la encina están basados en métodos dendrocronológicos y se encuentran focalizados en la subespecie *Q. ilex* ssp. *ilex*. En este sentido, hay que destacar el temprano estudio dendrocronológico de Susmel et al. (1976) en Orgosolo (Cerdeña, Italia), seguido por el de Cherubini et al. (2003) en Rapolano y Lajatico (Pisa, Italia). En la zona noreste de la Península Ibérica y sur de Francia se han realizado otros estudios dendrocronológicos en encina, como Zhang y Romane (1991) en Camp Redón (sur de Francia), Gené et al. (1993) en Prades (Cataluña, España), Ferrio et al. (2003) en diferentes localizaciones de Cataluña y Aragón (España), Campelo et al. (2010) en El Garraf (Cataluña, España), Camarero et al. (2010) en Huesca (Aragón, España), y Nijland et al. (2011) en Herault (sur de Francia). En esta región también se han realizado otros estudios de crecimiento diametral en encina por Cartan-Son et al. (1992) en Montpelier (sur de Francia), Mayor y Rodà (1994) y Ogaya et al. (2003) en Prades (Cataluña, España), y Rodríguez-Calcerrada et al. (2011) en Puéchabon (sur de Francia). En todos ellos se han utilizado mediciones realizadas con cinta métrica y estudiado el crecimiento a una escala anual. Pérez-Ramos et al. (2010) estudiaron el crecimiento diametral de la encina en Puéchabon (sur de Francia) a escala anual mediante mediciones consecutivas del diámetro a la altura del pecho, pero no indicaron en su estudio que instrumentos utilizaron. Campelo et al. (2007) y Gutiérrez et al. (2011) utilizaron métodos dendrocronológicos en combinación con mediciones realizadas con dendrómetros de banda en 10 encinas de El Garraf (Cataluña, España). En la zona centro, oeste y suroeste de la Península Ibérica, donde predomina la subespecie *Quercus ilex* ssp. *ballota*, destacan los estudios dendrocronológicos de Nabais et al. (1998-1999) en varias localizaciones del norte de Portugal, Campelo et al. (2009) y Abrantes et al. (2013) en Alqueva (sureste de Portugal), y de Gea-Izquierdo et al. (2009, 2011) en varias zonas del centro y oeste de España. Recientemente, Natalini et al. (2013) realizaron un estudio dendrocronológico en varias encinas localizadas en dos de las parcelas experimentales que se han estudiado en la presente tesis.

Sin embargo, hasta el momento no se han realizado estudios sobre el crecimiento diametral de *Quercus ilex* ssp. *ballota* en el suroeste de la Península Ibérica a escala intraanual mediante el uso de dendrómetros, ni se ha estudiado la influencia de variables ecológicas tales como la competencia entre árboles, parámetros hidrológicos o el efecto del tamaño del árbol, no habiéndose realizado hasta la actualidad ningún estudio sobre el patrón de crecimiento intraanual de la encina con el suficiente número de años y árboles necesarios para conocer su respuesta frente a la alta variabilidad del clima mediterráneo. Tampoco se ha publicado hasta el momento ningún estudio que haya abordado el análisis de *trade-offs* entre crecimiento diametral y producción de bellota en encina a nivel de árbol individual, tal y como se ha analizado en otros estudios sobre *Quercus* spp. de Norteamérica (Knops et al. 2007; Barringer et al. 2012; Knops y Koenig 2012), siendo la primera vez que se han analizado estas relaciones endógenas en árboles a escala intraanual. Asimismo, no se han publicado otros estudios que hayan evaluado el efecto de las prácticas tradicionales de gestión sobre el crecimiento intraanual de la encina, ni analizado el efecto de las podas sobre el crecimiento de esta especie.

2. Objetivos y estructura de esta tesis

2. - OBJETIVOS Y ESTRUCTURA DE ESTA TESIS

El objetivo principal de esta tesis doctoral es profundizar en el conocimiento del crecimiento diametral de la encina, *Quercus ilex* ssp. *ballota* (Desf.) Samp., y de los factores que lo condicionan, en el caso concreto de las dehesas y el monte mediterráneo del suroeste de España.

Para conseguir este objetivo general se definen nueve objetivos específicos que se abordan en cuatro subproyectos dirigidos a investigar cada uno de estos aspectos. Los resultados obtenidos en tres de estos trabajos han sido ya publicados como artículos en revistas científicas con índice de impacto, y el cuarto se encuentra en proceso de revisión. Estos trabajos se presentan en el Apéndice 1. Al final de cada objetivo específico se indica en qué artículo ha sido tratado.

1º.- Modelizar el patrón de crecimiento diametral intraanual de la encina a escala mensual (Artículo 1).

2º.- Definir las fases intraanuales de crecimiento y su variabilidad interanual (Artículos 1 y 2)

3º.- Conocer los ciclos diarios de variación del diámetro del tronco y del crecimiento diametral diario, para cuantificar de forma más precisa el proceso de crecimiento (Artículo 2).

4º.- Cuantificar el efecto que ejercen las variables climáticas en el crecimiento a escala mensual (Artículo 1).

5º.- Cuantificar el efecto de las variables climáticas sobre el crecimiento a escala diaria (Artículo 2).

6º.- Cuantificar el efecto que tienen la competencia, los parámetros hidrológicos y el tamaño del árbol sobre la variabilidad individual en el crecimiento mensual (Artículo 1).

7º.- Determinar la existencia de competencia interna en la asignación de recursos o *trade-offs* entre el crecimiento y la producción de bellota (Artículo 3).

8º.- Conocer el efecto de tratamientos tradicionales de mejora de suelo sobre el crecimiento mensual (Artículo 4).

9º.- Conocer la influencia de las podas sobre el crecimiento mensual (Artículo 4).

3. Materiales y métodos

3. – MATERIALES Y MÉTODOS

En esta sección se muestran de forma sucinta los materiales y métodos que se han empleado durante el desarrollo de esta tesis. La descripción más detallada de los mismos se desarrolla en la sección Materiales y métodos de cada uno de los artículos incluidos en el Apéndice 1.

3.1. – Sitio de estudio y parcelas experimentales

La presente tesis se ha desarrollado en la provincia de Huelva, suroeste de España, donde se han establecido cuatro parcelas experimentales. Tres parcelas se situaron en dehesas de encina de la comarca del Andévalo Occidental, y la cuarta en un monte mediterráneo de la comarca de Doñana. La parcela de Huerto Ramírez (HR) se estableció en una dehesa en el término municipal de Villanueva de los Castillejos (UTM 29S X:644288 m; Y:4161376 m), la parcela de San Bartolomé (SB) en una dehesa en el término municipal de San Bartolomé de la Torre (UTM 29S X:669638 m; Y:4145966 m), la parcela de Calañas (CA) en una dehesa en el término municipal de Calañas (UTM 29S X:681349 m; Y:4156557 m), y la parcela de Hinojos (HI) se situó en un monte mediterráneo mixto de encina y alcornoque en el término municipal de Hinojos (UTM 29S X:728082 m; Y:4133575 m) (Fig. 3.1). Las características de las parcelas experimentales se encuentran descritas detalladamente en la sección Materiales y métodos de los Artículos 1 y 4. En la parcela de CA se detectaron síntomas de *seca* en tres árboles durante el periodo de estudio. Los datos de crecimiento de esos árboles fueron eliminados de los análisis estadísticos.

El clima de estas parcelas es mediterráneo, aunque influenciado por la proximidad del océano Atlántico, con fuertes oscilaciones intraanuales e interanuales en el régimen de precipitaciones. Según datos de la AEMET, estación de Huelva, la precipitación media anual en el periodo 1920-2010 fue de 488 mm, transcurriendo el periodo de mayor precipitación entre los meses de noviembre y enero. La temperatura media de las mínimas del mes más frío en este periodo fue de 6.4 ºC, y la temperatura media de las máximas del mes más cálido fue de 32.0 ºC. Las variaciones de temperaturas en las parcelas experimentales en los años en los que se desarrolló este estudio (2003-2012) no fueron tan notables como las de precipitaciones, pero sí existió una alta variabilidad de las temperaturas de invierno y verano entre los diferentes años. Las características climáticas de las parcelas experimentales se encuentran descritas detalladamente en la sección Materiales y métodos de los Artículos 1, 2 y 4.

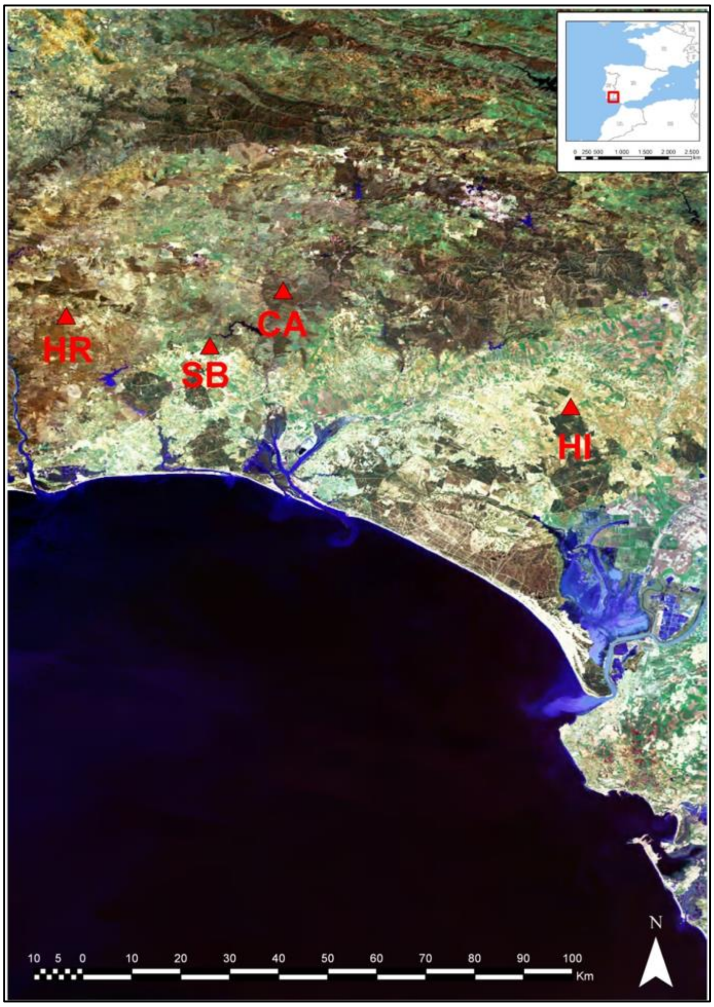

Fig. 3.1 Sitio de estudio y localización de las parcelas experimentales. Mapa: ESRI Data and Maps 2006

3.2. – Medición del crecimiento diametral

Para el estudio del crecimiento se han utilizado dendrómetros de banda de lectura manual y dendrómetros electrónicos automáticos. Los dendrómetros de banda se instalaron en un total de 128 árboles que se midieron mensualmente con un calibre digital de 0.01 mm de precisión (Fig. 3.2). Estos dendrómetros se distribuyeron de la siguiente manera entre parcelas: 55 en HR, 32 en SB, 32 en CA y 9 en HI, instalados en árboles seleccionados por muestreo aleatorio estratificado en función del diámetro, y en su caso, de los tratamientos de poda o mejora de suelo que se describen en los apartados 3.6 y 3.7. La descripción detallada de los dendrómetros de banda se encuentra en la sección Materiales y métodos de los Artículos 1, 3 y 4. Los dendrómetros electrónicos se instalaron en 9 árboles de la parcela HR (Fig. 3.3), seleccionados también por muestreo aleatorio estratificado. Las características de los dendrómetros electrónicos se encuentran detalladas en la sección Materiales y métodos del Artículo 2.

Fig. 3.2 Detalle de la medición de un dendrómetro de banda en la parcela SB. Autor: Daniel Martín Pérez

Fig. 3.3 Detalle del sensor de un dendrómetro electrónico en la parcela HR. Autor: Daniel Martín Pérez

El periodo de estudio abarca desde mayo de 2003 hasta diciembre de 2011 para las mediciones con dendrómetros de banda, y desde octubre de 2005 hasta octubre de 2012 para las mediciones con dendrómetros electrónicos. En la sección Materiales y métodos de cada uno de los artículos se indica el periodo concreto de estudio para cada parcela experimental.

3.3. – Medición de las variables climáticas y de la humedad y temperatura de suelo

Para la obtención de los datos climáticos se instalaron en las parcelas HR e HI dos estaciones meteorológicas automáticas. En la parcela SB los datos climáticos se obtuvieron de la estación meteorológica de Gibraleón (37º 24' 49" N; 7º 03' 31" W; 169 m.s.n.m.), perteneciente a la Red de Información Agroclimática de Andalucía (Junta de Andalucía).

La humedad de suelo en HR se midió con 7 sensores (ECH2O-20, Decagon Devices Inc.), repartidos de forma homogénea por la superficie de la parcela. De estos sensores, 4 fueron colocados a una profundidad de 5-25 cm, y los otros 3 sensores se colocaron a una profundidad de 25-45 cm en tres de las cuatro localizaciones correspondientes a los sensores más superficiales (Fig. 3.4).

Fig. 3.4 Instalación de un sensor de humedad de suelo en HR. Autora: Reyes Alejano

En SB se colocaron dos sensores de humedad de suelo, el primero a 5-25 cm, y el segundo a 25-45 cm de profundidad, en la misma localización que el anterior. Todos los sensores fueron calibrados mediante las correspondientes curvas de calibración, realizadas en laboratorio con muestras de suelo extraídas de los puntos donde fueron instalados, siguiendo la metodología descrita por Cobos (2010). En HI se colocaron dos sensores de humedad de suelo (C-Probe Corp.), el primero a 10 cm y el segundo a 30 cm de profundidad.

La temperatura de suelo se midió en todas las parcelas experimentales mediante sensores instalados a una profundidad de 10 cm en las mismas localizaciones donde se encontraban situados los sensores de humedad. Las características de las estaciones meteorológicas y sus dispositivos, así como las de los sensores de humedad de suelo, se encuentran detalladas en la sección Materiales y métodos de los Artículos 1 y 2.

3.4. – Medición del tamaño del árbol, los parámetros hidrológicos y la competencia

Durante la instalación de cada parcela experimental se midieron para cada árbol los valores de altura total y circunferencia normal del tronco. Asimismo, se realizó el levantamiento topográfico de todos los árboles de las parcelas con una estación total (Sokkia 3B) y de aquellos exteriores próximos que pudieran ejercer un efecto competitivo sobre los árboles muestreados.

Para el cálculo de los parámetros hidrológicos se realizó un modelo digital del terreno (MDT) de cada parcela a partir de los datos del levantamiento topográfico y del Modelo Digital del Terreno de Andalucía del año 2005 (Junta de Andalucía 2005, Fig. 3.5). Los parámetros hidrológicos analizados fueron la longitud de ladera (SL), el área de drenaje específica (BA) y el índice de humedad Wetness Index (WI) (Barling et al. 1994). El cálculo del MDT y de los parámetros hidrológicos fue realizado con ArcGis 9.2.

Para la estimación de la competencia, con las coordenadas de cada árbol proporcionadas por el levantamiento topográfico y el valor de su circunferencia a la altura del pecho se calcularon 725 índices de competencia para cada árbol. Los detalles del cálculo de los parámetros hidrológicos y de los índices de competencia se describen en la sección Materiales y Métodos del Artículo 1.

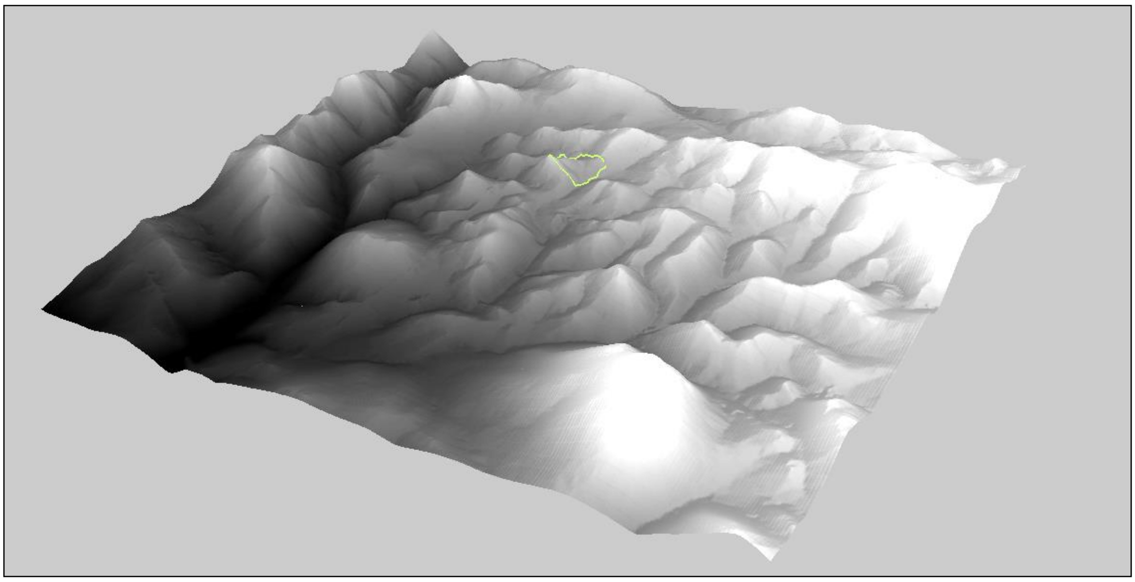

Fig. 3.5 Modelo digital del terreno de la parcela HR (línea verde) y su cuenca de influencia. Escala vertical Z multiplicada por factor k = 5. Elaboración propia mediante ArcGis 9.2.

3.5. – Estimación de la producción de bellota

La producción de bellota se estimó utilizando el método de los contenedores, método contrastado para la zona como buen estimador de la producción total por árbol (Alejano et al. 2008). Para ello se instalaron en 18 árboles de HR y en 13 de SB, todos con dendrómetros de banda, cuatro contenedores de goma de 45 cm de diámetro en su parte superior localizados en las direcciones norte, sur, este y oeste, a una distancia del tronco de ¾ del radio de la copa en esa dirección. La bellota fue recogida de los contenedores cada dos semanas durante la época de diseminación o montanera, es decir, de septiembre a enero del siguiente año. A continuación las bellotas eran trasladadas al laboratorio donde se procedió a pesarlas para estimar la producción de cada árbol en g de peso fresco de bellota por m^2 de área de copa. En total se estudió la producción de bellota durante 6 temporadas de diseminación entre los años 2006-2007 y 2011-2012. Los detalles sobre el procedimiento para la estimación de la producción de bellota se encuentran en la sección Materiales y métodos del Artículo 3.

3.6. - Tratamientos de mejora de suelo

En la parcela HR se realizaron dos tipos de tratamientos de mejora de suelo durante el otoño de 2005, que fueron repetidos posteriormente en el otoño de 2008, dejando zonas sin tratamiento que actuaron como control en el estudio. La parcela fue dividida en 9 subparcelas, de superficies y número de árboles similares (Fig. 3.6).

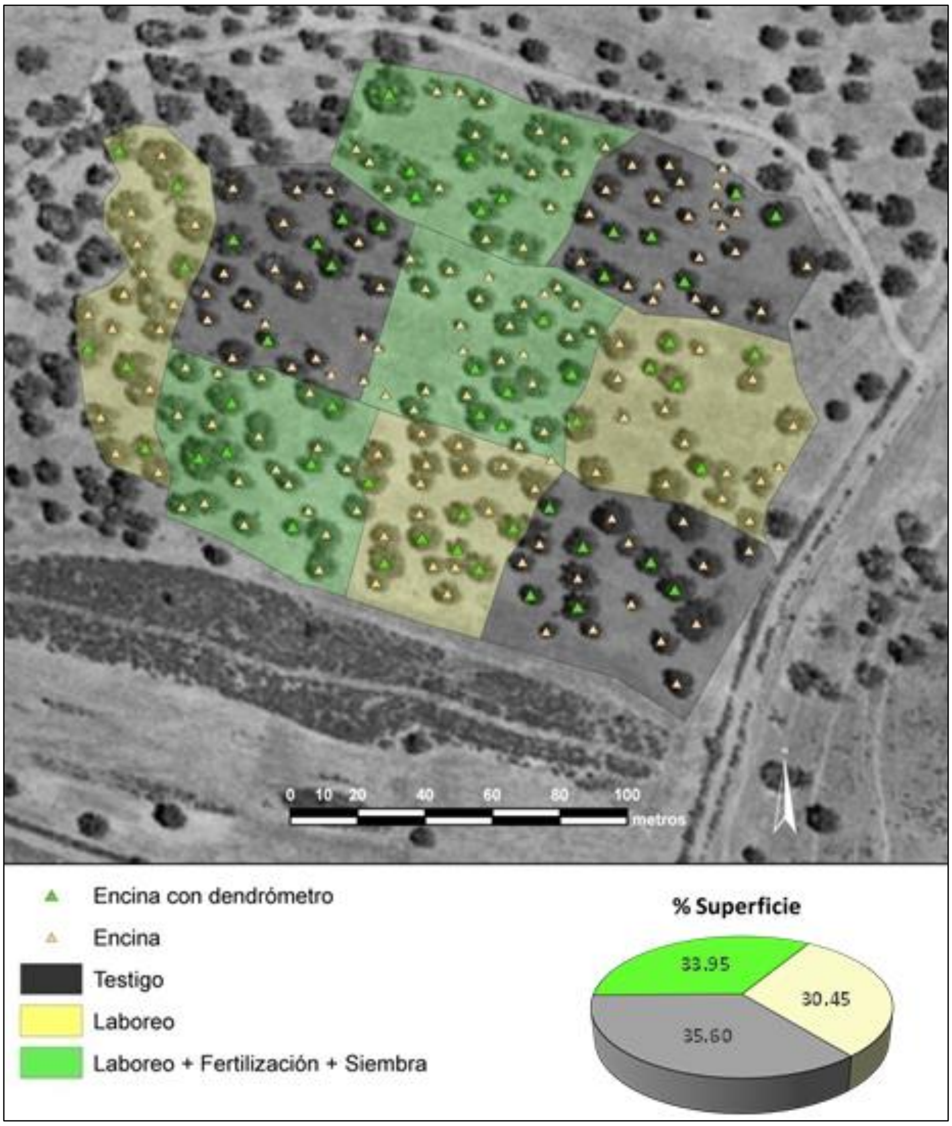

Fig. 3.6 Distribución de los tratamientos de mejora de suelo en la parcela HR. Foto: Ortofotografía digital pancromática de Andalucía 2001

En 3 subparcelas se llevó a cabo una labor superficial del terreno con un tractor agrícola y un arado de grada de discos. En otras 3 subparcelas se aplicó el mismo tratamiento que en las anteriores, y se realizó adicionalmente una fertilización inorgánica con superfosfato de cal y una siembra con la leguminosa herbácea conocida localmente como tremosilla (*Lupinus luteus*), por medio de una sembradora mecanizada. En las últimas 3 subparcelas no se realizó ninguna actuación, manteniéndose como grupo de control para evaluar el efecto de los tratamientos sobre el crecimiento. Las características de los tratamientos de suelo se muestran de forma detallada en la sección Materiales y métodos del Artículo 4.

3.7. - Tratamientos de poda

Los árboles de las parcelas CA y SB fueron sometidos a un tratamiento de poda tradicional de tres intensidades diferentes: ligera, moderada y fuerte, manteniendo un conjunto de árboles sin podar como grupo de control (Fig. 3.7). La poda ligera consistió únicamente en la eliminación de los brotes epicórmicos o chupones, la poda fuerte en la eliminación de hasta 1/3 de la copa, y la poda moderada se realizó con una intensidad intermedia entre las dos anteriores. Los detalles sobre la ejecución y la cuantificación de cada intensidad de poda son descritos en la sección Materiales y métodos del Artículo 4.

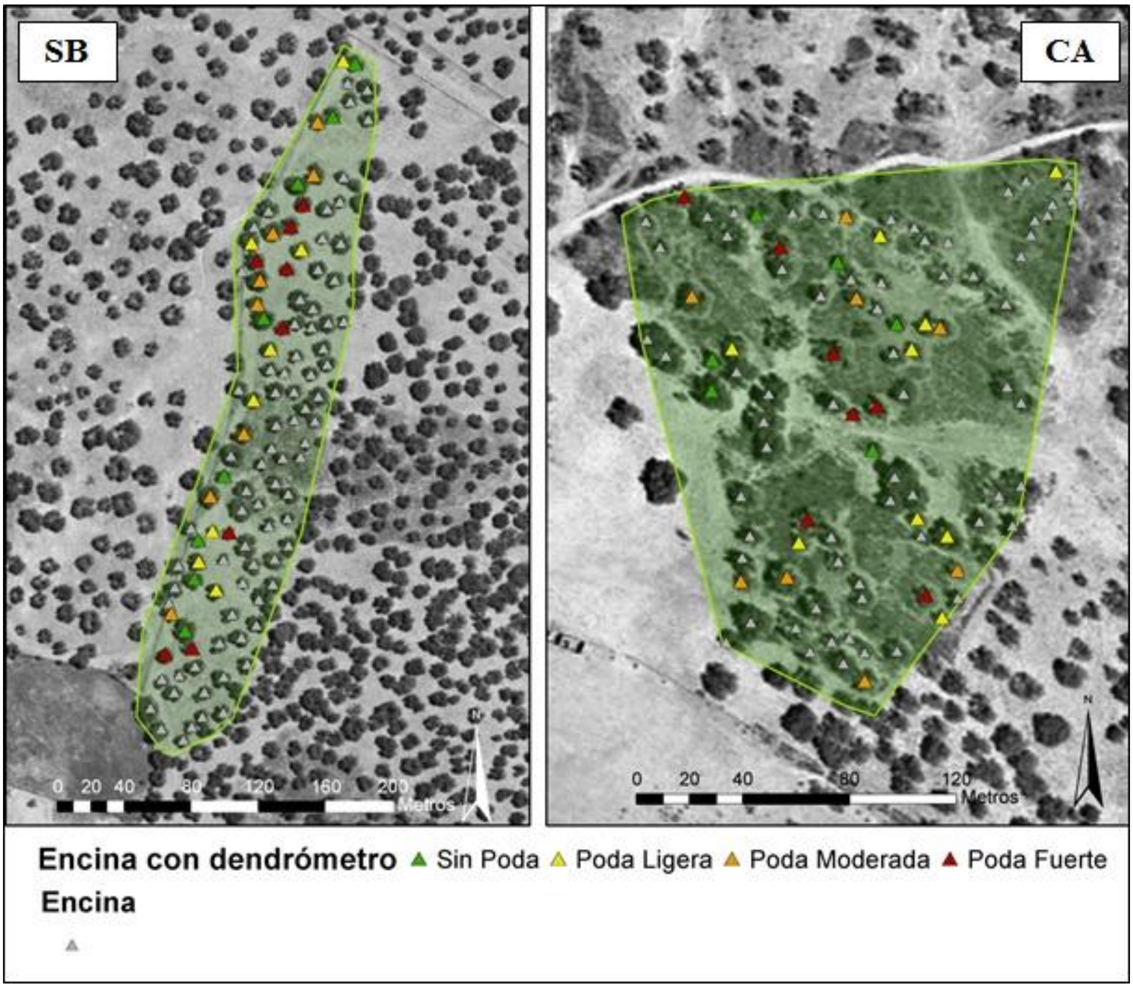

Fig. 3.7 Distribución de los tratamientos de poda en las parcelas SB y CA. Foto: Ortofotografía digital pancromática de Andalucía 2001

3.8. – Análisis de datos

Los datos de crecimiento a nivel mensual de analizaron en los Artículos 1 y 4 mediante el ajuste de varios modelos lineales mixtos de efectos fijos y aleatorios a nivel de árbol individual. Como efectos fijos se consideraron el mes, el año, la parcela, los tratamientos de suelo y las podas en su caso, y todas sus posibles interacciones dobles, triples o cuádruples, y como efecto aleatorio se consideró el árbol. Se evaluaron distintas estructuras de matrices de varianza-covarianza, atendiendo a la posible existencia de correlación temporal y espacial entre las observaciones, y de heterocedasticidad en la varianza. Los componentes de la varianza de cada estructura fueron estimados por máxima verosimilitud restringida (REML) (Patterson y Thompson 1971), y la selección de los modelos ser realizó en base al criterio de información de Akaike (AIC, Akaike 1974). Una vez seleccionados los mejores modelos, se evaluó el efecto que ejercieron sobre el crecimiento diametral mensual las diferentes variables climáticas, la humedad y temperatura del suelo, la competencia, el diámetro del tronco, los parámetros hidrológicos, los tratamientos de suelo, y las podas, introduciéndolas en los modelos como covariables de forma lineal.

En el Artículo 3 los datos de crecimiento mensuales de cada árbol se agruparon en datos anuales y estacionales, y se ajustó un modelo lineal mixto para el crecimiento anual, otro para el crecimiento de invierno y primavera, y un tercer modelo para el crecimiento de final de verano y otoño, siguiendo el mismo procedimiento realizado en los Artículos 1 y 4. Una vez seleccionado el mejor modelo para cada uno de estos periodos, se introdujeron los datos estimados de producción de bellota para cada árbol como covariable de forma lineal y se evalúo su efecto sobre el crecimiento.

En la sección Materiales y métodos de los Artículos 1, 2 y 4 se detalla el proceso de ajuste y selección de cada modelo lineal mixto.

En el Artículo 2 se calcularon los datos de crecimiento diametral diario a partir de las series de ciclos diarios de expansión y contracción del tronco. Mediante el examen visual de las series de crecimiento se determinaron las cuatro fases características del crecimiento intraanual de la encina dentro de cada uno de los 7 años de datos. Una vez determinadas estas fases se evaluó el efecto de las variables climáticas a nivel diario mediante análisis de correlación de Pearson. En la sección Materiales y métodos del

Artículo 2 se detalla la selección de las fases de crecimiento intraanual y la evaluación de las variables climáticas a nivel diario.

4. Discusión general

4. – DISCUSIÓN GENERAL

4.1. - Patrón de crecimiento diametral intraanual y ciclos de crecimiento diario

Los resultados muestran que el clima es el principal factor que dirige el crecimiento diametral de la encina en el suroeste de España, tal y como también indican estudios dendrocronológicos realizados en esta región (Natalini et al. 2013) y en otras cercanas, como el centro y el oeste de la Península Ibérica (p. ej. Nabais et al. 1998-1999; Campelo et al. 2009; Gea-Izquierdo 2009, 2011). El clima mediterráneo provoca a nivel intraanual dos periodos de crecimiento muy característicos que coinciden con las condiciones climáticas más favorables para el crecimiento (Mitrakos et al. 1980; Terradas y Savé 1992); y entre ellos dos periodos de letargo, con bajas tasas de crecimiento, parada o incluso contracciones del tronco. El periodo principal de crecimiento ocurre generalmente desde finales del invierno hasta finales de la primavera, y es producido por el suave aumento de las temperaturas y la presencia de agua disponible en el suelo, que fomentan un aumento en las tasas de actividad fotosintética, asimilación de carbono y conductividad hidráulica xilemática de la encina (Corcuera et al. 2004, 2005; Carevic et al. 2010, 2014), formándose además grandes vasos conductores en el xilema (Campelo et al. 2010). Durante este periodo se observan con claridad en los ciclos diarios de expansión y contracción del tronco crecimientos diametrales netos, producidos por la división celular en el cambium y por la expansión irreversible de las células y las paredes celulares del xilema (Tatarinov y Čermák 1999; Deslauriers et al. 2003). A medida que avanza la primavera el estrés hídrico provoca una disminución de las tasas de crecimiento (Herzog et al. 1995; Abe y Nakai 1999; Oberhuber et al. 2014), hasta que se detiene el incremento del diámetro del tronco y comienza un periodo de letargo en el crecimiento (Cherubini et al. 2003), que abarca generalmente desde finales de la primavera hasta finales del verano. Esta fase de letargo se caracteriza por un primer periodo donde los dendrómetros electrónicos detectan que se produce una contracción progresiva del tronco a medida que éste se deshidrata, seguido de un segundo periodo de estabilización, donde el cierre estomático reduce las pérdidas de agua (Carevic et al. 2010) y el tronco mantiene su tamaño. A finales del verano y coincidiendo con las primeras lluvias, el estado hídrico de la encina se recupera (Corcuera et al. 2004), produciéndose una rápida rehidratación del tronco y una reactivación del proceso de crecimiento. Los dendrómetros digitales mostraron una respuesta súbita e intensa en el incremento del tamaño del tronco al inicio de este periodo. Posteriormente, tanto los dendrómetros de banda como los electrónicos detectaron una tendencia del incremento del tronco positiva durante todo el periodo, con valores máximos de radio o circunferencia mayores que los anteriores máximos de primavera, apareciendo ciclos diarios con crecimiento diametral neto, al igual que en el primer periodo de crecimiento. Este periodo de crecimiento se encuentra fomentando por un aumento en las tasas de actividad fotosintética, conductividad hidráulica, y una mayor densidad de vasos xilemáticos (Carevic et al. 2014). Además, varios estudios han encontrado una relación entre este segundo periodo de crecimiento y la aparición de fluctuaciones intraanuales de densidad en la madera y/o *dobles anillos o falsos anillos*, que forman una banda difusa de crecimiento extra al final de cada anillo, tanto en la encina como en muchas otras especies mediterráneas (p.ej. Cherubini et al. 2003; Campelo et al. 2007; Battipaglia et al. 2010, 2014). A medida que transcurre el otoño, las temperaturas descienden y las tasas de crecimiento disminuyen, al mismo tiempo que se reducen las tasas fotosintéticas (Corcuera et al. 2005), comenzando un periodo donde generalmente el tamaño del tronco se mantiene estable. Esta parada invernal de la encina también ha sido encontrada en otros estudios anteriores realizados en zonas más frías (p. ej. Zhang y Romane 1991; Campelo et al. 2007), aunque en algunos años del presente estudio no se detectó una clara parada invernal en el crecimiento. El inicio, duración y cese de estos cuatro periodos o fases de crecimiento y parada varió significativamente entre años, mostrando como también la característica variabilidad a nivel interanual del clima mediterráneo puede modular el patrón de crecimiento intraanual de la encina. En ese sentido, hay que destacar la mayor sensibilidad con la que los dendrómetros electrónicos permitieron diferenciar el inicio y la finalización de cada fase intrannual de crecimiento, habiéndose determinado las mismas con una precisión diaria.

4.2. – Efectos del clima sobre el crecimiento diametral mensual y los ciclos de crecimiento diario

Los resultados de los dendrómetros de banda y electrónicos indican que el clima afecta significativamente tanto al crecimiento diametral mensual como a los ciclos diarios de crecimiento de la encina, y que el efecto de las variables climáticas varía notablemente entre los diferentes periodos intraanuales de crecimiento. En el caso del crecimiento mensual, la mayoría de las variables climáticas y de humedad y temperatura de suelo analizadas explicaron de forma significativa parte de las diferencias de crecimiento a nivel parcela x año x mes, año x mes y parcela x mes. El efecto del clima también resultó significativo en el caso del crecimiento diario, aunque los resultados mostraron que su efecto fue más destacado durante los dos principales periodos de crecimiento que durante las dos fases de letargo o parada, sugiriendo en estas últimas una pausa en la actividad del cambium. La falta de respuesta del crecimiento diario frente a la precipitación y a la humedad del suelo durante el verano indica que la encina en el suroeste de España se comporta frente al estrés hídrico severo como una *especie evitadora*, a pesar de ser tradicionalmente considerada como una *especie tolerante*, que mantiene tasas de crecimiento constantes, aunque reducidas, durante todo el verano (Ogaya et al. 2003). A pesar de esto, los resultados mostraron que la precipitación y la humedad del suelo son los principales factores climáticos limitantes para el crecimiento de la encina, tal y como ha sido encontrado en otros estudios (Campelo et al. 2009; Gea-Izquierdo 2009; Gutiérrez et al. 2011). Sin embargo, el crecimiento no estuvo significativamente correlacionado con el nivel de humedad en el suelo a nivel diario, lo que sugiere una respuesta no lineal del crecimiento frente a la cantidad de agua del suelo. La evapotranspiración de referencia afectó negativamente al crecimiento mensual a nivel parcela x año x mes y año x mes, y al diario durante todas las fases intraanuales, poniendo de relieve el efecto limitante que el estrés hídrico tiene sobre el crecimiento, incluso durante los periodos en los que se encuentra agua abundante disponible en el suelo. Las causas del estrés hídrico no se limitan a la escasez de humedad edáfica, sino que puede también estar producido por las bajas temperaturas de invierno (Corcuera et al. 2004; Cavender-Bares et al. 2005), o incluso por la hipoxia de las raíces debido al encharcamiento del suelo (Tatarinov y Čermák 1999). El efecto positivo de la humedad relativa, tanto en el crecimiento mensual como en el diario, probablemente se encuentre relacionado con la disminución de las perdidas de agua por transpiración (Köcher et al. 2012). En este sentido, la radiación solar también afectó negativamente al crecimiento, tanto por sus efectos sobre el incremento de la transpiración, como por el fenómeno de fotoinhibición en las hojas (Corcuera et al. 2005). Las correlaciones negativas entre temperatura y crecimiento diario, y mensual a nivel parcela x año x mes y año x mes, se producen probablemente debido también a su efecto sobre el incremento de la transpiración y del control estomático durante periodos de altas temperaturas (Carevic et al. 2010). A pesar de esto, el crecimiento se reduce en otoño e invierno debido a las bajas temperaturas, explicando por qué no se encontró una correlación negativa significativa entre temperatura y crecimiento diario durante esta fase. Por otra parte, en el caso del crecimiento mensual, los resultados mostraron que a nivel parcela x mes las temperaturas influenciaron de forma significativa y positiva al crecimiento. Este resultado probablemente sea debido a que la introducción de esta interacción en el modelo provocó una mayor reducción de la varianza en el mes de marzo, y también a que se incluyó a la parcela de HI, en la que se registraron en invierno temperaturas sensiblemente inferiores a las temperaturas de las otras parcelas, y donde los datos mostraron un retraso en el inicio del crecimiento, produciéndose generalmente en esta parcela durante el mes de abril. Del mismo modo, esta hipótesis explicaría también el efecto significativo y positivo de la evapotranspiración de referencia en el crecimiento mensual a nivel parcela x mes debido a la fuerte correlación existente entre temperatura y evapotranspiración, y a que en el mes de marzo se encuentra generalmente una abundante cantidad de agua disponible en el suelo. En otras regiones más frías, análisis dendrocronológicos han encontrado una correlación positiva entre la temperatura de primavera y el crecimiento de encina (Nijland et al. 2011).

4.3. – Efectos del tamaño del árbol, parámetros hidrológicos y competencia en el crecimiento diametral mensual

A pesar de que los resultados indican que el clima es el principal factor que controla el crecimiento diametral de la encina, existe una alta variabilidad a nivel individual en el crecimiento. El tamaño del árbol, en este caso representado por su circunferencia a la altura del pecho, explica parcialmente estas diferencias, presentando mayores crecimientos aquellos individuos con un mayor tamaño. Los árboles con un mayor tamaño poseen un sistema radical más desarrollado, y por lo tanto son capaces de extraer agua a una mayor profundidad, la cual no se encuentra disponible para aquellos árboles más pequeños. La influencia positiva del tamaño de la encina en su crecimiento diametral también ha sido anteriormente encontrada por Cartan-Son et al. (1992).

Los parámetros hidrológicos no explicaron las diferencias individuales en el crecimiento, indicando que no existieron zonas más favorables que otras dentro de cada parcela. Esta falta de respuesta se explica debido a la suave topografía del sitio de estudio, siendo las diferencias de pendiente reducidas. El efecto de la topografía sobre el crecimiento de la encina no ha sido evaluado en estudios anteriores, aunque Miller et al. (2001) sí encontraron un efecto significativo de la topografía sobre el crecimiento *Prosopis glandulosa* en bosques abiertos de Texas con fuerte pendiente.

Algunos de los índices de competencia evaluados explicaron de forma significativa las diferencias de crecimiento entre individuos, mostrando que aquellos árboles con menor competencia presentan mayores crecimientos. Las masas forestales bajo condiciones climáticas y edáficas limitantes, como es el caso de las dehesas y los bosques de encina, se ven sometidas frecuentemente a procesos de competencia por los recursos hídricos y los nutrientes, que ejercen una influencia negativa significativa sobre el crecimiento diametral (Rodríguez-Calcerrada et al. 2011).

4.4. – *Trade-offs* entre crecimiento diametral y producción de bellota

La producción de bellota explicó parte de la variabilidad individual encontrada en el crecimiento de encina, indicando que aquellos árboles con una mayor producción de bellota crecieron menos que aquellos árboles cuya producción fue menor, aunque solamente en aquellos años de abundante cosecha y durante la época en la que se produce el engorde de las bellotas. Estos resultados indican la existencia de *trade-offs* en la asignación de recursos entre reproducción y crecimiento en encina, tal y como ha sido encontrado en otras especies (Mund et al. 2010; Staudhammer et al. 2013). En años en los que las condiciones climáticas son favorables, los árboles utilizan esa abundancia de recursos para asegurar una efectiva reproducción, derivando incluso parte de los recursos asignados inicialmente al crecimiento para aumentar la producción de bellota. Esa derivación de recursos o *resource switching* (Kelly y Sork 2002; Monks y Kelly 2006) provocó un menor crecimiento diametral en aquellos árboles que produjeron más bellota, a pesar de que en términos generales, tanto la producción de bellota como el crecimiento resultaron beneficiados por esas favorables condiciones climáticas, apareciendo por tanto un fenómeno de *resource matching* (Norton y Kelly 1988) a nivel parcela entre crecimiento diametral y producción de bellota, tal y como ha sido encontrado anteriormente para encina por Pérez-Ramos et al. (2010). Por el contrario, en años en los que los recursos fueron más escasos, los resultaron no mostraron una correlación negativa a nivel individual entre crecimiento diametral y producción de bellota, resultando ambos procesos limitados. Los resultados indicaron que este *trade-off* entre crecimiento diametral y producción de bellota puede variar entre diferentes localizaciones, mostrando la influencia que pueden ejercer las diferentes variables ecológicas del medio o las características de la masa sobre estas relaciones endógenas (Reznick 1985; Staudhammer et al. 2013).

4.5. – Efecto de los tratamientos de suelo sobre el crecimiento mensual

Los tratamientos de mejora de suelo no tuvieron ningún efecto significativo en el crecimiento diametral de la encina. El tratamiento de laboreo reduce la compactación en la parte superior del suelo, mejora la infiltración de agua, y elimina la competencia del matorral y de la vegetación herbácea, por lo que estos resultados indican que ninguno de estos factores limita el crecimiento de la encina en esta zona de estudio, a pesar de que Rolo y Moreno (2011) encontraron que en las dehesas el matorral compite intensamente con la encina, afectando negativamente a su estado nutricional, conductancia estomática y actividad fotosintética. Por otra parte, Moreno et al. (2007) encontraron que el matorral también ejerce una influencia negativa en el crecimiento de los ramillos de encina. Estos resultados contradictorios probablemente sean debidos a que en el presente estudio el matorral existente en las parcelas de control se encontraba poco desarrollado debido a los tratamientos de laboreo que se realizaban antes del comienzo del estudio. El segundo tratamiento aplicado, fertilización y siembra de tremosilla, tampoco tuvo un efecto significativo sobre el crecimiento. La encina es una especie esclerófila adaptada a una baja disponibilidad de nutrientes (Monk 1966), y tiene una alta eficiencia en el uso de recursos, y en consecuencia una baja tasa de crecimiento (Aerts 1995). Otros autores han encontrado una baja respuesta del crecimiento de la encina a la fertilización (Cartan-Son et al. 1992; Mayor y Rodà 1994). Como se ha discutido anteriormente, la disponibilidad de agua es el principal factor limitante del crecimiento de la encina, y de acuerdo con Landsberg (1986), la fertilización no es correctamente aprovechada por las plantas en condiciones de sequía. Por otra parte, a pesar de que la tremosilla es una planta leguminosa que fija N_2 atmosférico en el suelo, Rivest et al. (2011) encontraron que la cantidad de N_2 fijado por leguminosas en las dehesas de encina es muy reducida e insuficiente para compensar su consumo por parte del pasto. La tremosilla es una especie con un sistema radical poco profundo (Bramley et al. 2009), mientras que la encina tiene una baja dependencia de los recursos localizados en las capas más superficiales del suelo (Moreno et al. 2007). Por lo tanto, esto explicaría por qué la fijación de N_2 atmosférico en el suelo no tuvo un efecto significativo sobre el crecimiento diametral de la encina.

4.6. – Efecto de las podas sobre el crecimiento mensual

El efecto de los tratamientos de poda sobre el crecimiento varió sensiblemente dependiendo del mes de crecimiento, de la intensidad de la poda, y de la parcela en la que se ejecutaron. La poda fuerte redujo ligeramente el crecimiento en los meses de primavera. Estudios previos en *Pinus ponderosa* (Gyenge et al. 2010) y *Acacia nilotica* (Siddiqui et al. 2010) encontraron que las podas intensas tienen un efecto negativo en el crecimiento diametral. La eliminación de una parte significativa de la copa de los árboles altera el equilibrio entre las raíces y la parte aérea, provocando una desviación de los recursos para la restauración de la biomasa sustraída (Cañellas et al. 2007), lo que hace disminuir aquellos disponibles para el crecimiento diametral. La eliminación de una parte de la copa también provoca una reducción en la capacidad fotosintética del árbol (Gyenge et al. 2010) y en su asimilación de carbono (Balandier et al. 2000). Las mayores contracciones en verano y posteriores rehidrataciones de los árboles sometidos a poda moderada y fuerte en la parcela CA, pudieron ser provocadas por la mayor sensibilidad de estos árboles a los efectos del estrés hídrico (Jackson et al. 2000; Gyenge et al. 2009). Los árboles de esta parcela se encontraban sometidos a un mayor estrés hídrico provocado por presentar suelos más superficiales y menos desarrollados, y además posiblemente por la presencia de la enfermedad de *la seca* (Brasier 1995). Por el contrario, en la parcela SB, con suelos más desarrollados y profundos, las podas no tuvieron un efecto significativo en las contracciones de verano, y posterior rehidratación y crecimiento. A diferencia de las podas fuertes, las podas de intensidad moderada favorecieron ligeramente el crecimiento en los meses de primavera en esta parcela, mientras que los árboles con poda ligera no presentaron crecimientos significativamente diferentes de los árboles no podados.

5. Conclusiones

En aplicación de lo dispuesto en el artículo 38.1 del Reglamento de Estudios de Doctorado de la Universidad de Huelva, aprobado en Consejo de Gobierno de 23 de abril y modificado por Consejo de Gobierno de 19 de diciembre de 2012, se presentan las Conclusiones en lengua inglesa para la obtención de la mención de Doctor Internacional.

5. - CONCLUSIONS

1. The Mediterranean climate is the main driving force of the stem growth of holm oak. Climate provoked a characteristic intra-annual growth pattern with two main periods of stem growth. The first one occurred during late winter and spring, and the second one during late summer and autumn. Between these two growth periods there were two periods of low growth rates or growth cessation. These periods occurred in late spring and summer, when water stress increased, and in late autumn and winter, when temperatures decreased. Intra-annual growth patterns vary significantly among years because the high variability of Mediterranean climate, especially in relation to precipitation.

2. The main climatic variables that affected stem growth were those related with water status. Precipitation, soil moisture and relative humidity were positively correlated to stem growth, but solar radiation were negatively correlated to growth. Temperatures and reference evapotranspiration were, in general terms, negatively correlated to stem growth, probably because its negative influence in relation to increasing transpirations and water losses, despite low temperatures decreased growth rates in late autumn and winter.

3. All trees generally followed the same climate signal and were highly synchronized, but there was also a high individual variability that was not explained by climate. Tree size, represented by circumference at breast height, and some competition indexes explained part of that individual variability in growth rates, indicating a competence for the scarce resources in open woodlands and Mediterranean forests of holm oak. On the contrary, hydrological parameters were not significantly correlated to stem growth, probably due to experimental plots were located in sites with flat or smooth undulated topography.

4. Acorn production had a significant negative effect on stem growth at individual level, indicating that those trees with higher acorn crops grew lesser than the trees with a

smaller acorn production. This negative effect on stem growth was only significant at the time when acorns fattened, during late summer and autumn, and occurred only in years of high acorn production or mast years. These results showed the existence of *trade-offs* between stem growth and reproduction in holm oak, which explained part of individual variability in growth rates.

5. Soil treatments did not influence significantly the stem growth of holm oak, indicating a scarce relation of holm oak in that study site to shrubs competence, soil compaction and water infiltration, and nutrient improvement. These results show the low response of holm oak to uppermost soil features.

6. Intense pruning treatments have detrimental effects on stem growth. This negative influence was higher in trees located on poor soils and under other stress factors, increasing the effects of water stress on stem growth. These negative effects were significantly lesser in trees with good sanitary status located on better soils, despite intense pruning decreased slightly stem growth rates in spring. Therefore, intense pruning could affect the vigor and vegetative status of trees in areas where tree survival is already compromised.

6. Referencias

6. - REFERENCIAS

Abe H, Nakai T (1999) Effect of the water status within a tree on tracheid morphogenesis in *Cryptomeria japonica* D. Don. Trees 14:124–129

Abrantes J, Campelo F, García-González I, Nabais C (2013) Environmental control of vessel traits in *Quercus ilex* under Mediterranean climate: relating xylem anatomy to function. Trees 27:655–662

Aerts R (1995) The advantages of being evergreen. Trends Ecol Evol 10:402–407

Akaike H (1974) A new look at the statistical model identification. IEEE Trans Autom Control 19:716–723

Alejano R, Tapias R, Fernández M, Torres E, Alaejos E, Domingo J (2008) Influence of pruning and the climatic conditions on acorn production in holm oak (*Quercus ilex* L.) dehesas in SW Spain. Ann For Sci 65:209–217

Alejano R, Domingo JM, Fernández M et al. (2011) Manual para la gestión sostenible de las dehesas andaluzas. Foro Encinal-Universidad de Huelva, Huelva

Balandier P, Lacointe A, Le Roux X, Sinoquet H, Cruiziat P, Le Dizès S (2000) “SIMWAL”: a structure – function model simulating single walnut tree growth according to climate and pruning. Ann For Sci 57:571–585

Barling RD, Moore ID, Grayson RB (1994) A quasi-dynamic wetness index for characterising the spatial distribution of zones of surface saturation and soil water content. Water Resour Res 30:1029–1044

Barringer BC, Koenig WD, Knops JMH (2012) Interrelationships among life-history traits in three California oaks. Oecologia 171:129–139

Battipaglia G, De Micco V, Brand WA, Linke P, Aronne G, Saurer M, Cherubini P (2010) Variations of vessel diameter and d13C in false rings of *Arbutus unedo* L. reflect different environmental conditions. New Phytol 188:1099–1112

Battipaglia G, De Micco V, Brand WA, Sauren M, Aronne G, Linke P, Cherubini P (2014) Drought impact on water use efficiency and intra-annual density fluctuations in *Erica arborea* on Elba (Italy). Plant Cell Environ 37:382–391

Böhmerle K (1883) Die pfister´sche Zuwachsuhr. Zentralblatt für das Gesamte Forstwesen 9:83–93

Bormann FH, Kozlowski TT (1962) Measurements of tree growth with dial gage dendrometers and vernier tree ring bands. Ecology 43:289–294

Bramley H, Turner NC, Turner DW, Tyerman SD (2009) Roles of morphology, anatomy, and aquaporins in determining contrasting hydraulic behavior of roots. Plant Physiol 150:348–364

Brasier CM (1995) *Phytophthora cinnamomi* and oak decline in southern Europe. Environmental constraints including climate change. Ann For Sci 53:347–358

Bravo JA, Roig S, Serrada R (2008) Selvicultura en montes bajos y medios de *Quercus ilex* L., *Quercus pyrenaica* Willd. y *Quercus faginea* Lam. En: Serrada R, Montero G, Reque JA (eds) Compendio de Selvicultura aplicada en España. INIA, Madrid, pp 657-744

Breitsprecher A, Hughes W (1975) A recording dendrometer for humid environments. Biotropica 7:90–99

Camarero JJ, Albuixech J, López-Lozano R, Casterad MA, Montserrat-Martí G (2010) An increase in canopy cover leads to masting in *Quercus ilex*. Trees 24:909–918

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

Campelo F, Nabais C, García-González I, Cherubini P, Gutiérrez E, Freitas H (2009) Dendrochronology of *Quercus ilex* L. and its potential use for climate reconstruction in the Mediterranean region. Can J For Res 39:2486–2493

Campelo F, Nabais C, Gutiérrez E, Freitas H, García-González I (2010) Vessel features of *Quercus ilex* L. growing under Mediterranean climate have a better climatic signal than tree-ring width. Trees 24:463–470

Cañellas I, Roig S, Poblaciones MJ, Gea-Izquierdo G, Olea L (2007) An approach to acorn production in Iberian dehesas. Agroforest Syst 70:3–9

Carevic FS, Fernández M, Alejano R, Vázquez-Piqué J, Tapias R, Corral E, Domingo J (2010) Plant water relations and edapho-climatic conditions affecting acorn production in a holm oak (*Quercus ilex* L. ssp. *ballota*) open woodland. Agroforest Syst 78:299–308

Carevic F, Fernández M, Alejano R, Vázquez-Piqué J (2014) Xylem cavitation affects the recovery of plant water status and consequently acorn production in a holm oak open Woodland. Acta Physiol Plant 36:3283–3290

Carrete M, Doñázar JA (2005) Application of central-place foraging theory shows the importance of Mediterranean dehesas for the conservation of the cinereous vulture, *Aegypius monachus*. Biol Conserv 126:582–590

Cartan-Son M, Floret C, Galan MJ, Grandjanny M, Le Floc'h E, Maistre M, Perret P, Romane F (1992) Factors affecting radial growth of *Quercus ilex* L. in a coppice stand in southern France. Vegetatio 99–100:61–68

Cavender-Bares J, Cortes P, Rambal S, Joffre R, Miles B, Rocheteau A (2005) Summer and winter sensitivity of leaves and xylem to minimum freezing temperatures: a comparison of co-occurring Mediterranean oaks that differ in leaf lifespan. New Phytol 168:597–612

Cherubini P, Gartner BL, Tognetti R, Bräker OU, Schoch W, Innes JL (2003) Identification, measurement and interpretation of tree rings in woody species from mediterranean climates. Biol Rev 78:119–148

Cobos DR (2010) Calibrating ECH2O soil moisture sensors. http://www.decagon.com/assets/Uploads/13393-04-calibratingECH2OSoilMoistureProbes.pdf. Accessed 26 October 2012

Cook ER, Kairiukstis LA (1990) Methods of Dendrochronology. Applications in the Environmental Sciences. Kluwer Academic Publishers, Dordrecht

Corcuera L, Camarero JJ, Gil-Pelegrín E (2004) Effects of a severe drought on *Quercus ilex* radial growth and xylem anatomy. Trees 18:83–92

Corcuera L, Morales F, Abadía A, Gil-Pelegrín E (2005) Seasonal changes in photosynthesis and photoprotection in a *Quercus ilex* subsp. *ballota* woodland located in its upper altitudinal extreme in the Iberian Peninsula. Tree Physiol 25:599–608

Deslauriers A, Morin H, Urbinati C, Carrer M (2003) Daily weather response of balsam fir (*Abies balsamea* (L.) Mill.) stem radius increment from dendrometer analysis in the boreal forests of Québec (Canada). Trees 17:477–484

Deslauriers A, Rossi S, Anfodillo T (2007) Dendrometer and intra-annual tree growth: what kind of information can be inferred? Dendrochronologia 25:113–124

Downes G, Beadle C, Worledge D (1999) Daily stem growth patterns in irrigated *Eucalyptus globulus* and *E. nitens* in relation to climate. Trees 14:102–111

Eichhorn MP, Paris P, Herzog F, Incoll LD, Liagre F, Mantzanas K, Mayus M, Moreno G, Papanastasis VP, Pilbeam DJ, Pisanelli A, Dupraz C (2006) Silvoarable systems in Europe — past, present and future prospects. Agroforest Syst 67:29–50

Environmental Systems Research Institute ESRI (2006) ESRI data and maps: 2006 world, Europe, United States, Canada, and Mexico. ESRI, Redlands

Fernández-Rebollo P, Porras CJ (1998) La dehesa. Algunos aspectos para la regeneración del arbolado. Informaciones Técnicas 58/98. Dirección General de Investigación y Formación Agraria, Servicio de Publicaciones y divulgación, Sevilla

Ferrio JP, Florit A, Vega A, Serrano L, Voltas J (2003) Δ^{13}C and tree-ring width reflect different drought responses in *Quercus ilex* and *Pinus halepensis*. Oecologia 442:512–518

Friedrich J (1890) Zuwachsmesser. Die pfister´sche Zuwachsuhr. Zentralblatt für das Gesamte Forstwesen 16:174–179

Friedrich J (1905) Zuwachsautograph. Zentralblatt für das Gesamte Forstwesen 31:456–461

Fritts HC (1976) Tree rings and climate. Academic Press, London

Fritts HC, Fritts EC (1955) A new dendrograph for recording radial changes of a tree. Forest Sci 1:271–276

Gea-Izquierdo G, Martín-Benito D, Cherubini P, Cañellas I (2009) Climate-growth variability in *Quercus ilex* L. west Iberian open woodlands of different stand density. Ann Forest Sci 66:802

Gea-Izquierdo G, Cherubini P, Cañellas I (2011) Tree-rings reflect the impact of climate change on *Quercus ilex* L. along a temperature gradient in Spain over the last 100 years. Forest Ecol Manage 262:1807–1816

Gené C, Espelta JM, Gràcia, M, Retana J (1993) Identificación de los anillos anuales de crecimiento de la encina (*Quercus ilex* L.). Orsis 8:127–139

Gutiérrez E, Campelo F, Camarero JJ, Ribas M, Muntán E, Nabais C, Freitas H (2011) Climate controls act at different scales on the seasonal pattern of *Quercus ilex* L. stem radial increments in NE Spain. Trees 25:637–646

Gyenge JE, Fernández ME, Schlichter TM (2009) Effect of pruning on branch production and water relations in widely spaced ponderosa pines. Agroforest Syst 77:223–235

Gyenge JE, Fernández ME, Schlichter TM (2010) Effect of stand density and pruning on growth of ponderosa pines in NW Patagonia, Argentina. Agroforest Syst 78:233–241

Herzog KM, Häsler R, Thum R (1995) Diurnal changes in the radius of a subalpine Norway spruce stem: their relation to the sap flow and their use to estimate transpiration. Trees 10:94–101

Intergovernmental Panel on Climate Change IPCC (2007) Climate Change 2007: Synthesis Report. Cambridge University Press, Cambridge

Jackson NA, Wallace JS, Ong CK (2000) Tree pruning as a means of controlling water use in an agroforestry system in Kenia. For Ecol Manag 126:133–148

Junta de Andalucía (2001) Ortofotografía digital pancromática de Andalucía Año 2001. Servicio WMS: http://www.ideandalucia.es/wms/ortofoto2001?

Junta de Andalucía (2005) Modelo Digital del terreno de Andalucía. Relieve y orografía. Junta de Andalucía, Sevilla

Kelly D, Sork VL (2002) Mast seeding in perennial plants: why, how, where? Annu Rev Ecol Syst 33:427–47

Knops JMH, Koenig WD, Carmen WJ (2007) Negative correlation does not imply a tradeoff between growth and reproduction in California oaks. Proc Natl Acad Sci USA 104:16982–16985

Knops JMH, Koenig WD (2012) Sex allocation in California oaks: trade-offs or resource tracking? PLoS ONE 7(8): e43492

Köcher P, Horna V, Leuschner C (2012) Environmental control of daily stem growth patterns in five temperate broad-leaved tree species. Tree Physiol 32:1021–1032

Landsberg JJ (1986) Physiological ecology of forest production. Academic Press, New York

Loris K (1981) Dickenwachstum von Zirbe, Fichte, und Lärche an der alpinen Waldgrenze/Patscherkofel. Ergebnisse der Dendrometermessungen 1976/79. Mitteilungen der forstlichen Bundesversuchsanstalt. Wien 142:417–441

Lumaret R, Mir C, Michaud H, Raynal V (2002) Phytogeographical variation of chloroplast DNA in holm oak (*Quercus ilex* L.). Mol Ecol 11:2327–2336

MacDougal DT (1918). The dendrograph: a new instrument for recording growth and other variations in the dimensions of trees. Carnegie Inst Wash Year Bk 18:72–74

Mäkinen H, Seo J-W, Nöjd P, Schmitt U, Jalkanen R (2008) Seasonal dynamics of wood formation: a comparison between pinning, microcoring and dendrometer measurements. Eur J For Res 127:235–245

Martínez-Pastur GM, Lencinas MV, Cellini JM, Mundo I (2007) Diameter growth: can live trees decrease? Forestry 80:83–88

Mayor X, Rodà F (1994) Effects of irrigation and fertilization on stem diameter growth in a Mediterranean holm oak forest. For Ecol Manag 68:119–126

Miller D, Archer SR, Zitzer SF, Longnecker MT (2001) Annual rainfall, topoedaphic heterogeneity and growth of an arid land tree (*Prosopis glandulosa*). J Arid Environ 48:23–33

Mitrakos KA (1980) A theory for Mediterranean plant life. Acta Oecol 1:245–252

Monk CD (1966) An ecological significance of evergreenness. Ecology 47:504–505

Monks A, Kelly D (2006) Testing the resource-matching hypothesis in the mast seeding tree *Nothofagus truncata* (Fagaceae). Austral Ecol 31:366–375

Moreno G, Obrador JJ, García E, Cubera E, Montero MJ, Pulido FJ, Dupraz C (2007) Driving competitive and facilitative interactions in oak dehesas with management practices. Agroforest Syst 70:25–40

Mund M, Kutsch WL, Wirth C, Kahl T, Knohl A, Skomarkova MV, Schulze ED (2010) The influence of climate and fructification on the inter-annual variability of stem growth and net primary productivity in an old-growth, mixed beech forest. Tree Physiol. 30:689–704

Nabais C, Freitas H, Hagemeyer J (1998–1999) Tree-rings to climate relationships of *Quercus ilex* L. in NE-Portugal. Dendrochronologia 16–17:37–44

Natalini F, Alejano R, Vázquez-Piqué FJ, Cañellas I, Gea-Izquierdo G (2013) Growth trends and sensitivity to climate of declining Mediterranean open woodlands exhibiting widespread mortality in Southern Spain. Second American Dendrochronology Conference Ameridendron. Tucson, Arizona.

Nijland W, Jansma E, Addink EA, Domínguez-Delmás M, De Jong SM (2011) Relating ring width of Mediterranean evergreen species to seasonal and annual variations of precipitation and temperature. Biogeosciences 8:1141–1152

Nishimua TB, Suzuki E, Kohyama T, Tsuyuzaki S (2007) Mortality and growth of trees in peat-swamp and heath forests of Central Kalimantan after severe drought. Plant Ecol 188:165–177

Norton DA, Kelly D (1988) Mast seeding over 33 years by *Dacrydium cupressinum* Lamb. (rimu) (Podocarpaceae) in New Zealand: the importance of economies of scale. Funct Ecol 2:399–408

Oberhuber W, Gruber A, Kofler W, Swidrak I (2014) Radial stem growth in response to microclimate and soil moisture in a drought-prone mixed coniferous forest at an inner Alpine site. Eur J For Res 133:467–479

Ogaya R, Peñuelas J, Martínez-Vilalta J, Mangirón M (2003) Effect of drought on diameter increment of *Quercus ilex*, *Phillyrea latifolia*, and *Arbutus unedo* in a holm oak forest of NE Spain. Forest Ecol Manage 180:175–184

Olea L, López-Bellido RJ, Poblaciones MJ (2005) Europe types of silvopastoral systems in the Mediterranean area: dehesa. En: Mosquera MR, Rigueiro-Rodríguez A, McAdam J (eds) Silvopastoralism and Sustainable Land Management. CABI Publishing, Wallingford, pp 30-35

Patterson HD, Thompson R (1971) Recovery of inter-block information when block sizes are unequal. Biometrika 58:545–554

Pérez-Ramos IM, Ourcival JM, Limousin JM, Rambal S (2010) Mast seeding under increasing drought: results from a long-term dataset and from a rainfall exclusion experiment. Ecology 91:3057–3068

Quézel P, Médail F (2003) Ecologie et biogéographie des forêts du bassin méditerranéen. Elsevier, Paris

Reznick R (1985) Cost of reproduction: An evaluation of the empirical evidence. Oikos 44:257–267

Rivas-Martínez S (1987) Memoria del mapa de series de vegetación de España. ICONA, Madrid

Rivest D, Rolo V, López-Díaz ML, Moreno G (2011) Belowground competition for nutrients in shrub-encroached Mediterranean dehesas. Nutr Cycl Agroecosys 90:347–354

Rodà F, Vayreda J, Ninyerola M (2009) Encinares de *Quercus ilex* y *Quercus rotundifolia*. En: VV. AA. Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España. Ministerio de Medio Ambiente y Medio Rural y Marino, Madrid, pp 94

Rodríguez-Calcerrada J, Pérez-Ramos IM, Ourcival JM, Limousin JM, Joffre R, Rambal S (2011) Is selective thinning an adequate practice for adapting *Quercus ilex* coppices to climate change? Ann Forest Sci 68:575–585

Rolo V, Moreno G (2011) Shrub species affect distinctively the functioning of scattered *Quercus ilex* trees in Mediterranean open woodlands. For Ecol Manag 261:1750–1759

Rossi S, Deslauriers A, Anfodillo T (2006) Assessment of cambial activity and xylogenesis by microsampling tree species: an example at the Alpine timberline. IAWA J 27:383–394

Ruiz de la Torre J (Dir.) (2005) Mapa Forestal de España Escala 1:1.000.000. Ministerio de Medio Ambiente, Madrid

Ruiz de la Torre J (2006) Flora Mayor. Organismo Autónomo de Parques Nacionales, Madrid

San Miguel A (1994) La dehesa española: origen, tipología, características y gestión. Fundación Conde del Valle de Salazar, Madrid

Sarris D, Christodoulakis D, Körner C (2007) Recent decline in precipitation and tree growth in the eastern Mediterranean. Glob Change Biol 13:1–14

Schmitt U, Möller R, Eckstein D (2000) Seasonal wood formation dynamics of beech (*Fagus sylvatica* L.) and black locust (*Robinia pseudoacacia* L.) as determined by the “pinning” technique. J Appl Bot 74:10–16

Seo J-W, Eckstein D, Schmitt U (2007) The pinning method: from pinning to data preparation. Dendrochronologia 25:79–86

Serrada R, San Miguel A (2008) Selvicultura de la Dehesa. En: Serrada R, Montero G, Reque JA (eds) Compendio de Selvicultura aplicada en España. INIA, Madrid, pp 861-879

Siddiqui T, Farrakh-Nawaz M, Ahmed I (2010) Effect of different pruning intensities on the growth of *Acacia nilotica* (Kikar). Agrociencia 44:93–97

Speer JH (2010) Fundamentals of tree-ring research. University of Arizona Press, Tucson

Staudhammer CL, Wadt LHO, Kainer AK (2013) Tradeoffs in basal area growth and reproduction shift over the lifetime of a long-lived tropical species. Oecologia 173:45–57

Stearns SC (1989) Trade-offs in life-history evolution. Funct Ecol 3:259–268

Steppe K, De Pauw DJW, Lemeur R, Vanrolleghem PA (2006) A mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth. Tree Physiol 26:257–273

Stevenson AC, Harrison RJ (1992) Ancient forests in Spain: a model for land-use and dry forest management in south-west Spain from 4000 BC to 1900 AD. Prehist Soc 58:227–247

Susmel L, Viola F, Bassato F (1976) Ecología della lecceta del Supramonte di Orgosolo (Sardegna centro-orientale). Annali del Centro di Economia Montana delle Venezie 10:1–261

Tardif J, Flannigan M, Bergeron Y (2001) An analysis of the daily radial activity of 7 boreal tree species, northwestern Quebec. Environ Monit Assess 67:141–160

Tatarinov F, Čermák J (1999) Daily and seasonal variation of stem radius in oak. Ann For Sci 56: 579–590

Terradas J, Savé R (1992) The influence of summer and winter stress and water relationships on the distribution of *Quercus ilex* L. Vegetatio 99–100:137–145

Wang Z, Yang B, Deslauriers A, Bräuning A (2015) Intra-annual stem radial increment response of Qilian juniper to temperature and precipitation along an altitudinal gradient in northwestern China. Trees 29:25–34

Wolter KE (1968) A new method for marking xylem growth. For Sci 14:102–104

Zhang SH, Romane F (1991) Variations de la croissance radiale de *Quercus ilex* L. enfonction du climat. Ann Forest Sci 48:225–234

Zweifel R, Eugster W, Etzold S, Dobbertin M, Buchmann N, Häsler R (2010) Link between continuous stem radius changes and net ecosystem productivity of a subalpine Norway spruce forest in the Swiss Alps. New Phytol 187:819–830

Apéndice 1. Artículos incluidos en esta tesis

Artículo 1. Effect of ecological factors on intra-annual stem girth increment of holm oak

Artículo publicado como: Martín D, Vázquez-Piqué J, Fernández M, Alejano R (2014) Effect of ecological factors on intra-annual stem girth increment of holm oak. Trees 28:1367-1381.

Effect of ecological factors on intra-annual stem girth increment of holm oak

Abstract

Holm oak (*Quercus ilex* ssp. *ballota* (Desf.) Samp.) is the most widespread species in the Iberian peninsula, being one of the most representative trees in forests and open woodlands. The analysis of stem girth increment of holm oak may provide valuable information about how Mediterranean ecosystems will respond to the forecasted climate changes. However, due to the variability of the Mediterranean climate, the knowledge of intra-annual patterns of growth is needed for a better understanding of the influence of the climatic variables at this scale. To this end, we used band dendrometers to measure monthly stem girth increments of 96 holm oak trees from 2003 to 2010, located in open woodlands and dense Mediterranean forests in southwestern Spain. We assessed the effects of climate, competition, topography, and initial stem diameter on stem girth increment. The major stem increment periods were in spring and autumn whereas increment rates were very low or even negative in winter and summer. Spring was not every year the season with the higher stem increments, but autumn when spring was very dry. Higher precipitation, soil moisture, and relative humidity had significant positive effects on stem increment, whereas higher temperature, reference evapotranspiration, and solar radiation had significant negative effects. Initial tree diameter and competition from nearby trees partly explained significant differences in stem increment of individual trees. Therefore, the forecasted climatic changes, in which decreased rainfall in spring and increased summer drought are expected in the Mediterranean region, may be a significant threat to the *Q. ilex* ecosystems.

Keywords: Ecological modeling, *Quercus ilex,* stem growth, climate, competition.

Key message: The intra-annual stem girth increment of *Quercus ilex* is mainly driven by water availability and secondly by temperature. Tree size and competition modulate the growth response to climate.

Introduction

Holm oak (*Quercus ilex* L.) is the most widespread *Quercus* species in the Iberian Peninsula where it covers an area of about 3 million ha (Bravo et al. 2008). It occurs in different ecosystems, from sea level up to 2,000 m, in limestone to siliceous rock systems, and it is able to withstand the high temperatures and summer droughts of the Mediterranean region (Rodá et al. 1999). Forests of holm oak and cork oak (*Quercus suber* L.) in the western Iberian Peninsula have been transformed into open woodlands with densities of 20-100 trees ha^{-1} and canopy covers of 10% to 50%, with an understory of croplands, grasslands, and shrublands where cattle, sheep, pigs, and goats are raised (San Miguel 1994). These systems have been harvested (fuelwood, acorns, grasses, livestock) for more than 4,500 years (Stevenson and Harrison 1992) and even nowadays have an important role in local economies. Open woodlands of *Quercus* in southwestern Spain are also highly diverse ecosystems, create unique landscapes and constitute a refuge for many endangered species, including the Spanish imperial eagle *Aquila adalberti*, the cinereous vulture *Aegypius monachus*, and the Iberian lynx *Lynx pardinus* (Carrete and Doñázar 2005).

Forestry studies have traditionally measured tree growth as the main indicator of yield. However, measurements of tree growth also provide information about health and vigor of the forests, phenological processes, the influence of management systems, or resource competition (Gea-Izquierdo et al. 2009). In a time when climate change is affecting the dynamics of different ecosystems, the analysis of stem growth in a species such as holm oak may provide valuable information about how these ecosystems will respond to forecasted climate changes, where temperature will increase and precipitation will decrease (IPCC 2007). Hence, measurements of tree growth and its ecological determinants are essential for planning sustainable management of these Mediterranean ecosystems.

Some previous studies have examined *Quercus ilex* growth patterns, but many of them have been focused on *Quercus ilex* ssp. *ilex*, which is ecologically and genetically distinct and has a different and more limited geographical distribution than *Quercus ilex* ssp. *ballota* (Lumaret et al. 2002). Previous studies have employed dendrochronological techniques (e.g., Zhang and Romane 1991; Cherubini et al. 2003; Gea-Izquierdo et al. 2009, 2011; Campelo et al. 2010) for annual measurements of growth. However, monthly measurement of stem growth is needed to provide valuable information on growth during different seasons in a Mediterranean area where climate changes among and within years, and regarding the trade-offs of growth with other phenological processes such as acorn production (Mund et al. 2010). The analysis of the relationship of climatic variables with intra-annual stem growth of *Q. ilex* will provide a more complete understanding of the influence of climate on growth (Campelo et al. 2007; Gutiérrez et al. 2011) and of the possible effects of climatic change on phenology, structure, and even geographic distribution of this species.

Band dendrometers provide accurate measurements of stem girth increment at different temporal scales and are especially useful for intra-annual studies. These devices measure the increment of the entire cross section of a tree. Therefore, they are more accurate than techniques that sample one or two points of the stem only (e.g., analysis of cores or microcores, electronic point dendrometers), which can be affected by local changes in the xylem or bark (Gutiérrez et al. 2011). It is especially important to measure the entire cross-section in anatomically complex trees such as *Q. ilex* (Gea-Izquierdo et al. 2011), which has frequent medullar rays, false rings and growth eccentricity (Cherubini et al. 2003; Campelo et al. 2007). Nevertheless, band dendrometers also register expansions and contractions of the stem due to changes in hydration (Gutiérrez et al. 2011), so their use might be combined with other techniques, such as ecosystem net carbon and water vapour fluxes measurements with eddy covariance system (Mund et al. 2010) in order to determine accurately the timing of growth cycles. In addition, band dendrometers can also be useful to assess remote sensing data at monthly scale (e.g., NDVI values) and they have also been used in ecological studies and dendrocronology (Campelo et al. 2007).

In harsh environmental conditions, such as the Mediterranean ones, resource competition (especially for water) can reduce the growth rates of trees. If forest managers understand how trees respond to competition, they can adjust stand density according to the climatic conditions and thereby improve the response to drought (Moreno and Cubera 2008). Moreover, *Q. ilex* trees in hydrologically favorable locations will respond differently to climatic factors such as drought (Miller et al. 2001). Nevertheless, no studies have yet employed intra-annual measurements of tree growth to assess the importance of competition and microtopography in forest stands.

The main questions that we wanted to answer in this study were:

a) How is the intra-annual stem girth increment pattern of holm oak (*Quercus ilex* ssp. *ballota* (Desf.) Samp.) in open woodlands and forests of southwestern Spain?

b) Are there differences in intra-annual stem increment among plots? Do trees within each plot have similar increment rates?

c) How does climate drive intra-annual stem increment?

d) Do the competition, topography and stem size have a significant influence on intra-annual stem increment?

Materials and methods

Field plots

This study was performed in three experimental plots in the Huelva province of southwestern Spain (Table 1). The Huerto Ramirez (HR) plot is in open woodland of *Q. ilex* with soils characterized as acrisols, alisols and lixisols, or less developed as regosols and cambisols (IUSS Working Group WRB 2007); a sparse understory of mainly *Cistus ladanifer* and *Cistus crispus*, and an abundant herbaceous layer of mainly grasses. The San Bartolomé (SB) plot is in an open woodland of *Q. ilex* that is characterized by soils that are endoleptic regosols (episkeletic) or deeper profiles as endoleptic luvisols (dystric) in depositional or concave areas (IUSS Working Group WRB 2007); a very scarce understory due to frequent tillage, and an abundant herbaceous layer of mainly grasses.

Plot	Coordinates (UTM. Zone 29)	Area (ha)	Density (trees ha^{-1})	Mean diameter ± SD (cm)	Mean height ± SD (m)	Sample size (trees)	Species
Huerto Ramírez	X:644288 m Y:4161376 m	2.94	73.0	30.02 ± 7.68	6.58 ± 1.58	55	*Q. ilex*
San Bartolomé	X:669638 m Y:4145966 m	2.70	36.0	35.40 ± 7.23	6.54 ±1.08	32	*Q. ilex*
Hinojos	X:728082 m Y:4133575 m	1.78	17.3	24.34 ± 9.11	7.20 ± 1.90	9	*Q. ilex*
			84.4	28.39 ± 8.39	8.11 ± 1.93	n.a.	*Q. suber*

Table 1 General and dendrometric characteristics of study plots. SD: standard deviation; *Q. ilex*: *Quercus ilex*; *Q. suber*: *Quercus suber*; n.a.: not applicable

The Hinojos (HI) plot is in a *Quercus suber* stand that has some scattered *Q. ilex* trees and is characterized by soils with complex profiles classified as haplic regosol (dystric) over stagnic regosol (dystric) (IUSS Working Group WRB 2007), and an understory that consists of a dense layer of *Cistus salvifolius* and *Halimium halimifolium* with scattered individuals of *Pistacia lentiscus, Phillyrea angustifolia* and *Chamaerops humilis*.

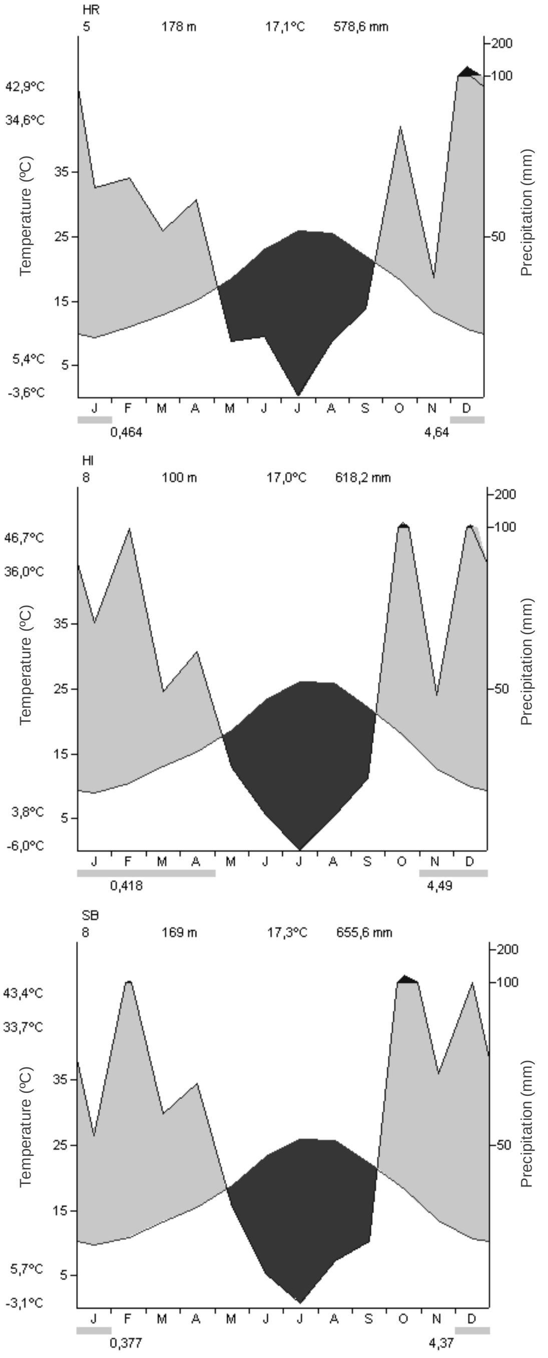

Fig. 1 Walter-Lieth climate diagrams of plots in Huerto Ramírez (HR), Hinojos (HI) and San Bartolomé (SB) during the study period

The climate of all three plots is Mediterranean, with highly variable temperature and rainfall within and among years. The nearby ocean modulates temperature and increases the precipitation with respect to more continental areas. There were no large monthly variations in temperature during the study period (2003-2010), but there were large monthly and annual changes in precipitation (Figs. 1, 2; Table 2). In particular, the annual precipitation in HI was only 274 mm (156 mm in autumn) during 2005, but was 1,011 mm in 2010. The year 2009 was also remarkable: in HI (651 mm total rain) more than 50% of the rain was in December and only 36 mm was in the spring.

Measurement of stem girth increment

A total of 96 aluminum band dendrometers (system developed by the University of Huelva) were installed at breast height (1.30 m), with care taken to avoid stem deformities. Details of band dendrometers theory and construction are available in Keeland and Young (2014). Trees were selected within plots by use of stratified sampling so that different

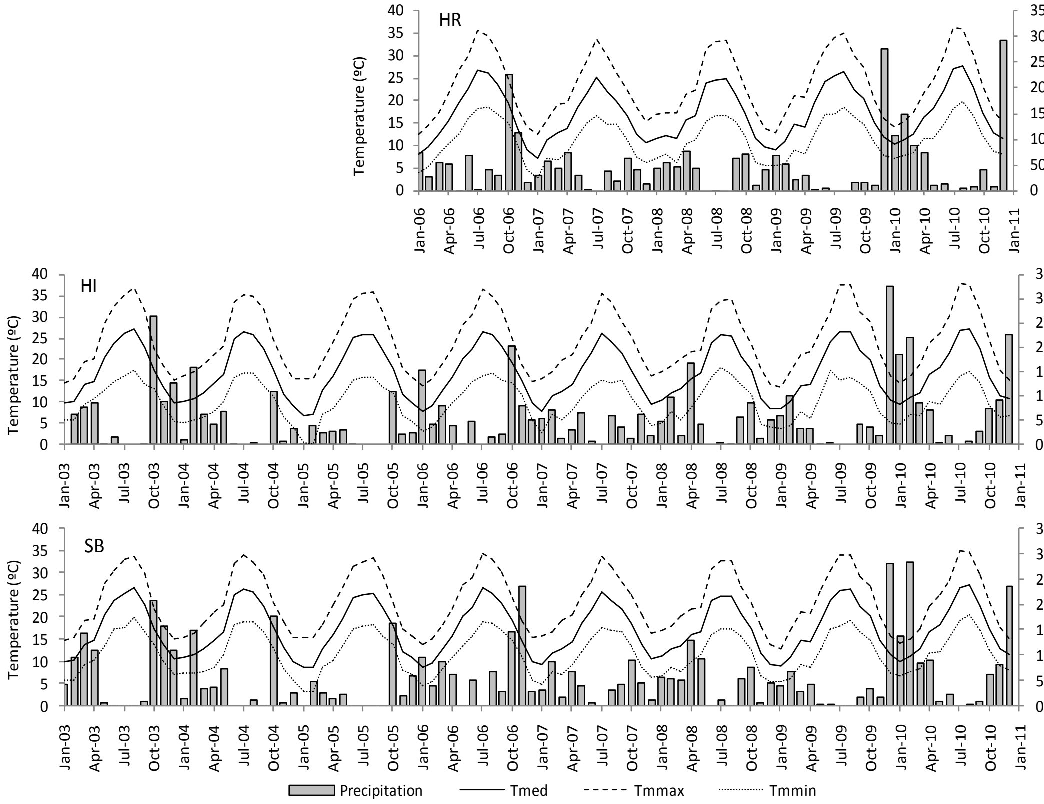

Fig. 2 Monthly precipitation and temperatures of plots in Huerto Ramírez (HR), Hinojos (HI) and San Bartolomé (SB) during the study period. Tmed: mean temperature; Tmmax: mean of the maximum daily temperature; Tmmin: mean of the minimum daily temperature

Year	Precipitation (mm)						Temperature (ºC)								
	HI		SB		HR		HI			SB			HR		
	P	Pmn	P	Pmn	P	Pmn	TM	Tm	T	TM	Tm	T	TM	Tm	T
2003	780	534	884	634			36.9	5.8	17.5	33.8	5.8	17.5			
2004	495	293	536	343			35.3	4.0	16.9	33.9	6.0	17.4			
2005	274	212	362	251			35.9	0.0	16.8	33.3	3.2	17.1			
2006	734	487	850	684	713	592	36.5	3.0	17.3	34.4	4.5	17.4	35.6	4.2	17.4
2007	424	280	473	341	421	318	35.7	2.5	16.5	33.8	4.9	17.0	33.9	2.8	16.1
2008	577	381	584	424	458	315	34.4	3.9	16.7	32.7	5.7	17.0	33.4	5.5	16.8
2009	651	166	538	150	502	108	37.6	3.6	17.5	34.1	5.7	17.9	35.0	5.7	18.0
2010	1011	378	1019	362	799	252	38.0	4.7	16.9	34.9	6.7	17.5	36.3	7.1	17.6

Table 2 Precipitation and temperature of the three plots during the study period. P: annual precipitation; Pmn: precipitation from March to November; TM: mean of the maximum temperatures of the hottest month; Tm: mean of the minimum temperatures of the coldest month; T: mean annual temperature

diametric classes were considered. There were 55 trees in HR, 32 trees in SB, and 9 trees in HI (which only had scattered *Q. ilex* trees) (Table 1). Measurements were recorded each month with a digital caliper (0.01 mm accuracy) in SB and HI from 2003-2010, and in HR from 2006-2010. Because there were differences in measurement dates and in the number of days per month, average daily increments for each tree between the first day and the last day of each month were calculated. Girth increment data were not transformed into diameter increment because *Q. ilex* is a species with high within-tree variability in girth stem growth and then the stems were not enough cylindrical to assume diameter transformation. Hence, girth increment data of entire cross-sections were used instead of diameter increment.

Dendrometry, hydrological parameters and competition indexes

Stem diameter at breast height, tree height (using a Vertex III, Haglöf Sweden AB), and topographic location (using a topographical total station Sokkia 3B) were measured for all 96 trees. Based on topographic location and a digital elevation model (Junta de Andalucía 2005), three hydrological parameters were calculated for each tree: flow length (FL), specific catchment area (SCA), and wetness index (WI) (Barling et al. 1994). Based on the location and size of each tree, 725 distance-dependent competition indexes were calculated in four groups, as described by Vázquez-Piqué and Pereira (2004): Area Overlapping Indexes (AOI), Distance-weighted Ratio (DR), Punctual Density (PD), and Area Potentially Available (APA). Hydrological parameters were calculated with ArcGIS ver. 9.2 (ESRI) and competition indexes with INCO ver. 1.0 (Vázquez-Piqué et al. 2001).

Climatic parameters and soil moisture

Two meteorological stations (HOBOH21-001) were installed in HR and HI for recording of temperature and relative humidity (Onset sensor S-THB-M002), precipitation (Onset rain gauge S-RGB-M002), wind speed and direction (Onset sensor S-WCA-M003), and photosynthetically active radiation (PAR, Onset silicon pyranometer S-LIB-M003) every 15 min. Reference evapotranspiration (ET_0) was calculated by the FAO Penman-Monteith method (Allen et al. 1998). Climatic data for SB were from the nearby Gibraleón meteorological station (37º 24' 49" N; 7º 03' 31" W; 169 m.a.s.l., Agroclimatic Information Network, Junta de Andalucía, available at http://www.juntadeandalucia.es/agriculturaypesca/ifapa/ria/servlet/FrontController?action=Static&url=coordenadas.jsp&c_provincia=21&c_estacion=3), which provided temperature, precipitation, relative humidity (daily average, maximum, and minimum), wind speed and direction, PAR, and ET_0.

For analysis of soil moisture, 9 moisture sensors (ECH2O-20, Decagon Devices Inc.) were placed at 4 locations in the HR plot at depths of 5-25 cm, at 3 locations (matching the locations above) at depths of 25-45 cm, and at one location in SB at depths of 5-25 cm and 25-45 cm. Data were registered every 30 min. Calibration curves were performed in laboratory (8 calibration points per sensor) as described by Cobos (2010) to obtain percent soil moisture from measured voltage. The regression r^2 values of calibration ranged from 0.92 to 0.99. At HI, two capacitance sensors (C-Probe Corp.) were placed at depths of 10 cm and 30 cm at the same locations, and soil moisture was measured every 15 min. Relative Extractable Water (REW) (Granier 1987) was calculated between 5-25 cm, 25-45 cm, and 5- 45 cm depths as:

$$\mathrm{REW} = \frac{\mathrm{WC} - \mathrm{WC_{min}}}{\mathrm{WC_{FC}} - \mathrm{WC_{min}}} \tag{1}$$

where WC is the water content (mm), WC_{min} is the minimum water content (mm) registered during the study period, and WC_{FC} is the water content at field capacity (mm), i.e., the registered water content 48 h after an intense rainfall event that saturated the soil. Soil temperature at 10 cm depth was measured simultaneously in all plots.

Data analysis

A linear mixed model with the following initial structure was used for data analysis:

$$y_{ijlm} = \mu + b_{i(j)} + \alpha_j + \gamma_l + \tau_m + (\alpha|\gamma|\tau)_{jlm} + e_{ijlm} \tag{2}$$

where y_{ijlm} is the girth increase of tree i at plot j in the month l of year m (mm day^{-1}); μ is the general mean; $b_{i(j)}$ is a tree random effect within each plot with i = 1, 2,..., 55 and j = 1, 2, 3 under the hypothesis $b_{i(j)} \sim N(0, \mathbf{G})$; α_j is a plot fixed-effect with j = 1, 2, 3; γ_l is a month fixed-effect with l = 1, 2,..., 12; τ_m is a year fixed-effect with m = 2003, 2004,...., 2010; $(\alpha|\gamma|\tau)_{jlm}$ is all possible double and triple interactions between fixed effects; and e_{ijlm} is residual error under the hypothesis $e_{ijlm} \sim$ N(0, **R**).The initial hypothesis of the independence of observations is not logical because spatial correlations can occur in the growth of trees from the same plot and temporal correlations can occur because observations in consecutive months have more similar growth values than those from non-consecutive months.

The following procedure was used to select the best model structure:

1. The model was adjusted by consideration of tree random effect, the presence of temporal correlations between observations of different months for each tree and year, and the presence of heterogeneous variances in different months of the year. Hence, **G** was initially considered as a diagonal matrix and **R** as a block diagonal matrix, with each block corresponding to a 12 × 12 submatrix of observations taken in one year in each tree. We considered the following alternatives for the structure of blocks in the **R** matrix: autoregressive order 1, autoregressive heterogeneous, Toeplitz up to 5 bands, heterogeneous Toeplitz up to 5 bands, unstructured up to 5 bands, Huynh-Feldt, compound symmetry, compound symmetry heterogeneous, dependent covariance, and first order factor analytic (Littell et al. 2006).Variance components for each structure were estimated by restricted maximum likelihood (REML) (Patterson and Thompson 1971) and model selection was based on the Akaike information criterion (AIC, Akaike 1974).

2. The significance of the tree random effect was determined by a likelihood ratio test, as the reduction of the statistic -2 × log likelihood (-2LL), after introducing the tree random effect follows χ^2 distribution with 1 degree of freedom. An α value of 0.05 was considered an indication of improvement in the covariance structure.

3. If the tree random effect was significant, the presence of spatial correlation was determined. In particular, the following isotropic power covariance model was used:

$$\mathrm{cov}\,(b_{i(j)}, b_{i'(j)}) = \sigma^2{}_b\,\rho^{d_{ii'}} \qquad (3)$$

where $d_{ii'}$ is the distance between trees i and i' in location j; $\sigma^2{}_b$ is the variance component at tree level; and ρ is a parameter to be estimated with $|\rho| < 1$. Spatial covariance between observations of different locations was considered zero.

4. After selection of the best variance–covariance structure, the fixed effects were estimated by generalized least squares (GLS) (Searle 1971) and the significance of each effect was determined with an *F* test. Only significant effects ($\alpha = 0.05$) were retained in the model. Comparisons among levels of significant effects were determined by the Scheffe´ test.

5. If there was a significant tree effect, then tree diameter at breast height, hydrological parameters, and competition indexes were introduced to the model as covariates as additive linear effects and each significance was assessed with an *F* test. To analyze the significance of covariates variance components were estimated by maximum likelihood (ML).

6. If there was a significant year × month, plot × month, or plot × year × month interaction, climatic and soil moisture data were added to the model as covariates at each of the significance levels to explain the categorical effects. Climatic variables at the month, year, or plot level were not introduced due to the small degrees of freedom at those levels. The covariates were introduced as additive linear effects after deleting the fixed categorical interaction at that level. All statistical analysis was performed with SAS/ETS (ver. 9.2).

Results

Pattern of stem girth increment

We ultimately selected a mixed model with a significant tree random effect, significant plot, year, and month fixed effects, and significant plot × month, year × month, plot × year, and plot × year × month interactions. The structure of the variance-covariance matrix for the 12 × 12 blocks of the **R** matrix is unstructured with 4 bands. This indicates that the variance of observations was different for different months (heterogeneous structure) and that there was a temporal correlation for groups of 4 consecutive months. Structures of heterogeneous variances had smaller AIC than structures of homogeneous, indicating clear heteroscedasticity of stem increment. All fixed effects and their interactions were highly significant ($p < 0.0001$) (Table 3). Tree random effect was also significant ($p < 0.0001$), but spatial covariance was not ($p = 0.934$). In other words, the significant stem increment differences among trees cannot be explained by their relative positions within the plots.

Monthly variance was higher in the spring (March, April, and May), when most stem increment occurred (Table 4). In August, when the stem increment rate was very low, variability among trees was smaller. In September, October, and November, variability among trees increased, although it was less than during the spring. The fixed effects that accounted for most of the variance were month and the interaction of year × month. These two factors accounted for 31.5% and 43.6% of the total variance, respectively. The estimated correlations among months within a tree were greater for consecutive months during the spring (April and May, $\rho = 0.62$). The correlations between non-consecutive months were more irregular and not dependent on proximity (data not shown).

Effect	*F*	*Pr > F*
month	145.74	<0.0001
year	58.07	<0.0001
year x month	48.17	<0.0001
plot	30.66	<0.0001
plot x year x month	14.82	<0.0001
plot x month	14.53	<0.0001
plot x year	8.29	<0.0001

Table 3 Significant fixed effects in the final model. *F*: *F*-statistic

Month	Variance components			% of variance absorbed by tree effect
	Tree	Month	Total	
Jan	0.017	0.244	0.261	6.52
Feb		0.444	0.461	3.69
Mar		0.676	0.693	2.45
Apr		0.846	0.863	1.97
May		0.623	0.640	2.66
Jun		0.426	0.442	3.84
Jul		0.243	0.260	6.53
Aug		0.132	0.149	11.43
Sep		0.311	0.328	5.18
Oct		0.361	0.378	4.49
Nov		0.352	0.369	4.60
Dec		0.154	0.171	9.95

Table 4 Variance components in the final model and percentage of variance accounted for by tree effect

Stem girth increment varied significantly throughout the year, with peaks in all plots during the spring and autumn, but with some differences in the timing among the three plots (Fig. 3) and in different years (Fig. 4). In all plots and years, there was little increment or even stem contraction during the summer. The stem increment during the spring was typically greater and lasted longer than that during the autumn (Figs. 3, 4).

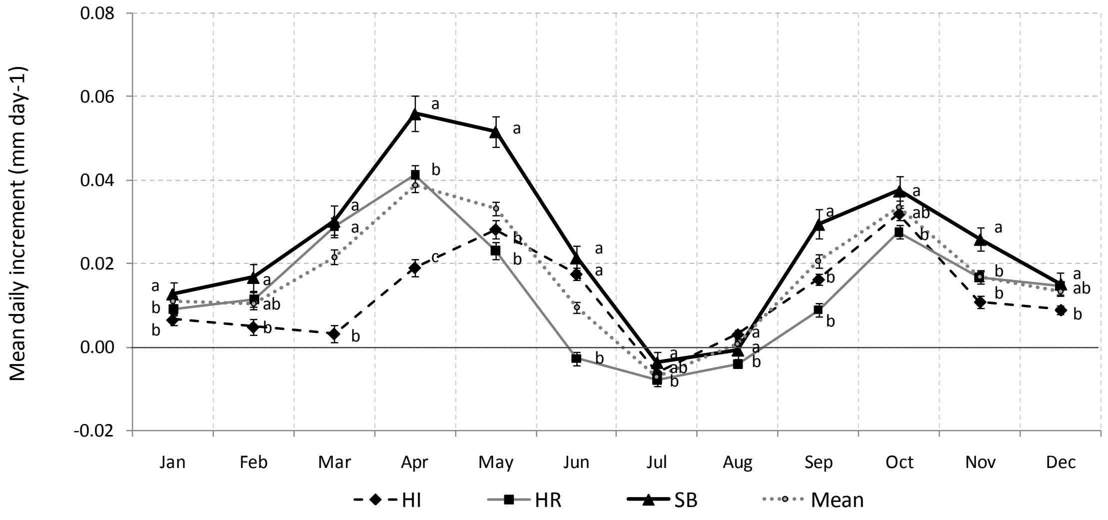

Fig. 3 Least squares means of daily girth increment rate (mm day^{-1} ± standard error) of holm oak trees in the study plots during different months of the study period (2003-2010). Different letters indicate significant differences between plots in a month ($p < 0.05$)

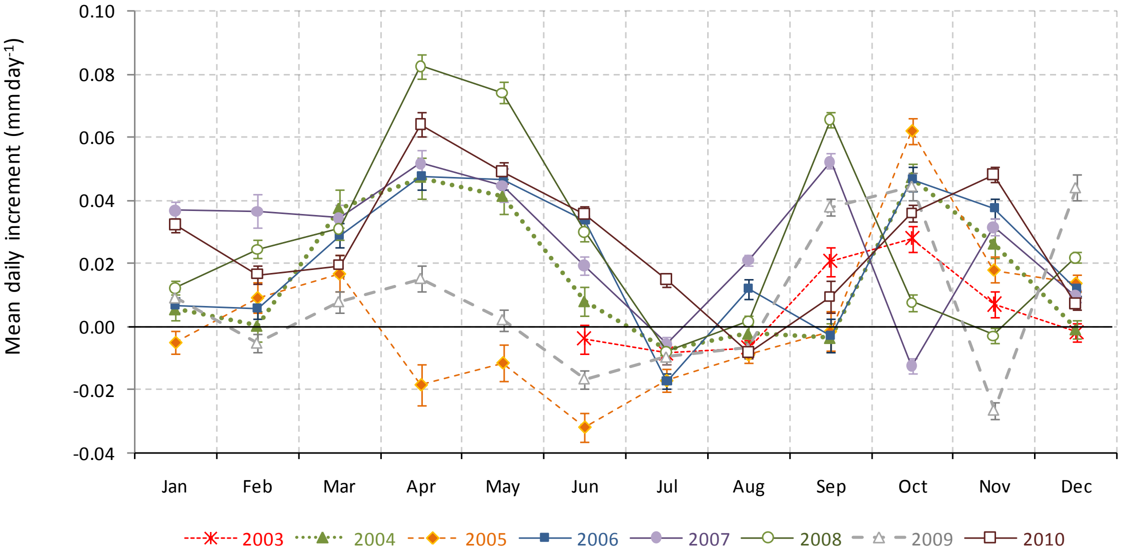

Fig. 4 Least squares means of daily girth increment rate (mm day^{-1} ± standard error) of holm oak trees in different months during each year of the study period

However, in 2005 spring increment was about 0.02 mm day^{-1}, stem contraction occurred from April to September, increment was about 0.062 mm day^{-1} in October, and then it declined until December. The same trend, but not so strong, occurred in 2009. In 2005 and 2009, most of the annual stem increment was in the autumn, with 78% and 69%, respectively, contrary to common years where spring growth accounted for 66-75% of annual stem increment.

Comparison of the three plots indicated that stem increment started in February in SB and HR (Fig. 3) and reached a maximum in April. In contrast, stem increment in HI was very small until April, and reached a peak in May. Trees stopped growing in June in HR and in July in SB and HI. Larger stem contractions always occurred in July. Stem increment increased during September and October in all plots, and then decreased until December, with similar growth rates in SB and HR, and slightly smaller growth rates in HI. The average annual estimated increment of stem girth for the three plots was 8.98 ± 0.34 mm year $^{-1}$ in SB, 5.18 ± 0.30 mm year^{-1} in HR, and 4.43 ± 0.65 mm year^{-1} in HI. All differences at the plot level were significant ($p < 0.0001$).

The stem increment differences between plots varied in different years. For example, in 2008 growth in SB was much larger than in HR and HI; but in years with small average growth (e.g., 2009), these differences were smaller. The smallest average annual increment in all plots occurred during 2005 and 2009. In 2005, annual stem contraction exceeded annual stem increment in HI (Figs. 5, 6).

Effect of climatic parameters and soil moisture

Table 5 shows the climatic parameters that explain the plot × year × month interaction. The results indicate that high precipitation and relative humidity were correlated to higher stem increment and that high air and soil temperature and high solar radiation were correlated with lower increment. At the year × month interaction level (Table 5; Fig. 4), the same results occurred, but soil moisture and relative extractable water at every soil layer were also significant. This indicates a positive association of soil moisture and stem increment. ET_0 was also significant at this level, and higher values correlated with lower increment. The climatic and soil parameters that explained the plot × month interaction (Table 5; Fig. 3) were similar to those that explained the year × month interaction, but ET_0 and the temperature (mean, minimum, mean of the maximum, and soil temperature) had an effect opposite to that of the year × month interaction, in that higher ET_0 and temperature were correlated with greater stem increment. Fig. 7 shows that the response of stem increment to precipitation was positive and rapid, except during December and February.

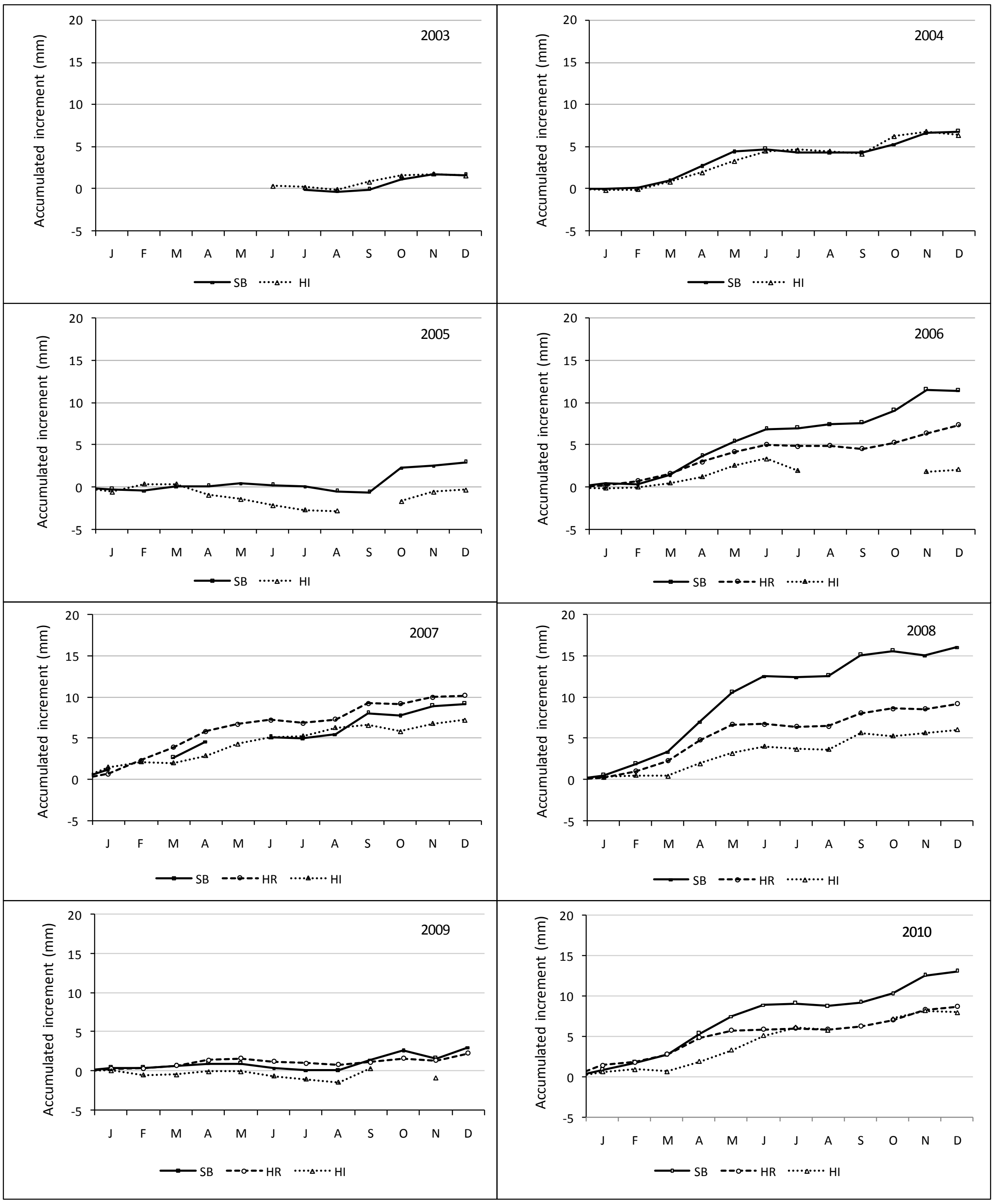

Fig. 5 Least squares means of accumulated monthly change in girth (mm) of holm oak trees in each plot during each year of the study period

Level	Parameter	*F* value	*Pr* > *F*	Coefficient
Plot x year x month	P	59.51	<0.0001	0.1329
	Tsm	57.05	<0.0001	-4.0409
	RHmmax	38.07	<0.0001	1.1180
	Tmmin	36.78	<0.0001	-4.5827
	Tm	36.24	<0.0001	-5.3178
	Rm	13.35	0.0003	-1.1286
	Tmax	11.79	0.0006	-1.7911
	Tmmax	11.27	0.0008	-2.6691
Year x month	RHmmin	410.48	<0.0001	0.9424
	RHm	319.01	<0.0001	0.9947
	P	314.60	<0.0001	0.0976
	Tmmax	262.70	<0.0001	-3.2221
	Rm	260.55	<0.0001	-3.5810
	RHmmax	251.45	<0.0001	1.5560
	REWTm	237.41	<0.0001	38.6659
	SM1m	200.92	<0.0001	0.4733
	SMTm	199.42	<0.0001	0.2158
	REW1m	198.89	<0.0001	31.6205
	ET_0	169.37	<0.0001	-12.8304
	Tmax	158.4	<0.0001	-1.8464
	REW2m	143.45	<0.0001	33.5260
	Tm	105.08	<0.0001	-2.5767
	SM2m	103.42	<0.0001	0.2660
	Tmin	91.85	<0.0001	-1.5855
	Tsm	24.87	<0.0001	-0.8929
Plot x month	RHmmin	35.36	<0.0001	0.3463
	REW1m	32.99	<0.0001	23.8818
	REW2m	22.55	<0.0001	24.2224
	RHm	20.36	<0.0001	0.4844
	Tm	19.66	<0.0001	6.4210
	RHmmax	18.74	<0.0001	1.4202
	SM2m	17.83	<0.0001	0.2706
	REWTm	15.98	<0.0001	20.1011
	Tmin	11.86	0.0006	1.3414
	ET_0	10.45	0.0012	5.8344
	Tsm	8.65	0.0033	0.5022
	SMTm	8.55	0.0035	0.1095
	SM1m	5.91	0.0151	0.2874
	P	5.29	0.0215	0.0661
	Tmmax	5.04	0.0248	1.4479

Table 5 Climatic covariates that are significant at the plot × year × month, year x month and plot x month level. P: precipitation; Tsm: mean soil temperature; RHmmax: mean of the daily maximum relative humidity; Tmmin: mean of the minimum daily temperature; Tm: mean temperature; Rm: mean solar radiation; Tmax: maximum temperature; Tmmax: mean of the maximum daily temperature; RHmmin: mean of the minimum relative humidity; RHm: mean relative humidity; REWTm: mean relative extractable water at 5-45 cm; SM1m: mean soil moisture at 5-25 cm; SMTm: mean soil moisture at 5-45 cm; REW1m: mean relative extractable water at 5-25 cm; ET_0: reference evapotranspiration; REW2m: mean relative extractable water at 25-45 cm; SM2m: mean soil moisture at 25-45 cm; Tmin: minimum temperature

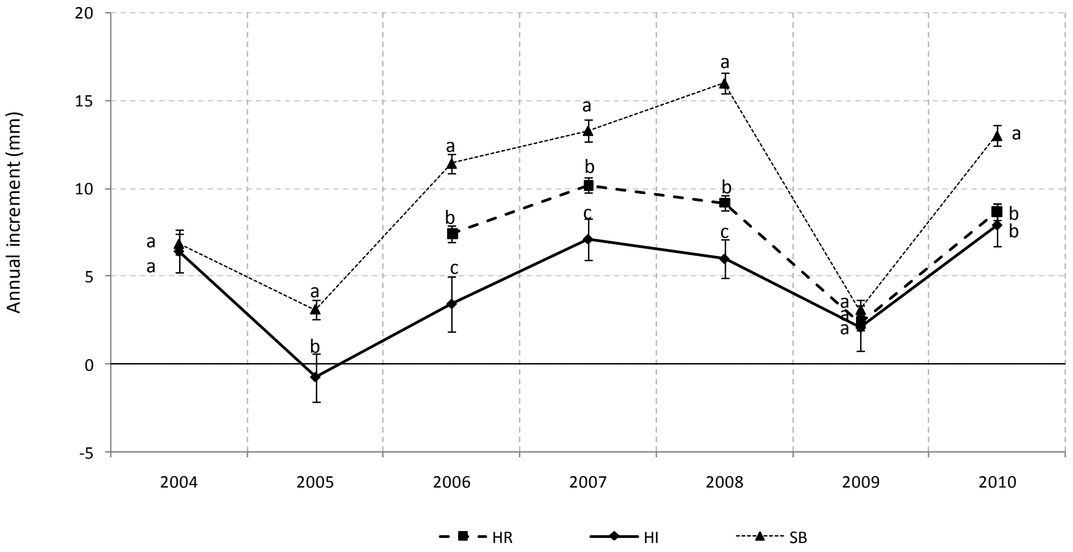

Fig. 6 Least squares means of annual girth increment rate (mm year^{-1} ± standard error) of holm oak trees from 2004-2010 in the three different plots. Different letters indicate significant differences between plots in a year ($p < 0.05$)

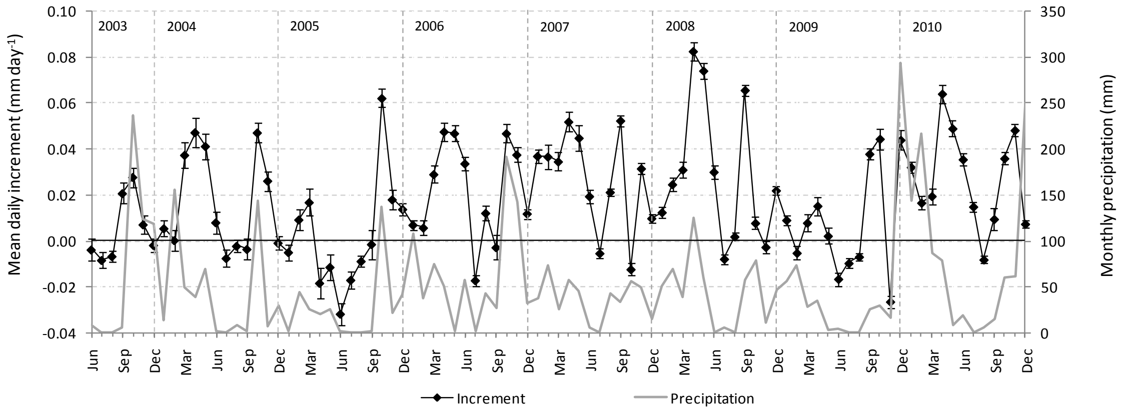

Fig. 7 Least squares means of daily girth increment rate (mm day^{-1} ± standard error) of holm oak trees and monthly precipitation (mm) during the study period

Effect of initial diameter, hydrological parameters and competition

Initial tree diameter was a significant covariate ($p = 0.0038$, coefficient = 0.056), and explained 12% of the variance at the tree level. This indicates that thicker trees have greater stem increment. None of the hydrological parameters were significant (FL: $p = 0.54$, coefficient = -0.019, SCA: $p = 0.11$, coefficient = -0.004, WI: $p = 0.989$, coefficient = -0.0038).

Five of the 725 competition indexes used in our model were significant, with each accounting for 12-25% of the total variance at the tree level (Table 6). The APA index, with weight factor k = 4 (the highest value that we tested), was the most significant. An APA index with k = 0 was also significant, but model convergence was not possible with k = 1, 2, or 3. The positive values of the APA coefficients show that as the potentially available area is larger, tree stem increment is greater. Three Distance-weighted ratio (DR) indexes were also significant and they use the basal area factor (BAF) as the criterion to select competitors. We tested four values of BAF (1, 2, 3 and 4 m^2 ha^{-1}) and the only significant indexes occurred with BAF = 1, that is the lowest we tested. A lower BAF is associated with a selection of more competitors for a tree, suggesting large competition zones in our plots. The negative coefficients of all three DR indexes indicate that higher competition is associated with less stem increment.

Competition index	*k*	BAF	Distance function	Laterality	*F* value	*Pr* > *F*	Coefficient
Area potentially available	4	n.a.	n.a.	n.a.	8.53	0.004	0.0099
Area potentially available	0	n.a.	n.a.	n.a.	5.22	0.022	0.021
Distance weighted ratio	1	1	$1/dist_{ij}$	Bilateral	4.29	0.038	-65.513
Distance weighted ratio	1	1	$1/dist_{ij}$	Unilateral	4.29	0.039	-46.758
Distance weighted ratio	2	1	$1/dist_{ij}$	Unilateral	4.23	0.040	-29.877

Table 6 Significant competition indexes from the final model. *k*: weighting factor; BAF: Basal area factor (m^2 ha^{-1}); n.a.: not applicable. Unilateral indexes do not consider trees smaller in diameter at breast height as competitors for a subject tree

Discussion

Patterns of stem girth increment

In this study, we observed that *Q. ilex* had a bimodal stem increment pattern with maximum stem increment rates during the spring and a lesser peak during autumn, as also reported in other studies (Campelo et al. 2007; Gutiérrez et al. 2011). Thus, the maximum vegetative activity is synchronized with the most favorable conditions for growth, when water is available and temperatures are moderate (Pinto et al. 2011). However, in years with a dry spring (e.g., 2005, 2009), this pattern changed and the second period accounted for most of the total annual increment. This finding does not agree with former studies

(Campelo et al. 2007; Gutiérrez et al. 2011) probably because our study sites were in drier and warmer locations.

The stem increment of holm oak begins to increase at the end of the winter and increases more significantly during the spring, in parallel with the significant increase in photosynthetic activity (Corcuera et al. 2005) and the formation of large xylem vessels (Campelo et al. 2010). The spring stem increment rates in our plots were higher than those reported by other authors for this species (e.g., Campelo et al. 2007; Gutiérrez et al. 2011). As summer comes, the stem increment rates decreases because of water stress, and xylem vessels become narrower (Corcuera et al. 2004; Nijland et al. 2011) and there is apparently a cessation of vegetative growth. As suggested by Cherubini et al. (2003), this should be considered as a "resting period" due to water stress rather than actual vegetative inactivity. Nevertheless, when stem contractions by water stress occur, we cannot determine with band dendrometers whether cambial activity stopped completely. Our results indicate that the duration of this summer rest period had a strong inter-annual variability and was strongly dependent on the length of the drought. This period can start as early as the beginning of spring and can last until the end of the summer, as occurred in 2005 and 2009. In 2005, only February and March accounted for the first stem increment period and stem contraction lasted until October because scarce precipitation in spring and summer.

At the end of summer and matching with the first rains, the water status of *Q. ilex* improves, as reported by Corcuera et al. (2004). Rapid stem increments detected were probably because stem hydration. However, after that phenomenon, the stems diameter was stabilized and increments occurred throughout the autumn, with a maximum accumulated stem increments at the end of each year higher than the previous spring maximum. It suggests that, despite water stem expansion occurred, there were a true second growth period, also reported by Campelo et al. (2007) and Gutiérrez et al. (2011). The results of these authors and Cherubini et al. (2003), who studied *Q. ilex* trees in central Italy, suggest that there is a relationship between this second growth period and the appearance of intra-annual density fluctuations in the wood and/or the existence of double rings, especially when there is a severe summer drought followed by high precipitation events at the end of summer and the start of autumn. Similar results for *Arbutus unedo* and *Erica arborea* in other Mediterranean regions have been reported by Battipaglia et al. (2010, 2014).

The stem increment rates slowed down at the end of autumn and the start of winter, with low values from December to February. Nevertheless, there was not a complete cessation of growth in the winters of 2006, 2007, 2008, and 2010. These results do not agree with other works of holm oak in colder and more continental areas (e.g., Gutiérrez et al. 2011; Nijland et al. 2011).

Effect of climate and soil moisture on stem increment

Stem increment of holm oak is strongly influenced by precipitation, especially in the spring. The amount of precipitation was significant in the three levels of the model in which this variable was tested, especially in the year × month interaction. Similar results have been reported in other Mediterranean regions in the south of France (Nijland et al. 2011), central Italy (Cherubini et al. 2003), and the Iberian Peninsula (Campelo et al. 2007; Gea-Izquierdo et al. 2011). Hence, an increase in the frequency and intensity of a spring drought could have a significant negative impact on holm oak, as suggested by Rodríguez-Calcerrada et al. (2011). We also found a significant and positive effect of soil moisture on stem girth increment indicating the importance of water availability. Soil moisture variables did not explain the plot × year × month interaction; however, this interaction accounted for more variability in autumn and winter months, when water availability was not limiting for growth.

Holm oak has a high capacity for physiological recovery following summer drought, and the stem increment rate increased significantly in subsequent precipitation events. Corcuera et al. (2004) indicated that this species can maintain a relatively high hydraulic conductivity with very low xylem water potential (50% of hydraulic conductivity at -5.6 MPa). Holm oak xylem contains a combination of large conductive vessels, which efficiently transport water but are vulnerable to cavitation, and small vessels, which are less efficient in water transport but less vulnerable to cavitation (Davis et al. 1999). Nevertheless, water stress can still lead to the loss of cambial cell turgor and low cellular division rates (Wimmer et al. 2000). The loss of cambial cell turgor may be an important cause of the stem contraction that we observed during certain dry periods. After the summer drought, precipitation seems to be the main factor responsible for stem increment, as suggested by Campelo et al. (2007). Nevertheless, the influence of precipitation in this period apparently does not occur in colder and more continental areas (e.g., Zhang and Romane 1991; Nijland et al. 2011). We also found a significant and positive effect of relative humidity on stem increment. This effect may be due to a positive correlation of relative humidity and precipitation, or because leaf stomata close during drought to avoid water loss, leading to a reduction of photosynthesis and growth.

The mean daily temperature, maximum daily temperature, mean of the maximum daily temperature, and soil temperature, all had negative effects on stem increment at the plot × year × month and year × month levels. This could be explained because there was a stronger negative effect of high temperature during dry springs and summers than positive effect during autumn and winter. Campelo et al. (2009) reported that low precipitation associated with higher temperatures in May, increased evapotranspiration, and the formation of narrow rings. Campelo et al. (2007) and Gutiérrez et al. (2011) reported similar results regarding the influence of high summer temperatures on growth of holm oak in the Iberian Peninsula. Nevertheless, the minimum daily temperature and the mean of the minimum temperature also had a negative effect at year × month and plot × year × month levels respectively. This could be because temperatures were never very low in our study area, with the exception of occasional events during the winter (Fig. 2; Table 2). Cavender-Bares et al. (2005) reported that *Q. ilex* can maintain most of its hydraulic conductivity at a temperature of -5ºC. We recorded temperatures below -5ºC only on a few days in the HI plot, so our trees were probably not affected by embolisms. The maintenance of photosynthetic and vascular function during the winter gives evergreens as holm oaks a competitive advantage over deciduous ones (Cavender-Bares et al. 2005).

The reference evapotranspiration (ET_0) negatively influenced stem increment at the year × month level. This negative relationship can be considered a combined effect of temperature and radiation, because the highest values of both occur in the middle of the summer, when water availability limits growth. The negative effect of solar radiation on stem increment may be explained by the induction of phototoxic reactions due to excessive radiation. However, we believe a more plausible explanation is that high radiation leads to increased evapotranspiration. On the contrary, ET_0 and the temperature had positive effects on stem increment at the plot × month level. This may be because the plot × month level interactions account for more variability during March, when there is a higher temperature (and hence higher ET_0) and readily available water.

Effect of initial diameter, hydrological parameters and competition

We were not able to attribute the significant stem increment differences of trees to spatial processes or hydrological parameters, suggesting that all locations in our plots were about equally favorable for growth. This could be due to the flat or smooth undulated topography of our plots. Holm oak has a well-developed root system, with a main penetrating root and several secondary and extending roots (Ruiz de la Torre 2006), getting deeper soil layers when growing with competition (Rolo and Moreno 2012). This could allow trees to take water from the surface and at different depths, hence minimizing the effect of topography. No previous studies of *Q. ilex* evaluated this hypothesis. However, Miller et al. (2001) reported that topography modulated the effect of precipitation on growth in *Prosopis glandulosa* in a subtropical savanna-type forest in Texas, with a higher growth at lower elevations.

Trees with larger potentially available area (i.e., more access to resources such as water, nutrients, and light) had higher stem increment rates. In addition, the selected index in the category of distance-weighted ratio indexes indicated that there was a large radius of competition for each tree. This suggests that the severe water limitation in these ecosystems leads to competition among trees mainly at the root level. On the other hand, our results indicated a significant and positive influence of initial stem diameter on stem increment. This may be because larger trees have more developed root systems, so are better able to extract water from deeper soil layers. If so, this would give larger trees an extended growth period during the end of spring and beginning of summer. The positive influence of stem size on growth of *Q. ilex* has also been reported by Cartan-Son et al. (1992). As reported by Rivest et al. (2011), trees in forests with limiting climatic and soil conditions, such as the open woodlands of the Mediterranean, frequently compete for water and nutrients at the intra- and inter-specific levels. Trees in the HI plot had lower stem increment rates than those in the other two plots, and this may be due to higher competition for resources in HI. According to Pulido and Díaz (2005), in water-limited ecosystems, the suppression of a proportion of the trees due to limited water availability means that the standing trees have a larger soil volume available per tree, leading to improved productivity and health and increased growth. This has also been reported for *Q. ilex* (Rodríguez-Calcerrada et al. 2011). Nevertheless, most of the indexes that we tested were not significant, so the validity of these results should be evaluated in further studies.

We explained 12% of the stem increment variability among trees by the stem diameter and 12% to 25% by competition. However, *Q. ilex* forests have high genetic variability, so there could also be physiological adaptations at the tree level that modify the response of cambial activity to climate (Baas 1976).

Conclusions

Q. ilex showed two periods of intra-annual stem increment, typically with a higher peak in spring and a lesser peak in autumn. However in years with a dry spring this pattern changed, with autumn stem increment being higher than spring increment. In some years, stem increment did not completely stop in winter. Stem contractions always occurred in summer because water stress, but we cannot determine whether cambial activity stopped entirely. Stem increment varied significantly among plots and among trees within each plot.

Climate strongly drives the stem increment pattern within and among years, with water availability acting as the main climatic factor. This species appears to respond to the summer drought by simply decreasing its vegetative activity. The effect of temperature was mostly related to the reduced water availability due to evapotranspiration rather than reduced vegetative activity during the winter. Therefore, the forecasted climatic change, in which decreased rainfall in spring and increased summer drought are expected in the Mediterranean region, may be a significant threat to the forests and open woodlands of *Quercus ilex*. The intra-annual stem increment model developed in this study might be used to build large-scale ecological models or geographical mapping of vulnerable areas under different climate change scenarios.

Tree size and competition can modulate the growth response to climate. These findings might help to design silvicultural management practices to mitigate some of those climate effects by reducing competition (i.e., selective harvesting), especially in dense forests.

Acknowledgments

This work was supported by the Department of Innovation, Science and Business of the Regional Government of Andalusia, Spain [C03-192]; and the Ministerio de

Educación y Ciencia – Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Spain [SUM2006-00026-00-00]. We thank Rocío Macías, Enrique Andivia and Felipe Carevic for their help with the fieldwork.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Akaike H (1974) A new look at the statistical model identification. IEEE Trans Autom Control 19:716–723

Allen RG, Pereira LS, Raes D, Smith M (1998) Crop Evapotranspiration: Guidelines for Computing Crop Water Requirements, FAO Irrigation and Drainage Paper 56. FAO, Rome

Baas P (1976) Some functional and adaptative aspects of vessel members morphology. In: Baas P, Bolton AJ, Catling DM (eds) Wood structure in biological and technological research. Leiden Bot Series 3, The Hague, pp 157–181

Barling RD, Moore ID, Grayson RB (1994) A quasi-dynamic wetness index for characterising the spatial distribution of zones of surface saturation and soil water content. Water Resour Res 30:1029–1044

Battipaglia G, De Micco V, Brand WA, Linke P, Aronne G, Saurer M, Cherubini P (2010) Variations of vessel diameter and d13C in false rings of *Arbutus unedo* L. reflect different environmental conditions. New Phytol 188:1099–1112

Battipaglia G, De Micco V, Brand WA, Sauren M, Aronne G, Linke P, Cherubini P (2014) Drought impact on water use efficiency and intra-annual density fluctuations in *Erica arborea* on Elba (Italy). Plant Cell Environ 37:382–391

Bravo JA, Roig S, Serrada R (2008) Selvicultura en montes bajos y medios de *Quercus ilex* L., *Quercus pyrenaica* Willd. y *Quercus faginea* Lam. In: Serrada R, Montero G, Reque JA (eds) Compendio de Selvicultura aplicada en España. INIA, Madrid, pp 657–744

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

Campelo F, Nabais C, García-González I, Cherubini P, Gutiérrez E, Freitas H (2009) Dendrochronology of *Quercus ilex* L. and its potential use for climate reconstruction in the Mediterranean region. Can J For Res 39:2486–2493

Campelo F, Nabais C, Gutiérrez E, Freitas H, García-González I (2010) Vessel features of *Quercus ilex* L. growing under Mediterranean climate have a better climatic signal than tree-ring width. Trees 24:463–470

Carrete M, Doñázar JA (2005) Application of central-place foraging theory shows the importance of Mediterranean dehesas for the conservation of the cinereous vulture, *Aegypius monachus*. Biol Conserv 126:582–590

Cartan-Son M, Floret C, Galan MJ, Grandjanny M, Le Floc'h E, Maistre M, Perret P, Romane F (1992) Factors affecting radial growth of *Quercus ilex* L. in a coppice stand in southern France. Vegetatio 99–100:61–68

Cavender-Bares J, Cortes P, Rambal S, Joffre R, Miles B, Rocheteau A (2005) Summer and winter sensitivity of leaves and xylem to minimum freezing temperatures: a comparison of co-occurring Mediterranean oaks that differ in leaf lifespan. New Phytol 168:597–612

Cherubini P, Gartner BL, Tognetti R, Bräker OU, Schoch W, Innes JL (2003) Identification, measurement and interpretation of tree rings in woody species from mediterranean climates. Biol Rev 78:119–148

Cobos DR (2010) Calibrating ECH2O soil moisture sensors. http://www.decagon.com/assets/Uploads/13393-04-calibratingECH2OSoilMoistureProbes.pdf. Accessed 26 October 2012

Corcuera L, Camarero JJ, Gil-Pelegrín E (2004) Effects of a severe drought on *Quercus ilex* radial growth and xylem anatomy. Trees 18:83–92

Corcuera L, Morales F, Abadía A, Gil-Pelegrín E (2005) Seasonal changes in photosynthesis and photoprotection in a *Quercus ilex* subsp. *ballota* woodland located in its upper altitudinal extreme in the Iberian Peninsula. Tree Physiol 25:599–608

Davis SD, Sperry JS, Hacke UG (1999) The relationship between xylem conduit diameter and cavitation caused by freezing. Am J Bot 86:1367–1372

Gea-Izquierdo G, Martín-Benito D, Cherubini P, Cañellas I (2009) Climate-growth variability in *Quercus ilex* L. west Iberian open woodlands of different stand density. Ann Forest Sci 66:802

Gea-Izquierdo G, Cherubini P, Cañellas I (2011) Tree-rings reflect the impact of climate change on *Quercus ilex* L. along a temperature gradient in Spain over the last 100 years. Forest Ecol Manage 262:1807–1816

Granier A (1987) Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. Tree Physiol 3:309–320

Gutiérrez E, Campelo F, Camarero JJ, Ribas M, Muntán E, Nabais C, Freitas H (2011) Climate controls act at different scales on the seasonal pattern of *Quercus ilex* L. stem radial increments in NE Spain. Trees 25:637–646

Intergovernmental Panel on Climate Change IPCC (2007) Climate Change 2007: Synthesis Report. Cambridge University Press, Cambridge

IUSS Working Group WRB (2007) World Reference Base for Soil Resources 2006, first update 2007. World Soil Resources Reports No. 103. FAO, Rome

Junta de Andalucía (2005) Modelo Digital del terreno de Andalucía. Relieve y orografía. Junta de Andalucía, Sevilla

Keeland BD, Young PJ (2014) Installation of traditional dendrometer bands. U.S. Geological Survey. National Wetlands Research Center. http://www.nwrc.usgs.gov/topics/Dendrometer/. Accessed 23 January 2014

Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O (2006) SAS system for mixed models. SAS Institute, Cary

Lumaret R, Mir C, Michaud H, Raynal V (2002) Phytogeographical variation of chloroplast DNA in holm oak (*Quercus ilex* L.). Mol Ecol 11:2327–2336

Miller D, Archer SR, Zitzer SF, Longnecker MT (2001) Annual rainfall, topoedaphic heterogeneity and growth of an arid land tree (*Prosopis glandulosa*). J Arid Environ 48:23–33

Moreno G, Cubera E (2008) Impact of stand density on water status and leaf gas exchange in *Quercus ilex*. Forest Ecol Manage 254:74–84

Mund M, Kutsch WL, Wirth C, Kahl T, Knohl A, Skomarkova MV, Schulze ED (2010) The influence of climate and fructification on the inter-annual variability of stem growth and net primary productivity in an old-growth, mixed beech forest. Tree Physiol 30:689–704

Nijland W, Jansma E, Addink EA, Domínguez-Delmás M, De Jong SM (2011) Relating ring width of Mediterranean evergreen species to seasonal and annual variations of precipitation and temperature. Biogeosciences 8:1141–1152

Patterson HD, Thompson R (1971) Recovery of inter-block information when block sizes are unequal. Biometrika 58:545–554

Pinto CA, Henriques MO, Figueiredo JP, David JS, Abreu FG, Pereira JS, Correia I, David TS (2011) Phenology and growth dynamics in Mediterranean evergreen oaks: effects of environmental conditions and water relations. Forest Ecol Manage 262:500–508

Pulido F, Díaz M (2005) Regeneration of a Mediterranean oak: a whole cycle approach. Ecoscience 12:92–102

Rivest D, Rolo V, López-Díaz ML, Moreno G (2011) Belowground competition for nutrients in shrub-encroached Mediterranean dehesas. Nutr Cycl Agroecosys 90:347–354

Rodà F, Retana J, Gracia CA, Bellot J (1999) Ecology of Mediterranean evergreen oak forests. Springer, New York

Rodríguez-Calcerrada J, Pérez-Ramos IM, Ourcival JM, Limousin JM, Joffre R, Rambal S (2011) Is selective thinning an adequate practice for adapting *Quercus ilex* coppices to climate change? Ann Forest Sci 68:575–585

Rolo V, Moreno G (2012) Interspecific competition induces asymmetrical rooting profile adjustments in shrub-encroached open oak woodlands. Trees 26:997–1006

Ruiz de la Torre J (2006) Flora Mayor. Organismo Autónomo de Parques Nacionales, Madrid

San Miguel A (1994) La dehesa española. Origen, tipología, características y gestión. Fundación Conde del Valle de Salazar, Escuela Técnica Superior de Ingenieros de Montes, Madrid

Searle SR (1971) Linear Models. John Wiley & Sons, New York

Stevenson AC, Harrison RJ (1992) Ancient forests in Spain: a model for land-use and dry forest management in south-west Spain from 4000 BC to 1900 AD. Prehist Soc 58:227–247

Vázquez-Piqué J, Lago J, González A (2001) INCO: Programa para el cálculo de índices de competencia de especies forestales. In: Junta de Andalucía (ed) Proceedings of the III Congreso Forestal Español. Sevilla, pp 517–524

Vázquez-Piqué J, Pereira H (2004) Modelos de crecimiento en diámetro para alcornocales del centro y sur de Portugal. Cuad Soc Esp Cienc For 18:219–226

Wimmer R, Strumia G, Holawe F (2000) Use of false rings in Austrian pine to reconstruct early growing season precipitation. Can J For Res 30:1691–1697

Zhang SH, Romane F (1991) Variations de la croissance radiale de *Quercus ilex* L. en fonction du climat. Ann Forest Sci 48:225–234

Artículo 2. Seven year study of the effect of climate on daily radial stem variations of holm oaks in an open woodland forest in Southwestern Spain

Artículo enviado para su publicación como: Martín D, Vázquez-Piqué J, Alejano R. Seven year study of the effect of climate on daily radial stem variations of holm oaks in an open woodland forest in Southwestern Spain. New Phytol.

Seven year study of the effect of climate on daily radial stem variations of holm oaks in an open woodland forest in Southwestern Spain

Abstract

Holm oak (*Quercus ilex* ssp. *ballota* (Desf.) Samp.) is the most widespread tree in the Iberian peninsula, and is an ecologically important species in the open woodland forests of this region. We used high-resolution automatic point dendrometers to assess the daily changes in stem radius and the effects of climate on intra-annual growth patterns in nine holm oak trees in an open woodland forest in SW Spain over seven years. Our results showed that *Q. ilex* has characteristic daily cycles of stem swelling and shrinkage that are closely associated with changes in ecophysiological processes. There were two main intra-annual growth phases: during late-winter and spring and during the late-summer and autumn. An interval of low growth rates or cessation occurred between each of these growth phases. Reference evapotranspiration negatively affected stem increase during all phases. Precipitation had positive effects during the two main growth phases, and solar radiation had negative effects during these phases. High temperature had negative effects except in the phase with the coldest months. High relative humidity had positive effects during the same phases. Greater daily variations of relative extractable water had positive effects except during late spring and summer.

Keywords: dendrometers, *dehesa*, radial stem variation, climatic factors, soil moisture, weather-growth relations

Introduction

Measurements of tree growth provide important information about overall tree health and the vigor of forests (Nishimua et al. 2007; Martínez-Pastur et al. 2007), phenological processes (Pinto et al. 2011), effects of climate (Gea-Izquierdo et al. 2011; Martín et al. 2014; Jiang et al. 2015), physiology and water status (Herzog et al. 1995; Steppe et al. 2006; Zweifel et al. 2010), competition (Gea-Izquierdo et al. 2011; Rodríguez-Calcerrada et al. 2011; Martín et al. 2014), resource allocation (Mund et al. 2010; Martín et al. 2015a), and the influence of management practices (Cartan-Son 1992; Gyenge et al. 2010; Martín et al. 2015b).

The patterns of daily radial stem growth provide insight into growth process and the responses of trees to short-term changes in environmental conditions such as temperature, soil water content, and precipitation (Deslauriers et al. 2007). Variations in daily stem radius are closely related to water flow through the stem (Zweifel et al. 2000; Steppe et al. 2006). Stem shrinkage usually occurs during daytime when transpiration exceeds the capacity of roots to take up water, leading to a reversible dehydration and contraction of elastic tissues in the stem, mainly phloem, cambium, and parenchyma in the bark (Tatarinov and Čermák 1999; Zweifel et al. 2000). Overnight, when transpiration and sap flow decrease, the stem swells because of tissue rehydration (Kozlowski and Winget 1964; Herzog et al. 1995; Zweifel et al. 2001). Variations in stem diameter also depend on growth processes (Steppe et al. 2006). Growth is a result of cell division and irreversible enlargement of the cambium and the zone of differentiation of xylem (Steppe et al. 2006; Drew et al. 2009). According to Deslauriers et al. (2003), cell enlargement is the major driving force of daily stem growth.

Dendrometers provide continuous and non-destructive measurements of changes in stem radius (Zweifel et al. 2000). Dendrometers and dendrographs were first developed in the late 19th century (Böhmerle 1883; Friedrich 1890), and improved throughout the 20th century (Friedrich 1905; MacDougal 1918; Fritts and Fritts 1955). Since the 1970s, researchers have used electronic dendrometers with dataloggers to continuously measure stem radial variations (Breitsprecher and Hughes 1975). Electronic dendrometers, in combination with in situ automatic weather stations and other physiological and environmental measuring devices, are powerful tools for assessment of the influence of climate on tree growth. These methods are currently used in many species in diverse geographic regions (Drew et al. 2009; Oberhuber et al. 2014; Jiang et al. 2015), and have provided valuable insights about the effects of climate change on ecosystems (IPCC 2007).

Quercus ilex L. (holm oak) is a widespread evergreen species in the Mediterranean Basin that covers more than 6.5 million ha (Quézel and Médail 2003). It is one of the dominant species in *dehesas*, traditional agroforestry systems composed of open woodland forests (10–60 trees ha^{-1}) with herbaceous layers (Cubera and Moreno 2007) that have great ecological, social, and economical importance. These unique open woodland forests in Southwestern Spain are highly diverse ecosystems, and are refuges for many endangered species, including the Spanish imperial eagle (*Aquila adalberti*), the cinereous vulture (*Aegypius monachus*), and the Iberian lynx (*Lynx pardinus*) (Carrete and Doñázar 2005).

Previous studies that used electronic dendrometers to measure daily changes in the radial stem width of trees usually ranged from several weeks to 1-2 years in duration (Downes et al. 1999; Zweifel et al. 2000; Wang et al. 2015), and typically focused on the main growth phase (Tardif et al. 2001; Köcher et al. 2012; Oberhuber et al. 2014). However, the large intra-annual and inter-annual growth variability of *Q. ilex* (Gutiérrez et al. 2011; Martín et al. 2014), means that it is necessary to perform high-resolution studies over several complete years to completely understand its growth patterns and identify climatic factors that drive changes in growth.

We present a study on the daily radial stem variations of *Quercus ilex* ssp. *ballota* (Desf.) Samp. that was conducted over seven years in an open woodland forest plot in the SW Iberian Peninsula. This study addresses three main questions. First, what is the pattern of daily radial stem variations in *Q. ilex?* Second, when is the onset and cessation of stem growth during the year? Third, what climatic factors drive changes in daily stem growth and influence the patters of intra-annual growth?

Materials and Methods

Field plot

This study was conducted in an experimental plot in the Huelva province, SW Spain (UTM X: 644288 m; Y: 4161376 m; Zone 29). This plot was in an open woodland forest of holm oak where sheep and Iberian pigs are raised. The area of the study plot was 2.94 ha, the tree density was 73 trees ha^{-1}, the mean stem diameter was 30.02 ± 7.68 cm, and the mean tree height was 6.58 ± 1.58 m. Measurements of tree ring data of 12 trees indicated an average age of 110 ± 31 years (Natalini et al. 2013). The soils have different degrees of development from acrisols, alisols and lixisols, to regosols and cambisols (IUSS Working Group WRB 2007). There is a sparse understory of mainly rock-rose (*Cistus ladanifer*) and curly rock-rose (*Cistus crispus*) and an abundant herbaceous layer of mainly grasses. The climate is Mediterranean, with highly variable temperature and precipitation within and among years. The mean annual temperature during the study period (2005-2012) was 17.2ºC and the mean annual precipitation was 572 mm. There were no large monthly variations in temperature among years, but there were large monthly and annual changes in precipitation (Fig 1; Table 1).

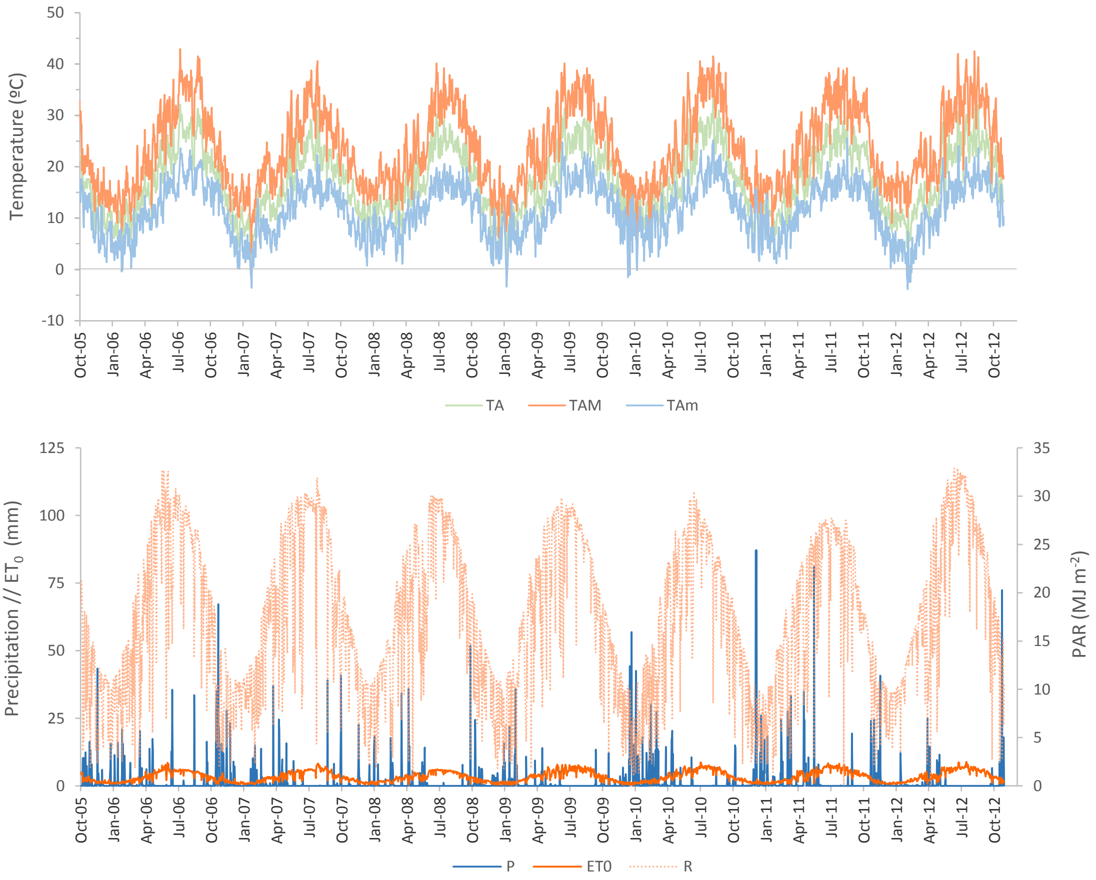

Fig. 1 Main climatic variables of the experimental plot during the study period (October 2006-October 2012). Top: TA: daily mean temperature (ºC); TAM: daily maximum temperature (ºC); TAm: daily minimum temperature (ºC). Bottom: P: daily precipitation (mm); ET$_0$: daily reference evapotranspiration (mm); R: daily PAR (MJ m^{-2})

Year	Precipitation (mm)					Temperature (ºC)		
	Pwi	Psp	Psm	Pat	P	TM	Tm	T
2006	160	123	74	356	713	35.6	4.2	17.4
2007	133	110	60	118	421	33.9	2.8	16.1
2008	147	121	64	126	458	33.4	5.5	16.8
2009	143	38	16	305	502	35.0	5.7	18.0
2010	344	99	14	342	799	36.3	7.1	17.6
2011	236	226	31	158	651	33.2	6.0	17.4
2012	55	91	11	303	460	34.6	2.4	17.0

Table 1 Precipitation and temperature in the experimental plot during the study period (2005-2012). Pwi: winter precipitation; Psp: spring precipitation; Psm: summer precipitation; Pat: autumn precipitation; P: annual precipitation; TM: mean maximum temperature of the hottest month; Tm: mean minimum temperature of the coldest month; T: mean annual temperature

Measurement of radial stem growth

Nine healthy *Q. ilex* trees with well-developed crowns were randomly selected in October 2005. The sampled trees had a mean diameter at breast height of 33.44 ± 4.03 cm and a mean height of 7.55 ± 1.33 m. A high-resolution automatic point dendrometers with a resolution of 2.44 µm (Depfor, University of Huelva, details are available in Vázquez-Piqué et al. 2009) was placed on each tree at breast height, with care taken to avoid stem deformities. Before installation, the outer dead bark was brushed off without wounding the cambial zone to reduce hygroscopic swelling and shrinkage of the bark. The manufacturer did not provide thermal expansion-contraction information, but Tardif et al. (2001) and Deslauriers et al. (2007) reported that the effect of temperature on dendrometers measurements is negligible relative to stem variations. Dataloggers were set up to record stem radius at 30 min intervals (48 records day^{-1}). Time-series of raw data were visually verified, and data resulting from equipment malfunctions were removed.

Downes et al. (1999) reported that trees typically undergo shrinkage from early morning to early afternoon, and swelling during the late afternoon and evening. Each shrinkage and swelling phase was calculated with a routine written in SAS/ETS (ver. 9.2), derived from a routine by Deslauriers et al. (2011). Daily stem growth was defined as the part of the swelling phase from the time the stem radius exceeded the maximum of the previous day (Deslauriers et al. 2007). If the current cycle did not reach the previous maximum (i.e., the difference of consecutive maxima was negative), this indicated daily stem contraction.

Measurement of climatic parameters and soil moisture

An automatic meteorological station (HOBO H21-001, Onset Computer Corporation) was installed in the plot to record temperature and relative humidity (Onset sensor S-THB-M002), precipitation (Onset rain gauge S-RGB-M002), wind speed and direction (Onset sensor S-WCA-M003), and photosynthetically active radiation (PAR, Onset silicon pyranometer S-LIB-M003) every 15 min. Reference evapotranspiration (ET_0) was calculated by the FAO Penman-Monteith method (Allen et al. 1998). For the analysis of soil moisture, 7 moisture sensors (ECH2O-20, Decagon Devices Inc.) were placed at 4 locations near sample trees at depths of 5-25 cm, and at 3 locations (matching the locations above) at depths of 25-45 cm. Data were registered every 30 min, at the same times as stem radius measurement. Calibration curves were performed in the laboratory (8 calibration points per sensor) as described by Cobos (2010) to obtain percent soil moisture from measured voltage; the r^2 values ranged from 0.92 to 0.99. Relative extractable water (REW) (Granier 1987) was calculated between 5-25 cm, 25-45 cm, and 5-45 cm depths as:

$$\mathrm{REW} = \frac{\mathrm{WC}-\mathrm{WC}_{\mathrm{min}}}{\mathrm{WC}_{\mathrm{FC}}-\mathrm{WC}_{\mathrm{min}}} \tag{1}$$

where WC is the water content (mm), $\mathrm{WC}_{\mathrm{min}}$ is the minimum water content (mm) registered during the study period, and $\mathrm{WC}_{\mathrm{FC}}$ is the water content at field capacity (mm), i.e., the water content at 48 h after an intense precipitation event that saturated the soil. Soil temperature at a depth of 10 cm was measured simultaneously in the same 4 locations as soil moisture.

Data analysis

Time-series of daily stem growth were visually assessed as described previously (Kozlowski and Winget 1964; Tardif et al. 2001) to determine the onset and cessation of the 4 phases of stem growth during each year: (I) late winter-spring growth, (II) late spring-summer shrinkage, (III) late summer-autumn rapid rehydration and subsequent growth, and (IV) late autumn-winter low-growth or cessation of growth (Martín et al. 2014). For comparison among years, all growth series were set to zero on the first day of each year. Once the intra-annual growth phases were defined, Pearson´s correlation analysis was performed within each phase to evaluate the effects of climatic and soil moisture variables on daily stem growth (Table 2), as previously performed by Wang et al. (2012) in *Sabina przewalskii* and Jiang et al. (2015) in *Platycladus orientalis*. All statistical analyses were performed with SAS/ETS (ver. 9.2).

Variable	Abbreviation	Unit
Daily precipitation	P	mm
Daily precipitation of previous day	P_{d-1}	mm
Accumulated precipitation of current and previous 4 days	P5	mm
Daily mean soil moisture at 5-25 cm depth	SM1	mm
Daily mean soil moisture at 25-45 cm depth	SM2	mm
Daily mean soil moisture at 5-45 cm depth	SMT	mm
Daily mean relative extractable water at 5-25 cm depth	REW1	%
Daily mean relative extractable water at 25-45 cm depth	REW2	%
Daily mean relative extractable water at 5-45 cm depth	REWT	%
Daily variation of relative extractable water at 5-25 cm depth	REW1P	%
Daily variation of relative extractable water at 25-45 cm depth	REW2P	%
Daily variation of relative extractable water at 5-45 cm depth	REWTP	%
Daily mean soil temperature at 10 cm depth	TS	ºC
Daily maximum soil temperature at 10 cm depth	TSM	ºC
Daily minimum soil temperature at 10 cm depth	TSm	ºC
Daily mean air temperature	TA	ºC
Daily maximum air temperature	TAM	ºC
Daily minimum air temperature	TAm	ºC
Daily reference evapotranspiration	ET_0	mm
Daily solar radiation (PAR)	R	MJ m^{-2}
Daily mean relative humidity	RH	%
Daily maximum relative humidity	RHM	%
Daily minimum relative humidity	RHm	%
Daily mean wind speed	WS	m s^{-1}

Table 2 Climatic variables assessed in this study

Results

Daily cycles and intra-annual patterns of stem growth

The dendrometer data showed daily cycles of stem swelling and shrinkage, as expected. Stem swelling occurred typically from late afternoon to early morning, reaching at that time the daily maximum value of radius. After that, the stems started to shrink until they reached the daily minimum value in late afternoon. During the main intra-annual phases of stem growth (I and III), the maximum radius on each day exceeded the maximum radius of the previous day (Fig. 2A), indicating radial stem growth. On the contrary, during phases of growth cessation or stem shrinkage (II and IV), there were many days when the maximum radius was less than the maximum radius of the previous day, resulting in daily contractions (Fig. 2B). During precipitation events, there was little or no daily stem shrinkage (Fig. 2C).

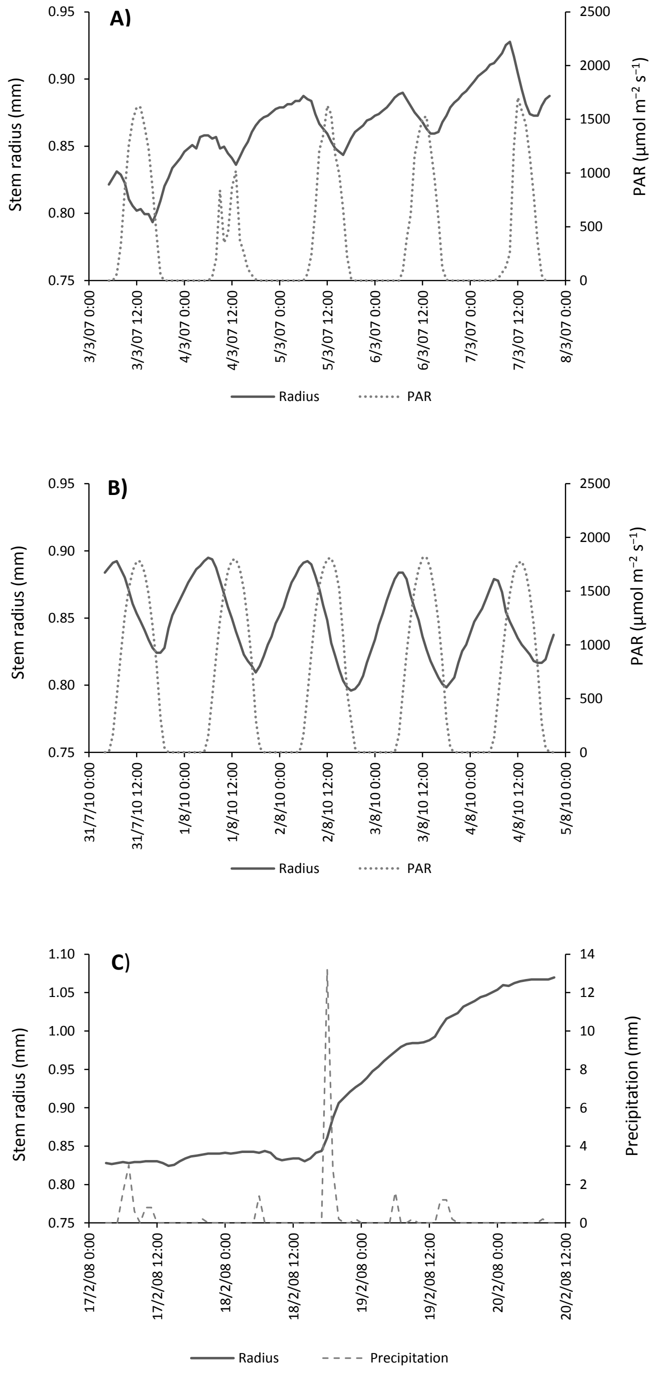

Fig. 2 A): Daily cycles of stem radius variations of a representative tree during a period of net stem growth. B): Daily cycles of stem radius variations of a representative tree during a period of stem contraction. C): Daily cycles of stem radius variations of a representative tree during a period of high precipitation. Radius: change of stem radius (mm/h); PAR: average PAR (µmol m^{-2} s^{-1}) over 1 h; P: hourly precipitation (mm)

The 4 typical intra-annual growth phases of *Q. ilex* were evident during all years, but their onset, duration, and cessation were highly variable (Fig. 3). Daily stem growth during phase I was more regular and steady than those during phase III, although there were several growth peaks during precipitation events. The mean daily stem growth during phase I was 0.0113 ± 0.0335 mm day^{-1}, with peaks up to 0.3748 mm day^{-1}. The duration of phase I, generally from late February to late May or early June, was also longer than phase III. The year 2012 had a very dry and cold winter (Fig. 1; Table 1), and phase I started in early April of that year. Phase II was characterized by progressive daily contractions of the stems followed by a plateau until phase III. Summer precipitation events led to rapid swelling of stems during this phase, but the radii generally returned to their previous values after a few days. The mean daily contraction during this phase was 0.0010 ± 0.0021 mm day^{-1}, with peaks up to 0.1245 mm day^{-1}. This phase occurred generally from late May or early June to late August or September, although it lasted until October during 2006, 2010, 2011 and 2012. Phase III started with a rapid swelling of stems due to precipitation, followed by stabilization over several days. After that, the radii increased until daily growth rates progressively declined as autumn progressed. During this phase, there were events of rapid stem swelling and shrinkage, but the growth rates of most of trees generally had positive trends during all years, especially in 2005, 2006, 2008, and 2010. The mean daily stem growth during this phase was 0.0093 ± 0.0029 mm day^{-1}, with peaks up to 0.3735 mm day^{-1}.The timing of phase III was highly variable; it began from August to October and ended from October to December. Phase IV was characterized by a plateau or slight increases and decreases of stem radii, with the exception of some peaks and a marked decrease during the winters of 2010, 2011, and 2012. The mean daily stem growth during this phase was 0.0013 ± 0.0039 mm day^{-1}, with peaks up to 0.2173 mm day^{-1}. This phase occurred generally from December to February, but in 2008 it started at the end of October; and in 2010 and 2012 it ended in March.

The changes in growth of all trees generally had the same trends and were quite synchronized, although the nine trees differed in absolute growth. However, some trees exhibited unique patterns in some phases of certain years (e.g., E8 in December 2006 and E7 from February to May 2009).

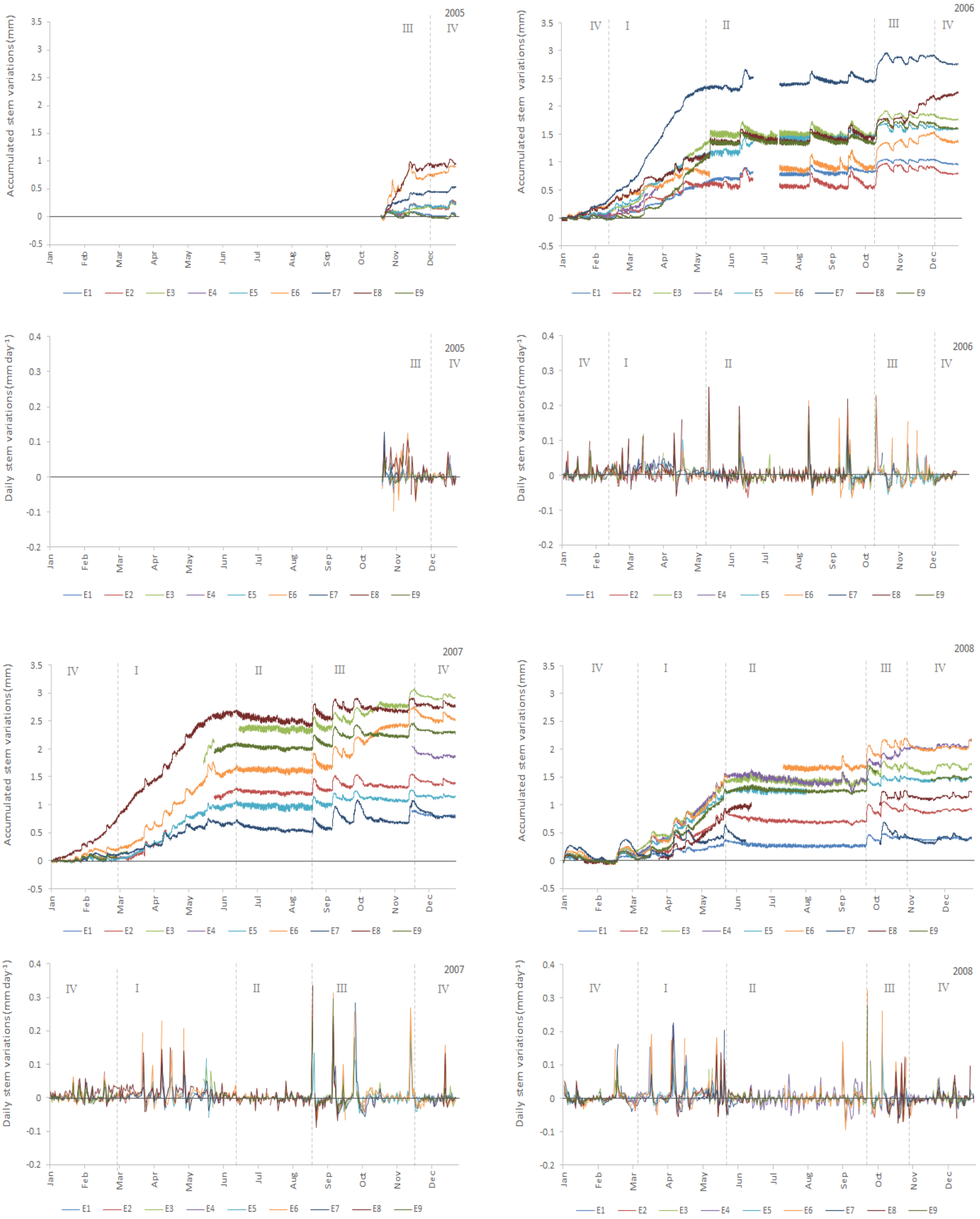

Fig. 3 Accumulated stem radius variations (mm) and daily stem radius variations (mm) of the 9 sample trees (E1-E9) in each year of the study period. Vertical bars and Roman numerals denote each intra-annual growth phase (years 2005-2008)

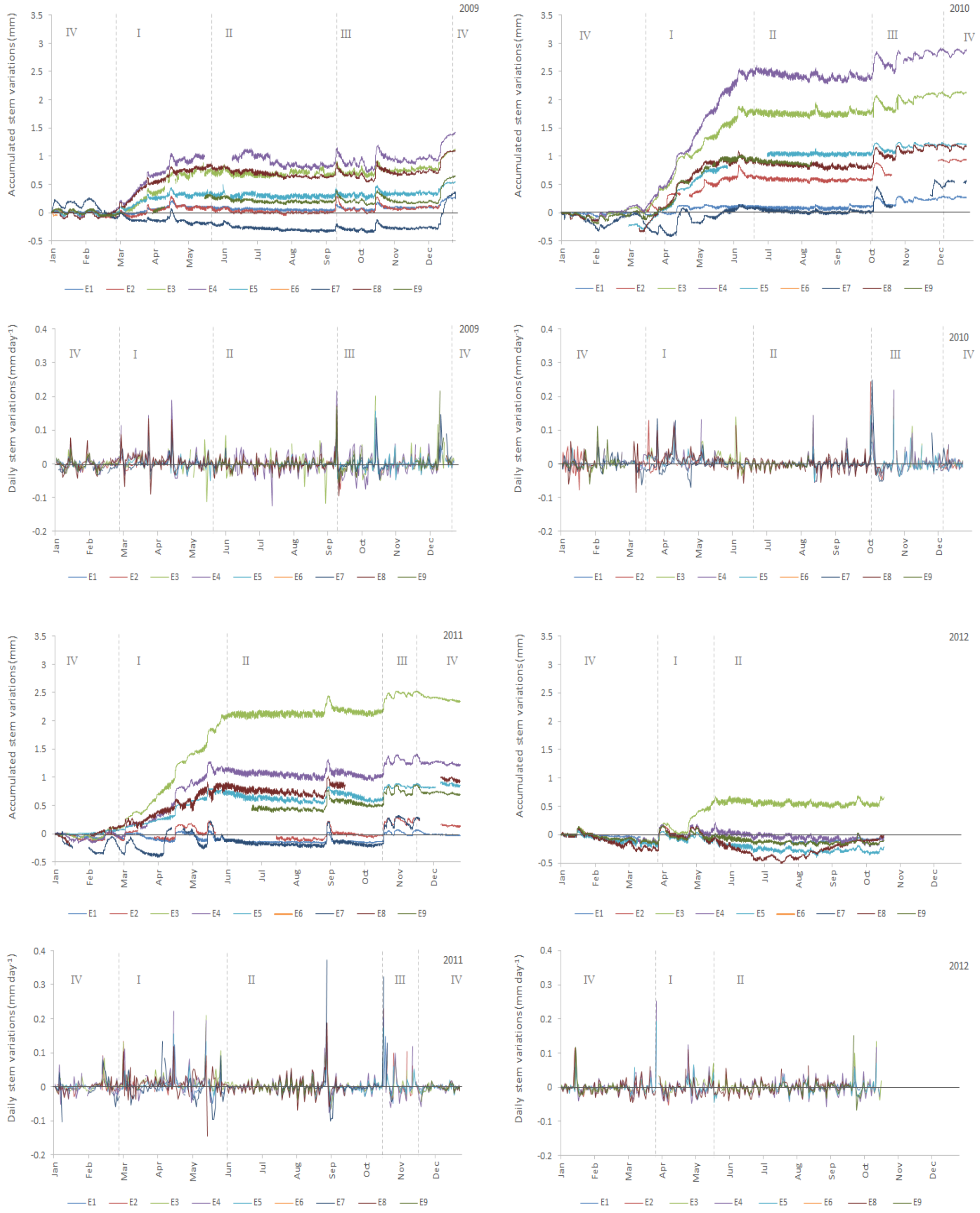

Fig. 3 (cont.) Accumulated stem radius variations (mm) and daily stem radius variations (mm) of the 9 sample trees (E1-E9) in each year of the study period. Vertical bars and Roman numerals denote each intra-annual growth phase (years 2009-2012)

Effect of climate and soil moisture on daily stem growth

Most climatic variables had significant influences on daily stem growth, but their effects varied greatly among the different intra-annual phases (Table 3).

Phase	Variable	*r* value	Prob > *r*
I	P	0.4880	<0.0001
	ET_0	-0.4218	<0.0001
	RHm	0.3542	<0.0001
	REW2P	0.3527	<0.0001
	R	-0.3503	<0.0001
	REWTP	0.3268	<0.0001
	RH	0.2665	<0.0001
	REW1P	0.2606	<0.0001
	P5	0.1836	0.0054
	TAM	-0.1823	<0.0001
	TA	-0.1081	0.0186
II	ET_0	-0.2403	<0.0001
	RHM	0.1631	0.0003
	RH	0.1511	0.0009
	TAM	-0.0997	0.0132
III	R	-0.3818	<0.0001
	ET_0	-0.3735	<0.0003
	RHm	0.3349	<0.0001
	REW2P	0.3231	<0.0001
	REWTP	0.3201	<0.0001
	REW1P	0.3087	<0.0001
	RH	0.2491	0.0007
	P	0.2256	0.0030
	TAM	-0.2118	0.0007
	WS	0.1428	0.0234
	TA	-0.1354	0.0317
IV	ET_0	-0.3408	<0.0001
	REWTP	0.0868	0.0218
	REW2P	0.0779	0.0395

Table 3 Climatic variables (Table 2) that had significant positive or negative Pearson correlation coefficients ($p < 0.05$) with daily stem growth during each intra-annual growth phase. *r* value: Pearson correlation coefficient

More climatic variables had significant impacts on the main growth phases (I and III) than on the phases of growth cessation (II and IV). High ET_0 had a significant negative effect on daily stem growth during all phases. High relative humidity had significant positive effects during phases I, II, and III and high daily maximum air temperature

(TAM) had significant negative effects during these phases. High daily mean air temperature (TA) also had significant negative effects, but only during phases I and III. There were positive correlations between daily stem growth and high daily minimum air temperature (TAm) during all phases, but these correlations were not statistically significant (data not shown). Despite this lack of significance, the growth series had marked daily contractions during very low temperature events (Fig. 4). Variables related to daily mean soil moisture and relative extractable water had no significant influence on daily stem growth. Nevertheless, increased daily variations of relative extractable water had significant positive effects during phases I, III, and IV. Greater daily solar PAR (R) had a significant negative effect and daily precipitation (P) had a significant positive effect only during the main growth phases (I and III). Greater accumulated precipitation of the current and previous 4 days (P5) had a significant positive effect only during phase I. Daily mean wind speed (WS) had a significant positive effect during phase III. During this phase, greater WS had a significant and positive correlation with RHm ($r = 0.2304$, $p = 0.0018$), and significant and negative correlations with R ($r = -0.3807$, $p < 0.0001$), TAM ($r = -0.2333$, $p = 0.0002$), and ET_0 ($r = -0.2536$, $p = 0.0159$).

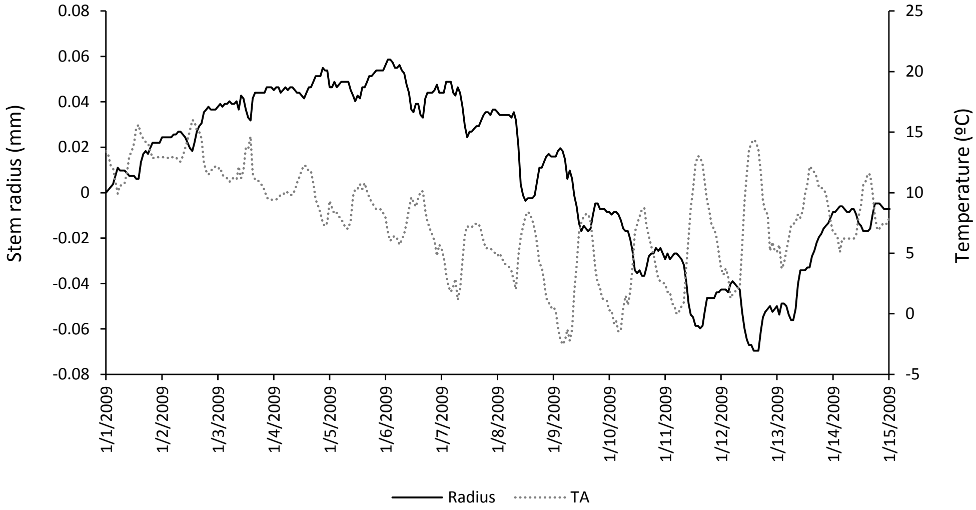

Fig. 4 Daily cycles of stem radius variations of a representative tree during a period of low winter temperatures. Radius: hourly change of stem radius (mm); TA: hourly temperature (ºC)

Discussion

Daily cycles of stem radius variation and intra-annual patterns of stem growth

Q. ilex exhibited typical daily cycles of stem swelling and shrinkage, with maximal values during the early morning and minimum values in the afternoon, as reported in many previous studies of different trees (Downes et al. (1999) in *Eucalyptus* spp.; Tatarinov and Čermák (1999) in *Quercus robur*; and Zweifel et al. (2010) in *Picea abies*).These circadian cycles are mainly caused by reversible changes in the water content of the living and elastic tissues of the stem (phloem, cambium, and parenchyma), which are mainly located in the bark (Tatarinov and Čermák 1999; Zweifel et al. 2000), and are triggered by the imbalance between leaf transpiration and water uptake by roots (Herzog et al. 1995). These changes in stem radius are closely related to the daily cycles of sap flow (Herzog et al. 1995; Steppe et al. 2006). In particular, during early morning (soon after sunrise), sap flow of *Q. ilex* increases because the increasing temperature and solar radiation increases transpiration. Sap flow reaches its maximum after midday, and decreases during the afternoon until it reaches a minimum after sunset (Barbeta et al. 2012; Fernández et al. 2013). Stem swelling occurs when water flow from the soil to the stem is greater than that lost by transpiration; shrinkage occurs when water loss exceeds uptake (Kozloswki and Winget 1964; Zweifel et al. 2001). Stored water in the bark acts as a buffer that allows the trees to respond rapidly to increased transpiration that cannot be accommodated by water uptake (Herzog et al. 1995; Steppe et al. 2006). Rapid radial flow of water from the bark to the xylem during periods of significant transpiration prevents water depletion of the xylem and subsequent cavitation (Herzog et al. 1995; Zweifel et al. 2001; Steppe et al. 2006). This stored water plays an important role during periods of drought, and indeed whenever water transport occurs within a tree, and accounts for most of the transpired water when transpiration is maximal (Zweifel et al. 2001).

When the radius of a tree reaches a daily maximum that is greater than that of the previous day, this indicates irreversible cell growth, and leads to a net increase of the stem radius (Drew et al. 2009; Jiang et al. 2015). Growth is due to the division and enlargement of cells (Tatarinov and Čermák 1999), and cell enlargement is the major driving force of the daily stem growth (Deslauriers et al. 2003). During stem swelling, the water content of cells increases and turgor pressure provokes enlargement of cells and inelastic expansion of cell walls (Proseus and Boyer 2005; Steppe et al. 2006; Köcher et al. 2012). Moreover, when the stem has a favorable water status, this promotes metabolic processes and cell division in the cambium (Molz et al. 1973; Köcher et al. 2012).

During significant precipitation events, the stem radii of holm oaks increased significantly. These peaks were frequently followed by contractions on subsequent days, as reported by Kozlowski et al. (1962) for *Quercus ellipsoidalis* and Downes et al. (1999) for *Eucalyptus* spp. Root hypoxia limits root respiration and water uptake immediately after significant precipitation (Tatarinov and Čermák 1999). Thus, when the rain stops, the stems shrink markedly because the increasing transpiration overwhelms water flow from soil. Stem shrinkage continues until soil water decreases, assuring sufficient aeration of roots (Tatarinov and Čermák 1999).

Daily contractions of the stems occurred during all of the intra-annual phases. In addition to the stem contraction after rain (discussed above), daily contractions occurred when water stress increased and the stems dehydrated, as reported by Herzog et al. (1995). Additionally, water stress reduces transpiration, photosynthesis, and carbon assimilation (Ogaya and Peñuelas 2003; Carevic et al. 2010, 2014), and inhibits cambial activity (Oberhuber et al. 2014). This leads to disruptions in the processes of cell enlargement and division (Abe and Nakai 1999; Zweifel et al. 2006).

During the cold months, *Q. ilex* did not undergo "inverse daily cycles" of frost-induced shrinkage and thaw-induced swelling (i.e., minimum radius in the morning and maximum in the afternoon) that is typical of trees in cold sites (Tardif et al. 2001; Deslauriers et al. 2007; Wang et al. 2012). Wang et al. (2012) reported that "inverse daily cycles" of *Sabina przewalskii* occurred when daily mean temperatures dropped below 0ºC and maximum temperatures remained above 0ºC, so they found significant positive correlations between daily minimum temperature and daily variations of stem radius. In our study site, temperatures never reached those low values, and no such correlations occurred.

At the intra-annual level, *Q. ilex* showed the 4 growth phases that are typical of trees in the SW Iberian Peninsula (Martín et al. 2014). Low temperatures in winter and drought in summer are the main unfavorable factors for this intra-annual growth pattern (Mitrakos 1980). The best growth occurred during phase I, when water was available and the temperatures were warm. Growth slowly declined near the end of this phase, as spring was ending. In phase I, *Q. ilex* had its maximal rates of photosynthesis, transpiration, xylem hydraulic conductivity, and carbon assimilation (Corcuera et al. 2005; Carevic et al. 2010, 2014). Contrary to previous studies of several tree species in colder sites (Tardif et al. 2001; Deslauriers et al. 2007; Zweifel et al. 2010), *Q. ilex* does not undergo a "rehydratation phase" in early spring because the winter temperatures at low altitudes in the SW Iberian Peninsula are not low enough to cause frost of soil water. Hence, there is no abrupt swelling due to thaw of frost.

Phase II commonly started with a series of progressive daily contractions during the late spring and early summer, followed by a period of stabilization with slight contractions during midsummer. Several previous studies have reported progressive daily stem contractions during dry seasons (Herzog et al. 1995; Drew et al. 2009). In contrast, Rossi et al. (2006) reported that conifers in cold regions had maximal stem growth rates during summer solstice. The daily contractions that we observed in phase II were probably due to water stress that provoked cessation of cambial growth and led to day-to-day stem shrinkage. In addition, high temperatures during summer increase respiration losses, resulting in decreased levels of carbohydrates that are needed for stem growth (Jiang et al. 2015). As summer continues, increasing stomatal closure and decreasing hydraulic conductivity reduce water losses (Carevic et al. 2010; Jiang et al. 2015), and this leads to less variability in stem radius. Summer water stress also provokes xylem cavitation (Corcuera et al. 2004; Carevic et al. 2014). The loss of hydraulic conductivity and reduced photosynthesis explains why summer precipitation did not cause net stem growth, although it did cause rapid swelling and shrinkage events. This midsummer behavior is characteristic of drought-avoiding species, even though *Q. ilex* is considered a drought-tolerant species (Ogaya et al. 2003).

Phase III generally started in late summer, in conjunction with the first significant precipitation and a moderation of temperature. During this phase, precipitation caused a swelling and shrinkage of stems (as phase II), but also triggered net positive growth with progressive increases in maximum and minimum radii after the trees stabilized following precipitation events. Carevic at al. (2014) studied *Q. ilex* in the same study site and reported increased net photosynthesis, xylem hydraulic conductivity, and xylem vessel density during phase III. The presence of intra-annual density fluctuations (IADF) and/or double rings, a common feature of Mediterranean species (Battipaglia et al. 2010), supports the presence of a second growth period in *Q. ilex* during the late summer and autumn (Campelo et al. 2007; Gutierrez et al. 2010; Martín et al. 2014).

During Phase IV, at the end of autumn and early winter, stem growth rates slowed and there was basically no growth, although in some years (e.g., 2012), there was significant stem shrinkage because of low temperatures and drought. On the contrary, there were slight growth increases throughout this phase during winter 2006 and in some trees during winter 2007. However, in general terms, *Q. ilex* stem growth stopped during winter, as reported also by Zhang and Romane (1991) and Campelo et al. (2007). The low temperatures and a short photoperiod during winter reduce photosynthesis and cambium activity (Corcuera et al. 2005; Gutiérrez et al. 2010). In addition, low temperatures also reduce water uptake and increase water stress and xylem cavitation (Kramer 1942; Corcuera et al. 2004). Thus, *Q. ilex* did not benefit from precipitation during the late autumn and early winter (Nijland et al. 2011).

Effect of climate and soil moisture on daily stem growth

Climate and soil moisture variables had notably different effects on daily stem growth during the different intra-annual phases. This supports the accuracy of our use of graphical definitions to determine the onset and ending of each intra-annual growth phase (Wang et al. 2012) and shows that the Mediterranean climate has a considerable influence on intra-annual variability of daily stem growth (Campelo et al. 2007; Martín et al. 2014). Most variables correlated significantly with daily stem growth during the main growth phases (I and III), but few variables were significant during phases of growth cessation (II and IV). As discussed above, water stress is the main constraint to plant growth in the Mediterranean region (Di Castri 1981). The lack of responsiveness to precipitation and soil moisture during phases II and IV indicates that *Q. ilex* behaved as a drought-avoiding species during severe water stress. This is supported by the presence of anatomical features in *Q. ilex* that are typical of drought-avoiding species: deep roots, phenotypic plasticity of leaves, rapid stomatal response to soil drying, and mesophyll parenchyma cells with a relatively rigid walls (Corcuera et al. 2004). Moreover, a dendrochronological analysis of *Q. ilex* at this study site by Natalini et al. (2013) reported that summer precipitation did not affect tree-ring width. This contrasts with studies of trees in more mesic sites, which indicated significant correlation of early and midsummer precipitation with tree-ring width (Campelo et al. 2007; Gea-Izquierdo et al. 2011). On the contrary, we found that precipitation had a significant positive correlation with stem growth during the main growth phases (I and III), as also reported by Campelo et al. (2007). This finding highlights the importance of water availability when the cambium is active (Molz et al. 1973; Köcher et al. 2012). The positive influence of precipitation is not simply due to increased water uptake by roots. Crown-wetting from precipitation can also have positive effects on stem water status even if there is no significant increase of soil water content (Herzog et al. 1995; Breshears et al. 2008), and this increases daily stem growth (Kozloswky and Widget 1964; Zweifel et al. 2006; Oberhuber et al. 2014).

Daily variations of relative extractable water were significant during phases I, III and IV, despite the lack of significance of daily mean soil moisture and relative extractable water. However, when mean soil moisture was great enough to cause hypoxia, the stems shrank and growth stopped because hypoxia inhibited water uptake by roots (Tatarinov and Čermák 1999). On the contrary, when soil moisture was below the threshold of drought, stomatal closure reduced the daily variations in stem radius. These results indicate a non-linear relationship between soil moisture and stem growth. In agreement, Zweifel et al. (2006) and Oberhuber et al. (2014) reported no significant correlations between current soil water content and daily stem growth for several tree species in dry sites. On the other hand, Martín et al. (2014) reported a significant positive correlation between monthly stem growth of *Q. ilex* and monthly mean soil moisture. These findings suggest that soil moisture may have a significant influence on stem growth when short-term effects are not considered.

Reference evapotranspiration had a significant negative correlation with daily stem growth during all phases, highlighting the importance of water stress on stem growth of *Q. ilex*, even during the wettest periods. When trees are unaffected by water stress, greater transpiration could enhance net carbon assimilation and induce greater stem growth, as reported by Zweifel et al. (2010). As discussed above, drought, low temperatures during the winter, and hypoxia of roots after a significant precipitation event can all trigger water stress. High atmospheric humidity had a significant positive effect on stem growth because transpiration declines as humidity increases (Köcher et al. 2012). This positive effect was not significant during phase IV, probably because the humidity remained high throughout late autumn and winter.

Daily mean air temperature and daily maximum air temperature had significant negative effects on daily stem growth during phases I, II, and III. High temperatures increase evapotranspiration and reduce soil moisture, thereby intensifying water stress. This result contrasts to a previous dendrochronological analysis in a colder site conducted by Nijland et al. (2011), which reported positive correlations between tree-ring width and spring temperatures, but negative correlations during the summer. These results are probably different because the spring temperatures in our study site were warm, and low temperature events during this period were rare. We found no significant effect of temperature on growth during phase IV. The declining temperatures during the coldest months probably reduced their effects on daily stem growth. Daily minimum temperatures did not significantly correlate with daily stem growth during any phase, even though graphical assessment of the growth series showed the existence of stem contractions during low temperature periods. These results suggest that higher temperatures were more detrimental for stem growth than lower temperatures, but these negative effects decreased when temperatures lowered. Natalini et al. (2013) studied holm oak trees at the same site and found that during the coldest months there was a significant positive correlation between minimum temperatures and tree-ring width and a significant negative correlation with maximum temperatures. These apparently different results may be because dendrochronological methods are not suitable for assessment of short-term relationships between climate and stem growth (Köcher et al. 2012).

Solar radiation had a significant negative effect on daily stem growth during the main growth phases (I and III). Corcuera et al. (2005) reported that leaf photoinhibition in *Q. ilex* occurred mainly during winter and summer. Therefore, the negative effects of solar radiation may be explained by its induction of increasing evapotranspiration. Wind speed had a significant negative effect on daily stem growth in phase III. High wind speed can have a negative impact on plant growth, although gentle breezes usually increase photosynthesis rates due to the decrease in the resistance to movement of CO_2 (Ennos 1997). However, our correlation analysis showed that wind speed during phase III correlated positively with daily minimum relative humidity and negatively with solar radiation, reference evapotranspiration, and daily maximum air temperature. Hence, the significant and positive influence of wind speed on stem growth is probably related to its effect on microclimate.

Conclusions

The daily radial stem variations of holm oak are closely associated with daily changes in environmental parameters. Net stem growth mainly occurs during two distinct intra-annual phases (Phase I and Phase III) due to the Mediterranean climate. Phase I is in late-winter and spring, and Phase III is in late-summer and autumn. Between these growth phases, there are phases of limited growth or cessation of growth, due to the summer drought (Phase II) and low winter temperatures (Phase IV).

Climate has different effects during each of the 4 intra-annual phases. It has a greater influence during the main growth phases (I and III) because it can increase or decrease tree water stress. Reference evapotranspiration negatively affects stem radial increase during all 4 phases, but precipitation only had positive effects during the two main growth phases; solar radiation has a negative effect on these phases. High temperatures had negative effects and high relative humidity had positive effects throughout the year, except during the coldest months. Relative humidity influence positively stem increases in the same phases. Daily variations of relative extractable water had positive effects throughout the year except during late spring and summer.

Acknowledgments

This work was supported by the Department of Innovation, Science and Business of the Regional Government of Andalusia, Spain [C03-192] and the Ministerio de Educación y Ciencia – Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Spain [SUM2006-00026-00-00]. We thank Rocío Macías, Enrique Andivia, and Felipe Carevic for their help with the fieldwork.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Abe H, Nakai T (1999) Effect of the water status within a tree on tracheid morphogenesis in *Cryptomeria japonica* D. Don. Trees 14:124–129

Allen RG, Pereira LS, Raes D, Smith M (1998) Crop Evapotranspiration: Guidelines for Computing Crop Water Requirements, FAO Irrigation and Drainage Paper 56. FAO, Rome

Barbeta A, Ogaya R, Peñuelas J (2012) Comparative study of diurnal and nocturnal sap flow of *Quercus ilex* and *Phillyrea latifolia* in a Mediterranean holm oak forest in Prades (Catalonia, NE Spain). Trees 26:1651–1659

Battipaglia G, De Micco V, Brand WA, Linke P, Aronne G, Saurer M, Cherubini P (2010) Variations of vessel diameter and d13C in false rings of *Arbutus unedo* L. reflect different environmental conditions. New Phytol 188:1099–1112

Böhmerle K (1883) Die pfister´sche Zuwachsuhr. Zentralblatt für das Gesamte Forstwesen 9:83–93

Breitsprecher A, Hughes W (1975) A recording dendrometer for humid environments. Biotropica 7:90–99

Breshears DD, McDowell NG, Goddard KL, Dayem KE, Martens SN, Meyer CW, Brown KM (2008) Foliar absorption of intercepted rainfall improves woody plant water status most during drought. Ecology 89:41–47

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

Carevic FS, Fernández M, Alejano R, Vázquez-Piqué J, Tapias R, Corral E, Domingo J (2010) Plant water relations and edapho-climatic conditions affecting acorn production in a holm oak (*Quercus ilex* L. ssp. *ballota*) open woodland. Agroforest Syst 78:299–308

Carevic F, Fernández M, Alejano R, Vázquez-Piqué J (2014) Xylem cavitation affects the recovery of plant water status and consequently acorn production in a holm oak open woodland. Acta Physiol Plant 36:3283–3290

Carrete M, Doñázar JA (2005) Application of central-place foraging theory shows the importance of Mediterranean dehesas for the conservation of the cinereous vulture, *Aegypius monachus*. Biol Conserv 126:582–590

Cartan-Son M, Floret C, Galan MJ, Grandjanny M, Le Floc'h E, Maistre M, Perret P, Romane F (1992) Factors affecting radial growth of *Quercus ilex* L. in a coppice stand in southern France. Vegetatio 99–100:61–68

Cobos DR (2010) Calibrating ECH2O soil moisture sensors. http://www.decagon.com/assets/Uploads/13393-04-calibratingECH2OSoilMoistureProbes.pdf. Accessed 26 October 2012

Corcuera L, Camarero JJ, Gil-Pelegrín E (2004) Effects of a severe drought on *Quercus ilex* radial growth and xylem anatomy. Trees 18:83–92

Corcuera L, Morales F, Abadía A, Gil-Pelegrín E (2005) Seasonal changes in photosynthesis and photoprotection in a *Quercus ilex* subsp. *ballota* woodland located in its upper altitudinal extreme in the Iberian Peninsula. Tree Physiol 25:599–608

Cubera E, Moreno G (2007) Effect of land-use on soil water dynamics in dehesas of Central-Western Spain. Catena 71:298–308

Deslauriers A, Morin H, Urbinati C, Carrer M (2003) Daily weather response of balsam fir (*Abies balsamea* (L.) Mill.) stem radius increment from dendrometer analysis in the boreal forests of Québec (Canada). Trees 17:477–484

Deslauriers A, Rossi S, Anfodillo T (2007) Dendrometer and intra-annual tree growth: what kind of information can be inferred? Dendrochronologia 25:113–124

Deslauriers A, Rossi S, Turcotte A, Morin H, Krause C (2011) A three-step procedure in SAS to analyze the time series from automatic dendrometers. Dendrochronologia 29:151–161

Di Castri F (1981) Mediterranean-type shrublands of the world. In: Di Castri F, Goodall DW, Specht RL (eds) Mediterranean-type shrublands. Elsevier, Amsterdam, pp 1-52

Downes G, Beadle C, Worledge D (1999) Daily stem growth patterns in irrigated *Eucalyptus globulus* and *E. nitens* in relation to climate. Trees 14:102–111

Drew DM, Downes G, Grzeskowiak V, Naidoo T (2009) Differences in daily stem size variation and growth in two hybrid eucalypt clones. Trees 23:585–595

Ennos AR (1997) Wind as an ecological factor. Trends Ecol Evol 12:108–11

Fernández M, Alejano R, Vázquez-Piqué J, Andivia E, Martín D (2013) Evolución estacional de la velocidad de flujo de savia en encinas de dehesas de la provincia de Huelva. Actas del VI Congreso Forestal Español, Vitoria-Gasteiz

Friedrich J (1890) Zuwachsmesser. Die pfister´sche Zuwachsuhr. Zentralblatt für das Gesamte Forstwesen 16:174–179

Friedrich J (1905) Zuwachsautograph. Zentralblatt für das Gesamte Forstwesen 31:456–461

Fritts HC, Fritts EC (1955) A new dendrograph for recording radial changes of a tree. Forest Sci 1:271–276

Gea-Izquierdo G, Cherubini P, Cañellas I (2011) Tree-rings reflect the impact of climate change on *Quercus ilex* L. along a temperature gradient in Spain over the last 100 years. Forest Ecol Manage 262:1807–1816

Granier A (1987) Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. Tree Physiol 3:309–320

Gutiérrez E, Campelo F, Camarero JJ, Ribas M, Muntán E, Nabais C, Freitas H (2011) Climate controls act at different scales on the seasonal pattern of *Quercus ilex* L. stem radial increments in NE Spain. Trees 25:637–646

Gyenge JE, Fernández ME, Schlichter TM (2010) Effect of stand density and pruning on growth of ponderosa pines in NW Patagonia, Argentina. Agroforest Syst 78:233–241

Herzog KM, Häsler R, Thum R (1995) Diurnal changes in the radius of a subalpine Norway spruce stem: their relation to the sap flow and their use to estimate transpiration. Trees 10:94–101

Intergovernmental Panel on Climate Change IPCC (2007) Climate Change 2007: Synthesis Report. Cambridge University Press, Cambridge

IUSS Working Group WRB (2007) World Reference Base for Soil Resources 2006, first update 2007. World Soil Resources Reports No. 103. FAO, Rome

Jiang Y, Wang BQ, Dong MY, Huang YM, Wang MC, Wang B (2015) Response of daily stem radial growth of *Platycladus orientalis* to environmental factors in a semi-arid area of North China. Trees 29:87–96

Köcher P, Horna V, Leuschner C (2012) Environmental control of daily stem growth patterns in five temperate broad-leaved tree species. Tree Physiol 32:1021–1032

Kozlowski TT, Winget CH, Torrie JH (1962) Daily radial growth of oak in relation to maximum and minimum temperature. Bot Gaz 124:9–17

Kozlowski TT, Winget CH (1964) Diurnal and seasonal variations in radii of tree stems. Ecology 45:149–155.

Kramer PJ (1942) Species differences with respect to water absorption at low soil. Am J Bot 29:828–832

MacDougal DT (1918). The dendrograph: a new instrument for recording growth and other variations in the dimensions of trees. Carnegie Inst Wash Year Bk 18:72–74

Martín D, Vázquez-Piqué J, Fernández M, Alejano R (2014) Effect of ecological factors on intra-annual stem girth increment of holm oak. Trees 28:1367–1381

Martín D, Vázquez-Piqué J, Carevic FS, Fernández M, Alejano R (2015a) Trade-off between stem growth and acorn production in holm oak. Trees 29:825–834

Martín D, Vázquez-Piqué J, Alejano R (2015b) Effect of pruning and soil treatments on stem growth of holm oak in open woodland forests. Agrofor Syst 89:599–609

Martínez-Pastur GM, Lencinas MV, Cellini JM, Mundo I (2007) Diameter growth: can live trees decrease? Forestry 80:83–88

Mitrakos KA (1980) A theory for Mediterranean plant life. Acta Oecol 1:245–252

Molz FJ, Klepper B, Peterson CM (1973) Rehydration versus growth-induced water uptake in plant tissues. Plant Physiol 51:859–862

Mund M, Kutsch WL, Wirth C, Kahl T, Knohl A, Skomarkova MV, Schulze ED (2010) The influence of climate and fructification on the inter-annual variability of stem growth and net primary productivity in an old-growth, mixed beech forest. Tree Physiol 30:689–704

Natalini F, Alejano R, Vázquez-Piqué FJ, Cañellas, I, Gea-Izquierdo G (2013) Growth trends and sensitivity to climate of declining Mediterranean open woodlands exhibiting widespread mortality in Southern Spain. Second American Dendrochronology Conference Ameridendron. Tucson, Arizona

Nijland W, Jansma E, Addink EA, Domínguez-Delmás M, De Jong SM (2011) Relating ring width of Mediterranean evergreen species to seasonal and annual variations of precipitation and temperature. Biogeosciences 8:1141–1152

Nishimua TB, Suzuki E, Kohyama T, Tsuyuzaki S (2007) Mortality and growth of trees in peat-swamp and heath forests of Central Kalimantan after severe drought. Plant Ecol 188:165–177

Oberhuber W, Gruber A, Kofler W, Swidrak I (2014) Radial stem growth in response to microclimate and soil moisture in a drought-prone mixed coniferous forest at an inner Alpine site. Eur J For Res 133:467–479

Ogaya R, Penuelas J (2003) Comparative field study of *Quercus ilex* and *Phillyrea latifolia*: photosynthetic response to experimental drought conditions. Environ Exp Bot 50:137–148

Ogaya R, Peñuelas J, Martínez-Vilalta J, Mangirón M (2003) Effect of drought on diameter increment of *Quercus ilex, Phillyrea latifolia*, and *Arbutus unedo* in a holm oak forest of NE Spain. Forest Ecol Manage 180:175–184

Pinto CA, Henriques MO, Figueiredo JP, David JS, Abreu FG, Pereira JS, Correia I, David TS (2011) Phenology and growth dynamics in Mediterranean evergreen oaks: effects of environmental conditions and water relations. Forest Ecol Manage 262:500–508

Proseus TE, Boyer JS (2005) Turgor pressure moves polysaccharides into growing cell walls of *Chara corallina*. Ann Bot 95:967–979

Quézel P, Médail F (2003) Ecologie et biogéographie des forêts du bassin méditerranéen. Elsevier, Paris

Rodríguez-Calcerrada J, Pérez-Ramos IM, Ourcival JM, Limousin JM, Joffre R, Rambal S (2011) Is selective thinning an adequate practice for adapting *Quercus ilex* coppices to climate change? Ann For Sci 68:575–585

Rossi S, Deslauriers A, Anfodillo T, Morin H, Saracino A, Motta R, Borghetti M (2006) Conifers in cold environments synchronize maximum growth rate of tree-ring formation with day length. New Phytol 169:279–290

Steppe K, De Pauw DJW, Lemeur R, Vanrolleghem PA (2006) A mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth. Tree Physiol 26:257–273

Tardif J, Flannigan M, Bergeron Y (2001) An analysis of the daily radial activity of 7 boreal tree species, northwestern Quebec. Environ Monit Assess 67:141–160

Tatarinov F, Čermák J (1999). Daily and seasonal variation of stem radius in oak. Ann For Sci 56:579–590

Vázquez-Piqué J, Tapias-Martín R, González-Pérez A (2009) Desarrollo, características y aplicaciones de un dendrómetro potenciométrico para la medición continua del crecimiento diametral de especies arbóreas. Actas del V Congreso Forestal Español, Ávila

Wang Z, Yang B, Deslauriers A, Qin C, He M, Shi F, Liu J (2012) Two phases of seasonal stem radius variations of *Sabina przewalskii* Kom. in northwestern China inferred from sub-diurnal shrinkage and expansion patterns. Trees 26:1747–1757

Wang Z, Yang B, Deslauriers A, Bräuning A (2015) Intra-annual stem radial increment response of Qilian juniper to temperature and precipitation along an altitudinal gradient in northwestern China. Trees 29:25–34

Zhang SH, Romane F (1991) Variations de la croissance radiale de *Quercus ilex* L. en function du climat. Ann For Sci 48:225–234

Zweifel R, Item H, Häsler R (2000) Stem radius changes and their relation to stored water in stems of young Norway spruce trees. Trees 15:50–57

Zweifel R, Item H, Häsler R (2001) Link between diurnal stem radius changes and tree water relations. Tree Physiol. 21:869–877

Zweifel R, Zimmermann L, Zeugin F, Newbery DM (2006) Intra-annual radial growth and water relations of trees: implications towards a growth mechanism. J Exp Bot 57:1445–1459

Zweifel R, Eugster W, Etzold S, Dobbertin M, Buchmann N, Häsler R (2010) Link between continuous stem radius changes and net ecosystem productivity of a subalpine Norway spruce forest in the Swiss Alps. New Phytol 187:819–830

Artículo 3. Trade-off between stem growth and acorn production in holm oak

Artículo publicado como: Martín D, Vázquez-Piqué J, Carevic FS, Fernández M, Alejano R (2015) Trade-off between stem growth and acorn production in holm oak. Trees 29:825-834

Trade-off between stem growth and acorn production in holm oak

Abstract

Reproduction in trees often requires significant resources, and previous studies have documented trade-offs between reproduction and growth in numerous tree species. In the present study, we assessed the relationships of acorn production with annual and intra-annual stem growth of *Quercus ilex* (holm oak) at the level of the individual tree over six years (2006-2011) at two study sites in southwestern Spain. There were negative correlations between acorn production and annual and late summer-autumn stem growth during masting years. In other words, the growth rates were lower in trees that had greater acorn production. These results suggest the existence of trade-offs between growth and reproduction in *Q. ilex*. However, there was no relationship between acorn production and winter-spring growth. Moreover, the costs of reproduction varied between the two study sites. There were negative correlations between acorn production and late summer-autumn growth in both study sites, but there were only negative correlations between acorn production and annual growth in one study site. Trade-offs appear to be greater in smaller trees living under more stressful conditions. These results show the importance of making intra-annual measurements of tree growth for appropriate interpretation of potential trade-offs.

Keywords: Holm oak, masting, reproduction, resource allocation.

Key message: There were trade-offs between acorn production and stem growth in individuals of *Quercus ilex*, and these occurred with annual and intra-annual periods. The costs of reproduction differed significantly between two study sites.

Introduction

The growth of forest trees is important for commercial wood production. In addition, growth also shows the response to ecological factors (Cook and Kairiukstis 1990), and it is an indicator of fitness and resources allocation in trees (Stearns 1989). Stem growth generally occupies a low position in the carbon allocation hierarchy (Hoff et al. 2002; Rodríguez-Calcerrada et al. 2011). Trade-offs represent the cost paid in the currency of fitness when a beneficial change in one trait is linked to a detrimental change in another (Stearns 1989).

Investment in reproduction may be considered a hierarchical process (Obeso 2004). Hence, it is expected that the extraordinary resource allocation to reproduction during “mast years” (when there is high production of seeds) could lead to reduced stem growth. Masting seems to be triggered by climate, in that more seeds are produced when the climate is better (resource matching hypothesis; Norton and Kelly 1988). However, masting may also be a strategy to reduce the percentage of seeds lost by predation (predator satiation hypothesis; Janzen 1971) or to synchronize seed production in good climatic years, and thereby improve seedling establishment (environmental prediction hypothesis; Kelly 1994). Masting as reproductive strategy requires a resource allocation mechanism that increases the variation of seed production among years (resource switching or trade-off hypothesis; Kelly and Sork 2002; Monks and Kelly 2006; Sánchez-Humanes et al. 2011). Thus, a large investment in reproduction could reduce the energy available for growth in long lived plants (Koenig and Knops 1998), such as *Quercus* species (Camarero et al. 2010; Sánchez-Humanes et al. 2011; Barringer et al. 2012). However, the phenomenon of resource switching is not a universal explanation for masting (Knops et al. 2007), and there can even be positive correlations between plant growth and reproduction (Despland and Houle 1997).

Quercus ilex L. (holm oak) is a widespread species in the Mediterranean Basin that covers more than 6.5 million ha (Quézel and Médail 2003). It is one of the dominant species in “dehesas”, traditional agroforestry systems consisting of an open woodland forest (10 to 60 trees ha^{-1}) and an herbaceous layer (Cubera and Moreno 2007). *Q. ilex* has highly variable annual acorn production (Alejano et al. 2011), as other oak species (Sork 1993; Sánchez-Humanes et al. 2011). Previous studies of trade-offs between growth and reproduction in trees have generally examined annual growth data, such as tree-ring width or annual dendrometer measurements (e.g., Monks and Kelly 2006; Knops et al. 2007; Barringer et al. 2012). However, *Q. ilex* typically has two intra-annual periods of stem growth (Gutiérrez et al. 2011; Martín et al. 2014); in late winter to early summer and after the summer drought throughout the autumn. This second period of growth overlaps with acorn fattening (Siscart et al. 1999), the period of maximal investment in acorns (Knops et al. 2007). Hence, the relations between stem growth and acorn production in holm oak may change at the intra-annual level.

Even when there is a negative correlation between life history traits, this may not be due to trade-offs due to resource limitation (Barringer et al. 2012). For example, Knops et al. (2007) reported the presence of a negative correlation between annual growth and acorn production in several *Quercus* species and they suggested that spring rainfall caused increased growth and decreased pollination (apparent or putative trade-offs). The analysis of trade-offs at the intra-annual level in holm oak, particularly during the second intra-annual growth period in late summer and autumn, could be a method to test the hypothesis of a true trade-offs between growth and reproduction, because late summer and autumn growth are not driven by spring rainfall, and autumn is the period of acorn fattening, when a large amount of tree resources could be derived to reproduction.

This study addressed two main questions. First, are there trade-offs at the level of the individual tree between acorn production and stem growth in *Q. ilex* at annual and intra-annual scales? Second, can the costs of reproduction in *Q. ilex* vary between study sites due to differences in ecological traits? Answering these questions will improve our understanding of the growth process and the influence of endogenous biological factors on individual growth variability of *Q. ilex* that is not driven by climate, competition, or microecological factors (Martín et al. 2014). Ultimately, understanding these issues will be useful for the development of accurate and full-parameterized growth models for Mediterranean ecosystems and will provide guidance for their sustainable management.

Materials and Methods

Field plots

This study was performed in two experimental plots in the Huelva province of southwestern Spain (Table 1). The Huerto Ramirez (HR) plot is in an open woodland of

Q. ilex where sheep and Iberian pigs are raised. Its soils have different degrees of development from acrisols, alisols, and lixisols to regosols and cambisols (IUSS Working Group WRB 2007). There is a sparse understory of mainly *Cistus ladanifer* and *C. crispus* and an abundant herbaceous layer of mainly grasses.

Plot	Coordinates (UTM. Zone 29)	Area (ha)	Density (trees ha^{-1})	Mean diameter ± SD (cm)	Mean height ± SD (m)
Huerto Ramírez (HR)	X:644288 m Y:4161376 m	2.94	73.0	30.0 ± 7.7	6.6 ± 1.6
San Bartolomé (SB)	X:669638 m Y:4145966 m	2.70	36.0	35.4 ± 7.2	6.5 ± 1.1

Table 1 Characteristics of the two study plots. SD: standard deviation

The San Bartolomé (SB) plot is in an open woodland of *Q. ilex* where bulls are raised. Its soils are endoleptic regosols (episkeletic) or endoleptic luvisols (dystric), with deeper soils in depositional or concave areas (IUSS Working Group WRB 2007). SB has a very sparse understory due to frequent tillage, and an abundant herbaceous layer of mainly grasses. Both plots were fenced-in to avoid predation on acorns and damage of field equipment. The climate of both plots is Mediterranean, with highly variable temperature and rainfall within and among years. The nearby ocean modulates air temperature and increases precipitation relative to the more continental areas in the Iberian Peninsula. There were no large variations in temperature during the study period (2006-2011), but there was large monthly and annual variability in precipitation (Figs. 1, 2; Table 2).

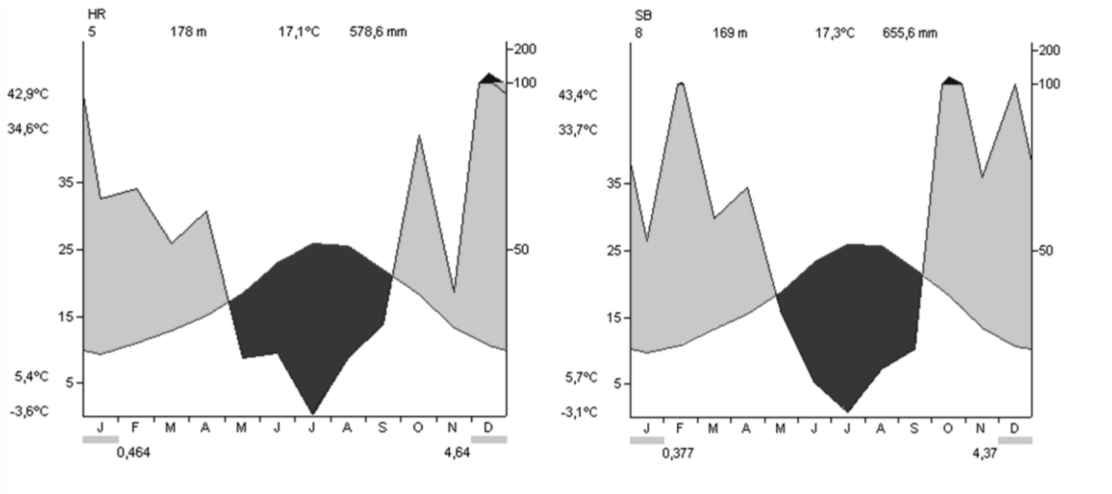

Fig. 1 Walter-Lieth climate diagrams of the plots in Huerto Ramírez (left) and San Bartolomé (right)

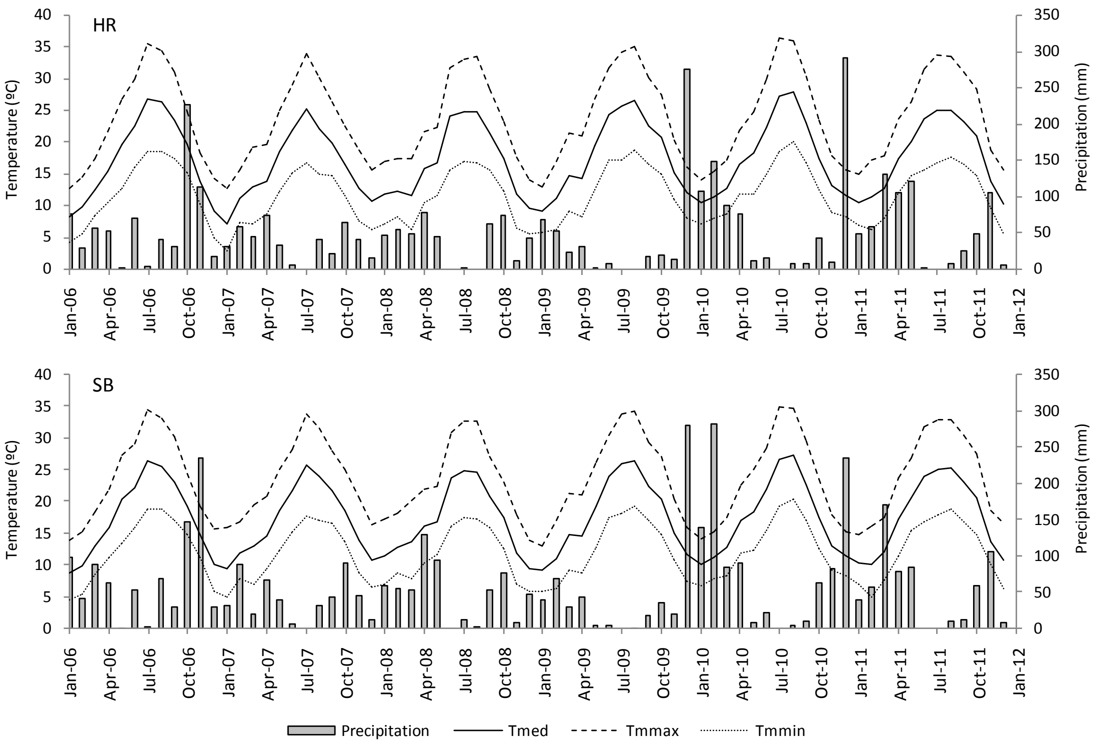

Fig. 2 Monthly precipitation and temperature of plots in Huerto Ramírez (top) and San Bartolomé (bottom) during the study period. Tmed: mean temperature; Tmmax: mean maximum daily temperature; Tmmin: mean minimum daily temperature

Year	Precipitation (mm)								Temperature (ºC)					
	SB				HR				SB			HR		
	Psp	Psm	Pat	P	Psp	Psm	Pat	P	TM	Tm	T	TM	Tm	T
2006	115	99	411	850	123	74	356	713	34.4	4.5	17.4	35.6	4.2	17.4
2007	111	75	148	473	110	60	118	421	33.8	4.9	17.0	33.9	2.8	16.1
2008	222	66	130	584	121	64	126	458	32.7	5.7	17.0	33.4	5.5	16.8
2009	50	18	334	538	38	16	305	502	34.1	5.7	17.9	35.0	5.7	18.0
2010	120	12	379	1019	99	14	342	799	34.9	6.7	17.5	36.3	7.1	17.6
2011	162	21	107	618	226	31	158	651	32.8	5.0	17.7	33.2	6.0	17.4

Table 2 Precipitation and temperature of the two plots during the study period. Psp: spring precipitation; Psm: summer precipitation; Pat: autumn precipitation; P: annual precipitation; TM: mean maximum temperature of the hottest month; Tm: mean minimum temperature of the coldest month; T: mean annual temperature

Measurement of stem growth and location of trees

31 aluminum band dendrometers that were developed by the University of Huelva were installed at breast height (1.30 m) on 18 trees in HR and 13 trees in SB, with care taken to avoid stem deformities. Trees were selected within plots by use of stratified

sampling so that different diametric classes were considered. Keeland and Young (2014) provide details of band dendrometer theory and construction.

Measurements were recorded each month with a digital caliper (0.01 mm precision) from 2006 to 2011 and these data were used to calculate annual stem girth increment (hereafter, annual growth), January to June stem girth increment (hereafter, winter-spring growth) and August to December stem girth increment (hereafter, late summer-autumn growth). The calculation of these values as the accumulation of monthly measurements is more accurate than single-season or annual measurements because it allowed detection and correction of measurement errors. Changes in girth were not transformed into diameter because *Q. ilex* has high within-tree variability in stem growth, and because the stems were not sufficiently cylindrical for this transformation. At the beginning of the study, the topographic location of each of the 31 trees was measured using a Sokkia 3B total station.

Estimation of acorn production

Acorns were harvested from the same trees whose growth was measured by use of a trapping method (Greenberg 2000). Four containers (0.45 m diameter at the top) were placed on the ground under selected trees at the north, south, east, and west positions, at three-quarters of the distance from the stem to the edge of the crown. This trapping method allowed sampling of a fraction of the projection of the crown surface where acorns were assumed to fall. Acorns were collected from each container every 2 weeks during the six dissemination periods (2006/2007 to 2011/2012) from September to January.

The acorns were transferred to the laboratory in polyethylene bags for counting and determination of fresh weight. Alejano et al. (2011) reported that acorn water content of *Q. ilex* did not vary significantly among trees in months and years. Thus, we used fresh acorn mass in this study, which we consider a more accurate measurement. Acorn production (AP) was calculated as fresh weight of acorns per m^2 of the orthogonal projection of the crown on the ground (g FM m^{-2}). Acorn production estimated by this container method is consistent with total acorn yield of the whole tree (Alejano et al. 2008).

Data analysis

We used three linear mixed models for data analysis: (a) a model for estimating annual growth; (b) a model for estimating winter-spring growth; and (c) a model for estimating late summer-autumn growth (when acorns fatten and disseminate). The initial structure of each model was:

$$y_{ijl} = \mu + b_{i(j)} + \alpha_j + \gamma_l + (\alpha\gamma)_{jl} + e_{ijl} \qquad (1)$$

where y_{ijl} is the girth increase (mm) of tree *i* at plot *j* of year *l* for the entire year in the annual model, from January to June of year *l* in the winter-spring model, or from August to December of year *l* in the late summer-autumn model; μ is the general mean; $b_{i(j)}$ is a tree random effect within each plot with i = 1, 2,....,18 and j = 1, 2 under the hypothesis $b_{i(j)} \sim$ N(0, **G**); α_j is a plot fixed-effect with j = 1, 2; γ_l is a year fixed-effect with l = 2006, 2007,..., 2011; $(\alpha\gamma)_{jl}$ is the plot × year interaction (fixed effect); and e_{ijl} is the residual error under the hypothesis $e_{ijl} \sim$ N(0, **R**).

The following procedure was used to select the best model structure:

First, the models were adjusted by consideration of tree random effect, temporal correlations between observations of different years for each tree, and different variances for different years. Hence, **G** was initially considered a diagonal matrix and **R** a block diagonal matrix, with each block corresponding to a 6 × 6 submatrix of observations for each tree. We considered the following alternatives for the structure of blocks in the **R** matrix: autoregressive order 1, autoregressive heterogeneous, Toeplitz up to 6 bands, heterogeneous Toeplitz up to 6 bands, unstructured up to 6 bands, Huynh-Feldt, compound symmetry, compound symmetry heterogeneous, dependent covariance, and first-order factor analytic (Littell et al. 2006). Variance components for each structure were estimated by restricted maximum likelihood (REML, Patterson and Thompson 1971) and model selection was based on the Akaike information criterion (AIC, Akaike 1974).

Second, the significance of the tree random effect was determined by a likelihood ratio test, as the reduction of the statistic -2 x log likelihood (-2LL), after introducing the tree random effect, which follows a χ^2 distribution with 1 degree of freedom. An α value of 0.05 was considered to indicate an improved covariance structure.

Third, if the tree random effect was significant, the presence of spatial correlation was determined by use of the following isotropic power covariance model:

$$\mathrm{cov}\,(b_{i(j)}, b_{i'(j)}) = \sigma^2{}_b\,\rho^{d_{ii'}} \qquad (2)$$

where $d_{ii'}$ is the distance between trees i and i' in location j; $\sigma^2{}_b$ is the variance component at the tree level; and ρ is a parameter to be estimated with $|\rho| < 1$. The spatial covariance between observations at different locations was considered zero.

Fourth, after selection of the best variance-covariance structure, the fixed effects were estimated by a generalized least squares (GLS) equation (Searle 1971), and the significance of each effect was determined with an F test. Only significant effects ($\alpha = 0.05$) were retained in the model. Different levels of significance were compared by the Scheffe´ test.

Fifth, acorn production of the current year was introduced into the models as an additive linear effects covariate, and its significance was assessed with an F test. To analyze the significance of covariates, the variance components were estimated by maximum likelihood (ML). Significant covariates were tested in each plot by covariance analysis (Littell et al. 2006). All statistical analysis was performed with SAS/ETS (ver. 9.2).

Results

Annual growth

The best structure of the variance-covariance matrix for the 6 × 6 blocks of the **R** matrix was heterogeneous Toeplitz with 5 bands. The tree random effect was significant ($p = 0.039$), but spatial covariance was not ($p = 0.499$ in HR; $p = 0.145$ in SB). This indicates the presence of significant growth differences between trees that is not explained by tree location within a plot. The selected model indicates that plot effect, year effect, and the plot × year interaction were highly significant (Table 3). In other words, the annual stem growth varied between plots and among years. For both plots, annual growth was greatest during 2007 and 2008 (12.71 ± 0.91 mm in 2007 and 12.75 ± 0.65 mm in

2008) and lowest during 2009 (2.51 ± 0.39 mm, Fig. 3). At the plot level, growth in the SB plot was significantly greater than in the HR plot (11.30 ± 0.82 vs. 7.51 ± 0.60 mm, $p = 0.001$). Growth was greatest during 2008 in the SB plot (16.23 ± 0.99 mm) and during 2007 in the HR plot (10.21 ± 1.19 mm). Growth was lowest during 2009 for both plots (3.12 ± 0.60 mm in SB and 1.90 ± 0.51 mm in HR). Trees in the SB plot grew more than trees in the HR plot during all 6 years (Fig. 4).

	Annual growth			Winter-spring growth			Late summer-autumn growth		
Effect	Year	Plot	Plot × year	Year	Plot	Plot × year	Year	Plot	Plot × year
F value	107.11	12.42	6.85	88.18	3.82	9.25	20.57	24.78	2.56
Pr > *F*	<0.0001	0.0014	<0.0001	<0.0001	0.0604	<0.0001	<0.0001	<0.0001	0.0301

Table 3 Significance of fixed effects in the annual, winter-spring, and late summer-autumn growth models

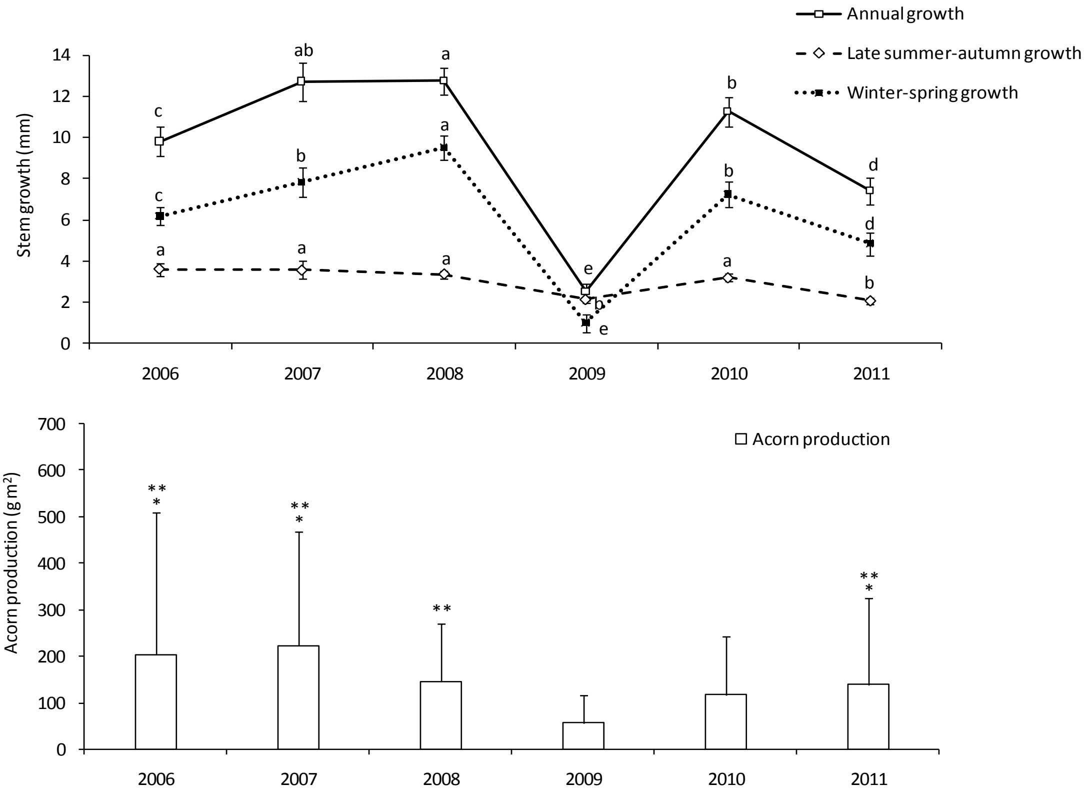

Fig. 3 Top: Least squares estimates of the mean annual, winter-spring, and late summer-autumn stem growth per tree (mm ± standard error) at both plots. Different letters indicate significant differences for each growth model ($p < 0.05$). Bottom: Mean annual acorn production per tree at both plots (g m^{-2} ± standard error). * indicates that acorn production reduced the variance of annual stem growth at the individual tree level. ** indicates that acorn production reduced the variance of late summer-autumn growth at the individual tree level

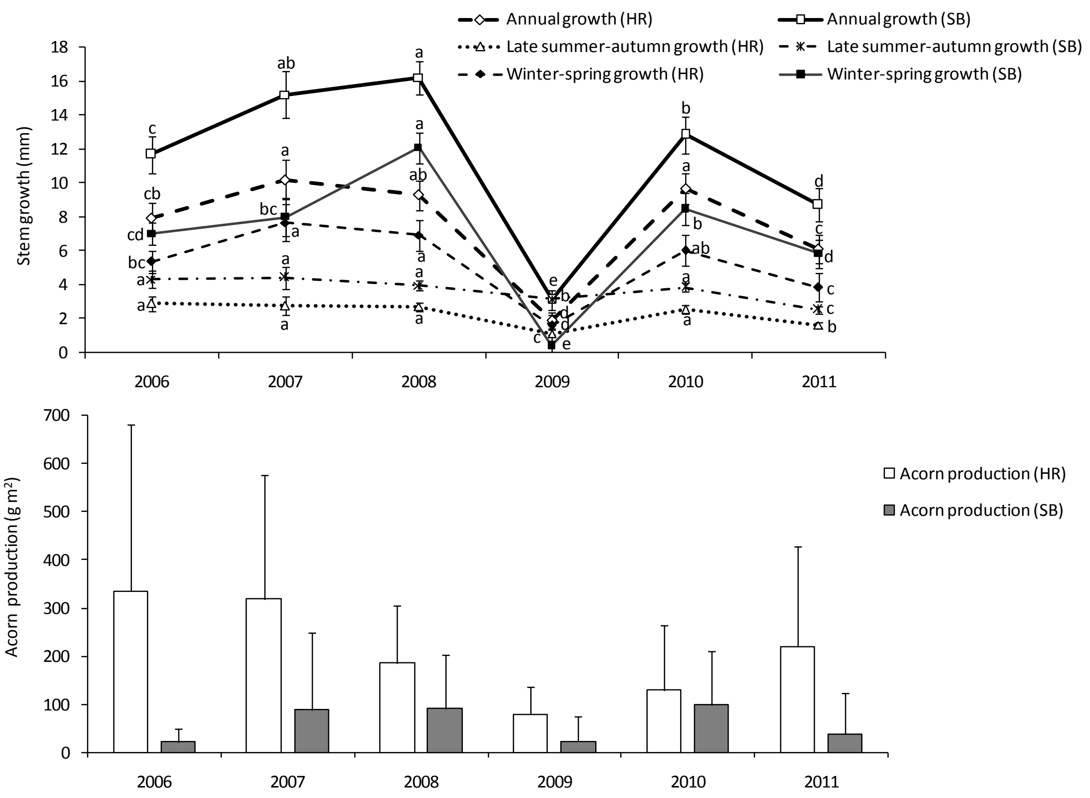

Fig. 4 Top: Least squares mean estimates of annual, winter-spring, and late summer-autumn stem growth per tree (mm ± standard error) at each plot. Different letters indicate significant differences for each growth model ($p < 0.05$). Bottom: Mean annual acorn production per tree at each plot (g m^{-2} ± standard error)

Winter-spring growth

The best structure of the variance-covariance matrix for the 6 × 6 blocks of the **R** matrix was heterogeneous Toeplitz with 5 bands. The tree random effect was significant ($p = 0.027$), indicating significant growth differences among trees. We could not calculate spatial covariance because the model did not converge. The selected model indicates that the year effect and the plot × year interaction were highly significant, but the plot effect was not significant (Table 3). Thus, there were generally no significant differences in winter-spring growth between study sites, but these differences were significant in some years. For both plots, winter-spring growth was greatest during 2006 (9.51 ± 0.59 mm) and lowest during 2009 (0.99 ± 0.42 mm, Fig. 3). At the plot × year level, winter-spring growth was maximal during 2008 at SB (12.08 ± 0.91 mm) and during 2007 at HR (7.67 ± 0.94 mm). Both plots had the lowest growth during 2009 (0.42 ± 0.64 mm in SB and 1.57 ± 0.55 mm in HR). Trees in the SB plot grew more than trees in the HR plot in all years except 2009 (Fig. 4).

Late summer-autumn growth

The best structure of the variance-covariance matrix for the 6 × 6 blocks of the **R** matrix was heterogeneous autoregressive of order 1. The tree random effect was significant ($p = 0.036$), indicating significant growth differences among trees. We could not calculate spatial covariance because the model did not converge. The selected model indicates that plot effect, year effect, and plot × year interaction were highly significant (Table 3). For both plots, late summer-autumn growth was greatest during 2006 (3.60 ± 0.32 mm) and lowest during 2011 (2.08 ± 0.15 mm, Fig. 3). At the plot level, the growth at SB was significantly greater than growth at HR (3.71 ± 0.22 vs. 2.28 ± 0.19 mm, $p < 0.001$). At the plot × year level, late summer-autumn growth was greatest during 2007 at SB (4.43 ± 0.64 mm) and during 2006 at HR (2.89 ± 0.41 mm). The lowest growth was during 2011 at SB (2.55 ± 0.22 mm) and during 2009 at HR (1.13 ± 0.19 mm). Trees in the SB plot grew more than trees in the HR plot in all years (Fig. 4).

Trade-off between acorn production and growth

There were negative correlations between acorn production and annual growth ($p = 0.038$; coefficient = -0.003), and between acorn production and late summer-autumn growth ($p = 0.037$; coefficient = -0.014). The introduction of this effect reduced the variance of the annual model in years 2006, 2007 and 2011, and reduced the variance of the late summer-autumn model in years 2006, 2007, 2008 and 2011 (Table 4). These results indicate that acorn production explained part of the variability of growth between individual trees in these years. The reduction of variance was remarkable during 2006 (the year of maximal acorn production) in the model of annual growth and in the model of late summer-autumn growth. In contrast, the variance did not decrease after the introduction of this effect in both models during the years of less acorn production, e.g., 2009, indicating that acorn production did not influence stem growth in these years (Table 4; Fig. 3). Covariance analysis showed no significant differences between the plots in the late summer-autumn model ($p = 0.079$), but the presence of significant differences in annual model ($p = 0.019$). Specifically, acorn production was significantly and negatively correlated with growth at HR ($p = 0.001$; coefficient = -0.004), but not at SB ($p = 0.161$). On the contrary, acorn production had no significant effect on winter-spring growth ($p = 0.238$).

Year	Annual growth	Late summer-autumn growth
2006	6.68	8.26
2007	5.56	2.23
2008	-1.32	3.22
2009	-9.17	-4.57
2010	-3.96	-4.18
2011	0.33	1.59

Table 4 Effect of acorn production on the variance in annual and late summer-autumn growth models during each year (% reduction of variance)

Discussion

Patterns of stem growth

The stem growth of *Q. ilex* varied significantly during the 6-year study period, with the highest growth rates in 2007 and 2008, and the lowest rates in 2009. Most of the annual growth occurred during spring, as reported in previous studies of this species (e.g., Gutiérrez et al. 2011; Martín et al. 2014), but there was also a second (generally shorter) growth period after the summer drought that correlated with late summer or early autumn precipitation. However, in years with dry springs and rainy autumns, such as 2009, most annual stem growth can occur during the second growth period, indicating that intra-annual growth patterns can be switched by climate.

Martín et al. (2014) reported high between-year variability in the timing of the second period of stem growth in holm oak. However, our results indicated that between-year variability in the late summer-autumn growth was less marked than annual growth and winter-spring growth, even though precipitation and temperature variability were greater during summer and autumn than spring. Hence, other biological processes during this period, such as acorn production, apparently compete for the limited available resources, and this modulates the between-year variability in late summer-autumn growth.

Our results showed that differences of growth among trees were not due to spatial differences. In other words, different locations within a plot were about equally favorable for growth. Thus, processes such as acorn production may explain the observed growth differences. The growth differences between plots, both annual growth and late-summer growth, are probably related to the greater soil depth and development, reduced competition (because of lower stand density), and better climate (higher precipitation, milder temperatures) at SB than HR. On the contrary, during winter-spring, growth differences between the plots were not significant. Spring is generally the most favorable season for growth in Mediterranean climates because of the mild temperatures and greater water availability (Gea-Izquierdo et al. 2011). Hence, a greater water supply due to precipitation may have modulated the differences in soil type and stand density.

Trade-offs between acorn production and growth

Our results showed negative correlations between acorn production and annual growth and late summer-autumn growth in good masting years (2006, 2007, 2008, and 2011). Camarero et al. (2010) reported similar results for *Q. ilex* and Barringer et al. (2012) reported similar results for *Q. lobata*, *Q. douglasii*, and *Q. agrifolia*. However, we found no correlations between acorn production and winter-spring growth. Several studies (e.g., Kelly and Sork 2002; Monks and Kelly 2006; Mund et al. 2010) suggested that a negative correlation between fruit production and stem growth generally indicates a switching of resources from vegetative growth to reproduction. Hence, when a tree of *Q. ilex* produces a large acorn crop during a mast year, this leads to reduced allocation for stem growth.

It may be questionable whether our observed negative correlations between acorn production and stem growth were due to a true trade-off of resources, or occurred simply because different environmental factors regulate growth and reproduction. Knops et al. (2007) suggested that the negative correlation between acorn production and annual stem growth in California oaks may be explained by this later mechanism. Thus, spring precipitation could enhance stem growth, but have a detrimental effect on pollination, leading to low acorn production and greater growth in years with more rainfall (Knops et al. 2007). Alejano et al. (2011) found that April precipitation was associated with decreased acorn production of *Q. ilex* in dehesas of southwestern Spain. However, we found negative correlations between stem growth and acorn production in late summer-autumn growth, which probably does not depend on spring rainfall, but there were no such correlations between winter-spring stem growth and acorn production. These results are not consistent with the hypothesis of Knops et al. (2007), and suggest that the negative correlations between growth and reproduction were not driven by spring rainfall. Mund et al. (2010) found negative correlations between stem growth and fruit production in

Fagus sylvatica, and demonstrated that this was a causal relationship, rejecting the existence of apparent or putative trade-offs.

Our results showed that the negative correlation between growth and reproduction was greater during intra-annual growth periods, when there was greater resource allocation to reproduction. Late summer-autumn growth partially overlaps with the fattening of acorns, which is typically a period of lower resource availability (Carevic et. al. 2010). In spring, only male flowering overlaps with stem growth. In general, female plants invest up to 10-fold more resources to reproduction than males (Obeso 2004), and in oaks, female allocation closely matches total reproductive allocation (Knops and Koenig 2012). Although summer and autumn had a higher intra-annual variability than spring in terms of climate (especially precipitation), the growth differences between years were smaller for late summer-autumn growth than for winter-spring and annual growth. On the contrary, variation in acorn production between years closely followed variations in precipitation. For example, greater acorn production occurred in years with greater summer and autumn precipitation (e.g., 2006), and lower production occurred in years with scarce summer and autumn precipitation (e.g., 2009). Stem growth has a low position in the resource allocation hierarchy (Hoff et al. 2002; Rodríguez-Calcerrada et al. 2011), and has lower sink strength than fruit production (Mund et al. 2010). Therefore, a resource allocation hierarchy may explain the greater inter-year variability in acorn production than in late summer-autumn stem growth in holm oak. In years of high resource availability, trees seem to devote the surplus to reproduction, while sustaining stem growth at moderate rates (as in normal years); however, in years with low resource availability (e.g., 2009) stem growth and acorn production were both low and a negative correlation between growth and reproduction could not explain the between-tree differences in stem growth. According to Kelly and Sork (2002), when resources are very scarce, there can even be positive correlations between growth and reproduction.

Our results clearly indicated negative correlations between acorn production and stem growth at the level of the individual tree. However, our results also showed that years with greater acorn production at the population level had greater stem growth, and that years with lower acorn production at the population level had lower stem growth. Pérez-Ramos et al. (2010) found a positive correlation between acorn production and stem growth at the population level in a stand of *Q. ilex* in Southern France, consistent with the resource matching hypothesis (Norton and Kelly 1988). Our study indicates that acorn production and stem growth in *Q. ilex* may be mainly driven by climate at the population level, consistent with the resource matching hypothesis. However, trade-offs between growth and reproduction occur at the level of the individual tree, with less stem growth in trees that allocate more resources to acorn production, consistent with the resource switching hypothesis (Kelly and Sork 2002; Monks and Kelly 2006; Sánchez-Humanes et al. 2011). Hence, resource switching and resource matching can co-occur in different periods and at different ecological levels, depending on resource availability. These findings highlight the importance of analyzing data at the individual level in examination of potential trade-offs, as suggested by Barringer et al. (2012).

Regarding the association of limited resources with trade-offs, growth in oaks is not limited by carbon availability even in mast years (Korner 2003). However, the costs of reproduction may be measured by considering photosynthesis, respiration, and resorption (Reekie and Bazzaz 1987; Ashman 1994; Sánchez-Humanes et al. 2011), which can be limited by water availability (Escudero et al. 1992; Sala and Tenhunen 1994; Mediavilla and Escudero 2003). Water shortage can cause premature abortion of fruits at the beginning of seed development (Larcher 2003; Carevic et al. 2010), thereby decreasing acorn production (Alejano et al. 2011). Stem growth is also highly dependent on water availability, especially during summer and autumn (Zhang and Romane 1991; Gutiérrez et al. 2011; Martín et al. 2014). Therefore, it is likely that the trade-offs we identified were related to intra-plant competition between stem growth and acorn production for water.

At the plot × year level, there were significant differences in stem growth and acorn production between the two plots, and covariance analyses showed that acorn production only correlated with annual stem growth in HR. HR had greater acorn production and lower stem growth than SB in all years, suggesting a preference in resource allocation to acorn production, especially in mast years. The HR plot has shallower and less developed soils and a slightly drier climate, with hotter summers and colder winters. There was also greater competition among trees in HR because of the twofold greater stand density. According to Reznick (1985), the costs of reproduction are greater in ecosystems with limited resource availability or other causes of stress. Thus, stress in the HR plot could increase resource allocation to acorn production, thereby ensuring successful reproduction and a greater density of seedlings (Kelly 1994). On the other hand, trees in HR were smaller than those in SB. Staudhammer et al. (2013) studied a tropical tree species and found that trade-offs between growth and reproduction decreased as trees increased in size and maturity; thus a phase in which there is a trade-off between growth and reproduction may slowly shift to a phase in which growth and reproduction are more independent. Acorn production was negatively correlated with late summer-autumn growth in both of our plots, and this suggests that in this more critical growth period (when acorns fatten) there were trade-offs in both plots.

Conclusions

1. During masting years, there were trade-offs between stem growth and acorn production at the level of the individual tree in *Q. ilex*.
2. The trade-offs between stem growth and acorn production occurred during late summer-autumn growth (the period of acorn fattening), but not during winter-spring growth. This result shows the importance of making intra-annual measurements of tree growth for appropriate interpretation of potential trade-offs.
3. The costs of reproduction differed between the two study sites. Trade-offs between stem growth and acorn production were greater in smaller trees that grew in conditions with greater pedological and climatic stress.

Acknowledgements

This work was supported by the Department of Innovation, Science and Business of the Regional Government of Andalusia, Spain [C03-192] and the Science and Education Ministry-National Institute of Food and Agriculture Research and Technology, Spain [SUM2006-00026-00-00]. We also thank Rocío Macías and Enrique Andivia for their help with the fieldwork.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Akaike H (1974) A new look at the statistical model identification. IEEE Trans Autom Control 19:716–723

Alejano R, Tapias R, Fernández M, Torres E, Alaejos E, Domingo J (2008) Influence of pruning and the climatic conditions on acorn production in holm oak (*Quercus ilex* L.) dehesas in SW Spain. Ann For Sci 65:209–217

Alejano R, Vázquez-Piqué J, Carevic F, Fernández M (2011) Do ecological and silvicultural factors influence acorn mass in Holm Oak (southwestern Spain)? Agrofor Syst 83:25–39

Ashman TL (1994) A dynamic perspective on the physiological cost of reproduction in plants. Am Nat 144:300–316

Barringer BC, Koenig WD, Knops JMH (2012) Interrelationships among life-history traits in three California oaks. Oecologia 171:129–139

Camarero JJ, Albuixech J, López-Lozano R, Casterad MA, Montserrat-Martí G (2010) An increase in canopy cover leads to masting in *Quercus ilex*. Trees 24:909–918

Carevic FS, Fernández M, Alejano R, Vázquez-Piqué J, Tapias R, Corral E, Domingo J (2010) Plant water relations and edapho-climatic conditions affecting acorn production in a holm oak (*Quercus ilex* L. ssp. *ballota*) open woodland. Agrofor Syst 78:299–308

Cook ER, Kairiukstis LA (1990) Methods of Dendrochronology. Applications in the Environmental Sciences. Kluwer Academic Publishers, Dordrecht

Cubera E, Moreno G (2007) Effect of land-use on soil water dynamics in dehesas of Central-Western Spain. Catena 71:298–308

Despland E, Houle G (1997) Climate influences on growth and reproduction of *Pinus banksiana* (Pinaceae) at the limit of the species distribution in eastern North America. Am J Bot 84:928–37

Escudero A, del Arco JM, Sanz IC, Ayala J (1992) Effects of leaf longevity and retranslocation efficiency on the retention time of nutrients in the leaf biomass of different wood species. Oecologia 90:80–87

Gea-Izquierdo G, Cherubini P, Cañellas I (2011) Tree-rings reflect the impact of climate change on *Quercus ilex* L. along a temperature gradient in Spain over the last 100 years. Forest Ecol Manage 262:1807–1816

Greenberg CH (2000) Individual variation in acorn production by five species of Southern Appalachian oaks. Forest Ecol Manage 132:199–210

Gutiérrez E, Campelo F, Camarero JJ, Ribas M, Muntán E, Nabais C, Freitas H (2011) Climate controls act at different scales on the seasonal pattern of *Quercus ilex* L. stem radial increments in NE Spain. Trees 25:637–646

Hoff C, Rambal S, Joffre R (2002) Simulating carbon and water flows and growth in a Mediterranean evergreen *Quercus ilex* coppice using the FOREST-BGC model. Forest Ecol Manage 164:121–136

IUSS Working Group WRB (2007) World Reference Base for Soil Resources 2006, first update 2007. World Soil Resources Reports No. 103. FAO, Rome

Janzen DH (1971) Seed predation by animals. Annu Rev Ecol Evol Syst 2:465–92

Keeland BD, Young PJ (2014) Installation of traditional dendrometer bands. U.S. Geological Survey. National Wetlands Research Center. http://www.nwrc.usgs.gov/topics/Dendrometer/. Accessed 23 January 2014

Kelly D (1994) The evolutionary ecology of mast seeding. Trends Ecol Evol 9:465–70

Kelly D, Sork VL (2002) Mast seeding in perennial plants: why, how, where? Annu Rev Ecol Syst 33:427–47

Knops JMH, Koenig WD, Carmen WJ (2007) Negative correlation does not imply a tradeoff between growth and reproduction in California oaks. Proc Natl Acad Sci USA 104:16982–16985

Knops JMH, Koenig WD (2012) Sex allocation in California oaks: trade-offs or resource tracking? PLoS ONE 7(8): e43492

Koenig WD, Knops JMH (1998) Scale of mast-seeding and tree-ring growth. Nature 396:225–226

Korner C (2003) Carbon limitation in trees. J Ecol 91:4–17

Larcher W (2003) Physiological plant ecology. Springer, Berlin

Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O (2006) SAS system for mixed models. SAS Institute, Cary

Martín D, Vázquez-Piqué J, Fernández M, Alejano R (2014) Effect of ecological factors on intra-annual stem girth increment of holm oak. Trees 28:1367–1381

Mediavilla S, Escudero A (2003) Stomatal responses to drought at a mediterranean site: a comparative study of co-ocurring woody species differing in leaf longevity. Tree Physiol 23:987–996

Monks A, Kelly D (2006) Testing the resource-matching hypothesis in the mast seeding tree *Nothofagus truncata* (Fagaceae). Austral Ecol 31:366–375

Mund M, Kutsch WL, Wirth C, Kahl T, Knohl A, Skomarkova MV, Schulze ED (2010) The influence of climate and fructification on the inter-annual variability of stem growth and net primary productivity in an old-growth, mixed beech forest. Tree Physiol. 30:689–704

Norton DA, Kelly D (1988) Mast seeding over 33 years by *Dacrydium cupressinum* Lamb. (rimu) (Podocarpaceae) in New Zealand: the importance of economies of scale. Funct Ecol 2:399–408

Obeso JR (2004) A hierarchical perspective in allocation to reproduction from whole plant to fruit and seed level. Perspect Plant Ecol Evol Syst 6:217–225

Patterson HD, Thompson R (1971) Recovery of inter-block information when block sizes are unequal. Biometrika 58**:**545–554

Pérez-Ramos IM, Ourcival JM, Limousin JM, Rambal S (2010) Mast seeding under increasing drought: results from a long-term dataset and from a rainfall exclusion experiment. Ecology 91:3057–3068

Quézel P, Médail F (2003) Ecologie et biogéographie des forêts du bassin méditerranéen. Elsevier, Paris

Reekie EG, Bazzaz FA (1987) Reproductive effort in plants. 1. Carbon allocation to reproduction. Am Nat 129: 876–896

Reznick R (1985) Cost of reproduction: An evaluation of the empirical evidence. Oikos 44:257–267

Rodríguez-Calcerrada J, Pérez-Ramos IM, Ourcival JM, Limousin JM, Joffre R, Rambal S (2011) Is selective thinning an adequate practice for adapting *Quercus ilex* coppices to climate change? Ann Forest Sci 68:575–585

Sala A, Tenhunen JD (1994) Site-specific water relations and stomatal response of *Quercus ilex* L. in a Mediterranean watershed. Tree Physiol 14:601–617

Sánchez-Humanes B, Sork VL, Espelta JM (2011) Trade-offs between vegetative growth and acorn production in *Quercus lobata* during a mast year: the relevance of crop size and hierarchical level within the canopy. Oecologia 166:101–110

Searle SR (1971) Linear Models. John Wiley & Sons, New York

Siscart D, Diego V, Lloret F (1999) Acorn ecology. In: Rodà F, Retana J, Gracia CA, Bellot J (eds) Ecology of Mediterranean evergreen oak forests. Springer-Verlag, Berlin, pp 75–86

Sork VL (1993) Evolutionary ecology of mast-seeding in temperate and tropical oaks (*Quercus* spp.). Plant Ecol 107–108:133–147

Staudhammer CL, Wadt LHO, Kainer AK (2013) Tradeoffs in basal area growth and reproduction shift over the lifetime of a long-lived tropical species. Oecologia 173:45–57

Stearns SC (1989) Trade-offs in life-history evolution. Funct Ecol 3:259–268

Zhang SH, Romane F (1991) Variations de la croissance radiale de *Quercus ilex* L. en fonction du climat. Ann Forest Sci 48:225–234

Artículo 4. Effect of pruning and soil treatments on stem growth of holm oak in open woodland forests

Artículo publicado como: Martín D, Vázquez-Piqué J, Alejano R (2015) Effect of pruning and soil treatments on stem growth of holm oak in open woodland forests. Agrofor Syst 89:599-609

Effect of pruning and soil treatments on stem growth of holm oak in open woodland forests

Abstract

The growth rate of forest trees indicates the rate of wood production and overall tree health. Tree growth also indicates resource distribution and the effect of ecological variables. Silvicultural treatments may improve the growth and resilience of trees growing in dehesas (Mediterranean open-woodland forests). These ecosystems are expected to experience increased temperature and decreased precipitation due to global climate change; hence their management is a key factor contributing to the adaption, and hence the conservation of these systems. In this paper, we analyzed the effect of traditional silvicultural treatments on the intra-annual stem growth of holm oak (*Quercus ilex* L.) in three dehesa plots in SW Spain: *(i)* soil treatments (ploughing, or ploughing + fertilization with calcium superphosphate + sowing with the legume yellow lupin, *Lupinus luteus*) and *(ii)* pruning intensity (heavy, moderate, or light). The soil treatments had no significant effects on growth, but pruning significantly affected growth patterns. Heavy pruning slightly reduced growth during the spring, and moderate and heavy pruning increased the normal stem contractions (due to water stress during drought) and expansions (due to rehydration after drought) in sites with poorly developed soils or other stress causes. Hence, heavy pruning could affect the vigor and vegetative status of trees in areas where tree survival is already compromised. Light pruning did not affect tree growth, so this treatment may be acceptable if the extraction of firewood or biomass is one of the management objectives.

Keywords: *Quercus ilex*, traditional management, pruning, fertilization, stem growth, dehesas

Introduction

Growth is an important biological parameter of forestry species because it indicates the amount of wood production and overall tree health. Growth is also an indicator of resource distribution, because it influences phenological processes and indicates response to ecological variables, such as climate and soil (Fritts 1976). Therefore, knowledge of tree growth processes and their ecological determinants is a key issue for planning sustainable management programs in the presence of global climate change.

Holm oak (*Quercus ilex* L.) is a widespread species in the Mediterranean Basin and covers more than 6.5 million ha in total (Quézel and Médail 2003), including 3 million ha in the Iberian Peninsula (Bravo et al. 2008). It is one of the dominant species in *"dehesas"*, traditional agroforestry systems composed of an open woodland forest (10-60 trees ha^{-1}) and an herbaceous layer (Cubera and Moreno 2007a). Dehesas are widely distributed in Spain and cover about 3 to 3.5 million ha (San Miguel 1994). Previous studies have shown that the stem growth of holm oak is strongly driven by climate, especially water availability (Campelo et al. 2009; Gea-Izquierdo et al. 2011; Martín et al. 2014). Improvement of soil and stand characteristics by certain management practices such as ploughing, fertilization or pruning, could increase the growth and resilience of holm oak in Mediterranean open woodland forests, which are expected to experience increased temperature and decreased precipitation in the coming years (IPCC 2007). However, it is unclear whether the traditional management practices applied in Mediterranean open woodland forests enhance or reduce tree growth.

Ploughing is one of the most common soil treatments traditionally performed in dehesas. Ploughing improves water infiltration and movement of organic matter and also removes woody shrubs and parched herbaceous plants, allowing the development of new pasture (Eichhorn et al. 2006). Nevertheless, in the Mediterranean area, ploughing also increases the vulnerability of soils to erosion and negatively influences tree regeneration because it removes oak seedlings and the shrub species that can protect them (Pulido and Díaz 2005). Fertilization is also commonly applied in dehesas to enhance pasture and crops production. N and P are the most limiting nutrients in Mediterranean ecosystems (Gallardo et al. 2009) so their addition is widely used in the Mediterranean area to enhance pasture production. On the other hand, because of soil acidity, dehesas respond positively to Ca fertilization (San Miguel 1994). Sowing of leguminous species, such as yellow lupin (*Lupinus luteus*) is also frequently applied because these species can be harvested as livestock feed (Serrano et al. 2010) and they fix atmospheric N_2. However, previous studies in diverse forests and open woodlands throughout the world have showed mixed and contradictory results of the response of tree growth to fertilization and atmospheric nitrogen fixation (e.g., McMaster et al. 1982; Cartan-Son et al. 1992; Scowcroft et al. 2007; Markewitz et al. 2012).

Pruning is a widespread multipurpose silvicultural practice applied to a large variety of tree species in distant regions (e.g., Pinkard and Beadle 1998; Balandier et al. 2000; Gyenge et al. 2010). Holm oak trees have traditionally been subjected to crown pruning for collection of fuelwood and improvement of acorn production (Alejano et al. 2011). Nevertheless, several researchers have questioned the benefits of pruning on acorn production (e.g., Cañellas et al. 2007; Alejano et al. 2008, 2011). This objection, along with the decreasing market value of fuelwood and the possibly negative influence of pruning on the spread of phytopathogenic agents involved in the “oak decline” of SW Spain (see Navarro 2011), have motivated reconsideration of the usefulness of pruning in holm oak. On the other hand, pruned trees are more sensitivity to water stress in dry regions (Gyenge et al. 2009). Hence, pruning could increase the vulnerability of holm oak to climate change, leading to a higher tree dieback.

Currently, the main management challenges of Mediterranean forest systems, and especially dehesas, is the survival and maintenance of productivity in the presence of oak decline, climate change and misuse of resources (Navarro 2011). In a former study (Martín et al. 2014), we studied the patterns of intra-annual stem growth of holm oak in open woodland forest and dense Mediterranean forests of SW Spain, and assessed the effects of climate, competition, tree size and microecological factors on stem increments. Now, the aim of the present study is to answer the following research questions:

i) Do the traditional soil management practices performed in open woodlands have a beneficial effect on the intra-annual stem growth of holm oak in this region?

ii) Do the pruning treatments have a detrimental effect on stem growth?

Our results will increase the basic scientific knowledge of Mediterranean open woodland forests and will therefore contribute to the development of methods for sustainable management based on established scientific criteria.

Materials and Methods

Field plots

This study was performed in three experimental plots in the Huelva province of SW Spain (Table 1). The Huerto Ramirez (HR) plot was in an open woodland of holm oak where sheep and Iberian pig were raised. Its soils had different degrees of development from acrisols, alisols and lixisols, to regosols and cambisols (IUSS Working Group WRB 2007). There was a sparse understory of mainly rock-rose (*Cistus ladanifer*) and curly rock-rose (*Cistus crispus*) and an abundant herbaceous layer of mainly grasses. The San Bartolomé (SB) plot was in an open woodland of holm oak where bulls were raised. Its soils were endoleptic regosols (episkeletic) or endoleptic luvisols (dystric) in depositional or concave areas (IUSS Working Group WRB 2007). There was a very scarce understory due to frequent tillage, and an abundant herbaceous layer of mainly grasses. The Calañas (CA) plot was in an open woodland of holm oak with a highly developed understory layer composed of rock-rose and Montpelier cistus (*Cistus monspeliensis*) that was mainly used for hunting and extensive sheep herding. Its soils had a low profile development and were classified as cambisols (dystric, chromic) and leptic regosols (IUSS Working Group WRB 2007). In CA symptoms of oak decline were detected in three trees during the study and dendrometer data of these trees were removed.

Plot	Coordinates (UTM. Zone 29)	Area (ha)	Density (trees ha^{-1})	Mean diameter ± SE (cm)	Mean height ± SE (m)
Huerto Ramírez (HR)	X:644288 m Y:4161376 m	2.94	73.0	30.0 ± 1.4	6.6 ± 0.2
San Bartolomé (SB)	X:669638 m Y:4145966 m	2.70	36.0	35.4 ± 1.3	6.5 ± 0.2
Calañas (CA)	X:681349 m Y:4156557 m	2.90	34.5	32.6 ± 1.9	6.1 ± 0.3

Table 1 Characteristics of the three study plots in dehesas of SW Spain. SE: standard error

Age of trees was very difficult to assess because it requires cutting trees to obtain cross sections for accurately estimate this data (Gea-Izquierdo et al. 2011). Even though we don’t have information for assessing the age structure of the plots, measurements in

30 sections in CA plot gave a result of an average age of 95 ± 12 years (oldest tree 113 years) and the measurement in 12 sections in HR plot gave a result of an average age of 110 ± 31 (oldest tree 149 years) (Natalini et al. 2013)

The climate of all three plots is Mediterranean, with highly variable temperature and rainfall within and among years. The mean annual temperature in the study period (2003-2011) was 17.1ºC in HR, 17.3ºC in SB and 17.7ºC in CA. Mean annual precipitation was 578 mm in HR, 655 mm in SB and 623 mm in CA (data from the Andalusian agroclimatic weather stations network). There were no large monthly variations in temperature across years, but there were large monthly and annual changes in precipitation. In particular, the annual precipitation in CA was only 306 mm during 2005, but was 1019 mm in SB during 2010. A more detailed description of the climate of this location is available in Martín et al. (2014).

Soil treatments

The HR plot was fenced and divided into 9 subplots, with a mean area of 3340 ± 305 m^2 and including 20-25 trees per subplot. Two soil treatments were applied randomly to 3 subplots each during autumn of 2005 and repeated during autumn of 2008: *(i)* ploughing (PL) or *(ii)* ploughing + fertilization + yellow lupin sowing (PFS). The other 3 subplots were used as controls. All ploughing was done with a disk harrow on a farm tractor. For the PFS treatment, ploughing was performed as in the PL treatment, and the soil was then fertilized with calcium superphosphate (P_2O_5 18%; CaO 28%; SO_3 25%) and sowed with yellow lupin using a mechanical seeder. Calcium superphosphate increases soil P without lowering pH, because CaO reduces the acidifying effect of phosphate. Yellow lupin provides soil N without the need for artificial fertilization. This species fixes atmospheric N_2, is well adapted to local conditions, and is traditionally grown in this area. A total of 300 kg ha^{-1} of fertilizer (54 kg ha^{-1} of P_2O_5, 84 kg ha^{-1} of CaO, 75 kg ha^{-1} of SO_3) and 60 kg ha^{-1} of seeds were used on each treatment date (100 kg of fertilizer and 20 kg of seeds in each PFS subplot, approximately). These particular treatments are traditional soil practices that have been frequently used in the dehesas of SW Spain. Control plots were progressively covered by an understory of shrubs, mainly rock-rose.

Pruning treatments

Trees in the CA and SB plots were subjected to traditional pruning at three different intensities (light, moderate, or heavy) or were left unpruned (controls). Pruning was performed in January 2001 in the CA plot and in February 2003 in the SB plot. All trees had been pruned every 6-7 years before we began our study. Pruning treatments were randomly assigned to trees at both sites. The pruning intensity was established as follows: light pruning involved removing sucker and dead branches only; heavy pruning, much stronger, removed up to 1/3 of the crown volume cutting branches thinner than 15 cm diameter and coincided with the usual practice in the area; and moderate pruning was in between the previous two. In order to ensure homogeneity in treatment intensity, each worker was assigned a single treatment type and supervised by a member of the research group. Once pruning was completed, pruning intensity was estimated based on the dry weight in kg of pruned branches (DW) and tree diameter in cm (D), as DW/D. A DW/D of 0.8 kg cm $^{-1}$ or less was considered light pruning, a DW/D greater than 1.7 kg cm^{-1} was considered heavy pruning, and intermediate DW/D values were considered moderate pruning. Details of the estimation of pruning intensities are available in Alejano et al. (2008).

Measurement of stem growth

A total of 119 aluminum band dendrometers (system developed by the University of Huelva) were installed at breast height (1.30 m), with care taken to avoid stem deformities. Details of band dendrometers theory and construction are available in Keeland and Young (2014). Trees were randomly sampled within each pruning or soil treatment. There were 55 trees in HR (18 control, 19 PL and 18 PFS), 32 trees in SB, and 32 trees in CA (8 control and 8 by pruning intensity in each plot). Measurements of every band dendrometer were recorded each month with a digital caliper (0.01 mm accuracy) from 2003-2010 in SB, from 2003-2006 in CA, and from 2006-2011 in HR. Because there were differences in measurement dates and in the number of days per month, average daily increments for each tree between the first day and the last day of each month were calculated. Girth increment data were not transformed into diameter increment because holm oak is a species with high within-tree variability in stem growth and then the stems were not enough cylindrical to assume diameter transformation. Hence, girth increment data of entire cross-sections were used instead of diameter increment. At the beginning of the study, the topographic location of each of the 119 trees was measured using a total station (Sokkia 3B).

Data analysis

We used two different models to analyze the effect of different pruning treatments on growth in the CA and SB plots and the effect of different soil treatments on growth in the HR plot. Each model was a linear mixed model in which we considered tree as a random effect and the following fixed effects: month, year, treatment (pruning or soil treatment), plot (only in the pruning experiment), and all interactions. Thus, the initial structure of the soil treatment model was:

$$y_{ijlm} = \mu + b_i + \alpha_j + \gamma_l + \tau_m + (\alpha \mid \gamma \mid \tau)_{jlm} + e_{ijlm} \qquad (1)$$

where y_{ijlm} is the girth increase (mm day^{-1}) of tree *i* in month *j* of year *l* under soil treatment *m*; μ is the general mean; b_i is a tree random effect with i = 1, 2,..., 55 under the hypothesis $b_i \sim$ N(0, **G**); α_j is a month fixed-effect with j = 1, 2,…, 12; γ_l is a year fixed-effect with l = 1, 2,..., 6; τ_m is a treatment fixed-effect with m = 1, 2, 3; $(\alpha|\gamma|\tau)_{jlm}$ is all possible double and triple interactions between fixed effects; and e_{ijlm} is the residual error under the hypothesis $e_{ijlm} \sim$ N(0, **R**).

The initial structure of the pruning treatment model is:

$$y_{iklmt} = \mu + b_{i(k)} + \alpha_k + \gamma_l + \tau_m + \lambda_t + (\alpha \mid \gamma \mid \tau \mid \lambda)_{klmt} + e_{iklmt} \qquad (2)$$

where y_{iklmt} is the girth increase (mm day^{-1}) of tree *i* at plot *k* in the month *l* of year *m* under pruning treatment *t*; μ is the general mean; $b_{i(k)}$ is a tree random effect within each plot with i = 1, 2,..., 32 and k = 1, 2 under the hypothesis $b_{i(k)} \sim$ N(0, **G**); α_k is a plot fixed-effect with k = 1, 2; γ_l is a month fixed-effect with l = 1, 2,..., 12; τ_m is a year fixed-effect with m = 1, 2,…, 8; λ_t is the treatment fixed effect with t = 1, 2, 3, 4; $(\alpha \mid \gamma \mid \tau \mid \lambda)_{klmt}$ is all possible double, triple and quadruple interactions between fixed effects; and e_{iklmt} is residual error under the hypothesis $e_{iklmt} \sim$ N(0, **R**).

The following procedure was used to select the best model structure:

1. The models were adjusted by consideration of tree random effect, the presence of temporal correlations between observations of different months for each tree and year, and the presence of heterogeneous variances in different months of the year. Hence, **G** was initially considered as a diagonal matrix and **R** as a block diagonal matrix, with each block corresponding to a 12 × 12 submatrix of observations for one year in each tree. We considered the following alternatives for the structure of blocks in the **R** matrix: autoregressive order 1, autoregressive heterogeneous, Toeplitz up to 5 bands, heterogeneous Toeplitz up to 5 bands, unstructured up to 5 bands, Huynh-Feldt, compound symmetry, compound symmetry heterogeneous, dependent covariance, and first-order factor analytic (Littell et al. 2006). Variance components for each structure were estimated by restricted maximum likelihood (REML) (Patterson and Thompson 1971) and model selection was based on the Akaike information criterion (AIC, Akaike 1974).

2. The significance of the tree random effect was determined by a likelihood ratio test, as the reduction of the statistic -2 x log likelihood (-2LL), after introducing the tree random effect, follows a χ^2 distribution with 1 degree of freedom. An α value of 0.05 was considered to indicate an improvement in the covariance structure.

3. If the tree random effect was significant, the presence of spatial correlation was determined. In particular, the following isotropic power covariance model was used:

$$\text{cov}\,(b_{i(j)}, b_{i'(j)}) = \sigma^2{}_b\,\rho^{d_{ii'}} \tag{3}$$

where $d_{ii'}$ is the distance between trees i and i' in location j; $\sigma^2{}_b$ is the variance component at tree level; and ρ is a parameter to be estimated with $|\rho| < 1$. The spatial covariance between observations at different locations in the pruning treatment model was considered zero.

4. After selection of the best variance–covariance structure, the fixed effects were estimated by a generalized least squares (GLS) equation (Searle 1971) and the significance of each effect was determined with an *F* test. The significance of all differences was determined with the Scheffe´ test. All statistical analysis was performed with SAS/ETS (ver. 9.2).

Results

Soil treatments

The best structure of the variance-covariance matrix for the 12 × 12 blocks of the **R** matrix was unstructured with 4 bands. This indicates that the variance of observations was different in different months (heterogeneous structure) and that there was a temporal correlation for groups in 4 consecutive months. The tree random effect was highly significant ($p < 0.0001$), but spatial covariance was not ($p = 0.934$), indicating the presence of significant growth differences among trees but that these differences cannot be explained by tree location within the plots.

The results of the soil treatment model indicates that there were no significant differences in growth among the different soil treatment groups or in any interaction in which soil treatment is included (Table 2).

Effect	*F* value	*Pr* > *F*
month	154.04	<0.0001
month x year	57.90	<0.0001
year	52.44	<0.0001
treatment	1.20	0.3018
treatment x month x year	1.11	0.2147
treatment x month	0.91	0.5819
treatment x year	0.49	0.8953

Table 2 Significance of fixed effects in the soil treatment model

Pruning treatments

The best structure of the variance-covariance matrix for the 12 × 12 blocks of the **R** matrix was unstructured with 2 bands. We found a highly significant tree effect ($p < 0.0001$), but could not determine the presence of spatial covariance due the lack of convergence.

The selected model indicated that pruning treatment was not significant, although there were significant treatment × month and treatment × month × plot interactions (Table 3). In other words, this model indicates that pruning is associated with significant differences in growth during some months, and that plots behave differently under some treatments during some months. These two interactions only account for a small amount of the total variance (Table 4), and in some months is even negative, indicating that variance does not decrease after the introduction of this interaction in some months. However, in July, October, and November the treatment × month × plot effect explained 1.09% to 3.94% of the residual variance, and in October the treatment × month effect explained 1% of the variance (Table 4).

Effect	*F* value	*Pr* > *F*
month	160.20	<0.0001
plot	122.78	<0.0001
year	98.65	<0.0001
year x month	56.84	<0.0001
year x plot	26.03	<0.0001
plot x month	22.89	<0.0001
plot x month x year	13.72	<0.0001
treatment x month	1.80	0.0034
treatment x month x plot	1.61	0.0151
treatment	1.39	0.2444
treatment x month x year x plot	0.98	0.5306
treatment x year x month	0.97	0.6157
treatment x year	0.91	0.5831
plot x treatment	0.82	0.4809
treatment x year x plot	0.42	0.9278

Table 3 Significance of fixed effects in the pruning treatment model

Month	Variation of variance (%)		
	treatment x month x plot	treatment x month	All fixed effects
Jan	-0.40	0.10	64.39
Feb	0.87	-0.97	53.27
Mar	-0.30	0.35	49.47
Apr	-0.15	0.41	70.99
May	-0.44	0.32	62.65
Jun	0.43	0.11	61.32
Jul	1.23	-0.28	70.70
Aug	-0.29	-0.26	81.29
Sep	0.00	0.24	76.87
Oct	3.94	1.00	74.18
Nov	1.09	-0.18	73.67
Dec	-0.30	-0.18	59.70

Table 4 Variation of variance in each month after entering significant interactions that include pruning treatments and percentage of variance accounted from all fixed effects (%). Positives values indicate a reduction of variance

Growth differences throughout the year were more evident in the CA plot (Fig. 1). For example, in July there was greater stem contraction in trees given heavy pruning (-0.030 mm day^{-1}) than in control trees (-0.019 mm day^{-1}) and in trees given light pruning (-0.017 mm day^{-1}). In September, when stems rapidly rehydrate, this trend was just the opposite being the growth greater in trees given heavy pruning (0.025 mm day^{-1}) than in those given light pruning (0.017 mm day^{-1}) or control trees (0.014 mm day^{-1}). The differences in growth rates of the three groups were greatest during October, when heavy pruning was associated with the greatest growth rate. These differences were maintained in November, although they were not as pronounced.

In the SB plot, the differences between treatments throughout the year were less evident (Fig. 2). In this plot, trees given heavy pruning generally had the slowest growth, and trees given moderate pruning had the greatest growth, although there were only significant differences in February, March, April, and September.

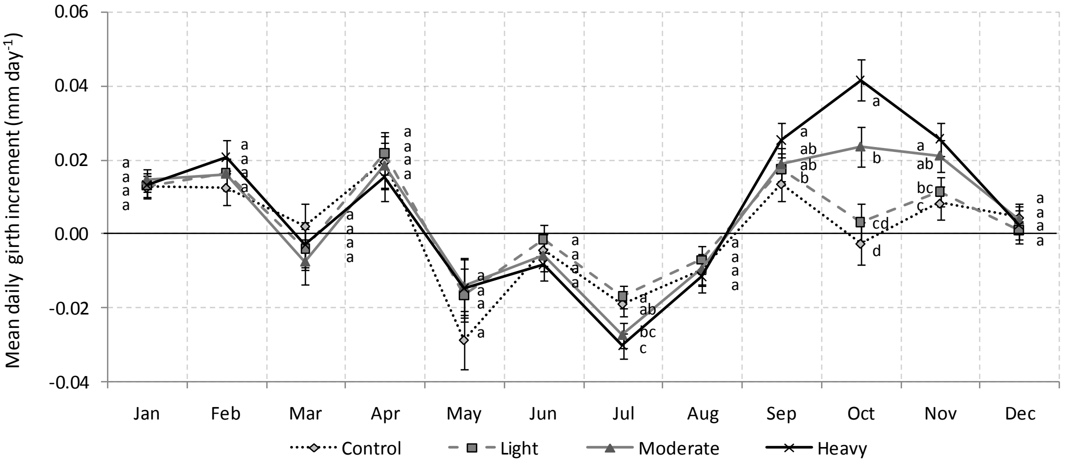

Fig. 1 Least squares mean estimates of daily girth increment (mm day^{-1} ± standard error) in the CA plot during different months for trees given different pruning treatments. Different letters indicate significant differences between treatments in a month (p < 0.05)

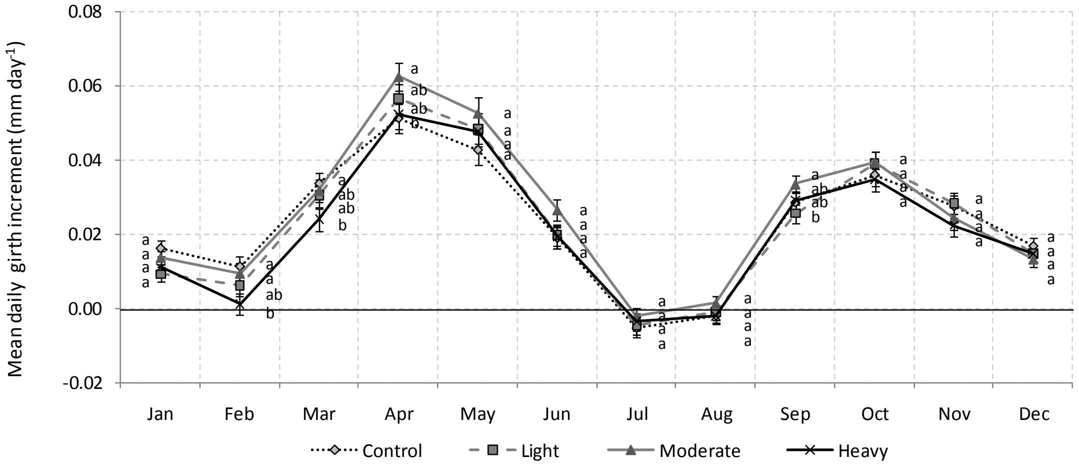

Fig. 2 Least squares mean estimates of daily girth increment (mm day^{-1} ± standard error) in the SB plot during different months for trees given different pruning treatments. Different letters indicate significant differences between treatments in a month (p < 0.05)

The lack of significance of the treatment × year effect, and of all interactions that include the treatment × year effect suggests that the effect of pruning on growth did not vary throughout the study period.

Discussion

Effect of soil treatments

Ploughing decreases competition from herbaceous plants and woody shrubs, improves water infiltration, and reduces short term compaction of the uppermost soil layer in heavy grazed areas (Coelho et al. 2004; Cubera and Moreno 2007a). Ploughing enhances stem and height growth of holm oak samplings (Sánchez-Andrés et al. 2006), which are highly dependent on soil features to survival (Gómez-Aparicio et al. 2008). In our study plots, soil treatments did not influence stem growth in holm oak, suggesting that water infiltration and shrub competition were not limiting factors for growth of adult trees. Rolo and Moreno (2011) reported that holm oak and rock-rose compete intensely for water and nutrients. Rock-rose can reduce the long-term survival of trees in densely shrub encroached stands, because it affects the water and nutritional status of trees, and has negative effects on stomatal conductance and photosynthesis. Furthermore, Moreno et al. (2007) found that shrub encroachment of dehesas reduced growth of holm oak shoots and reduced soil moisture. In our study, the similarity of growth in the ploughed and control plots may be explained by the scarcely developed shrub layer in our control plots, because treatments were carried out in 2006, but every plot was ploughed previously. On the other hand, because of the low density of trees in dehesas, holm oak does not use all available water in the soil (Cubera and Moreno 2007b). In shrub encroached dehesas, this water could be partly used by shrubs, because holm oak roots are very sparse (Moreno et al. 2005). Therefore, the low density of shrubs did not have a significant detrimental effect on holm oak growth in our plots. In addition, the existence of a low density shrub layer promotes tree regeneration in dehesas (Pulido and Díaz 2005), so ploughing cannot be recommended extensively in these open woodland forests.

Our other soil treatment (PFS) also had no significant effect on stem growth. Holm oak and other sclerophyllous species are adapted to low nutrient availability (Monk 1966), are highly efficient in nutrient use, and consequently have long-living tissues and low growth rates (Aerts 1995). Mayor and Rodà (1994) reported no positive effects of N and P fertilization on the stem growth of holm oak. These authors suggested that holm oak preferentially allocates increased soil nutrient resources to leaf and shoot production instead of stem growth. However, Moreno et al. (2007) reported that NPK fertilization did not improve the shoot growth of holm oak. On the other hand, trees cannot efficiently use added nutrients without sufficient irrigation or rainfall. Cartan-Son et al. (1992) reported that fertilization only had a positive effect on the stem growth of holm oak during years with significant rain. According to Landsberg (1986), drought can affect the uptake and translocation of nutrients and disrupt the response to fertilizer. Fertilization may even be detrimental in arid sites because it decreases water availability. Cubera and Moreno (2007a) found that fertilization reduced the availability of soil water in dehesas during spring and early summer. However, the analysis of the physiological status and water potential of fertilized holm oak trees in this plot indicated no significant differences between control and PFS plots (Carevic et al. 2010). This supports our finding that fertilization had no significant effect on stem growth. Fertilization is not widely used in forestry because it is very expensive, but its use to enhance pasture and crop production and quality in dehesas would not affect tree growth.

Sowing of yellow lupin also had no effect on tree growth. Cubera and Moreno (2007a) suggested that addition of N increases water use efficiency and photosynthesis of holm oak, but Rivest et al. (2011) found that the amount of N_2 fixed by leguminous plants in dehesas is very limited, and insufficient to compensate for the lack of this nutrient caused by pasture consumption. Yellow lupin has a shallow root system (Bramley et al. 2009), and holm oak is low dependent on resources of the uppermost soil layers (Moreno et al. 2007). Thus, the addition of N by growth of leguminous plants did not affect tree growth. On the other hand, recent phytopathology studies (Serrano et al. 2010) reported that yellow lupin can act as a vector of *Phytophthora cinnamomi*, one of the main agents responsible for “oak decline”. This serious pathogen is widespread at the regional scale, so planting of yellow lupin may have detrimental effects on holm oak in certain sites.

The lack of significance of spatial covariance indicates that tree location did not influence stem growth, suggesting that there were not better or worse locations within the plot for growth. The lack of significance of treatment x month, treatment x year, and treatment x month x year interactions, indicated that the effect of soil treatments on stem growth did not vary throughout the study period. Contrary, Cartan-Son et al. (1992) found a delayed positive effect of fertilization on the stem growth of holm oak to in a year with significant rain. In our study, we have repeated the application of the treatments every 3 years, and the study period comprised dry and wet years. Hence, our results suggested that there were not delayed or accumulative effects of fertilization on the growth of holm oak in our study site. Regarding the ploughing treatment, the lack of significance of this treatment throughout the whole study period could be explained by the slow shrub encroachment of control plots. Ramírez and Díaz (2008) found low covers of rock-rose in abandoned dehesas with less of 10 years of no grazing.

Effect of pruning

Pruning did not have a significant overall effect on stem growth in our plots, but it did have some significant effects during certain months (Figs. 1, 2).

Our results indicated that heavy pruning slightly decreased stem growth during the spring growth period. Previous studies on *Pinus ponderosa* (Gyenge et al. 2010) and *Acacia nilotica* (Siddiqui et al. 2010) indicated that pruning had a negative effect on diameter growth. The removal of a significant part of the crown disrupts the balance of the roots and crown and provokes the reallocation of resources to rebuild the aboveground biomass (Cañellas et al. 2007). This probably decreases the resources that would otherwise be used for stem growth. Intense pruning can also decrease total photosynthesis (Balandier et al. 2000) and carbon fixation of trees (Gyenge et al. 2010). Our observation of greater stem contractions in heavy pruned trees during the summer drought in CA plot is possible related to a greater response of pruned trees to water stress.

In addition, heavy pruning can interrupt the normal translocation of water and nutrients (Dagit and Downer 2002), exacerbating the effects of water stress (Jackson et al. 2000; Gyenge et al. 2009). From an ecophysiological standpoint, the foliar area/sapwood ratio of a tree depends on water availability, and pruning can imbalance the hydraulic system at the level of the whole plant (Gyenge et al. 2010). According to Dagit and Downer (2002), heavy pruning may also negatively affect the roots, because it may deprive them of nutrients normally synthesized in the leaves. Water recycling of heavily pruned trees then becomes less efficient, adversely affecting the feedback between roots and crown (Ringgenberg 2001). Trees in CA could be additionally stressed by shallow and poorly developed soils, and even by an overall influence of oak decline (Brasier

1995), increasing therefore the sensitivity of pruned trees, despites the trees included in the study did not show visible symptoms during the study period.

Holm oak rehydrate quickly after the first rain events following the summer drought, provoking water stem expansions followed by true stem growth (Campelo et al. 2007; Gutiérrez et al. 2011; Martín et al. 2014). Hence, the greater stem increments of moderately pruned and heavily pruned trees in the CA plot in autumn after the marked summer stem contractions (Fig. 1) could be explained by the greater sensitivity of pruned trees to climate (Balandier et al. 2000; Gyenge et al. 2010), leading to changes in intra-annual growth patterns, with lower temporal autocorrelation among months. On the contrary, in the SB plot, where soils were more developed and oak decline was no detected , no significant stem contractions occurred during the summer and there were no significant differences between pruned and control trees during the autumn (Fig. 2).

Tree growth following moderate pruning was slightly greater during spring at the SB plot (Fig. 2), indicating that moderate pruning had no negative effect on growth in an area with better soils and good sanitary status (Pinkard and Beadle 1998). Growth of lightly pruned trees at the same plot was not significantly different from unpruned (control) trees, indicating that removal of small, suppressed, or dead branches did not affect the photosynthetic capacity or hydraulic balance of these trees (Gyenge et al. 2010).

The effects of the pruning treatments remained throughout the study period, as showed the lack of significance of the treatment × year effect, and of all interactions that include the treatment × year effect. According to Pinkard and Beadle (1998), the effects of pruning on growth are related to the growth rates of the species. The slower the growth rate, the longer will be the period to crown restoration following pruning. Holm oak is a slow-growing species and this could explain the long-lasting effects of the pruning treatments, despite they were applied only once before the beginning of the study.

Conclusions

Soil treatments had no effect on stem growth of holm oak. These treatments mainly alter the upper layer of soil, where holm oak roots are sparse. Fertilization and atmospheric nitrogen fixation by yellow lupin also had no significant effect on stem growth. Thus, its use to enhance pasture and crop production and quality would not affect tree growth.

Pruning affected the stem growth of holm oak during some months (February, March, April, July, September, October, and November), but depending on plot features. Heavy pruning slightly reduced growth during the spring, and moderate and heavy pruning increased the normal stem contractions (due to water stress during drought) and expansions (due to rehydration after drought) in sites with poorly developed soils or other stress causes. Hence, heavy pruning could affect the vigor and vegetative status of trees in areas where tree survival is already compromised. Light pruning did not affect tree growth, so this treatment may be acceptable if the extraction of fuelwood or biomass is one of the management objectives.

Acknowledgments

This study was sponsored by the Department of Innovation, Science and Business of the Regional Government of Andalusia, Spain (ref: C03-192) and by MEC-INIA, Spain (ref: SUM2006-00026-00-00).

References

Aerts R (1995) The advantages of being evergreen. Trends Ecol Evol 10:402–407

Akaike H (1974) A new look at the statistical model identification. IEEE Trans Autom Control 19:716–723

Alejano R, Tapias R, Fernández M, Torres E, Alaejos J, Domingo J (2008) Influence of pruning and the climatic conditions on acorn production in Holm oak (*Quercus ilex* L.) dehesas in SW Spain. Ann For Sci 65:209–217

Alejano R, Vázquez-Piqué J, Carevic F, Fernández M (2011) Do ecological and silvicultural factors influence acorn mass in Holm Oak (southwestern Spain)? Agroforest Syst 83:25–39

Balandier P, Lacointe A, Le Roux X, Sinoquet H, Cruiziat P, Le Dizès S (2000) “SIMWAL”: a structure – function model simulating single walnut tree growth according to climate and pruning. Ann For Sci 57:571–585

Bramley H, Turner NC, Turner DW, Tyerman SD (2009) Roles of morphology, anatomy, and aquaporins in determining contrasting hydraulic behavior of roots. Plant Physiol 150:348–364

Brasier CM (1995) *Phytophthora cinnamomi* and oak decline in southern Europe. Environmental constraints including climate change. Ann For Sci 53:347–358

Bravo JA, Roig S, Serrada R (2008) Selvicultura en montes bajos y medios de *Quercus ilex* L., *Quercus pyrenaica* Willd. y *Quercus faginea* Lam. In: Serrada R, Montero G, Reque JA (eds) Compendio de Selvicultura aplicada en España. INIA, Madrid, pp 657-744

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

Campelo F, Nabais C, García-González I, Cherubini P, Gutiérrez E, Freitas H (2009) Dendrochronology of *Quercus ilex* L. and its potential use for climate reconstruction in the Mediterranean region. Can J For Res 39:2486–2493

Cañellas I, Roig S, Poblaciones MJ, Gea-Izquierdo G, Olea L (2007) An approach to acorn production in Iberian dehesas. Agroforest Syst 70:3–9

Carevic FS, Fernández M, Alejano R, Vázquez-Piqué J, Tapias R, Corral E, Domingo J (2010) Plant water relations and edapho-climatic conditions affecting acorn production in a holm oak (*Quercus ilex* L. ssp. *ballota*) open woodland. Agroforest Syst 78:299–308

Cartan-Son M, Floret C, Galan, MJ, Grandjanny M, Le Floc’h E, Maistre M, Perret P, Romane F (1992) Factors affecting radial growth of *Quercus ilex* L. in a coppice stand in southern France. Vegetatio 99–100:61–68

Coelho COA, Ferreira AJD, Laouina A, Hamza A, Chaker M, Naafa R, Regaya K, Boulet AK, Keizer JJ, Carvalho TMM (2004) Changes in land use and land management practices affecting land degradation within forest and grazing ecosystems in the Western Mediterranean. In: Schnabel S, Ferreira A (eds) Advances in GeoEcology 37: Sustainability of Agrosilvopastoral Systems—Dehesas, Montados. Catena Verlag, Reiskirchen, pp 137–153

Cubera E, Moreno G (2007a) Effect of land-use on soil water dynamics in dehesas of Central-Western Spain. Catena 71:298–308

Cubera E, Moreno G (2007b) Effect of single *Quercus ilex* trees upon spatial and seasonal changes in soil water content in dehesas of central western Spain. Ann For Sci 64:355–364

Dagit R, Downer J (2002) To Prune or Not to Prune: Responses of Coast Live Oaks (*Quercus agrifolia*) to Canopy Retention during Transplanting. USDA Forest Service. http://www.californiaoaks.org/ExtAssets/RosiDagitTransplantingOaks.pdf. Accessed 20 August 2011

Eichhorn MP, Paris P, Herzog F, Incoll LD, Liagre F, Mantzanas K, Mayus M, Moreno G, Papanastasis VP, Pilbeam DJ, Pisanelli A, Dupraz C (2006) Silvoarable systems in Europe — past, present and future prospects. Agroforest Syst 67:29–50

Fritts HC (1976) Tree rings and climate. Academic Press, London

Gallardo A, Covelo F, Morillas L, Delgado M (2009) Ciclos de nutrientes y procesos edáficos en los ecosistemas terrestres: especificidades del caso mediterráneo y sus implicaciones para las relaciones suelo-planta. Ecosistemas 18:4–19

Gea-Izquierdo G, Cherubini P, Cañellas I (2011) Tree-rings reflect the impact of climate change on *Quercus ilex* L. along a temperature gradient in Spain over the last 100 years. Forest Ecol Manage 262:1807–1816

Gómez-Aparicio L, Pérez-Ramos IM, Mendoza I, Quero JL, Matías L, Castro J, Zamora R, Marañón T (2008) Oak seedling survival and growth along resource gradients in Mediterranean forests: implications for regeneration under current and future environmental scenarios. Oikos 117:1683–1699

Gutiérrez E, Campelo F, Camarero JJ, Ribas M, Muntán E, Nabais C, Freitas H (2011) Climate controls act at different scales on the seasonal pattern of *Quercus ilex* L. stem radial increments in NE Spain. Trees 25:637–646

Gyenge JE, Fernández ME, Schlichter TM (2009) Effect of pruning on branch production and water relations in widely spaced ponderosa pines. Agroforest Syst 77:223–235

Gyenge JE, Fernández ME, Schlichter TM (2010) Effect of stand density and pruning on growth of ponderosa pines in NW Patagonia, Argentina. Agroforest Syst 78:233–241

IPCC (2007) Climate change 2007: synthesis report. In: Core Writing Team, Pachauri RK, Reisinger A (eds) Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, pp 104

IUSS Working Group WRB (2007) World Reference Base for Soil Resources 2006, first update 2007, World Soil Resources Reports No. 103. FAO, Rome

Jackson NA, Wallace JS, Ong CK (2000) Tree pruning as a means of controlling water use in an agroforestry system in Kenia. For Ecol Manag 126:133–148

Keeland BD, Young PJ (2014) Installation of traditional dendrometer bands. U.S. Geological Survey. National Wetlands Research Center. http://www.nwrc.usgs.gov/topics/Dendrometer/. Accessed 23 January 2014

Landsberg JJ (1986) Physiological ecology of forest production. Academic Press, New York

Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O (2006) SAS system for mixed models. SAS Institute, Cary

Markewitz D, Figueiredo RDO, Reis de Carvalho CJ, Davidson EA (2012) Soil and tree response to P fertilization in a secondary tropical forest supported by an Oxisol. Biol Fertil Soils 48:665–678

Martín D, Vázquez-Piqué J, Fernández M, Alejano R (2014) Effect of ecological factors on intra-annual stem girth increment of holm oak. Trees 28:1367–1381

Mayor X, Rodà F (1994) Effects of irrigation and fertilization on stem diameter growth in a Mediterranean holm oak forest. For Ecol Manag 68:119–126

McMaster GS, Jow WM, Kummerow J (1982) Response of *Adenostoma fasciculatum* and *Ceanothus greggii* Chaparral to Nutrient Additions. J Ecol 70:745–756

Monk CD (1966) An ecological significance of evergreenness. Ecology 47:504–505

Moreno G, Obrador JJ, Cubera E, Dupraz C (2005) Fine root distribution in dehesas of Central–Western Spain. Plant Soil 277:153–162

Moreno G, Obrador JJ, García E, Cubera E, Montero MJ, Pulido FJ, Dupraz C (2007) Driving competitive and facilitative interactions in oak dehesas with management practices. Agroforest Syst 70:25–40

Natalini F, Alejano R, Vázquez-Piqué FJ, Cañellas I, Gea-Izquierdo G (2013) Growth trends and sensitivity to climate of declining Mediterranean open woodlands exhibiting widespread mortality in Southern Spain. Second American Dendrochronology Conference Ameridendron. Tucson, Arizona.

Navarro R (2011) Situación actual de las dehesas. In: Alejano R, Domingo JM, Fernández M (eds) Manual para la gestión sostenible de las dehesas andaluzas. Foro Encinal - Universidad de Huelva, Huelva, pp 62–68

Patterson HD, Thompson R (1971) Recovery of inter-block information when block sizes are unequal. Biometrika 58:545–554

Pinkard EA, Beadle CL (1998) Effects of green pruning on growth and stem shape of *Eucalyptus nitens* Deane and Maiden (Maiden). New Forest 15:107–126

Pulido FJ, Díaz M (2005) Regeneration of a Mediterranean oak: a whole cycle approach. EcoScience 12:92–102

Quézel P, Médail F (2003) Ecologie et biogéographie des forêts du bassin méditerranéen. Elsevier, Paris

Ramírez JA, Díaz M (2008) The role of temporal shrub encroachment for the maintenance of Spanish holm oak *Quercus ilex* dehesas. For Ecol Manag 255:1976–1983

Ringgenberg S (2001) Flow in the phloem. Arborist News 10:21–26

Rivest D, Rolo V, López-Díaz ML, Moreno G (2011) Belowground competition for nutrients in shrub-encroached Mediterranean dehesas. Nutr Cycl Agroecosys 90:347–354

Rolo V, Moreno G (2011) Shrub species affect distinctively the functioning of scattered *Quercus ilex* trees in Mediterranean open woodlands. For Ecol Manag 261:1750–1759

San Miguel A (1994) La dehesa española: origen, tipología, características y gestión. Fundación Conde del Valle de Salazar, Madrid

Sánchez-Andrés R, Sánchez-Carrillo S, Benítez M, Sánchez-López A (2006) Tillage induced differential morphometric responses and growth patterns in afforestation with *Quercus ilex*. Soil Till Res 90:50–62

Scowcroft PG, Friday JB, Idol T, Dudley N, Haraguchi J, Meason D (2007) Growth response of *Acacia koa* trees to thinning, grass control, and phosphorus fertilization in a secondary forest in Hawai‘i. Forest Ecol Manage 239:69–80

Searle SR (1971) Linear Models. John Wiley & Sons, New York

Serrano MS, Fernández P, De Vita P, Carbonero MD, Trapero A, Sánchez ME (2010) *Lupinus luteus*, a new host of *Phytophthora cinnamomi* in Spanish oak-rangelands ecosystems. Eur J Plant Pathol 128:149–152

Siddiqui T, Farrakh-Nawaz M, Ahmed I (2010) Effect of different pruning intensities on the growth of *Acacia nilotica* (Kikar). Agrociencia 44:93–97

Apéndice 2. Índice de Impacto de los artículos publicados

Artículo 1.

Autores: Martín D, Vázquez-Piqué J, Fernández M, Alejano R.

Título: Effect of ecological factors on intra-annual stem girth increment of holm oak.

Referencia completa: Trees 28:1367-1381. DOI 10.1007/s00468-014-1041-y.

Año: 2014.

ISSN: 0931-1890.

Índice de Impacto 2014 (Thomson Reuters Journal Citation Reports® 2015): 1.651.

Área y posición: Forestry, 18/65.

Artículo 3.

Autores: Martín D, Vázquez-Piqué J, Carevic FS, Fernández M, Alejano R.

Título: Trade-off between stem growth and acorn production in holm oak.

Referencia completa: Trees 29:825-834. DOI 10.1007/s00468-015-1162-y.

Año: 2015.

ISSN: 0931-1890.

Índice de Impacto 2014 (Thomson Reuters Journal Citation Reports® 2015): 1.651.

Área y posición: Forestry, 18/65.

Artículo 4.

Autores: Martín D, Vázquez-Piqué J, Alejano R.

Título: Effect of pruning and soil treatments on stem growth of holm oak in open woodland forests.

Referencia completa: Agroforest Syst 89:599-609. DOI 10.1007/s10457-015-9794-x.

Año: 2015.

ISSN: 0167-4366.

Índice de Impacto 2014 (Thomson Reuters Journal Citation Reports® 2015): 1.215.

Área y posición: Forestry, 29/65.