Universitat de Lleida

Relationships between primary and secondary growth in two Mediterranean Quercus species

Arben Q. Alla

Dipòsit Legal: L.1218-2012

http://hdl.handle.net/10803/94497

RELATIONSHIPS BETWEEN PRIMARY AND SECONDARY GROWTH IN TWO MEDITERRANEAN QUERCUS SPECIES

Arben Q. ALLA

Doctoral dissertation

IPE
INSTITUTO PIRENAICO DE ECOLOGÍA
CSIC

Universitat de Lleida

RELATIONSHIPS BETWEEN PRIMARY AND SECONDARY GROWTH IN TWO MEDITERRANEAN *QUERCUS* SPECIES

A PhD Proposal submitted to the Department of Crop and Forest Sciences
University of Lleida

Presented by
Arben Q. ALLA

Supervisors
Dr. Jesús Julio CAMARERO MARTÍNEZ
ARAID, Dpt. Conservation of Biodiversity and Ecosystem Restoration
Pyrenean Institute of Ecology (IPE-CSIC), Saragossa, Spain

Dr. Gabriel MONTSERRAT-MARTÍ
Dpt. Conservation of Biodiversity and Ecosystem Restoration
Pyrenean Institute of Ecology (IPE-CSIC), Saragossa, Spain

Tutor
Jordi VOLTAS VELASCO
Dpt. of Crop and Forest Sciences
University of Lleida, Lleida, Spain

May 2012

Prindërve të mi

ACKNOWLEDGEMENTS

I would like to express my gratefulness to all of the persons and institutions that contributed in the realization of this thesis. I am thankful to the *Spanish Agency for International Development Cooperation* (MAEC-AECID) for the financial funding and the *Pyrenean Institute of Ecology* (IPE-CSIC) for the technical support.

My sincere appreciation goes to my supervisors, Dr. Jesús Julio Camarero Martínez and Dr. Gabriel Montserrat Martí for all the professional and personal experiences, for their reliable advices, patience and dedication to teach and inspire me all along this period.

I am very grateful to Dr. Jordi Voltas Velasco, who facilitated me the administrative processes with the University of Lleida.

Many thanks to Dr. Melchor Maestro-Martínez and Dr. Sara Palacio for their kind help with the chemical analyses and improving the scientific papers.

I would like to thank all scientists, students, technicians and administration of IPE-CSIC for their all-kind-of-help, influencing directly or indirectly in the progress of this work.

Special thanks to Elena, Mondi, Jorge, Gabriel-ito, Diego, Victoria, Angela, Robin, Yacine, Ahmed, Pedro, for their unconditional support and encourage, making my stay in Saragossa very pleasant and feel like at home.

My endless thanks to my family for everything they have done giving me the possibility to go so far in my professional formation. Thanks to my parents and sisters for their willingness, efforts and patience to inspire me. Thanks to my beloved wife for her constant attendance and interest in all my problems, for the interminable patience, support, encouragement, affection and wisdom advices.

To all of you:

Many thanks, muchísimas gracias, moltíssimes gràcies, shumë faleminderit, tanemmirt atas atas!!!

INDEX

ABSTRACT

This study evaluated the relationships between primary and secondary growth in two coexisting Mediterranean *Quercus* species (*Q. ilex*, *Q. faginea*) during several years of contrasted climatic conditions. These relationships were investigated by performing several types of measurements (stem length and diameter, leaf area, acorn and apical bud mass, length and number of buds, radial growth, earlywood anatomy) and analyses (statistical: correlation, allometries, structural equation models, generalized linear mixed models; evaluations of nitrogen and carbohydrates concentrations in wood and leaves). It was found that the *Q. faginea* xylem responses (tree-ring width, earlywood anatomy) to climatic variability was plastic along a climatic gradient, particularly in xeric sites, which may confer this species a great ability to withstand the forecasted warmer and drier conditions. Secondary growth is linked to bud size through allometric relationships modulated by climate. Acorn production also depends on shoot secondary growth since in acorn-bearing shoots the stem cross-sectional area increased, whereas the total transversal area occupied by vessels decreased toward the apex. The maximum growth rate of buds was reached between late July and mid-August in both species and was positively affected by minimum air temperatures. The longer buds were mainly apical and presented a higher probability to burst than the shorter ones which were mostly axillary ones, although no size threshold was found below which bud bursting was impaired. Considering the fact that the different plant structures demonstrate distinct climatic thresholds for their development, the information concerning the temporal arrangement of phenophases becomes crucial for the interpretation of the plant functional responses.

Key words: primary growth, secondary growth, bud development, bud demography, bud size, acorns, current-year shoot, *Quercus ilex* subsp. *ballota*, *Quercus faginea*, Mediterranean climate.

RESUMEN

Relaciones entre los crecimientos primario y secundario en dos especies de *Quercus* mediterráneos.

Se estudiaron las relaciones entre los crecimientos primario y secundario en dos especies de *Quercus* mediterráneas (*Q. ilex*, *Q. faginea*) que coexisten durante varios años de características climáticas contrastadas. Se analizaron dichas relaciones por medio de la realización de diversos tipos de medidas (longitud y diámetro de tallos, área foliar, peso seco de bellotas y yemas apicales, número y longitud de yemas, crecimiento radial y anatomía de la madera temprana) y análisis (estadísticos: correlación, alometrías, modelos de ecuaciones estructurales, modelos lineares mixtos generalizados; evaluación de las concentraciones de nitrógeno y carbohidratos en la madera y hojas). Resulto que la respuesta del xilema (anchura del anillo, anatomía de la madera temprana) de *Q. faginea* a la variabilidad climática era plástica a lo largo de un gradiente climático, particularmente en el sitio xerico, que le puede otorgar a esta especie una gran abilidad para resistir a las condiciones calidas y secas predecidas. El crecimiento secundario está vinculado al tamaño de la yema por medio de relaciones alométricas moduladas por el clima. La producción de bellotas también dependió del crecimiento secundario de los tallos del año siendo que en los tallos portadores de bellota la sección transversal del tallo aumentó, mientras que el área transversal total ocupada por vasos disminuyó hacia el apice. La máxima tasa de crecimiento de las yemas se alcanzó en ambas especies entre el final de julio y la mitad de agosto y le afectó positivamente la temperatura mínima del aire. Las yemas más largas eran mayoriamente yemas apicales mostraron una mayor probabilidad de brotar que las más cortas que eran principalmente yemas axilares, aunque no se encontró ningún umbral de tamaño por debajo del cual la brotación resultara imposibilitada. Si consideramos que el desarrollo de las distintas estructuras de la planta presenta diferentes umbrales climáticos, la información sobre la organización temporal de los distintos eventos fenológicos (fenofases) resulta esencial para interpretar las respuestas funcionales de las plantas.

Palabras clave: crecimiento primario, crecimiento secundario, desarrollo de las yemas, demografía de las yemas, tamaño de las yemas, bellotas, brotes del año, *Quercus ilex* subsp. *ballota*, *Quercus faginea,* clima mediterráneo.

RESUM

Relacions entre els creixements primari i secundari en dues espècies de *Quercus* mediterranis.

Es van estudiar les relacions entre els creixements primari i secundari en dues espècies de *Quercus* mediterranis que coexisteixen (*Q. ilex, Q. faginea*) en una mateixa localitat durant varis anys de característiques climàtiques contrastades. Es van analitzar aquestes relacions per mitjà de la realització de diversos tipus de mesures (longitud i diàmetre de branques, àrea foliar, pes sec d’aglans i gemes apicals, número i longitud de gemes, creixement radial i anatomia de la fusta de primavera) i anàlisis (estadístics: correlació, al·lometries, models d’equacions estructurals, models lineals mixtes generalitzats; avaluacions de les concentracions de nitrogen i carbohidrats en fusta i fulles). Les respostes del xilema (amplària d’anell, anatomia de la fusta primerenca) de *Q. faginea* a la variabilitat climàtica va ser plàstica al llarg d’un gradient climàtic, particularment en llocs xèrics, la qual cosa pot conferir a aquestes espècies una gran habilitat per a resistir les condicions més càlides i seques pronosticades. El creixement secundari està vinculat a la grandària de les gemes per mitjà de relacions al·lomètriques, modulades pel clima. La producció d’aglans també va dependre del creixement secundari de les branques ja que en branques amb aglans l’àrea transversal de la tija va incrementar, mentre que l’àrea transversal total ocupada per vasos va decréixer cap a l’àpex. La màxima taxa de creixement de les gemes es va assolir en ambdues espècies entre finals de juliol i meitat d’agost, afectant-li positivament la temperatura mínima de l’aire. Les gemes més llargues van ser principalment apicals i van mostrar una major probabilitat de brollar que les més curtes que van ser majoritàriament axil·lars, encara que no es va trobar cap llindar de grandària per sota del qual la brotada esdevingués impossibilitada. Si considerem que el desenvolupament de les diferents estructures de la planta presenta diferents llindars climàtics, la informació sobre la organització temporal dels diferents events fenològics (fenofases) resulta essencial per interpretar les respostes funcionals de les plantes.

Paraules clau: creixement primari, creixement secundari, desenvolupament de les gemes, demografia de les gemes, tamany de les gemes, aglans, brots de l’any, *Quercus ilex* subsp. *ballota, Quercus faginea,* clima mediterrani.

PËRMBLEDHJE

Ndërveprimi midis rritjes parësore dhe dytësore në dy lloje dushqesh mesdhetarë

Ky studim vlerësoi ndërveprimin midis rritjes parësore dhe dytësore në dy lloje bashkërritëse dushqesh mesdhetarë (*Q. ilex*, *Q. faginea*) gjatë një periudhe të karakterizuar nga kushte klimatike të kontrastuara. Këto varësi u studiuan nëpërmjet kryerjes së matjeve (gjatësia dhe diametri i biskut, sipërfaqja gjethore, pesha e lendeve dhe sytheve te majës, gjatësia dhe numri i sytheve, rritja radiale, anatomia e drurit të hershëm) dhe analizave të ndryshme (statistikore: korrelacione, alometri, modelet e ekuacioneve strukturalë, modelet lineare miks të përgjithësuar; vlerësimi i përqëndrimeve të azotit dhe karbohidrateve në dru dhe gjetheve). Në *Q. faginea* rezultoi që përgjigja e ksilemës (gjerësia e rrethir vjetor, anatomia e drurit të hershëm) ndaj ndryshueshmërisë klimatike përgjatë një gradienti klimatik ishte plastike, veçanërisht në zonën kserike, që mund ti lejojë këtij lloji dushku një cilësi të lartë përshtatje ndaj kushteve të nxehta dhe të thata të parashikuara. Rritja dytësore është e lidhur me madhësinë e sythit nëpërmjet ndërveprimeve alometrike të cilat ndryshojnë në varësi të klimës. Prodhimi i lendeve gjithashtu varet nga rritja dytësore e bisqeve duke qenë se në bisqet me lende sipërfaqja tërthore e biskut u rrit, ndërsa sipërfaqja totale tërthore e enëve përçuese u zvogëlua në drejtim të majës. Maksimumi i shkallës së rritjes së sytheve u arrit midis fundit të korrikut dhe mezit të gushtit në të dyja llojet dhe u ndikua pozitivisht nga temperaturat minimale. Sythet më të gjatë ishin kryesisht ata të majës të cilët shfaqën një mundësi më të lartë çelje se sa më të shkurtërit që përgjithësisht ishin sythe ndihmës, megjithëse nuk u vu re kufi madhësie nën te cilin çelja e sytheve të mos kryej. Duke patur parasysh faktin që elementët e ndryshëm të bimës shfaqin kufij të ndryshëm klimatikë përsa i përket zhvillimit të tyre, informacioni i lidhur me përputhshmërinë kohore të fenofazave marrin një rëndësi të veçantë në shpjegimin e reagimeve funksionale të bimëve.

Fjalë kyçe: rritja parësore, rritja dytësore, zhvillimi i sytheve, popullsia e sytheve, madhësia e sytheve, lendet, bisqet vjetorë, *Quercus ilex* subsp. *ballota*, *Quercus faginea*, klima mesdhetare.

1. GENERAL INTRODUCTION

1. 1. Tree growth

The performance of trees depends greatly on their ability to develop primary and secondary growth, and both of them are constrained by many factors like tree variables (size, age), site conditions, climatic stresses, the availability of resources, etc. Primary growth is the extension of shoots and roots (Barthélémy and Caraglio 2007). Shoots have several mechanical and physiological functions: they exploit available space and allow leaves exchanging gases and performing photosynthesis, they bear flowers and fruits, and they support other shoots that allow crowns to expand (Takenaka 1997). On the other hand, secondary or radial growth of woody stems is essential to supply mechanical strength for supporting an aerial canopy, for storing carbohydrates and for hydraulic transport of water to the foliage (Bret-Harte et al. 2002).

Several studies have analysed the phenology and the seasonal changes of the canopy organs (Montserrat-Martí et al. 2009; Limousin et al. 2011). Phenomorphological studies deal with the annual and seasonal temporal changes in the crown organs (buds, shoots, leaves, flowers, fruits) related to climatic conditions and plant architecture, i.e. phenomorphology studies where and when each part of the plant originates and starts growing, how long it grows and what happens to it when it stops growing (Orshan 1989). However, the phenomorphological approach does not take into account the processes of the plant organs development and how their changing sizes are related among them or respond to climatic variables. The interactions among the structural components of the current-year shoots and the main tree stem along different seasons as related to crown development have been scarcely studied despite the understanding of the ecological significance of tree development is not fulfilled. The aim of the present thesis was to study with a phenomorphological basis, how primary and secondary growth interacts and drive the crown and stem development as related to climate in two Mediterranean oak species with contrasting leaf habit: *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. (evergreen species; hereafter abbreviated as *Q. ilex*) and *Q. faginea* Lam. (winter deciduous species). To fulfil this objective several variables (length, diameter, mass, amount, tree-ring width, earlywood anatomical features such as transversal vessel area and density) of tree organs (shoots, leaves, buds, fruits, trunk), were measured mainly in current-year stems. The two species were selected because they are among the most important Iberian oak species and they form mixed stands in NE Spain (Amaral Franco 1990).

Both bud and fruit growth are highly relevant for the persistence and colonization ability of Mediterranean oaks, as buds determine primary growth on the following year and acorns determine the species ability to regenerate and to colonize new sites. The patterns of secondary growth, bud and crown development, and acorn production are influenced by climate, and particularly by summer drought, in the studied Mediterranean oak species (Montserrat-Martí et al. 2009). Montserrat et al. (2009) summarized as follows the developmental patterns in *Q. ilex* and *Q. faginea*: (*i*) in spring, stems elongate quickly after budburst as in other temperate trees with preformed growth (Kozlowski 1971; Kikuzawa 1983; Harmer 1989); (*ii*) before shoot elongation is finished, secondary growth reaches its peak of development being followed by those of buds and acorns, respectively. These growth processes considerably overlap between them, so the development of ones can limit the development of others occurring simultaneously. Moreover, the climate and its variations can modify these interactions. Such findings highlight the need to know the precise time of development of each current-year shoot component, the interactions among them and with climate.

1. 2. Links between primary and secondary growth

The growth in size of a tree depends on the production of shoots and their conservation by secondary thickening as permanent supporting units. However, the links between primary and secondary growth have been little explored in plant and forest ecology despite these are the main growth types for understanding the development and persistence of trees. The inter-dependence between primary and secondary growth is the basis for the proper functioning and enlargement of the tree crown (Maillette 1982). For instance, in tropical tree species it has been observed that the influence of phenology on secondary growth is more relevant than the inverse relationship (Yáñez-Espinosa et al. 2006). Such influence of the phenology on radial growth occurs through growth regulators produced during budburst and implicated in the activity of the cambium (Aloni 1991).

Radial-growth variables (e.g., tree-ring, earlywood and latewood widths) and earlywood anatomical features (e.g., vessel transversal area and density) capture relevant and diverse ecological information mainly related to water availability during the period when the conduits are formed (Carlquist 1975; Baas and Schweingruber 1987). In the case of oaks from Mediterranean continental areas, where xylem development is constrained by low temperatures in winter and drought in summer, radial growth is usually enhanced by late winter-early spring rainfall (Tessier et al. 1994). Therefore, these oak species perform most of their growth in spring, before soil water availability decreases (Montserrat-Martí et al. 2009). In ring-porous and winter-deciduous trees such as *Q. faginea*, freezing-induced embolism of vessels during winter, which is proportional to the transversal conduit area, leads to a pronounced loss of hydraulic conductivity (Hacke and Sperry 2001; Zweifel et al. 2006). Accordingly, the tree must form new functional earlywood vessels every spring (Ellmore and Ewers 1986). Consequently, the transversal vessel area or lumen size of those vessels should be mainly driven by climatic conditions at the time of cell enlargement (Tardif and Conciatori 2006). The development of new wide earlywood vessels allows ring-porous species to transport more water for a first leaf flush during the early growing season after the winter dormancy (Tyree et al. 1994). The first earlywood vessels are formed a few weeks or just before budburst (M. Suzuki et al. 1996; Frankenstein et al. 2005; Zweifel et al. 2006).

1. 3. The importance of buds for tree growth

In species with preformed growth as oaks, buds are the plant investment for the crown growth in the next year (Vesk and Westoby 2004). In spring, once shoot extension is accomplished and leaves are almost totally developed, shoots presents a small hypsophyllary bud at the apex (Nitta and Ohsawa 1998). This bud develops many scales in the following weeks before the onset of summer drought, switching to a small scaled bud which grows actively up to early or mid-autumn, when they enter dormancy until the following spring (Puntieri et al. 2002; Arora et al. 2003; Horvath et al. 2003).

After overwintering, buds may die or remain dormant, becoming part of the bud bank (Maillette 1982). Usually, only some current-year buds grow out to form new shoots, which suggests that bud size in winter could be a valuable predictor of shoot production and bud fate in spring (Harmer 1991). The fate of buds along a shoot is generally dependent on shoot position in the crown. Remphrey et al. (2002) found that a greater proportion of buds produced lateral shoots and inflorescences in the top compared with the bottom of the crown, and the fewest developed towards the inside location. In many species, leafless buds at the base of the shoot remain small at the end of the growing season, they do not usually expand in the next spring and may persist for many years, enlarging as they grow outwards, and may eventually become embedded in the bark, becoming a source of epicormic shoots (Gill 1971; Harmer 1991; Wilson and Kelty 1994). Furthermore, bud size may also determine bud fate and explain the different patterns in bud production (Maillette 1982). Bud size may be driven to some extent by bud position within the shoot and the crown. For example, it is well known that apical buds exert a strong dominance, display higher vigour and have greater probabilities to produce shoots than the rest of buds (Maillette 1982; Harmer 1991, 1992; Buck-Sorlin and Bell 2000; Sabatier and Barthélémy 2001; Puntieri et al. 2002).

The adaptive value of scaled buds seems to be the protection of the shoot primordia against climatic stressors such as low temperatures during the cold winter season (Nitta and Ohsawa 1998). For instance, the buds formed within an individual tree and shoot show different sizes (Gill 1971). This suggests that bud development is modulated by several factors including the nature of the species, the individual tree size and vigour, and the position of the bud in the shoot (Remphrey and Powell 1984; Sabatier and Barthélémy 2001; Puntieri et al. 2002; Ishihara and Kikuzawa 2009). Among the endogenous factors that drive bud size development, secondary growth has been found to be a critical driver since changes in current-year stem cross-sectional area are linked to the stem hydraulic conductivity and the size of buds (Cochard et al. 2005). On the other hand, climate and species are two of the main exogenous factors affecting bud size in trees (Kozlowski et al. 1973; Salminen and Jalkanen 2004; Meier and Leuschner 2008; Sanz-Pérez and Castro-Díez 2010). However, it remains unclear when the secondary growth of trunks and current-year stems occur in relation to the pattern of bud development, whether these processes overlap in time, and if climate indirectly affects the development of buds through the control of secondary growth.

Current-year shoots have been mainly studied as organs for leaf arrangement (M. Suzuki and Hiura 2000). Several allometric studies have demonstrated that the mass and area of leaves per unit of stem length are greater in short shoots than in long ones

(Caesar and Macdonald 1984; Yagi and Kikuzawa 1999; Yagi 2000). Moreover, stem thickness is correlated with individual leaf size and with the total leaf mass or leaf area held by that stem (Preston and Ackerly 2003; Westoby and Wright 2003; Sun et al. 2006). Species with larger twigs hold substantially more leaf area per stem cross-sectional area than species with smaller twigs (Westoby and Wright 2003). However, the complex dynamics of secondary growth in current-year shoots, which also depend on primary growth, may affect bud development (Lauri et al. 2010). Cochard et al. (2005) found that the current-year shoot secondary growth is positively related with stem hydraulic conductivity and the number of leaf primordia preformed in the buds.

A mature oak tree has a huge population of renewal buds which can be classified into vegetative and reproductive buds (Wilson and Kelty 1994). Flowering takes place concurrently with shoot growth and acorn development is mainly produced between July and October (Montserrat-Martí et al. 2009). Stem diameter can determine to a great extent the fruit set and fruit size (George et al. 1996; Porter et al. 2002). Furthermore, reproduction represents an important resource sink for trees (Roff 1992). This resource investment can compete with other growth processes in trees leading to different trade-offs, for instance between vegetative growth and fruit production (Koenig and Knops 1998; Obeso 2002; Monks and Kelly 2006; Hirayama et al. 2007). However, current-year shoots hold buds and leaves (Kozlowski 1971) and they become net exporters of carbohydrates soon after bud break (Johnson and Lakso 1986; Hasegawa et al. 2003; Keel and Schädel 2010; Landhäusser 2010). Consequently, the development of flowers and fruits depends on photosynthates provided by the reproductive shoot and the neighbouring ones (Lauri et al. 1996; Hoch 2005).

1. 4. Study area

The study area was located in the Huesca province (Aragón) in north-eastern Spain where five sampling sites were selected in the Pre-Pyrenees, the Sierra de Alcubierre and Agüero. In the Pre-Pyrenees, three sites along an altitudinal gradient were selected: Pico del Águila (high-elevation cold site, H), Arguis (mid-elevation mesic site, M) and Nueno (low-elevation dry site, L) (see Chapters I and II). In the Sierra de Alcubierre, a fourth low-elevation xeric site (X) was selected (see Chapter I). Furthermore, a fifth site was included in Agüero (A) (see Chapters III, IV and V) (Fig. 1a). In all sites the climate is Mediterranean continental characterised by dry summers, cold winters and a high inter-annual variability of precipitation (Fig.1b). The study sites shared similar geological substrate (limestone) and aspects but presented contrasting vegetation types with dominant species: *Quercus ilex* L. subsp. *ballota* (Desf.) Samp., *Q. faginea* Lam., *Fagus sylvatica* L., *Pinus sylvestris* L., *Arbutus unedo* L., *Pinus halepensis* Mill., and other less abundant woody species.

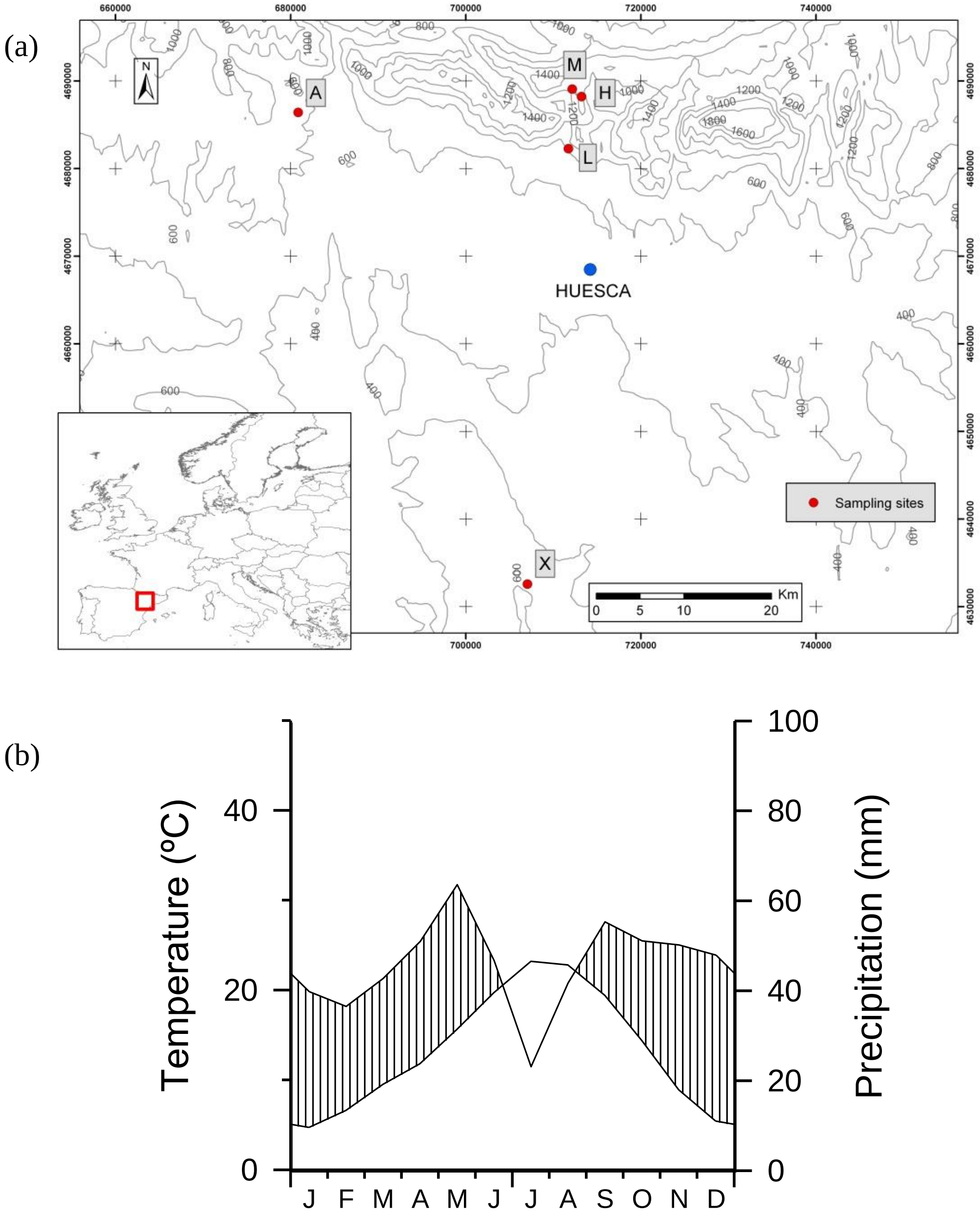


Figure 1. Study area and sampled sites (a) (A, Agüero; H, high-elevation site in Pico del Águila; M, mid-elevation site in Arguis; L, low-elevation site in Nueno; X, xeric site in Sierra de Alcubierre) and the climatic diagram (b) of Huesca.

1. 5. Study species

Quercus ilex L. subsp. *ballota* (Desf.) Samp. is an evergreen oak tree with semi-ring to diffuse porous wood (Campelo et al. 2007), which in Iberian Peninsula grows preferentially inland under continental conditions (Amaral Franco 1990). *Q. faginea* Lam. is a deciduous oak tree with ring-porous wood (Corcuera et al. 2004) and with a wide distribution in sub-Mediterranean areas of North Africa and the Iberian Peninsula, being dominant in NE Spain (Amaral Franco 1990). The study species frequently coexist in the study area and in many other sites of the Iberian Peninsula forming multi-stemmed trees (Fig. 2).

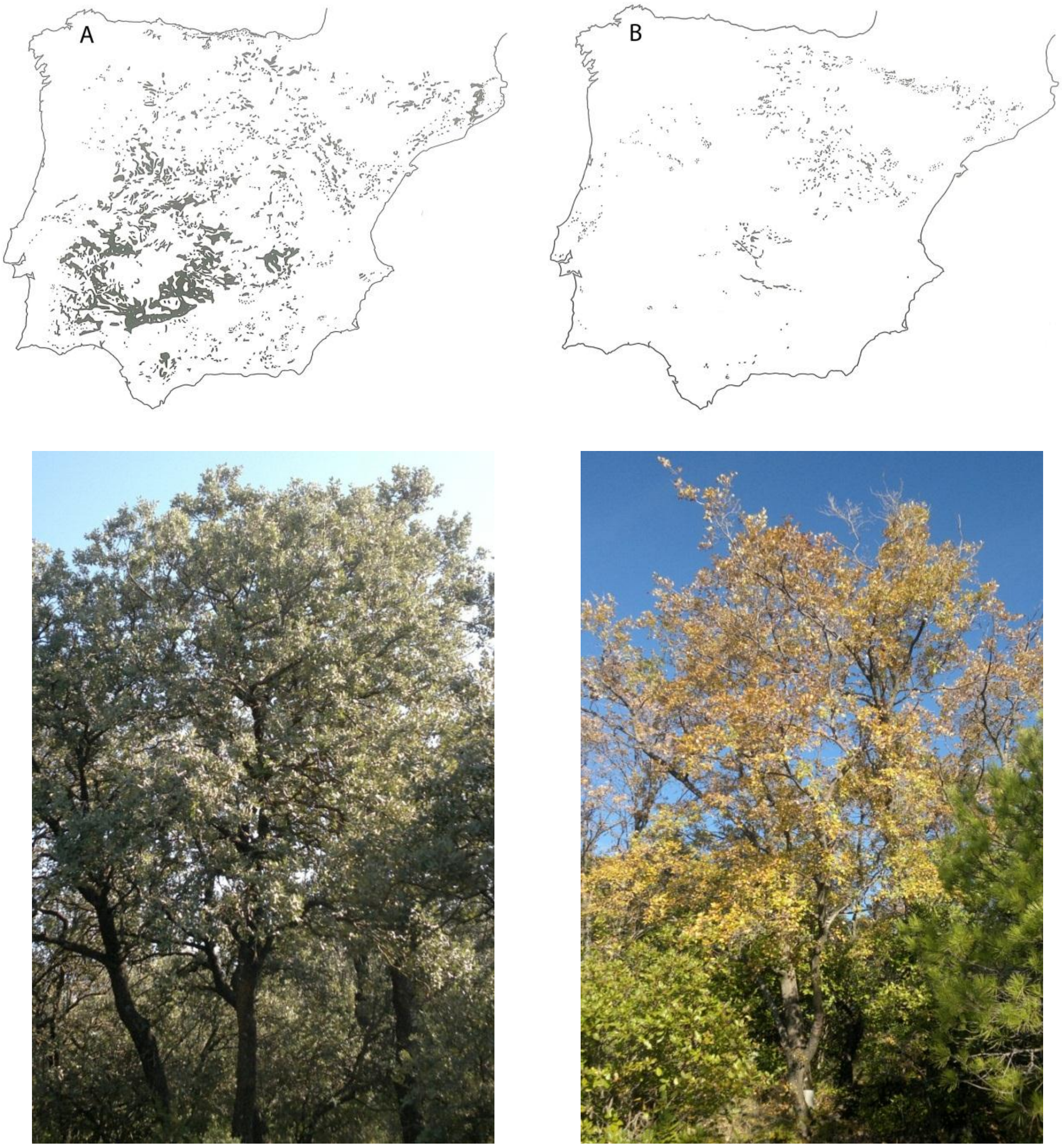


Figure 2. Distribution maps (upper figures) and representative trees (lower figures) of the two studied species: *Q. ilex* (left), *Q. faginea* (right). Source of maps: Blanco-Castro et al. (1997).

2. GENERAL OBJECTIVES

The main objective of the thesis was to understand the interactions between primary and secondary growth, how these relationships influence bud development and the implications that buds have on the phonological and architectural patterns of trees.

The specific objectives of the thesis were:

1. To quantify how radial growth and earlywood anatomy in a Mediterranean ring-porous winter deciduous oak (*Quercus faginea*) respond to climatic conditions (Chapter I).
2. To evaluate how climatic stress affects bud size and shoot secondary growth in *Q. faginea* (Chapter II).
3. To analyse the effect of acorn production on the current-year shoot growth and nitrogen and non-structural carbohydrate concentrations in *Q. ilex* and *Q. faginea* (Chapter III).
4. To describe the seasonal patterns of bud development and secondary growth of *Q. ilex* and *Q. faginea*, and to identify the climatic factors which most influence the inter-annual variability in bud size (Chapter IV).
5. To analyse the effect of bud size on the fate of buds located in different positions along shoots in *Q. ilex* and *Q. faginea* (Chapter V).

3. CHAPTERS

The present thesis is based on the following papers which will be referred hereafter as Chapters with their Roman numerals:

I. **Alla AQ, Camarero JJ. 2012.** Contrasting responses of radial growth and wood anatomy to climate in a Mediterranean ring-porous oak: implications for its future persistence or why the variance matters more than the mean. *European Journal of Forest Research*. In press. DOI: 10.1007/s10342-012-0621-x

II. **Alla AQ, Camarero JJ, Rivera P, Montserrat-Martí G. 2011.** Variant allometric scaling relationships between bud size and secondary shoot growth in *Quercus faginea*: implications for the climatic modulation of canopy growth. *Annals of Forest Science* **68**: 1245–1254. DOI: 10.1007/s13595-011-0093-z

III. **Alla AQ, Camarero JJ, Maestro-Martínez M, Montserrat-Martí G. 2012.** Acorn production is linked to secondary growth but not to declining carbohydrate concentrations in current-year shoots of two oak species. *Trees* **26**:841–850. DOI: 10.1007/s00468-011-0658-3

IV. **Alla AQ, Camarero JJ, Montserrat-Martí G. 2012.** Seasonal patterns of bud development and secondary growth as related to climate in two co-existing Mediterranean *Quercus* species.

V. **Alla AQ, Camarero JJ, Palacio S, Montserrat-Martí G. 2012.** The fate of buds revisited: bud mortality and bursting depend on bud size.

CHAPTER I

Contrasting responses of radial growth and wood anatomy to climate in a Mediterranean ring-porous oak: implications for its future persistence or why the variance matters more than the mean

I. Contrasting responses of radial growth and wood anatomy to climate in a Mediterranean ring-porous oak: implications for its future persistence or why the variance matters more than the mean

Abstract The plasticity of radial growth and earlywood anatomy to climate has not been properly assessed. To solve this, we evaluated how growth and earlywood anatomy changed in a Mediterranean ring-porous oak (*Quercus faginea*) across a climatic gradient. We hypothesized that the anatomical variables will be the most sensitive to climatic stress, particularly to water deficit in late winter and early spring when earlywood is formed. Testing these hypotheses allows evaluating whether such plasticity in growth and earlywood anatomy affects the projected responses of trees to climate forecasts. The gradient included high- (H), mid- (M), and low-elevation (L) sites and a xeric site (X). We used dendrochronology to quantify changes in growth and anatomy and to relate them to recent and twenty-first-century forecasted climatic data. The highest interannual variance was observed for the latewood width in the xeric site, where the earlywood vessel area reached the lowest values. Tree-ring width correlated positively with spring precipitation, whereas the earlywood vessel area correlated negatively with winter temperature. The predicted drier and warmer climate caused a 10 % reduction in tree-ring width and earlywood vessel area. Earlywood anatomical traits were less sensitive to climatic variability than radial-growth ones since the percentage of their variance explained by climate varied from 12 to 49 % in the former variables and from 15 to 57 % in the latter ones. The plastic xylem responses to climatic variability across time, mainly in the xeric site, indicate that this plasticity must be explicitly considered when forecasting changes in growth and persistence of trees.

Keywords Dendroecology, Earlywood, Hydraulic conductivity, Mediterranean climate, *Quercus faginea*, Tree-ring width, Vessels, Xylem.

Introduction

Xylem captures relevant and diverse ecological information in several of its anatomical features (e.g. area, density), usually measured across the transversal plane, which are assumed to be mainly related to water availability during the period when the conduits were formed (Carlquist 1975; Baas and Schweingruber 1987). The search for links between such wood-anatomical characteristics and climate may provide a functional proxy of tree performance over time (Wimmer 2002; Fonti et al. 2010). In angiosperm tree species, some studies have found climatic responses of the earliest-formed vessels in diffuse-porous (Sass and Eckstein 1995) and semi-ring-porous to ring-porous wood types (Pumijumnong and Park 1999; Fonti et al. 2006; Giantomasi et al. 2008; Campelo et al. 2010). For example, the area of earlywood vessels of a temperate oak decreased in response to reduced precipitation in the early growing season (García-González and Eckstein 2003).

In ring-porous and winter-deciduous trees from continental areas, freezing-induced embolism of vessels during winter, which is proportional to the transversal conduit area, leads to a pronounced loss of hydraulic conductivity (Hacke and Sperry 2001). Therefore, the tree must form new functional earlywood vessels every spring (Ellmore and Ewers 1986). Growth in these tree species resumes when buds start to break, which is a process shortly preceded by the differentiation of the first earlywood vessels formed by overwintering cambial derivatives (Suzuki et al. 1996; Frankenstein et al. 2005). Thus, the transversal vessel area (VA) or lumen size of those vessels should be mainly driven by climatic conditions at the time of cell enlargement (Tardif and Conciatori 2006). However, it may also depend on climatic constrains affecting carbohydrate synthesis and storage during the winter season preceding ring formation (Fonti and García-González 2004). For instance, narrow vessel lumens are expected to be formed in response to low water availability at the time of cambial resumption which may be induced by warm conditions during late winter and early spring in drought-prone areas (Woodcock 1989). However, it has also been observed that earlywood VA increased in response to warm conditions during spring, particularly in sites with reduced water deficit (WD), suggesting an earlier cambial resumption and enhanced vessel enlargement in response to mild climatic conditions (Tardif and Conciatori 2006). These contrasting findings have pushed the development of multiple studies to build continuous vessel chronologies and to quantify the responses of earlywood anatomical features to climate, mainly in ring-porous tree species (Fonti et al. 2010). A tenet of these research efforts is that measuring these features, which is usually time-consuming, might provide more relevant information on tree performance than obtaining more easily gathered dendrochronological variables such as tree-ring width (but see Tardif and Conciatori 2006).

In the case of winter-deciduous oaks from Mediterranean continental areas, where xylem development is constrained by low temperatures in winter and drought in summer, radial growth is usually enhanced by late winter–early spring rainfall (Tessier et al. 1994). The winter quiescence of these oak species forces them to perform most of their growth in spring before soil water availability decreases (Montserrat-Martí et al. 2009). Thus, the number and total area of earlywood vessels formed by these species and the latewood width might respond to the spring precipitation amount. In addition, the recently formed earlywood vessels are functionally very relevant to ring-porous oaks since they may account for ca. 95 % of the total hydraulic conductivity although latewood formation is still important for radial growth (Corcuera et al. 2006). However, little is known on the relative effects of late winter and early spring climatic conditions on earlywood anatomy and radial growth in Mediterranean ring-porous oaks from continental drought-prone areas subjected to this double climatic stress (Camarero et al. 2010). In the western Mediterranean Basin, both winter temperatures and summer drought may become more relevant for the future growth and persistence of these species since rising winter temperatures and decreasing spring precipitation have been observed and forecasted there (IPCC 2007). Furthermore, increased interannual variability is another component of climatic change whose effects on growth and earlywood anatomy have been rarely assessed. For instance, unusually severe spring droughts can induce a reduction in earlywood conductive area and in turn the production of rings without latewood (Corcuera et al. 2004; Eilmann et al. 2009). The lack of latewood formation in the outermost ring can have profound functional consequences when preceded by low temperatures which induce the cavitation of vulnerable earlywood vessels because in rings with few functional earlywood conduits latewood vessels may act as alternative paths for water transport (Granier et al. 1994).

In this study, we quantify how radial growth and earlywood anatomy in a Mediterranean ring-porous winterdeciduous oak (*Quercus faginea*) respond to climatic conditions before and during the year of tree-ring formation. We compare these responses in oak populations subjected to different climatic stressors which are considered as spatial analogues representing some of the expected future climatic conditions. This analogy was used to critically evaluate predictions on the potential distribution of tree species under future climates because these forecasts ignore the growth plasticity of trees (Camarero et al. 2010). We hypothesize that: (1) earlywood anatomical variables will be more sensitive to changes in precipitation than radial-growth variables, and (2) earlywood anatomy will respond more to changes in water availability during the previous winter and the current spring than to temperature variability, particularly in xeric sites. Testing the aforementioned two hypotheses will allow evaluating whether xylem plasticity modifies the forecasted potential distributions of tree species based on climate projections.

Materials and methods

Study species and sites

Quercus faginea Lam. is a deciduous ring-porous oak tree distributed in Mediterranean areas of the Iberian Peninsula and North Africa with basic soils (Loidi and Herrera 1990). To avoid confusion with coexisting oak hybrids, we only selected those individuals which presented clear leaf characteristics of Q. faginea (Amaral Franco 1990). We selected four study sites with such contrasting climatic conditions that they represent most of the climatic variability currently experienced by Iberian *Q. faginea* forests (Jiménez et al. 1998). The study sites were located in two different mountain ranges from Aragón, in north eastern Spain: the Pre-Pyrenees and the Sierra de Alcubierre, located at about 53 km southwards from the first range. In the Pre-Pyrenees, three sites along an altitudinal gradient were selected, the extreme sites being 8 km distant: Pico del Águila (high-elevation cold site, hereafter abbreviated as site H), Arguis (mid-elevation mesic site, hereafter abbreviated as site M) and Nueno (low-elevation dry site, hereafter abbreviated as site L) (Table 1; Fig. 1). In the Sierra de Alcubierre, a fourth low-elevation xeric site (hereafter abbreviated as site X) was selected. In all sites, the climate is Mediterranean continental with a summer drought and a cold winter (Fig. 1; see also Supporting Information, Table S1). The study sites shared similar geological substrate (limestone) and aspects but presented contrasting vegetation types (Table 1).

Table 1. Description of the four studied sites (H, high-elevation site, M, mid-elevation site; L, low-elevation site; X, xeric site) and sampled *Q. faginea* trees.

Site	Latitude (N)	Longitude (W)	Elevation (m)	Dbh (cm)	Height (m)	Mean annual temperature (ºC)	Total annual precipitation (mm)	Water balance (mm)	Main co-occurring tree species
H	42º 19′ 01″	0º 24′ 46″	1470	9.2 ± 0.8b	4.5 ± 0.3b	9.1	1,215	673	*Fagus sylvatica* L., *Pinus sylvestris* L.
M	42º 19′ 30″	0º 25′ 31″	1135	13.9 ± 1.0a	5.8 ± 0.4a	10.8	899	321	*Q. ilex* subsp. *ballota* (Desf.) Samp.
L	42º 15′ 50″	0º 25′ 59″	700	12.8 ± 1.1a	5.5 ± 0.3a	13.1	670	-3	*Q. ilex* subsp. *ballota* (Desf.)
X	41º 49′ 06″	0º 30′ 49″	550	15.0 ± 1.5a	6.4 ± 0.4a	15.0	395	-395	*Pinus halepensis* Mill.

Different letters correspond to significant ($P \leq 0.05$) differences between sites.

Field sampling and dendrochronological methods

In November 2007, 10 *Q. faginea* individuals were selected and tagged in each site, and their size was measured (Dbh, diameter at breast height, at 1.3 m; total height). Based on ring counts in basal samples, all sampled individuals were older than 30 years, which we regarded as an adequate age for avoiding ontogenetic effects on vessel features. At site X, seventeen additional individuals were sampled yielding a total of 27 trees at that site. From each individual, the main trunk was cored at 1.3 m using an increment borer and two cores from opposite directions were taken. All wood samples were prepared following standard dendrochronological methods and visually cross-dated (Fritts 1976). To perform further anatomical analyses, the cores were carefully cut until getting a level wood surface using a sledge microtome (G.S.L.1 microtome, Gärtner and Schweingruber, WSL, Birmensdorf, Switzerland). Earlywood (EW), latewood (LW) and tree-ring width (RW) were then measured at a precision of 0.001 mm using a LINTAB measuring system (Rinntech, Heidelberg, Germany). Visual cross-dating was evaluated using the COFECHA program, which calculates cross-correlations between individual series and a reference mean chronology (Holmes 1983).

Analyses of earlywood anatomical variables

To maximize the climatic signal of wood-anatomical variables, we focused on earlywood vessels. Prior to image acquisition, each cross-dated core was cleaned with pressurized air. Then, the cores were coloured black using a marker pen and rubbed with white chalk to fill the vessel lumina with chalk powder to improve the contrast of vessels. The measurement of vessel features was performed for the period 1985–2007 on images of the transversal wood surface along a tangential area 4.5-mm wide since it provides a representative selection of vessels for investigating their climatic response (García-González and Eckstein 2003; García-González and Fonti 2008). Two complementary methods were used. First, two cores from three trees per site were captured with a 4,800-dpi resolution scanner (Epson Perfection V750 Pro, Seiko Epson Corporation, Japan). Second, images of all wood samples of each site (ten trees, twenty cores) were captured at 2,048 9 1,536 resolution and 16–209 magnification using a stereomicroscope (MZ12.5 Leica, Heerbrugg, Switzerland) attached to a digital camera (Leica DFC290). To compare both methods, cores were analysed using scanned and stereomicroscope-acquired images. The estimates of VA based on scanned samples were usually greater than those derived from stereomicroscope measurements (Supporting Information, Figs. S1, S2). Thus, we only considered stereomicroscope-acquired images.

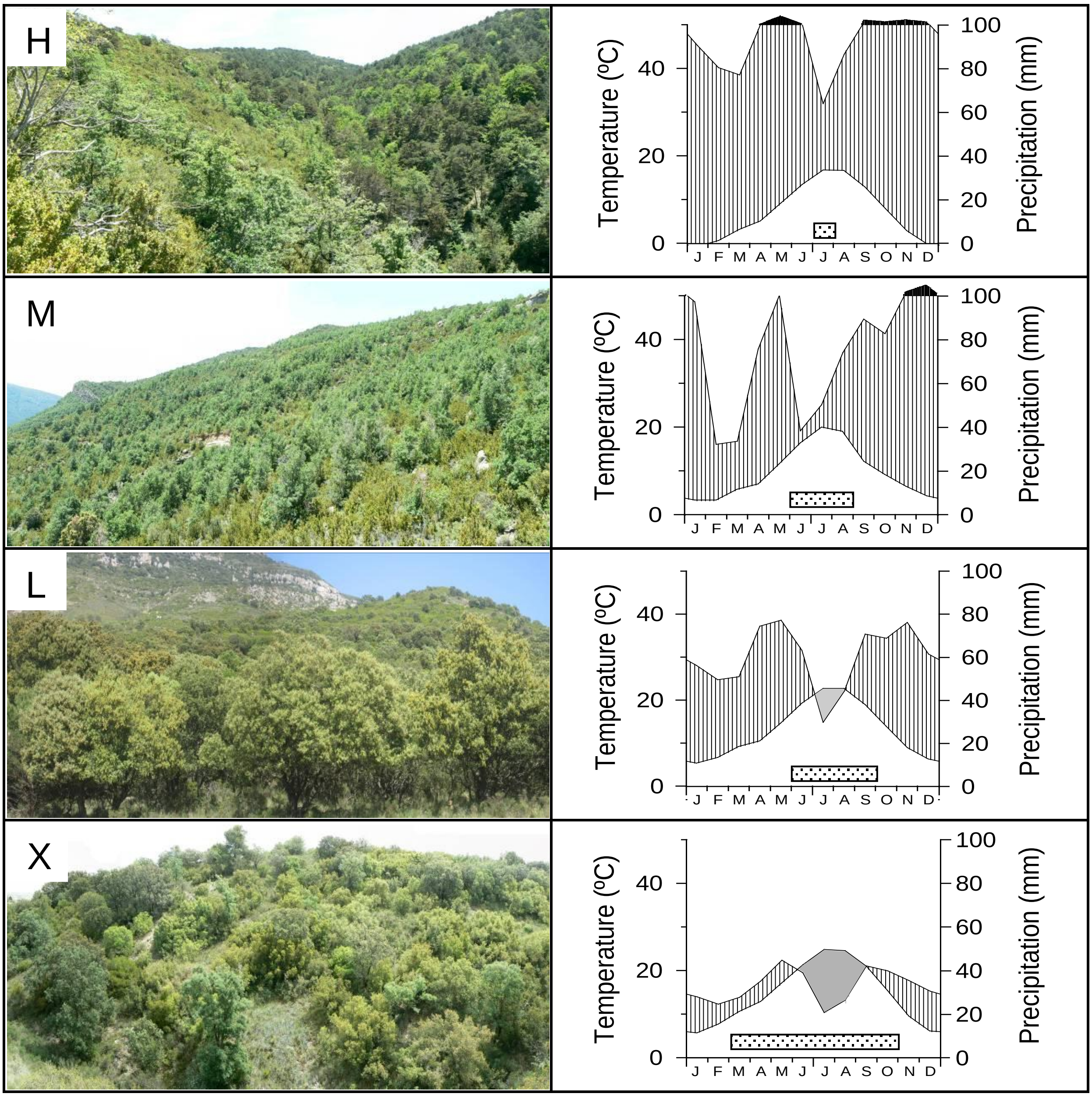


Figure 1. Representative images and climatic diagrams of the four study sites (*H* high-elevation site, *M* mid-elevation site, *L* low-elevation site, *X* xeric site). The *dotted* areas in the climatic diagrams indicate months with a negative water balance.

A total of 1,443 tree rings were captured (H, 384 rings; M, 365 rings; L, 357 rings; X, 337 rings), and 29,228 earlywood vessels were measured. Each image of each ring width was analysed using the WinCell PRO (version 2010a) software (Régents Instruments Inc., Québec, Canada). To define earlywood vessels, the minimum and maximum vessel lumen cross-sectional areas were set to 0.001 and 0.1 mm^2, respectively, based on previous studies on *Q. faginea* (Villar-Salvador et al. 1997; Corcuera et al. 2004). These thresholds included most earlywood vessels (Supporting Information, Fig. S3).

In all sites, selected vessel features (lumen cross-sectional area, number of vessels) were obtained in each ring for the common period 1985–2007. Vessel density (VD) was calculated as the ratio between the number of vessels and the total analysed area. The conductive area (CA) was calculated as the ratio between the total lumen area and the total analysed area. In addition to the mean cross-sectional VA of each tree ring, we also calculated the mean area of those vessels with areas above the 3rd quartile of lumen size (VA3q) and the mean area of the vessels located in the first half of the earlywood (VA1h), i.e., those located in the early earlywood (Supporting Information, Fig. S4). Previous studies suggested the selection of vessels according to their area or to their position within the ring for maximizing the climatic signal of vessel chronologies (García-González and Fonti 2006; Tardif and Conciatori 2006). Vessels were analysed for their frequency in 0.01 mm^2 area classes and for the relative contribution of each area class to the total predicted hydraulic conductance (Kh; mm^4), i.e., the sum of all the conduits diameters raised to the fourth power. Assuming that the vessel cross-sectional shape was circular, the predicted hydraulic conductance was calculated following the Hagen-Poiseuille law (Sperry et al. 1994).

To preserve the high-frequency variability in radial growth and earlywood anatomical features, tree-ring width and wood-anatomical series were standardized applying a two-step detrending using ARSTAN (Cook 1985). First, a negative exponential function was fitted to the measured values, and the second, detrending was based on a spline function with a 50 % frequency response of 20 years. Radial-growth and wood-anatomical indices were obtained by dividing the observed values by the expected ones estimated by the spline function. Then, autoregressive modelling was performed to remove the first-order temporal autocorrelation. Finally, a robust biweight mean was applied to average the standardized series and produce local residual chronologies of radial-growth (earlywood, latewood and tree-ring widths) and wood-anatomical (earlywood VA, CA and VD) variables. The quality of these chronologies was evaluated for the period 1985–2007 using the following dendrochronological statistics (Briffa and Cook 1990): the expressed population signal (EPS) which measures the tree-to-tree common growth variance and it is an indicator of chronology reliability as compared with a theoretical population’s chronology (Wigley et al. 1984); the mean correlation between trees (Rbt); the mean sensitivity (MSx) or the average relative difference between successive rings (Fritts 1976); the first-order autocorrelation coefficient (AR) based on raw data; the variance accounted for the first principal component (PC1); and the percentage of growth or wood anatomy variance explained by climate based on multiple linear regressions (R^2 climate).

Climatic data

Data from nearby meteorological stations were averaged for the period 1960–2007 to study growth–climate relationships (Supporting Information, Table S1). We built regional climatic time series due to the lack of local stations with complete records and to account for the spatial variability of climate across mountainous areas. Two regional series were built: one was used for the sites located along the altitudinal gradient (H, M, and L sites), and a second series was used for the xeric site (X). To estimate the missing data for each station and to combine them, we used the program MET from the Dendrochronology Program Library (Holmes 1992). For each station, monthly variables were transformed into normalized standard deviation to give each station the same weight in calculating the average values for each month and year. Regional climatic series were checked against spatially interpolated data for the Iberian Peninsula (Ninyerola et al. 2005). The temperature lapse rate (temperature = -0.06 elevation + 16.30, r = 0.88, P = 0.02) for the sites located in the altitudinal gradient was estimated using local climatic data. The precipitation amount increased upwards but the elevation–precipitation relationship was not linear. See additional details on climatic data in Alla et al. (2011).

To estimate the yearly water balance in each site, we used a modified Thornthwaite water-budget procedure (Willmott et al. 1985). Soil water balance was modelled by calculating soil–water withdrawal (actual evapotranspiration), recharge and surplus. Actual evapotranspiration was related to the percentage of the current soil at the available water capacity, and to potential evapotranspiration, which was estimated from monthly mean temperature data for the period 1960–2007. We calculated a site cumulative WD from previous September up to September of the year of tree ring formation, i.e., for the period when the studied species forms most of its tree ring and including previous months affecting growth the following year (Corcuera et al. 2004). Positive and negative WDs corresponded to dry and wet conditions, respectively. Calculations were done by using the AET software available at http://geography.uoregon.edu/envchange/pbl/software.html.

Simulated future climate

To assess the potential effects of future climatic conditions on growth and wood anatomy, we used projected monthly climatic data (mean temperature, total precipitation). We used climate predictions from two general circulation models (ECHAM4 and CGCM2) under two greenhouse gas emission scenarios covering half the range of the IPCC scenarios in terms of greenhouse gas (SRES scenarios A2 and B2). The IPCC (2007) recommends using a range of climate projections and emission scenarios to quantify uncertainty in predictive biological models. These are the emission scenarios and downscaled general circulation models currently available for the Iberian Peninsula (Brunet et al. 2007). These scenarios were also previously used to model the future distribution of *Q. faginea* in the Iberian Peninsula based on bioclimate models (Benito Garzón et al. 2008). Scenarios A2 and B2 correspond to a global intensive economy with rapid population increase and to local environmentally sustainable economies with lower population increase, respectively. The A2 and B2 scenarios would cause projected increases of 3–9 and 2–6 ºC of the mean summer temperature over the study area during the late twenty-first century (Brunet et al. 2007). Predicted climate data were obtained for the late twenty-first century (period 2071–2100), and they were downscaled using an analogue method and provided by the Spanish National Meteorological Agency (AEMET). Simulated climatic data for the twenty-first century were compared with modelled data of the second half of the twentieth century (1961–1990) and with regional climatic series based on local climatic data (period 1960–2000) to check for seasonal and monthly consistencies (Supporting Information, Fig. S5).

Statistical analyses

All variables were checked for normality using the Shapiro-Wilks test prior to performing statistical analyses. The following variables were log-transformed: tree-ring and latewood widths, total VA and CA. Comparisons between variables were assessed by hierarchical linear mixed models, based on Restricted Maximum Likelihood methods, considering trees (random factors) nested within sites (fixed factors) (Littell et al. 2006). Differences between site means were assessed by Bonferroni tests when variances were equal or Dunnett’s T3 tests otherwise. The relative contribution of the inter-annual variability and the factor ‘‘trees’’ to the overall variance of indexed radial-growth wood-anatomical variables was also estimated considering the period 1990–2007 when the maximum sample size (number of cores) was reached in all study sites. We also assessed differences in the distribution frequencies of earlywood vessels according to their area using the G-test. All these analyses were performed using the MIXED procedure in SPSS 19.0 (SPSS, Chicago, USA).

The Pearson correlation coefficient (*r*) was used: (1) to assess the relationships between radial-growth and wood-anatomical indexed variables, and (2) to quantify the associations between growth and wood-anatomical indices and monthly (mean temperature, total precipitation) climatic data for the period 1985–2007. These analyses were performed considering the year of ring formation (t) and the previous (t - 1) and subsequent years (t + 1) (Tardif and Conciatori 2006). In the case of growth–climate correlations, we used a temporal window of 13 months from of the previous (year *t* - 1) to the current September (year *t*). We also calculated bootstrapped response functions among growth indices and climatic variables using the program PRECON (version 5.16) (Fritts et al. 1991). To perform a reliable growth–climate analysis for earlywood anatomical variables, usually characterized by a generally low common signal (low correlation between trees, Rbt) (e.g., Fonti and García-González 2004), only those variables with Rbt > 0.05 were included. Finally, we selected the climatic variables more tightly related to growth and anatomical data to assess the changes in these variables for the late twenty-first century as a function of forecasted climatic conditions.

Results

Comparisons between growth and earlywood anatomical variables

The tree-ring, earlywood and latewood widths differed significantly among sites with the highest values observed in sites H and M and the lowest ones in sites L and X (Table 2). The mean area of earlywood vessels showed the lowest values in the X (0.020 mm^2) and H (0.027 mm^2) sites, whereas VD showed the highest value in the X site (ca. 8 vessels mm^{-2}). The percentage of earlywood CA reached the lowest and highest value in sites X (15.6 %) and M (19.5 %), respectively, with intermediate values in sites H and L (17.5 %). Considering all the study sites, the vessels with smallest area (lower than 0.01 mm^2) corresponded to 36 % of all measured earlywood vessels (Fig. 2), but they only contributed to about 1 % of the total predicted hydraulic conductance (*Kh*). These smallest area conduits comprised 44 % of all vessels in the X and L sites but only 26 % in the M and H sites (Fig. 2). Wide earlywood vessels with cross-sectional areas > 0.05 mm^2 were 15 % (accounting for 57 % of *Kh*) of all vessels in the H and M sites versus 6 % (accounting for 26 % of *Kh*) in the L and X sites, respectively. The distributions of the earlywood vessels according to their lumen area differed significantly among the four study sites (G = 32.5, P = 0.05).

Growth variables showed higher year-to-year variance than earlywood anatomical features which showed high tree-to-tree variance (Fig. 3). Indeed, the highest year-to-year variance was observed for the latewood width (46 %), whereas VA showed the highest tree-to-tree variance (46 %). CA and VD showed the lowest percentages of common year-to-year variance, being 7 and 8 %, respectively.

The mean between-trees correlation was higher in the radial-growth than in the wood-anatomical variables, reaching a maximum value of 0.66 for latewood width in site X (Table 3). The mean sensitivity of all measured variables was always higher in the X site than elsewhere, and it reached a maximum value of 0.63 for latewood width in that site

Table 2. Mean values of the growth and wood-anatomical variables for the common period 1985-2007.

Site	RW (mm)	EW (mm)	LW (mm)	VA (mm^2)	VD (mm^{-2})	CA (%)
H	1.85 ± 0.04a	0.66 ± 0.01a	1.19 ± 0.03a	0.027 ± 0.001b	6.8 ± 0.1b	17.5 ± 0.3b
M	1.62 ± 0.04b	0.62 ± 0.01b	0.99 ± 0.04b	0.034 ± 0.001a	6.2 ± 0.1c	19.5 ± 0.3a
L	1.37 ± 0.05c	0.47 ± 0.01d	0.89 ± 0.03c	0.032 ± 0.001a	6.1 ± 0.1c	17.5 ± 0.3b
X	1.27 ± 0.06d	0.53 ± 0.01c	0.74 ± 0.05d	0.020 ± 0.001c	8.3 ± 0.2a	15.6 ± 0.3c
F	**16.203**	**20.869**	**13.853**	**5.059**	2.842	1.671
P	**<0.001**	**<0.001**	**<0.001**	**0.031**	0.100	0.204

The last two lines show the statistical parameters (*F*; *P*, significance level) of a linear mixed model considering trees and sites as random and fixed factors, respectively. Variables abbreviations: *RW* tree-ring width, *EW* earlywood width, *LW* latewood width, *VA* vessel area, *VD* vessel density, *CA* conductive area. Different letters correspond to significant ($P \leq 0.05$) differences between sites. Significant effects are in bold. Sites are abbreviated as in Table 1.

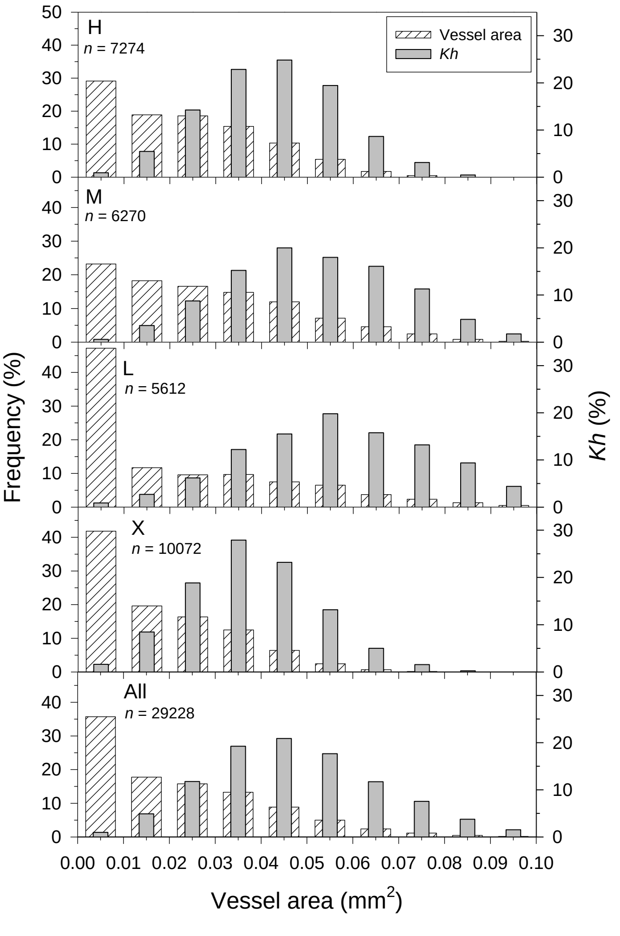


Figure 2. Distribution of earlywood vessels by lumen cross-sectional area for *Quercus faginea* compared with their relative contribution to the total predicted hydraulic conductance (*Kh*). Data are presented for each study site (*H*, *L*, *M*, *X*) and for the combined data set (All) indicating the number of measured earlywood vessels (sample size, *n*).

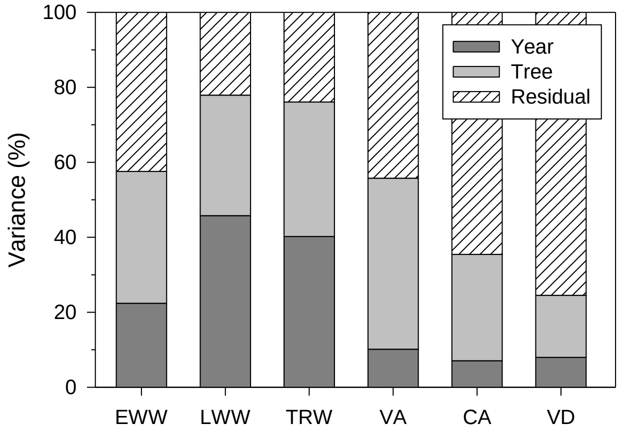


Figure 3. Estimated variance of the factors evaluated (year, tree) and residual variance for the following growth and wood-anatomical variables: earlywood width (*EW*), latewood width (*LW*), tree-ring width (*RW*), vessel area (*VA*), conductive area (*CA*), and vessel density (*VD*).

(Table 3). The variance amount accounted for the PC1 was also the highest in the case of latewood width in site X (82 %), while in the case of VA, this variance was highest in sites H and M (64–69 %). The highest percentages of growth variance explained by climate corresponded to latewood and ring widths in sites X, L and H (42–57 %), whereas the highest percentages for earlywood anatomical variables were found for VA and CA in these same sites (39–48 %). The maximum correlation among radial-growth site chronologies was obtained between sites H and M for latewood width (*r* = 0.69, *P* = 0.01), but in the case of wood-anatomical features, the strongest association was found between L and X sites for CA (*r* = 0.43, *P* = 0.04).

Considering all sampled trees (*n* = 57), the mean VA for the 1985–2007 period was positively related to tree diameter (*r* = 0.37, *P* = 0.04) and also with total height (*r* = 0.27, *P* = 0.045). The latewood width of the year of tree-ring formation was positively associated with the earlywood width of the following year in the H and M sites. The earlywood CA and the tree-ring or latewood widths were negatively related in all sites excepting for site X (Table 4). The mean area of all earlywood vessels was more tightly related to the mean area of the third-quartile earlywood vessels (VA3q) than to the mean area of the first half of earlywood vessels (VA1h) (Supporting Information, Fig. S4). The CA was positively and significantly related to mean VA and density only in the sites L and X. In site X, VA was also related to tree-ring or latewood width of the preceding year, whereas VD was also associated with previous radial growth in the site H.

Relationships among climate, growth and earlywood anatomy

All sites showed either increases or decreases in radial growth in response to wet (e.g. 1988 and 1997) or dry conditions in spring or early summer (e.g. 1995 and 2005), respectively. However, such changes in growth were always more pronounced in the X site than elsewhere (Fig. 4). In fact, tree-ring and latewood widths were positively related to current-year June precipitation in the H and M sites but also to May or April precipitation in the L and X sites (Figs. 5, 6). Current spring rainfall (e.g. in April) was also positively related to earlywood width in the X site, but earlywood formation also depended on October precipitation of the year before tree-ring formation in the H site. The earlywood width was negatively related to current May temperature, whereas the latewood and tree-ring widths responded negatively to June and July temperatures. In the X site, high March temperatures were associated with a low production of earlywood and latewood.

Cumulative WD was negatively related to tree-ring (mean r for all sites was = −0.50, P < 0.05 in all cases) and latewood widths (mean r = −0.51, P < 0.05) in all sites, whereas considering anatomical variables, WD was only significantly related in a negative way to the mean area of the biggest vessels (3rd quartile of lumen size) in site X (r = −0.50, P = 0.03). The chronologies of the widest earlywood vessels (VA3q) or those of the mean VA showed negative relationships with

Table 3. Statistics of radial-growth and wood-anatomical residual chronologies for the reference period 1985–2007.

	H	M	L	X	H	M	L	X	H	M	L	X
	EW				LW				RW			
Radial-growth variables												
First year EPS > 0.85	1975	1972	1968	1962	1975	1972	1968	1962	1975	1972	1968	1962
Rbt	0.31	0.20	0.15	0.43	0.64	0.46	0.47	0.66	0.60	0.45	0.45	0.65
MSx	0.21	0.10	0.25	0.32	0.46	0.44	0.41	0.63	0.31	0.31	0.31	0.45
AR	0.43	0.39	0.53	0.45	0.23	0.39	0.42	0.34	0.32	0.43	0.47	0.41
PC1 (%)	49.6	45.0	33.6	45.2	55.4	51.5	50.3	81.8	52.0	49.6	48.9	79.8
R^2 climate (%)	14.9	15.8	26.2	31.7	42.3	25.9	48.2	57.2	41.6	23.5	46.9	55.8
	VA				VD				CA			
Wood-anatomical variables												
First year EPS > 0.85	1988	1987	1985	1985	1988	1987	1985	1985	1988	1987	1985	1985
Rbt	0.20	0.19	0.22	0.25	0.08	0.11	0.09	0.08	0.37	0.02	0.10	0.30
MSx	0.17	0.16	0.24	0.26	0.20	0.16	0.21	0.22	0.19	0.15	0.20	0.22
AR	0.39	0.29	0.05	0.15	0.40	0.35	0.24	0.26	0.28	0.25	0.28	0.13
PC1 (%)	64.1	69.2	36.2	49.3	54.6	63.4	25.8	30.7	62.3	43.4	30.8	44.0
R^2 climate (%)	39.5	32.7	46.1	48.5	12.2	12.0	16.5	29.0	39.0	25.0	45.0	40.8

Variables are abbreviated as in Table 2. Sites are abbreviated as in Table 1. Statistics: the chronology segment with EPS > 0.85 is usually regarded as statistically reliable, where EPS is the expressed population signal which measures the tree-to-tree common growth variance (Wigley et al., 1984); Rbt, mean correlation between trees; MSx, mean sensitivity or the average relative difference between successive indices (Fritts, 1976); AR, first-order autocorrelation coefficient calculated from raw data; PC1, variance accounted for the first principal component; R^2 climate, percentage of growth or wood-anatomy variance explained by climate.

current February temperature in sites H, L and X (Table 5). The VA was also negatively related to previous December temperature in the site L and to current April temperature in the site X (Fig. 6). Nonetheless, the area of those vessels located in the first half of the earlywood (VA1h) was positively associated with February precipitation in the site X. The CA was negatively related to winter temperature and precipitation in site L, whereas winter and spring precipitation negatively affected CA and VD in site X. We found that tree-ring width in site H and earlywood VA in site X during the late twentieth century were significantly (ANOVA, tree-ring width $F = 31.2$, earlywood VA, $F = 75.5$; $P < 0.001$ in both cases) higher than those predicted for the late twenty-first century (Fig. 6). The decrease in growth and VA was a response to the forecasted drier June and warmer April conditions in the late twenty-first century.

Discussion

Most published studies on climate–vessel relationships are based on correlative approaches and not on mechanistic models. Such findings must be discussed taking into account vessel ontogenesis or at least the vessel position within the ring as has been done for tree species with ring-porous (Fonti and García-González 2004) and semi-ring-porous or diffuse-porous wood types (Giantomasi et al. 2008). For instance, poplar trees formed smaller vessel cells in response to WD in early summer while no response was detected in vessels formed later (Arend and Fromm 2007). In ring-porous species such as *Quercus robur*, the earlywood vessel lumen area was positively related to late winter–early spring rainfall (February–April) (García-González and Eckstein 2003). The aforementioned studies show the adjustment of the earlier vessels to water availability in late winter and early spring before tree growth resumes. Accordingly, the lumen area of earlywood vessels of *Q. faginea* responded to February precipitation in the xeric site. In addition, the earlywood width was positively related to April and May precipitation in sites with the highest WD where both earlywood and latewood widths were lower than elsewhere. The plasticity of the growth-climate response was also detected in latewood and tree-ring widths which responded positively to June precipitation in the high-elevation site and to May precipitation in the low-elevation site. Such discrepancies may be explained by an earlier cambial reactivation in *Q. faginea* stands at low elevation where the first earlywood vessels may be formed between late March and early April when radial growth resumes (Alla, personal observation). This is also in agreement with the rapid cell enlargement of earlywood cells in oaks which may last less than 10 days (Zasada and Zahner 1969). The plastic response of *Q. faginea* growth to climatic variability along the studied climatic gradient does not support the forecasts based on bioclimatic envelope models predicting the local extinction of *Q. faginea* forests in xeric sites for the late twenty-first century (Benito Garzón et al. 2008). The ring and wood-anatomical growth responses to climatic variability found in the xeric site, where January water availability determined earlywood CA and VA, were those expected for a drought-prone site. In addition, the lumen area of the first-formed earlywood vessels was also positively associated with February rainfall there, whereas the mean area of earlywood vessels was the lowest among all sites and consequently VD was the highest. As expected, the winter growth quiescence and deciduousness of *Q. faginea* and the Mediterranean summer drought confined most secondary growth to spring when climatic stress is low and consequently earlywood vessel area responded positively to late winter rainfall (Montserrat-Martí et al. 2009).

However, the response of earlywood vessel area to winter precipitation was detected at annual (cumulative WD) and monthly scales in the more drought-stressed sites, whereas late winter temperatures were negatively related to earlywood VA in most sites as has been found before in other tree species with ring-porous wood (Tardif and Conciatori 2006; Fonti et al. 2006). Furthermore, at an annual scale, cumulative WD affected tree-ring and latewood growth in all sites. Such discrepancies indicate that the growth and earlywood anatomical responses to climate depend on the analysed time scale.

The low responsiveness of the mesic mid-elevation site to climatic variability was observed in both ring and anatomical variables. Such low sensitivity, the response of VA to early summer climatic conditions (e.g., June rainfall) and the highest mean VA values observed for this mesic site suggest a longer earlywood growth period there than in the other sites. Additionally, these results indicate that the climatic stress in the mid-elevation site is the lowest of all compared sites for the radial growth of *Q. faginea*.

In *Q. faginea*, radial-growth variables such as tree-ring and latewood widths were more valuable than wood-anatomical variables. First, obtaining wood-anatomical variables was much more time-consuming than taking width-related measures. Second, the latter variables (e.g. latewood width) showed a higher mean correlation between trees, greater

Table 4. Significant ($P \leq 0.05$) correlations among ring-width and wood-anatomical chronologies for the reference period 1985–2007.

	RW*t*				EW*t*				LW*t*				VA*t*				VD*t*			
	H	M	L	X	H	M	L	X	H	M	L	X	H	M	L	X	H	M	L	X
RW*t*																				
EW*t*	–	0.61	–	0.60																
LW*t*	0.98	0.99	0.99	0.98	–	0.58	–	0.53												
VA*t*	–	–	–	–	–	–	–	–	–	–	–	–								
VD*t*	–	–	–	–	–	–	-0.41	-0.43	–	–	–	–	-0.54	-0.48	-0.68	-0.49				
CA*t*	-0.69	-0.51	-0.62	–	–	-0.50	–	-0.43	-0.69	-0.47	-0.62	–	–	–	0.66	0.70	–	–	0.74	0.58
RW*t*+1	–	–	–	–	-0.43	–	0.42	–	–	–	–	–	–	–	–	–	–	–	–	–
EW*t*+1	0.53	–	–	–	–	–	–	–	0.58	0.41	–	–	–	–	–	–	–	–	–	–
LW*t*+1	–	–	–	–	–	–	0.45	–	–	–	–	–	–	–	–	–	–	–	–	–
VA*t*+1	–	–	–	0.53	–	–	–	–	–	–	–	0.56	–	–	–	–	–	–	–	–
VD*t*+1	0.44	–	–	–	–	–	–	–	0.43	–	–	–	–	–	–	–	–	–	–	–
CA*t*+1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Variables are abbreviated as in Table 2 and they were calculated for the year of tree-ring formation (*t*) and the subsequent year (*t*+1). Sites are abbreviated as in Table 1.

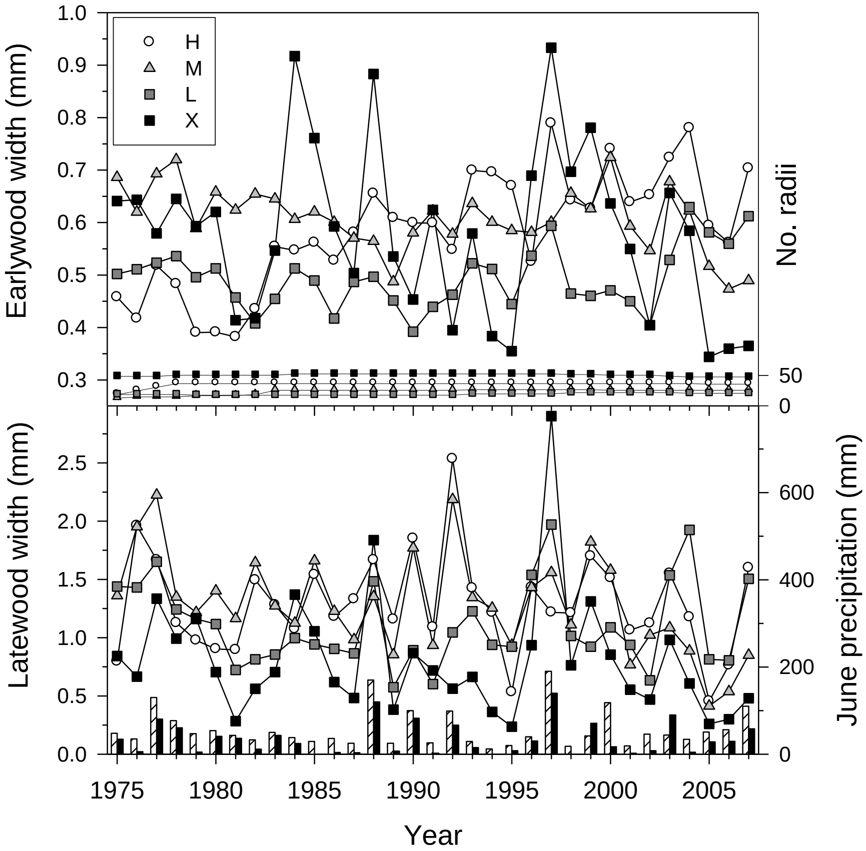


Figure 4. Trends of earlywood and latewood widths in the four study sites (*H* high-elevation site, *M* mid-elevation site, *L* low-elevation site, *X* xeric site) as related to June precipitation. Note the high year-to-year variability in the series of the xeric site (*X*). The right-side axis in the upper graph describes the yearly sample size (number of analysed radii) whereas June precipitation, the climatic variable more related with latewood width, is represented in the lower graph as bars (different fills correspond to the two regional climatic series for sites *H*, *M* and *L*, and for site *X*, respectively).

Table 5. Relationships observed between wood-anatomical variables and monthly climatic variables (*T*, mean temperature; *P*, total precipitation) for the period 1985-2007.

Variable	Site	Climatic variable	December	January	February	March	April	May
VA3q	H	T			-0.55			
	L	T	-0.63		-0.47			
VA	X	T			-0.50		-0.53	
VA1h	X	P			0.45			
CA	L	T	-0.64	-0.47				
	L	P		-0.57				
	X	P		-0.67	0.45			-0.54
Density	X	P			-0.45			

Only significant ($P<0.05$) Pearson correlations are displayed, and these were computed for months of the previous year (December) and the year of tree-ring formation (January, February, March, April). Variables' abbreviations: *VA3q*, mean area of the third-quartile earlywood vessels; *VA*, mean area of all earlywood vessels; *VA1h*, mean area of the vessels located in the first half of the earlywood; *CA*, conductive area. Sites are abbreviated as in Table 1.

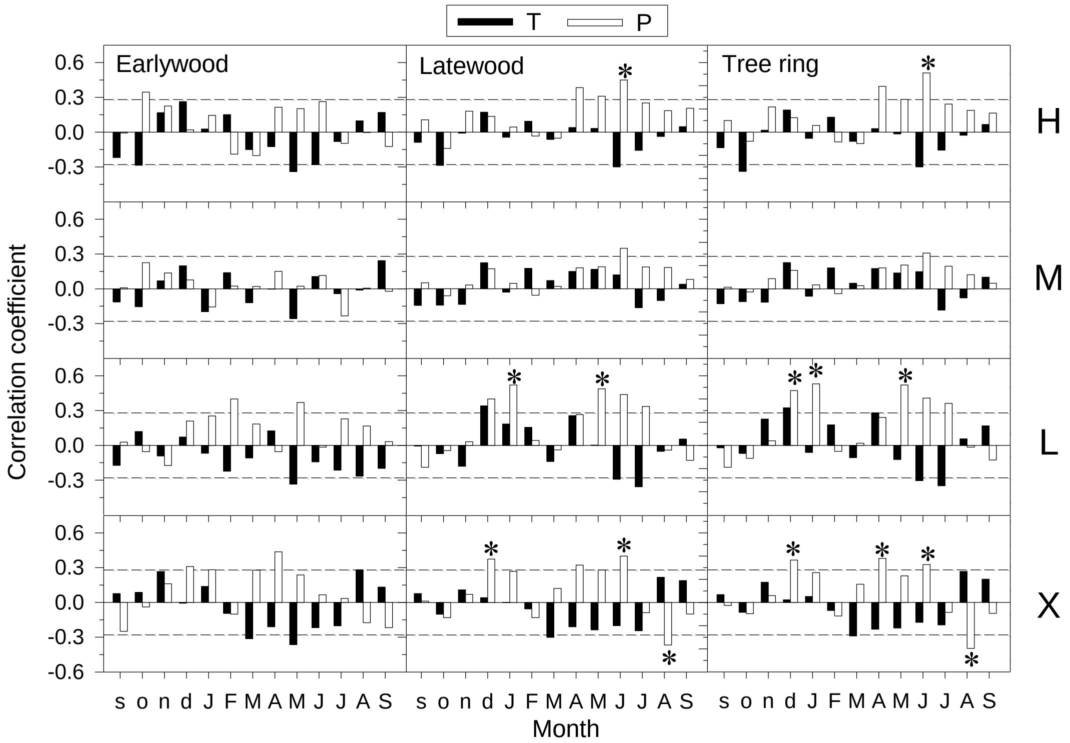


Figure 5. Correlation coefficients calculated between earlywood, latewood and tree-ring width indices and monthly climatic data (*T* mean temperature, black bars; *P* total precipitation, white bars). Pearson correlations were calculated considering climatic data of the year of tree-ring formation (*t*, months abbreviated by *capital letters*) and the preceding year (*t* - 1, months abbreviated by minor letters) in the four study sites. The dashed lines correspond to the significance ($P \leq 0.05$) thresholds for correlations and the asterisks indicate the significant bootstrapped regression coefficients.

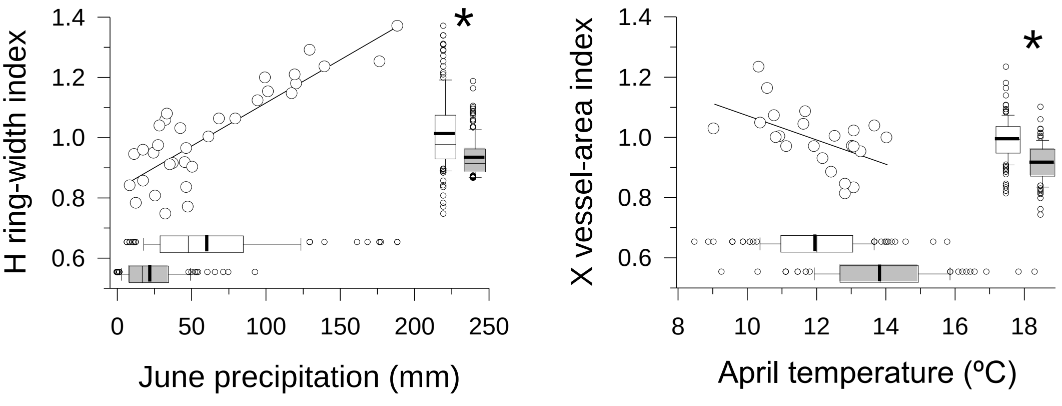


Figure 6. Predicted (twenty-first century) tree-ring width and earlywood vessel area in *Q. faginea* sites *H* (high-elevation site) and *X* (xeric site) based on the current (late twentieth century) associations between selected climatic variables and the mentioned growth and wood-anatomical variables. The associations were quantified using the displayed linear regressions. The graphs show current (*empty box* plots and *symbols*) and forecasted (*filled box* plots) climatic conditions (June precipitation, April temperature) for the last twenty-first century and corresponding tree-ring width and vessel area indices. In the *box* plots, the thin and *thick lines* correspond to the median and the mean, respectively. The *asterisks* indicate significant ($P < 0.001$) differences between observed and predicted growth and anatomical variables for the twentieth and twenty-first centuries, respectively.

variance accounted for the PC1, and a higher year-to-year variability (mean sensitivity) than vessel-related variables (Figs. 2, 4; Table 3). Third, latewood-width chronologies were more sensitive to climatic variability than vessel-area chronologies (Table 3). Thus, we concur with Tardif and Conciatori (2006) on the limited use of vessel-area chronologies as dendroclimatic records as compared with width-related measures. However, earlywood VA contained unique climatic information related to late winter temperature and precipitation, and the earlywood climatic sensitivity increased in the cold and more drought-stressed sites. Thus, the dendroclimatic potential of wood-anatomical studies in Mediterranean oaks is high in continental and xeric sites where climatic stress constrains growth through low winter temperatures and summer drought. Such anatomy–climate correlations present some weaknesses since (1) these relationships may not hold under future climatic regimes and (2) earlywood VA is a proxy of mechanistic variables as hydraulic conductivity. To amend these shortcomings, we advise (1) combining wood-anatomical studies with phenological measures (e.g. Eilmann et al. 2009) and (2) quantifying how unstable anatomy–climate associations are using series covering at least the past century.

The lower common signal in wood-anatomical features may seem to be a minute reward for the time-consuming task which involves generating well-replicated vessel series. Furthermore, if the climatic signal is weak, then the apparent new climatic information provided by wood anatomy may not be relevant for robust paleoclimatic reconstructions. Such limitation is probably derived from the lack of consensus and uniform procedures to select and define the most ‘‘climatically relevant’’ vessels which may contain different climatic information depending on vessel ontogenesis and location within the ring (Fonti and García-González 2004; García-González and Fonti 2006). Appropriate wood-anatomical information should be derived from the measurement of contemporaneous vessels with similar transversal area, if the ontogenetic origin of vessels may be properly discerned. In ring-porous wood, contemporaneous vessels are not perfectly arranged in tangential rows. The lack of a clear climatic signal in the *Q. faginea* vessel chronologies may be related to their shortness or to the fact that climate series were built using data from local stations situated along an altitudinal gradient. However, such methodological caveats do not explain our results since the common signal strength statistics, and the climatic responses of *Q. faginea* vessel chronologies were similar to those found in other study (Corcuera et al. 2004).

The high variability of vessel cross-sectional area found among *Q. faginea* stands located in sites with contrasting climatic conditions indicates that wood-anatomical features may be an additional and valuable source of information to assess how plastic growth and phenology responses are to local climatic variability. Moreover, the anatomical response of earlywood features to late winter temperatures could be used as a growth indicator of spring growth resumption in combination with phenological data such as bud burst and leaf unfolding (Čufar et al. 2008).

The presented results emphasize the site-to-site variability in the wood-anatomical response to climate. To improve the climatic information provided by the earlywood vessels, it has been suggested to select vessels according to their transversal area (García-González and Fonti 2006). Our results support this suggestion because most relationships between vessel area and climate in *Q. faginea* were more evident when using the widest earlywood vessels. Exclusively, the lumen area of the first-formed earlywood vessels responded positively to late winter rainfall in the xeric site. The reasons for the observed differences in our results compared with some previous works can probably be attributed to the dissimilarity between areas of study, the different species examined, the diverse different growing conditions and the contrasting internal controls of earlywood formation (Woodcock 1989).

In the case of *Q. faginea*, water shortage was the most important factor influencing xylem anatomy as compared with the co-occurring *Q. ilex* which is better adapted to summer drought (Villar-Salvador et al. 1997). However, we also found that the spring resumption of secondary growth in *Q. faginea* was also affected by conditions during the previous cold season since winter temperatures were negatively related to earlywood conductivity in the low-elevation site. We also uncovered a negative relationship between February temperature and earlywood vessel lumen area not only in high-elevation cold sites, but also in the dry low-elevation and xeric sites. Low temperature seems to be the main climatic stress in the uppermost site where *Q. faginea* grows close to its upper distribution limit. A possible explanation for the temperature-constraint found in the low-elevation site can be linked to the frequent thermal inversions and persistent fogs observed there where winter minimum temperatures are episodically lower than in upper sites (Alla et al. 2011). Late winter and early spring temperatures influence the physiological processes involved in spring xylogenesis such as vessel enlargement and differentiation, affecting the final lumen area of earlywood vessels in species with ring-porous wood (Fonti et al. 2006). The negative relationships between late winter temperatures before bud break and earlywood vessel lumen area may be due to an earlier differentiation of overwintering cambial derivatives (Aloni 1991). Warmer winter temperatures may cause cambium reactivation leading to the formation of narrower earlywood vessels with low theoretical hydraulic conductance.

Based on wood-anatomical and isotopic-discrimination data, Ponton et al. (2001) suggested that trees with a smaller earlywood VA might compensate the loss in hydraulic conductance through an increase in water-use efficiency. If the predicted climate warming for the Mediterranean Basin leads to warmer winters (IPCC 2007), the mean area of the widest earlywood vessels will decrease leading to a loss in total predicted hydraulic conductance which will negatively affect the development of the new canopy foliage in spring. Such loss in hydraulic conductance will be expected in *Q. faginea* stands similar to the cold high-elevation and xeric low-elevation study sites. However, if climate warming causes an increase in spring evapotranspiration, the earlywood CA will increase in drought-prone sites similar to the xeric sites. Therefore, winter temperatures can be the main climatic driver of the theoretical hydraulic conductance in Mediterranean deciduous oaks as *Q. faginea*.

Our results do not support the local disappearance of the Iberian *Q. faginea* populations near their xeric distribution limit in response to future warmer and drier conditions. The evidenced growth plasticity of these ‘‘rear-edge’’ populations from xeric sites in response to recent climatic variability suggests that they will persist during the twenty-first century unless thresholds even greater than those linked to climatic projections are surpassed. Climate-driven predictive models should introduce some measure of growth plasticity and variability to evaluate the uncertainty in their long-term forecasts of persistence of tree populations. The plastic responses of *Q. faginea* to climate among sites and across time indicate that when predicting changes in growth and wood anatomy of trees, the variance matters more than the mean.

Acknowledgements

A.Q. Alla thanks the support of CIHEAM-IAMZ and AECID grants. This study was supported by the projects CGL2007-66066-C04-02/BOS, CGL2008-04847-C02-01, RTA2005-00100-CO2-00 and SUM2006-00025-00-00 (Spanish Ministry of Science). JJC acknowledges funding by ARAID and collaborative effort within the Globimed network (http://www.globimed.net). We thank A.E. El-Kenawy for revising a previous version of the manuscript and J. Albuixech for his help in the field. We also thank the Spanish Meteorological Agency (AEMET) for providing climate data and predictions.

References

Alla AQ, Camarero JJ, Rivera P, Montserrat-Martí G (2011) Variant allometric scaling relationships between bud size and secondary shoot growth in *Quercus faginea*: implications for the climatic modulation of canopy growth. Ann For Sci 68:1245–1254.

Aloni R (1991) Wood formation in deciduous hardwood trees. In: Raghavendra AS (ed) Physiology of Trees. Wiley and Sons, New York, USA, pp 75–197.

Amaral Franco J (1990) *Quercus*. In: Castroviejo S, Laínz M, López González G, Montserrat P, Muñoz Garmendia F, Paiva J, Villar L (ed) Flora Iberica. Real Jardín Botánico, CSIC, Madrid, Spain, pp 15–36.

Arend M, Fromm J (2007) Seasonal change in the drought response of wood cell development in poplar. Tree Physiol 27:985–992.

Baas P, Schweingruber FH (1987) Ecological trends in the wood anatomy of trees, shrubs and climbers from Europe. IAWA Bull 8:245–274.

Benito Garzón M., Sánchez de Dios R, Sainz Ollero H (2008) Effects of climate change on the distributions of Iberian forest. Appl Veg Sci 11:169–178.

Briffa, K, Cook ER (1990) Methods of response function analysis. In Methods of Dendrochronology. Edited by Cook ER, Kairiuktsis LA, Kluwer, Dordrecht. pp 240–247.

Brunet M, Casado MJ, Castro M, et al. (2007) Generación de Escenarios de Cambio Climático Para España. Ministerio de Medio Ambiente, Madrid, Spain.

Camarero JJ, Olano JM, Parras A (2010) Plastic bimodal xylogenesis in conifers from continental Mediterranean climates. New Phytol 185:471–480.

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.

Carlquist S. 1975. Ecological Strategies of Xylem Evolution. Univ. California Press, Berkeley, USA.

Cook ER (1985) A Time Series Analysis Approach to Tree-ring Standardization. University of Arizona, Tucson, USA.

Corcuera L, Camarero JJ, Gil-Pelegrin E (2004) Effects of a severe drought on growth and wood anatomical properties of *Quercus faginea*. IAWA J 25:185–204.

Corcuera L, Camarero JJ, Sisó S, Gil-Pelegrin E (2006) Radial-growth and wood-anatomical changes in overaged *Quercus pyrenaica* coppice stands: functional responses in a new Mediterranean landscape. Trees 20:91–98.

Čufar K, Prislan P, de Luis M, Gričar J (2008) Tree-ring variation, wood formation and phenology of beech (*Fagus sylvatica*) from a representative site in Slovenia, SE Central Europe. Trees 22:749–758.

Eilmann B, Zweifel R, Buchmann N, Fonti P, Rigling A (2009) Drought-induced adaptation of the xylem in Scots pine and pubescent oak. Tree Physiol 29:1011–1020.

Ellmore GS, Ewers FW (1986) Fluid flow in the outermost xylem increment of a ring-porous tree, *Ulmus americana*. Am J Bot 73:1771–1774.

Fonti P, García-González I (2004) Suitability of chestnut earlywood vessel chronologies for ecological studies. New Phytol 163:77–86.

Fonti P, Solomonoff N, García-González I (2006) Earlywood vessels size of *Castanea sativa* records temperature before their formation. New Phytol 173:562–570.

Fonti P, von Arx G, García-González I, Eilmann B, Sass-Klaassen U, Gärtner H, Eckstein D (2010) Studying global change through investigation of the plastic responses of xylem anatomy in tree rings. New Phytol 185:42–53.

Frankenstein C, Eckstein D, Schmitt U (2005) The onset of cambium activity − A matter of agreement? Dendrochronologia 23:57–62.

Fritts HC. 1976. Tree Rings and Climate. Academic Press, New York.

Fritts HC, Vaganov EA, Sviderskaya IV, Shashkin AV (1991) Climatic variation and tree-ring structure in conifers: empirical and mechanistic models of tree-ring width, number of cells, cell-size, cell-wall thickness and wood density. Clim Res 1:97−116.

García-González I, Eckstein D (2003) Climatic signal of earlywood vessels of oak on a maritime site. Tree Physiol 23:497–504.

García-González I, Fonti P (2006) Selecting earlywood vessels to maximize their environmental signal. Tree Physiol 26:1289–1296.

García-González I, Fonti P (2008) Ensuring a representative sample of earlywood vessels for dendroecological studies: an example from two ring-porous species. Trees 22:237–244.

Giantomasi MA, Roig FA, Villagra PA, Srur AM (2008) Annual variation and influence of climate on the ring width and wood hydrosystem of *Prosopis flexuosa* DC trees using image analysis. Trees 23:117–126.

Granier A, Anfodillo T, Sabatti M, Cochard H, Dreyer E, Tomasi M, Valentini R, Bréda N (1994) Axial and radial water-flow in the trunks of oak trees–a quantitative and qualitative analysis. Tree Physiol 14:1383–1396.

Hacke UG, Sperry JS (2001) Functional and ecological xylem anatomy. Persp Plant Ecol Evol Syst 4:97–115

Holmes RL (1983) Computer-assisted quality control in tree ring dating and measurement. Tree-Ring Bull 43:69–78.

Holmes RL (1992) Dendrochronology Program Library. University of Arizona, Tucson, USA.

IPCC (2007) The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. University Press, Cambridge, UK.

Jiménez P, Díaz-Fernández PM, Martín S, Gil L (1998) Regiones de Procedencia de *Quercus pyrenaica W.*; *Quercus faginea Lam*. y *Quercus canariensis Willd*. Dirección General de Conservación de la Naturaleza, Madrid, Spain.

Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O (2006) SAS for Mixed Models. SAS Institute, Cary, USA.

Loidi J, Herrera M (1990) The *Quercus pubescens* and *Quercus faginea* forests in the Basque Country (Spain): distribution and typology in relation to climatic factors. Vegetatio 90:81–92

Montserrat-Martí G, Camarero JJ, Palacio S, Pérez-Rontomé C, Milla R, Albuixech J, Maestro M (2009) Summer-drought constrains the phenology and growth of two co-existing Mediterranean oaks with contrasting leaf habit: implications for their persistence and reproduction. Trees 4:787–799.

Ninyerola M, Pons X, Roure JM (2005) Atlas Climático Digital de la Península Ibérica. Metodología y aplicaciones en bioclimatología y geobotánica. Universidad Autónoma de Barcelona, Bellaterra, Spain.

Ponton S, Dupouey JL, Bréda N, Feuillat F, Bodénès C, Dreyer E (2001) Carbon isotope discrimination and wood anatomy variations in mixed stands of *Quercus robur* and *Quercus petraea*. Plant Cell Environ 24:861–868.

Pumijumnong N, Park WK (1999) Vessel chronologies from teak in Northern Thailand and their climatic signal. IAWA J 20:285–294.

Sass U, Eckstein D (1995) The variability of vessel size in beech (*Fagus sylvatica* L.) and its ecophysiological interpretation. Trees 9:247–252.

Sperry JS, Nichols KL, Sullivan JEM, Eastlack SE (1994) Xylem embolism in ring-porous, diffuse-porous and coniferous trees of northern Utah and interior Alaska. Ecology 75:1736–1752.

Suzuki M, Yoda K, Suzuki H (1996) Phenological comparison of the onset of vessel formation between ring-porous and diffuse-porous deciduous trees in a Japanese temperate forest. IAWA J 17:431–444.

Tardif JC, Conciatori F (2006) Influence of climate on tree rings and vessel features in red oak andwhite oak growing near their northern distribution limit, southwestern Quebec, Canada. Can J For Res 36:2317–2330.

Tessier L, Nola P, Serre-Bachet F (1994) Deciduous *Quercus* in the Mediterranean region: tree-ring/climate relationships. New Phytol 126:355–367.

Villar-Salvador P, Castro-Díez P, Pérez Rontomé C, Montserrat-Martí G (1997) Stem xylem features in three *Quercus* (Fagaceae) species along a climatic gradient in NE Spain. Trees 12:90–96.

Wigley TML, Briffa KR, Jones PD (1984) On the average value of correlated time-series, with applications in dendroclimatology and hydrometeorology. Int J Clim Appl Meteorol 23:201–213.

Willmott CJ, Rowe CM, Mintz Y (1985) Climatology of the terrestrial seasonal water cycle. J Climatol 5:589–606.

Wimmer R (2002) Wood anatomical features in tree-rings as indicators of environmental change. Dendrochronologia 20:21–36.

Woodcock D (1989) Climate sensitivity of wood-anatomical features in a ring-porous oak (*Quercus macrocarpa*). Can J For Res 19:639–644.

Zasada JC, Zahner R (1969) Vessel element development in earlywood of red oak (*Quercus rubra*). Can J Bot 47:1965–1971.

Supporting Information

Table S1. Meteorological stations used to compile the two regional climatic series for sites H, L and M (H, high-elevation site, M, mid-elevation site; L, low-elevation site) and for the site X (xeric site) of monthly mean temperature (T) and monthly total precipitation (P).

Site	Station[1]	Latitude (N)	Longitude (W)	Elevation (m)	Variable	Period (T / P)	Missing data T/P (%)	T (ºC)	P (mm)
H	Nocito	42º 20′	0º 15′	930	T / P	1950-2007 / 1950-2007	0.0 / 0.0	10.2	868
H	Aineto	42º 23′	0º 11′	985	T / P	—— / 1941-1994	—— / 0.0	——	892
H-M	Arguis-DGA	42º 19′	0º 26′	1039	T / P	1992-2007 / 1992-2007	47.2 / 9.9	10.3	899
M	Bernués	42º 29′	0º 35′	887	T / P	1970-2007 / 1931-2007	0.0 / 0.0	10.8	817
M	Yebra de Basa	42º 29′	0º 17′	880	T / P	1941-2007 / 1970-2007	0.0 / 0.0	10.5	887
M	Hostal de Ipiés	42º 26′	0º 24′	780	T / P	1973-2007 / 1949-2007	11.9 / 6.8	11.2	728
L	Embalse de la Sotonera	42º 06′	0º 40′	413	T / P	1939-2007 / 1919-2007	0.0 / 0.0	13.4	469
L	Huesca-Monflorite	42º 05′	0º 20′	541	T / P	1944-2007 / 1951-2007	0.0 / 0.0	13.5	549
L	Apies	42º 14′	0º 24′	680	T / P	1970-2007 / 1968-2004	4.0 / 3.6	13.5	686
L	Nueno	42º 16′	0º 26′	726	T / P	1973-2007 / 1962-2007	6.8 / 0.0	12.4	723
X	Alcubierre “Silo”	41º 49′	0º 27′	445	– / P	—— / 1929-2007	—— / 0.0	——	453
X	Leciñena	41º 48′	0º 37′	415	T / P	1966-2007 / 1970-2007	0.0 / 0.0	13.9	396
X	Pallaruelo de Monegros	41º 42′	0º 12′	356	T / P	1955-2007 / 1953-2007	0.0 / 0.0	14.7	378
X	Zaragoza-Aeropuerto	41º 40′	1º 00′	247	T / P	1910-2007 / 1906-2007	0.0 / 0.0	14.6	324

[1] Local climatic data were corrected for elevation differences with closest sampling sites using interpolated data from Ninyerola *et al.* (2005).

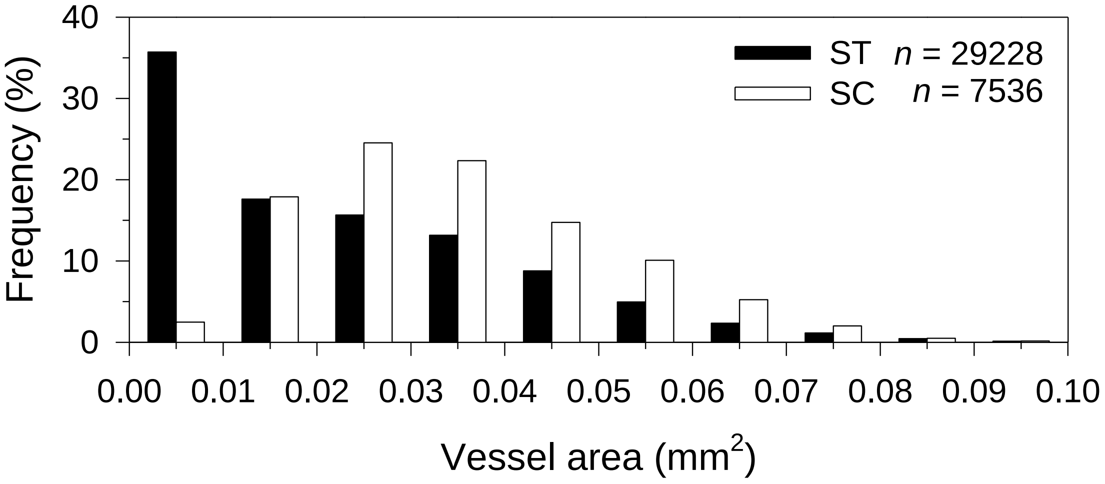


Figure S1. Distribution of all vessel areas measured using stereomicroscope (ST) or scanner (SC). Vessel area based on stereomicroscope analyses is usually smaller than area based on scanned samples. The two distributions differed significantly (*G*-test, G = 41.9, $P < 0.01$).

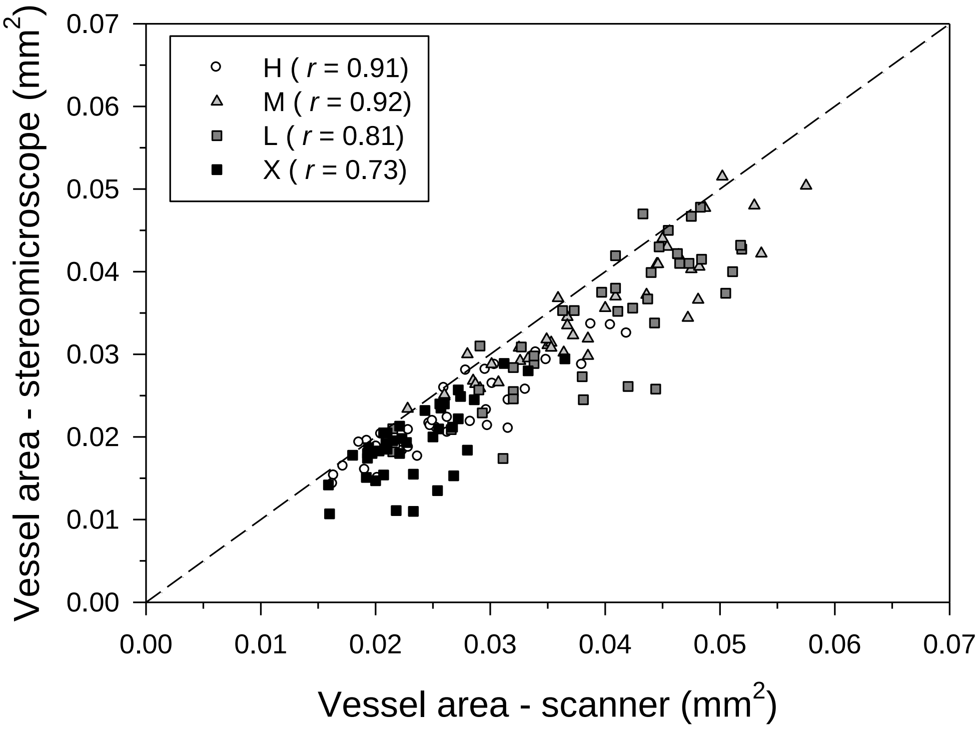


Figure S2. Relationships between mean vessel area measured using stereomicroscope or scanner for the same radii and years in the four study sites (H, high-elevation site; M, mid-elevation site; L, low-elevation site; X, xeric site). Vessel area based on stereomicroscope analyses is usually smaller than the vessel area based on scanned samples (the diagonal line corresponds to $x = y$). Pearson correlation values were highly significant ($P < 0.001$).

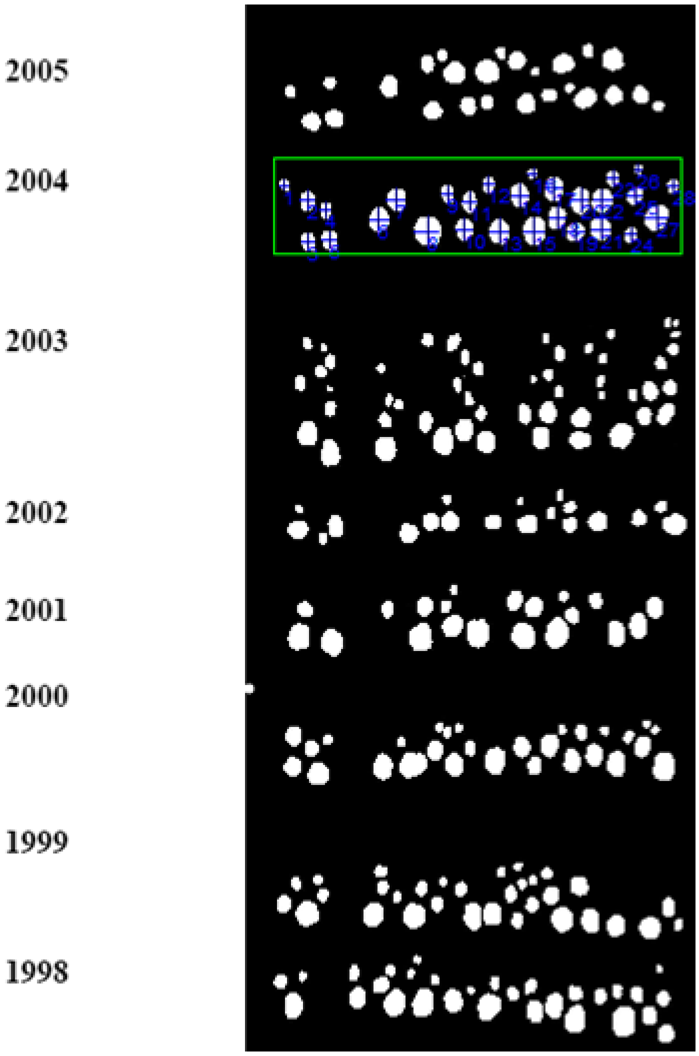


Figure S3. Image of a cross-dated and processed *Q. faginea* core used for earlywood-anatomical analyses. The analysed area (green rectangle) and vessels (note the two perpendicular diameters crossing each vessel lumen) are shown for the tree-ring formed in the year 2004 (*n* = 28 vessels). This sample was obtained from a tree growing in the xeric study site (site X).

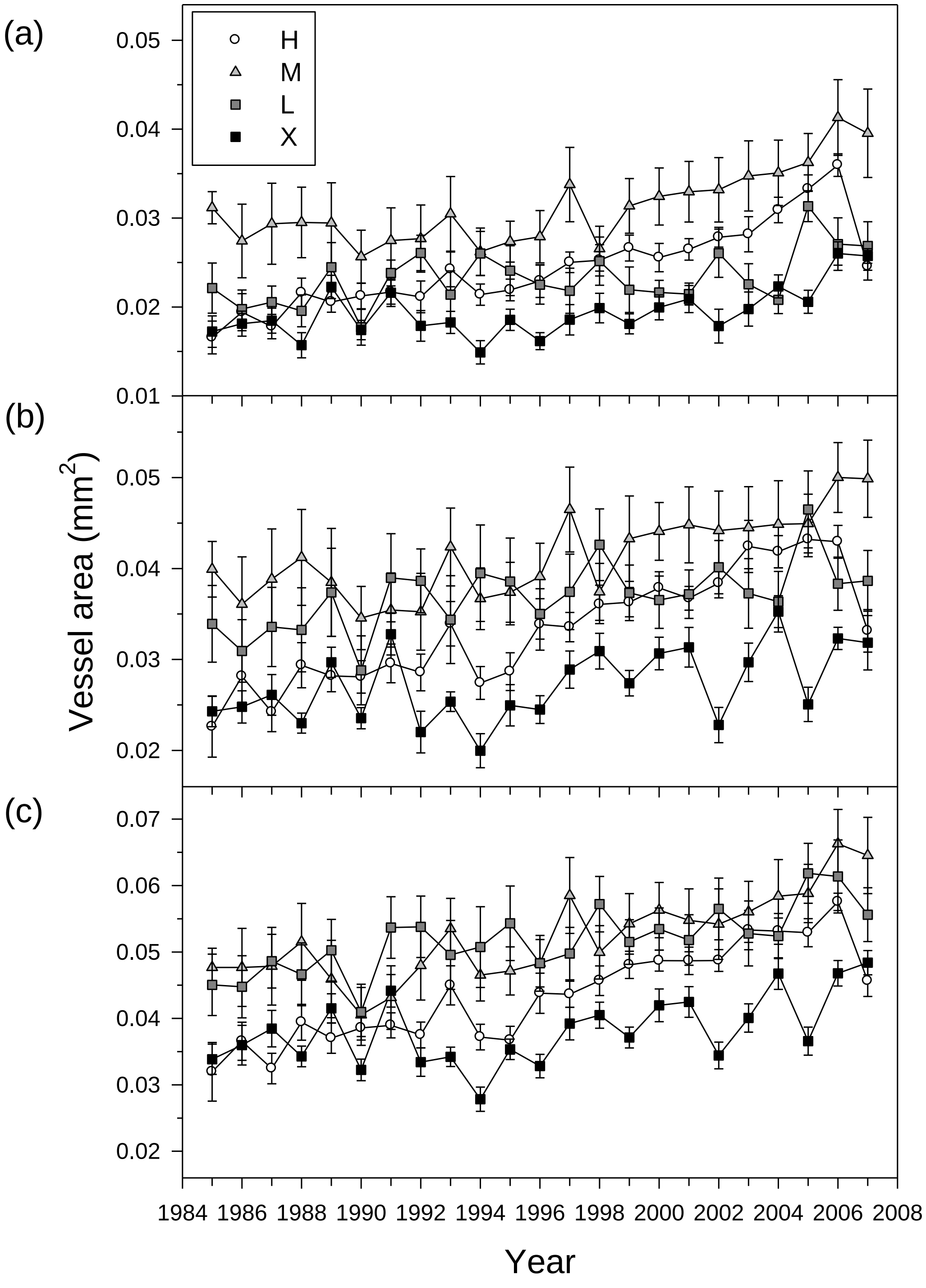


Figure S4. Changes in mean (± SE) earlywood vessel areas during the 1985-2007 period in the four study sites (H, high-elevation site, M, mid-elevation site; L, low-elevation site; X, xeric site) considering all vessels (a), only vessels located in the first half of the earlywood (b) and only vessels whose area was within the third upper quartile of vessel area (c). Note the different scales used in the three graphs. The error bars are standard errors.

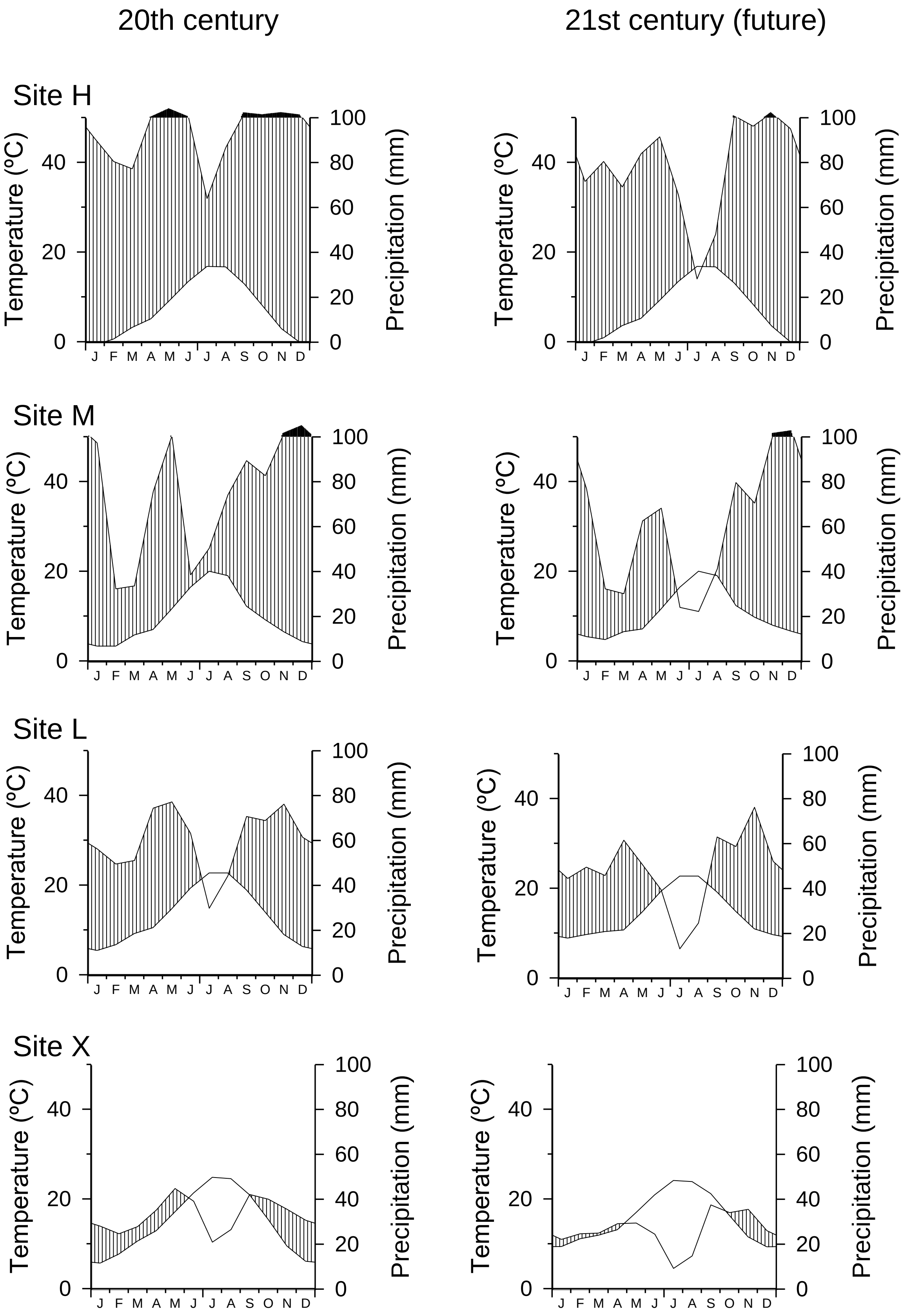


Figure S5. A schematic comparison of recent (20th century, period 1961-1990, see Fig. 1) and projected (21st century, period 2071-2100) climatic conditions in the four study sites.

CHAPTER II

Variant allometric scaling relationships between bud size and secondary shoot growth in Quercus faginea: implications for the climatic modulation of canopy growth

II. Variant allometric scaling relationships between bud size and secondary shoot growth in *Quercus faginea*: implications for the climatic modulation of canopy growth

Abstract The relationships between primary and secondary growth in tree populations of contrasting climates are poorly understood. We tested the hypotheses that bud size and stem cross-sectional area are related through allometric relationship in shoots and that their scaling slopes change in response to climatic stress. We sampled three *Quercus faginea* populations subjected to contrasting climates and elevations. The main components of the current-year shoots (length, cross-sectional area, apical bud mass, and number of buds) were measured in ten trees per site, and the relationships among them were analyzed using structural equation models. Cross-sectional area and apical bud mass were allometrically related and they were higher in the mid-elevation site than elsewhere. In the mid-elevation site, the relationship between cross-sectional area and apical bud mass was the strongest and its scaling slope was the highest. Hence, for a given increase in shoot cross-sectional area, trees from the mid-elevation site produced bigger buds than trees from the other sites. Trees from the mid-elevation site showed a greater potential for primary growth since mild temperatures and low-drought stress improve secondary shoot growth leading to an increased bud size. Therefore, secondary growth affects to a great extent bud size through allometric scaling which is modulated by climatic stress.

Keywords Allometry, Bud mass, Secondary growth, Mediterranean climate, Structural equation model.

Introduction

The search of links between form and function has fostered the research on tree attributes which often scale allometrically (Niklas 1994). For instance, the assessment of allometric relationships in current-year shoots has been focused on the leaf–stem size relationships, known as one of Corner’s rules (Brouat et al. 1998). Several studies have demonstrated that stem thickness is correlated with the total leaf area held by the stem (Westoby and Wright 2003). However, the allometric studies dealing with the structure of current-year shoots have rarely considered bud variables such as size or mass.

In species with preformed growth as oaks, buds are the plant investment for the crown growth in the next year (Vesk and Westoby 2004). A mature oak tree has a huge population of renewal buds which can be classified into three types: current-year large (vegetative) buds located in distal positions, small leafless (latent) buds located in proximal positions, and reproductive buds (Wilson and Kelty 1994). Usually, only some current-year buds grow out to form new shoots, which suggests that bud size in winter could be a valuable predictor of shoot production in spring (Harmer 1991). Such shoot–bud relationships might be also modulated by climatic stress along altitudinal gradients as has been found for leaf and stem cross-sectional areas (Sun et al. 2006).

Shoot growth differentiation and bud development are influenced by climatic stress (Barthélémy and Caraglio 2007). Therefore, the potentially allometric interactions among them might also be adjusted by climate. Current-year shoots have been mainly studied as organs for leaf arrangement being mostly made of primary tissues (Suzuki and Hiura 2000). However, the complex dynamics of secondary growth in current-year shoots, which also depend on primary growth, may affect bud development (Lauri et al. 2010). Furthermore, few studies have attempted to evaluate shoot-bud allometries at the intraspecific level in tree populations (but see Normand et al. 2008) subjected to contrasting climatic conditions.

We evaluate how climatic stress affects bud size and shoot secondary growth in Quercus faginea, a deciduous Mediterranean oak. In *Q. faginea*, stems extend quickly in early spring after bud burst. Once shoot extension is accomplished, buds start their main development up to early autumn while shoot secondary growth mostly occurs in spring and resumes in autumn (Montserrat-Martí et al. 2009). Thus, the sequential phenology or the overlapping of these developmental processes suggests potential causal relationships among current-year shoot components which may scale allometrically.

Since a higher conductivity in the vascular system is linked to an enhanced bud development and size (Cochard et al. 2005), we hypothesized that the stem cross-sectional area and vessel anatomy of current-year shoots, used here as surrogates of hydraulic conductivity (Sperry et al. 2006), must influence greatly the size of buds in *Q. faginea*. We also evaluated if the hypothesized association between stem cross-sectional area and apical bud mass is allometric, and if this relationship is modulated by climate. Following Niklas (1994), in this study, we considered allometry as a scaling relationship, as opposed to an isometric or nonscaling relationship, produced by size-correlated variations in shoot variables potentially reflecting different growth processes. We tested our hypothesis quantifying and characterizing the associations among several shoot variables (stem length and cross-sectional area, bud mass) in three *Q. faginea* populations subjected to contrasting climatic conditions along an altitudinal gradient.

Materials and methods

Study area

The study area is located in the Aragón Pre-Pyrenees (Spain) where climate is Mediterranean and continental being characterized by a dry summer and a cold winter (Table 1, Supplementary information, Fig. S1). Three sites along an altitudinal gradient were selected, the extreme sites being 8 km distant: Pico del Águila (high-elevation site, hereafter abbreviated as H site), Arguis (mid-elevation site, hereafter abbreviated as M site), and Nueno (low-elevation site, hereafter abbreviated as L site; Table 1). Climatic data were obtained from the closest meteorological stations (H and M sites, Arguis, 42° 19′ N, 0° 26′ W, 1,039 m; M site, Hostal de Ipiés, 42° 26′ N, 0° 24′ W, 780 m; L site, Nueno, 42° 16′ N, 0° 26′ W, 726 m). These values were corrected taking into account: (1) local data of air temperature and humidity recorded every 30 min during the year 2007 using one Hobo H8 Pro Series datalogger (Onset Co., USA) per site (Supplementary information, Fig. S1); (2) a linear regression between annual precipitation (y) and elevation (x) built using data from six meteorological stations located along the altitudinal gradient (y=189.96+0.697x; r^2=0.98, P<0.05; Supplementary information, Table S1). The sites shared similar soils, substrate (limestone) and aspect. We assumed that climatic stress was lower in the mesic M site than in the high- and low-elevation sites. Co-occurring trees in the H, M, and L sites were *Pinus sylvestris* L., *Quercus ilex* subsp. *ballota* (Desf.) Samp, and *Quercus coccifera* L., respectively.

Field sampling and variables measured

In March 2006, 20 *Q. faginea* trees of similar size were selected and tagged in each site. To avoid confusion with hybrids, we only selected those trees which presented clear *Q. faginea* morphological characteristics (Himrane et al. 2004). The size of all trees was measured considering that this species is usually multi-stemmed (diameter at breast height, total height, number of trunks per tree). To estimate tree age, rings were counted in radial cores taken at 1.3 m from the thickest stem with an increment borer. To determine the length of the vegetative period of tagged trees, the spring bud and shoot development were analyzed fortnightly. In each field record, the percentage of bursting buds and elongating shoots were visually estimated in some representative branches of the canopy. To extrapolate such percentages to the whole canopy, we divided the crown into its main branches and the percentages were estimated for each branch and the whole canopy. We estimated the day when 50% of the studied trees showed at least 50% of their crowns covered by sprouting buds or by extending shoots (Montserrat-Martí et al. 2009).

The sampling of current-year shoots was carried out at mid-August 2007 (summer) when leaves have fully developed and at mid-January 2008 (winter) to estimate bud size

Table 1. Characteristics of the three study sites (H, high elevation site; M, mid elevation site; L, low elevation site).

Site	Latitude (N)	Longitude (W)	Elevation (m)	Mean annual temperature (ºC)	Minimum and maximum air temperatures in 2007 (ºC)	Number of days with air temperatures below 0 ºC in 2007	Mean relative air humidity in 2007 (%)	Total annual precipitation (mm)	Estimated water deficit (mm) / period[a]
H	42º 19′ 01″	0º 24′ 46″	1470	9.1 ± 0.1c	-8.9 / 30.7	33	67.6 ± 0.2a	1215	0
M	42º 19′ 30″	0º 25′ 31″	1135	10.8 ± 0.1b	-6.8 / 32.3	14	65.4 ± 0.2b	899	0
L	42º 15′ 50″	0º 25′ 59″	700	13.1 ± 0.1a	-6.3 / 38.8	12	51.8 ± 0.2c	670	270/Jul-Aug

Values are means ± SE. Different letters correspond to significant ($P < 0.05$) differences between sites.
[a] Based on the difference between monthly mean temperature and two times the monthly total precipitation and considering data for the period 1970–2007 (see Supplementary information, Fig. S1).

(bud length) since buds are completely developed in winter (Montserrat-Martí et al. 2009). In August, ten trees per site were randomly selected. Six 3-year-old branches at the mid-crown on the southern exposure were harvested per tree. We randomly selected 10 current-year shoots in the whole current-year shoot sample of each branch, yielding a total of 600 current-year shoots per site. Shoots whose length was greater or lower than the site mean±2SD were excluded and also the lammas shoots. For each sampled shoot, we measured the stem length (SL) with a resolution of 0.5 mm and the stem diameter (the average of two perpendicular measures taken in the middle of the stem without nodes) with a centesimal caliper (Mitutoyo, Kawasaki, Japan). Diameter was transformed to stem cross-sectional area (SCA) assuming a circular shape. The number of leaves, leaf scars, and acorns were counted. Shoots were separated in their fractions (stem, leaves, and acorns) and oven-dried at 60°C to constant weight and the dry weight of each shoot fraction was obtained (stem mass, SM; leaf mass, LM; acorn mass, AM). To calculate mean leaf area, 30 leaves were randomly taken from each marked tree and their leaf area was measured individually with a Skye Analysis System (Skyeleaf 1.11, Powys, UK). Leaves were oven-dried at 60ºC to constant weight and their leaf mass per area ratio (LMA) was calculated by dividing the dry weight per leaf area.

The winter sampling was performed in the same tagged trees yielding also a total sample size of 1,800 shoots for the three sites. For each shoot, we measured stem length, stem diameter, number of acorn scars, and number of buds >1 mm, as only large buds produce new shoots (Harmer 1991). The apical bud of each shoot was removed at ×10 magnification under a stereomicroscope. Buds were oven-dried at 60°C to a constant weight before being individually weighted with a precision scale to obtain apical bud mass (ABM), which is highly related to bud length (r=0.97, n=290, P<0.001).

Wood anatomy of current-year shoots

In August 2007, we collected two current-year shoots per tree in each site. Stems were fixed in formaldehyde-ethanol-acetic acid solution and stored in 50% ethanol. Cross sections of the middle stem (10–20 μm thick) were cut with a sliding microtome (Anglia Scientific AS 200, UK). They were stained with safranin (0.5 g in 100 ml 96% ethanol) solution and mounted in Eukitt® (Merck, Germany). Mounted cross sections were photographed under a light microscope at ×100 magnification with a digital camera. Photographs were processed and converted into black-and-white images. The number of vessels and the area they occupied, excluding the pith, were analysed using the ImageJ software (Rasband 1997–2009) considering all vessels whose area was greater than 0.0001 mm^2 (Supplementary information, Fig. S2). Finally, we calculated the predicted hydraulic conductance (Kh) as the sum of the fourth power diameters of all the vessels from each section assuming that vessel areas had a circular shape (Sperry et al. 2006).

Statistical analyses

All variables were checked for normality using the Shapiro–Wilks test. Stem length and mass, leaf and acorns mass were log (x)-transformed, and stem diameter was $x^{1/3}$-transformed to follow normality. We assessed differences in the distribution frequencies of several variables using the *G* test (Sokal and Rohlf 1995). Differences between sites (fixed factor) were assessed using linear mixed models considering as random factors branches (nested in trees) and trees (nested in sites) (Littell et al. 2006). We used the restricted maximum-likelihood method and type III sum of squares within the MIXED procedure (SAS 9.0, Institute Inc., Cary, NY, USA). Mean values of sites were compared using Bonferroni tests when variances were equal or Dunnett’s T3 tests otherwise. Correlation analyses were performed to evaluate the relationship between growth variables based on Pearson coefficients (r) except those involving number of leaves and acorns which were analyzed using the rank Spearman coefficient (rs). Means are reported with their standard errors.

Allometries

In the case of allometric relationships, two variables (x, y) are related by a power equation ($y=b\ x^a$) which becomes linear after log-transformation ($\log y=\log b+a \log x$). Since we were more interested in the response of shoot variables to changes in shoot secondary growth, SCA was considered as the independent variable (x) in most of the analyses performed. The terms b and a are the y intercept and the slope of the relationship, respectively. The slope value determines if the relationships among plant traits are isometric (a=1) or allometric ($a\neq1$). To compare the y intercepts and the slopes of allometric equations between sites for selected variables, we performed model type II regression analyses. The slopes were calculated as standardized major axes because variables showed associated variation due to both measurement and sampling errors (Sokal and Rohlf 1995). Confidence intervals for individual regression slopes were calculated following Warton and Weber (2002) and Warton et al. (2006). The heterogeneity of regression slopes was assessed using analysis of variance and post hoc Tukey tests. The parameters of allometric equations were calculated using SMATR version 2.0 (Falster et al. 2006).

Structural equation models

Structural equation models (SEM) provides a robust framework to analyze the allometric relationships among plant traits (Shipley 2004). Researchers may enter information a priori and reformulate the models based on goodness-of-fit statistics thus allowing the use of both deductive and inductive approaches (Bollen 1989). This frame allows testing causal relationships and provides an assessment of direct and indirect influences among variables usually presented as standardized partial regression coefficients or path coefficients.

We built evaluated SEMs based on previously tested hypotheses and allometric relationships among the main shoot variables (stem length, stem cross-sectional area, apical bud dry mass, and number of buds per shoot). In addition, we used available phenological knowledge of *Q. faginea* to consider cause-and-effect relationships among variables (Montserrat-Martí et al. 2009). For instance, since shoot primary growth starts before secondary growth, stem length and cross-sectional area were considered as cause and effect, respectively. Note however that an appropriate testing of cause-effect relationships requires empirical approaches. First, we built a global SEM for the entire winter sample dataset (n=1,800), i.e., assuming that the relationships among stem variables did not differ among sites. Second, we hypothesized that the global SEM could be successfully fitted to the datasets of each site (*n*=600). We tested this hypothesis through a multigroup analysis evaluating the fitness of the global model to each site (Bentler 1995). Third, in the case that the last hypothesis was not supported by data, we should build different local SEMs for the three sites. Site SEMs were fitted using the multivariate Lagrange multiplier test on constrained parameters and the Wald W statistic (Bentler 1995).

To estimate SEMs we used the maximum likelihood method (Bentler 1995). The estimation of all the statistics and some indices considered from first up to fourth-order moments between variables. We used the following statistics and indices to evaluate the SEMs fitness: the Satorra-Bentler robust chi-square (χ^2 S-B), the robust root mean square error of approximation (R-RMSEA), the standardized root mean square residual (SRMR), the Goodness-of-Fit Index (GFI), the Adjusted Goodness-of-Fit Index (AGFI), and the Robust Comparative Fit Index (R-CFI; Jöreskog 1993). Values close to zero for the χ^2 S-B, R-RMSEA, SRMR statistics and values close to one for the GFI, AGFI, and RCFI indices would indicate that the evaluated models are consistent with the theoretical model. The use of several indices to evaluate the model fitness provides a robust assessment of the fitted model (Jöreskog 1993). SEMs were performed using the EQS program (Bentler 1995).

Results

Tree and shoot variables

The M site presented the largest trees and the H site the smallest ones (Table 2). The number of trunks per tree and the length of the vegetative period also decreased upwards. LMA was higher in the L site than elsewhere.

Several variables showed significant differences among sites (Table 3). The trees in the M site presented significantly longer and thicker stems, larger buds and higher stem and leaf mass values than the trees from the other two sites. Longer stems were more frequent in the M site than in the H and L sites (G=64.48, P<0.001; Fig. 1). In addition, the distribution of SCA in the M site was the most skewed towards thicker stems being significantly different from the other sites (G=358.63, P<0.001; Fig. 1). The number of buds per stem was higher in the H site than elsewhere. Finally, the stems tended to be shorter and thicker (in site H) in winter than in summer but differences in the mean values of SL and SCA were not significant between both sampling periods (P>0.05, data not shown).

Allometric relationships

Most of the current-year shoots' variables were significantly and positively related (Supplementary information, Table S2, Fig. S3). Summer LM was more tightly associated with SCA than SL and SM (Table 4). The SCA-SL relationship was stronger in winter than in summer. In winter, ABM was more highly related with SCA than with SL, but both relationships were stronger in the M site than elsewhere. The slopes of the SCA-ABM allometric relationship differed among sites, being highest for the M site (Fig. 2).

Vessel features

The vessels with the largest transversal areas were observed in the current-year shoots of M and L sites whereas the vessels with smallest areas were more frequent in the H site, and mean vessel areas significantly (P<0.05) differed between sites (Table 3). Among the largest vessels, those comprising a higher percentage of the total predicted hydraulic conductance were more frequent in the M site than in the other two sites (Fig. 3). In fact, the distributions of vessels according to their transversal areas differed significantly among the three studied sites (*G*=124.31, *P*<0.001).

SEM results

The global SEM provided a satisfactory fit of the entire dataset (χ^2 S-B=0.87, *P*=0.64; Table 5). However, the multigroup analyses did not reach satisfactory fits (χ^2 S-B=79.94, *P*<0.001) indicating that the three local site datasets would be more adequately described by different SEMs. Therefore, we evaluated different SEMs to the local datasets of each site which were successfully fitted (χ^2 S-B=2.75‑5.38, *P*=0.02‑0.13).

Considering the global SEM of the entire dataset, the strongest direct relationships were found between SL, SCA, and the number of buds, but no direct relationship between SCA and number of buds was observed. The

Table 2. Morphological and phenological features of trees according to the study sites (sites' codes are as in Table 1).

					Phenology		
Site	Dbh (cm)	Height (m)	Age (years)[a]	No. trunks ind^{-1}	Bud	Shoot	LMA (mg mm^{-2})
H	8.2 ± 0.8b	4.4 ± 0.3b	34.2 ± 0.7	1.6 ± 0.2b	4 May	19 May	12.0 ± 0.1b
M	14.2 ± 1.0a	5.8 ± 0.4a	33.3 ± 1.9	1.8 ± 0.3b	24 April	5 May	12.0 ± 0.1b
L	12.8 ± 1.1a	5.3 ± 0.3a	35.8 ± 2.7	2.6 ± 0.3a	2 April	10 April	12.3 ± 0.1a

Phenological phases indicate the estimated day when 50% of the studied trees showed at least half of their crowns covered by sprouting lateral buds or by extending shoots. Different letters correspond to significant (*P*<0.05) differences between sites. *Dbh* diameter at breast height, *LMA* leaf mass per area ratio. [a]Number of rings counted in wood cores sampled at 1.3 m.

Table 3. Mean (± SE) values of the studied variables and statistics (*F*, *P*) of the mixed models.

Season	Site	SL (mm)	SCA (mm^2)	SM (mg)	LM (mg)	ABM (mg)	No. buds per stem	VA (mm^2 10^{-4})
Summe	H	29.0 ± 0.6b	1.0 ± 0.1c	73.8 ± 2.1b	432.1 ± 8.4c	–	–	2.41 ± 0.02b
	M	35.0 ± 1.0a	1.5 ± 0.1a	135.4 ± 5.5a	757.7 ± 16.7a	–	–	2.52 ± 0.02a
	L	29.5 ± 0.7b	1.1 ± 0.1b	67.1 ± 1.9b	487.1 ± 9.5b	–	–	2.23 ± 0.02c
	F (*P*)	0.38 (0.68)	**26.96 (<0.0001)**	**10.27 (0.0005)**	**18.29 (<0.0001)**	–	–	**41.73 (<0.0001)**
Winter	H	28.4 ± 0.6b	1.3 ± 0.1b	–	–	8.9 ± 0.1b	6.0 ± 0.1a	–
	M	32.6 ± 0.8a	1.5 ± 0.1a	–	–	15.4 ± 0.3a	5.4 ± 0.1b	–
	L	26.8 ± 0.5c	1.1 ± 0.1c	–	–	6.8 ± 0.1c	4.0 ± 0.1c	–
	F (*P*)	1.35 (0.28)	**9.47 (0.0008)**	–	–	**17.03 (<0.0001)**	**10.11 (0.0005)**	–

Site was regarded as fixed factor, whereas tree (nested within site) and branch (nested within tree) were considered random factors. Sites' codes are as in Table 1. *SL* stem length, *SCA* stem cross sectional area, *SM* stem mass, *LM* leaf mass, *ABM* apical bud mass, *VA* vessel area. Different letters correspond to significant differences between sites (*P* < 0.05). Significant effects are in bold.

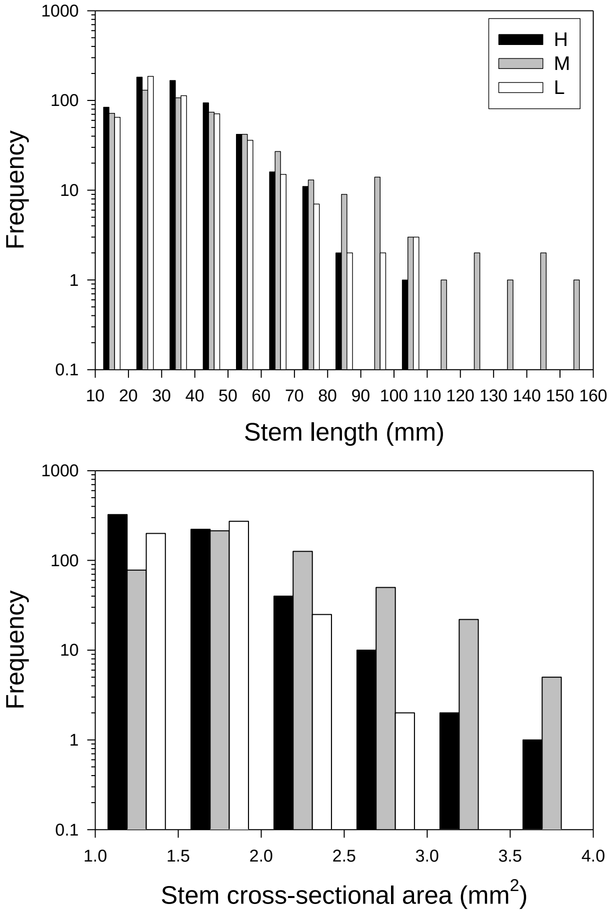


Figure 1. Distributions of stem length and stem cross sectional area according to their size in the three study sites (*H* high elevation site, *M* mid elevation site, *L* low elevation site). Note the logarithmic scale in both graphs.

variable most strongly related to ABM was SCA, followed by the number of buds (Fig. 4). In the case of the SEMs fitted to each site (Supplementary information, Fig. S4) dataset we found, as in the global model, strong positive effects of SL on SCA, and of the last variable on ABM. An additional positive effect of SCA on the number of buds was also detected in all site models. A direct effect of SL on ABM was found only for the M site, and a positive effect of the number of buds on ABM for the L site. For the H site, we also found a negative correlation between the variances not explained by the model of the number of buds and ABM.

Discussion

Functional interpretation of the allometric relationships

As predicted, we confirmed that the relationship between stem cross-sectional area and bud size followed a scaling relationship, i.e., it was allometric rather than isometric and the allometry was positive (Preston and Ackerly 2003). We found additional allometric relationships between shoot variables (length, number of buds) and apical bud mass. However, the variable which explained most variability in bud size in all sites was the stem cross-sectional area, being an association stronger in the mid-elevation site than elsewhere. The allometric relationship between bud size and stem cross-sectional area was not constant along the altitudinal gradient, i.e., it varied among different habitats showing different scaling slopes. Such variant allometric scaling relationship found in the present study indicates that bud size increases with stem cross-sectional area at a variable and disproportional rate, which could be mediated by the shoot secondary growth (Cochard et al. 2005). This suggests that developmental constraints, which restrict the change of the allometric scaling slope in response to environmental changes (Harvey and Pagel

Table 4. Summary of allometric analyses.

Variables *x*-*y* (season)	Site	Intercept	Slope	R^2
SCA-SL (summer)	H	1.42 ± 0.02a	1.66 ± 0.12b	0.15
	M	1.19 ± 0.03c	1.80 ± 0.14a	0.12
	L	1.37 ± 0.02b	1.97 ± 0.15a	0.21
SCA-SM (summer)	H	1.78 ± 0.02a	2.26 ± 0.14	0.31
	M	1.69 ± 0.04b	2.12 ± 0.16	0.24
	L	1.70 ± 0.01b	2.33 ± 0.15	0.52
SCA-LM (summer)	H	2.60 ± 0.01	1.72 ± 0.12a	0.38
	M	2.62 ± 0.02	1.38 ± 0.09b	0.34
	L	2.61 ± 0.01	1.78 ± 0.10a	0.56
SCA-SL (winter)	H	1.35 ± 0.01a	1.82 ± 0.10a	0.59
	M	1.13 ± 0.03c	1.89 ± 0.11a	0.48
	L	1.22 ± 0.02b	1.60 ± 0.10b	0.36
SCA-ABM (winter)	H	0.88 ± 0.01a	1.32 ± 0.10c	0.21
	M	0.89 ± 0.02a	1.78 ± 0.10a	0.38
	L	0.76 ± 0.02b	1.54 ± 0.10b	0.18
SL-ABM (winter)	H	0.09 ± 0.08b	0.72 ± 0.05b	0.15
	M	0.02 ± 0.08b	0.80 ± 0.05b	0.27
	L	0.50 ± 0.11a	0.96 ± 0.07a	0.12

Standardized major axes (SMA) regression parameters (intercept, slope) and statistics (R^2) for the three study sites (H, M, L). Abbreviations of sites and variables are as in Table 3. Means±95% confidence intervals. All the relationships were highly significant ($P<0.001$). Different letters correspond to significant ($P<0.05$) differences between sites.

1991), do not limit bud and shoot plasticity in the studied altitudinal gradient. Since the stem secondary growth mainly controls the vascular supply and the mechanical support for leaves, buds and other appendages, we argue that the stem cross-sectional area-bud size allometry should be a response to these functions (see a similar argument for leaf-stem allometries by Brouat and McKey 2001). The most plausible mechanism for the obtained positive allometry suggests that an improved vascular supply might be provided by thicker stems which would support buds whose size increases more than proportionally, i.e., allometrically, with stem cross-sectional area as climatic stress weakens. However, secondary growth may not be always a passive process following primary growth (Barnola and Crabbé 1993), which implied that the multiple correlations, here evaluated though structural equation models, may have different interpretations to those presented here.

Our results agree with the link suggested by Cochard et al. (2005) between the xylem growth of the parent shoot and the organogenesis of buds. Furthermore, our data also suggest that bud development is driven by the shoot hydraulic architecture since we

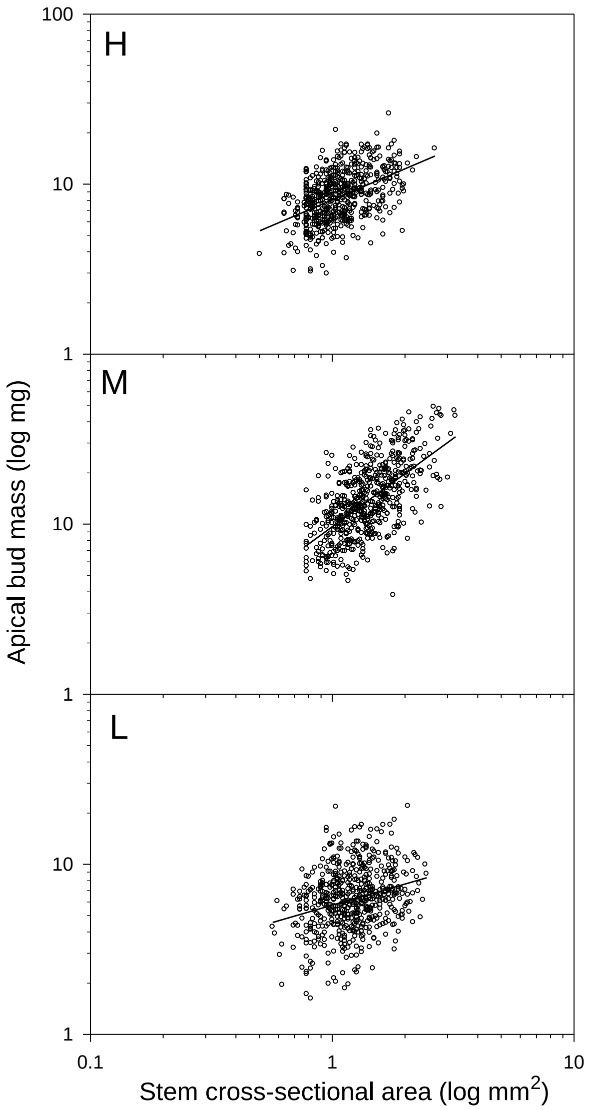


Figure 2. Allometric relationships between stem cross sectional area (SCA) and apical bud mass (ABM) in the three study sites (*H* high elevation site, *M* mid elevation site, *L* low elevation site). Note the logarithmic scales.

found that bud size was related to secondary shoot growth, i.e., bigger buds were found in thicker stems with wider vessels. As stated by these authors, this positive correlation indicates that a greater secondary growth is linked to an enhanced water availability and primary bud growth. A subsequent study by Lauri et al. (2008) in apple trees indicated that bud organogenesis may also depend on the size and hydraulic efficiency of the bud since larger buds had higher hydraulic efficiencies than smaller ones.

Environmental modulation of the scaling allometric relationships

We found that the largest buds, the thickest shoots and the widest vessels appeared in the mid-elevation site where climatic stress is lower than in the high- and low-elevation sites. For instance, trees from the low-elevation site presented higher LMA values than those from the other sites (but see the reverse association in Aspelmeier and Leuschner 2006) suggesting a high drought stress for leaf growth in spring

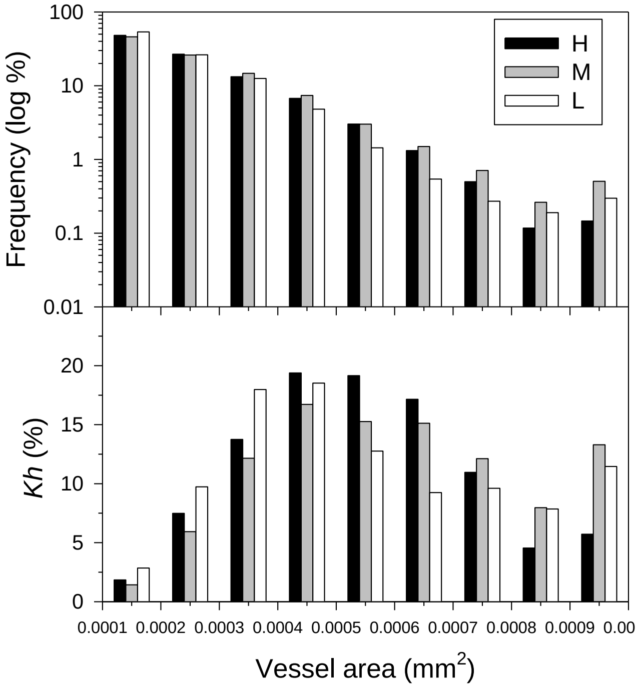


Figure 3. Distribution of vessels according to their area and to their relative (%) contribution to the total predicted hydraulic conductance (Kh) for the three study sites (sites' codes are as in Fig. 1). Note the logarithmic scale in the upper graph.

Table 5. Main goodness-of-fit statistics and indices of the selected structural equation models (see also Figure 4 and Supplementary information, Figure S4).

Model	*df*	*P*	χ^2 S-B	R-RMSEA	SRMR	GFI	AGFI	R-CFI
Global	2	0.641	0.872	0.000	0.005	1.000	0.999	1.000
Multigroup	6	0.000	79.941	0.143	0.041	0.978	0.888	0.962
Local (site H)	1	0.097	2.754	0.054	0.013	0.998	0.977	0.998
Local (site M)	1	0.132	2.269	0.046	0.013	0.999	0.998	0.998
Local (site L)	1	0.021	5.377	0.085	0.022	0.991	0.952	0.989

The degrees of freedom (*df*) and the significance level (*P*) of each model are indicated. Sites' codes are as in Table 1. Abbreviations of SEM statistics and indices: Satorra-Bentler robust Chi square (χ^2 S-B), robust root mean square error of approximation (R-RMSEA), standardized root mean square residual (SRMR), goodness-of-fit index (GFI), adjusted goodness-of-fit index (AGFI) and robust comparative fit index (R-CFI). Note: values close to zero for the χ^2 S-B, R-RMSEA, SRMR and SR indices and values close to one for the GFI, AGFI, and R-CFI indices would indicate that the evaluated models are consistent with the theoretical model generating the structures observed among the variables.

in that site (Castro-Díez et al. 1997). In the mid-elevation site, for a given increase in shoot crosssectional area trees enlarge their buds at a higher rate than trees from the other sites, which may explain that trees from the former site produced the biggest buds. Therefore, *Q. faginea* trees support bigger buds at a given twig cross-sectional area with decreasing climatic stress suggesting a higher hydraulic efficiency of current-year shoots in the mesic site, which agrees with the production of wider shoot vessels, bigger buds, and longer stems there than elsewhere.

Growth conditions for *Q. faginea* were better in the mid-elevation site than in the other sites since current-year shoots were larger and thicker and produced more leaf mass in that site than in the others. In addition, trees were bigger there than in the other two sites despite having similar ages. Furthermore, we have shown that shoots from this mesic mid-elevation site were more efficient organs for crown development than shoots from the high- and low-elevation sites since, for a given increase in secondary growth, in the former site shoots produced bigger buds than elsewhere. The functional divergence of bud size and the formation of short and long shoots will influence the crown architecture and its light-harvesting efficiency (Esteso-Martínez et al. 2006). Our models revealed additional links as the direct association of stem length and the number of buds on apical bud mass in the mid- and low-elevation sites, respectively. Nevertheless, in both cases, the association between bud mass and stem cross-sectional area was stronger than the others mentioned before. Furthermore, in the local models fitted to each site dataset, the stem cross-sectional area also influenced the number of buds

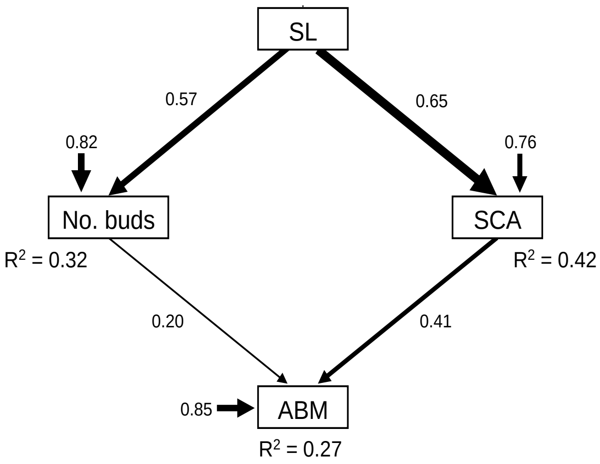


Figure 4. Selected structural equation model of primary and secondary growth variables for the global dataset. Boxes correspond to measured variables and directed arrows (paths) represent a causal influence. Path coefficients, corresponding to the estimated strength of one variable's influence on another, appear near arrows, and the arrow width is scaled proportionately. The arrows pointing measured variables indicate the error terms associated with their measurement, i.e. unexplained variance. The proportion of explained variance (R^2) is interpreted similarly to a regression analysis. Variables' abbreviations: SL, stem length; SCA, stem cross sectional area; No. buds, number of buds per shoot; ABM, apical bud dry mass. Significant ($P < 0.10$) path standardized coefficients, indicating the strength of one variable's influence on another appear next to arrows, and the arrow width is scaled proportionately.

formed per stem. This last association was stronger in the high-elevation site than elsewhere suggesting a higher control of shoot architecture by secondary growth, more than by the apical bud size, in cold sites with a short growing season.

Seasonal variation of the allometric relationships

We also found differences in the data recorded in summer and winter. For instance, in all sites, the stems tended to be shorter and thicker in winter than in summer, possibly as a consequence of selective shedding of long shoots and secondary growth during autumn. Such temporal, and plausibly spatial, variability in secondary growth of the stems would have functional implications for hydraulic conductivity through the canopy (Lauri et al. 2010). In *Q. faginea* many shoots bearing acorns, which usually are bigger than non-bearing shoots, are shed in winter (personal observation). This could also explain the stronger association between stem cross-sectional area and length in winter than in summer. Further studies are required to disentangle the structural causes of seasonal changes in stem size and their functional implications.

Our interpretation of the differences in bud size and number and in the bud‑shoot relationships observed among sites is based on the different climatic stress experienced by trees along the altitudinal gradient. In *Q. faginea*, the highest bud growth rate is observed in summer (Montserrat-Martí et al. 2009). In the low-elevation site, we postulate that water deficit in summer may constrain both bud size and the number of large buds (here regarded as those larger than 1 mm). However, in the high-elevation site a short growing season may limit the maximum size reached by buds. The production of the largest buds in the mid-elevation mesic site would be a consequence of a higher bud growth rate during a longer growing period as compared with the other two sites. In summary, the changing allometric slopes in *Q. faginea* shoots could be a response to the effects of different climatic stressors on each shoot organ. For instance, Sun et al. (2006) demonstrated that the allometric relationships between the stem cross-sectional area and leaf area in different plant species changed along an altitudinal gradient as climatic stress did. In *Q. faginea*, the mesic conditions in the mid-elevation site should enhance the formation of thick stems and big buds, which in turn will probably produce large shoots in the following spring. A more precise seasonal monitoring of bud and shoot development and a detailed assessment on how climatic stress constraints these phenological processes would increase our understanding of shoot-bud relationships as related to crown developmental patterns.

Conclusions

We found an allometric scaling relationship between stem cross-sectional area and apical bud mass in current-year shoots of *Q. faginea*. Such allometric association was stronger in the mesic mid-elevation site than in the other two sites, and the scaling slope of this association was also the highest in the first site. Thus, for a given increase in shoot cross-sectional area trees from the mesic site produced bigger buds with greater potential for primary shoot growth in the following season than trees from the other sites. Hence, it may be hypothesized that the greatest potential for shoot primary growth at a lowest cost in terms of secondary growth increases as climatic stress decreases. This speculation suggests a higher hydraulic efficiency of the vascular system of current-year shoots of trees in sites with low climatic stress, which agrees with the production of bigger buds and longer stems there than elsewhere. This hypothesis should be tested in further studies considering trees at different ages and interspecific comparisons.

Acknowledgements

AQ Alla acknowledges the support of CIHEAM-IAMZ and MAEC-AECID. This work was supported by the Spanish Ministerio de Ciencia e Innovación (grant numbers CGL2007-66066-C04-02/BOS, CGL2008-04847-C02-01, RTA2005-00100-CO2-00, SUM2006-00025-00-00 to JCC and GM-M). We thank the constructive comments provided by two anonymous reviewers and the editor. We thank the support of the Globimed network (www.globimed.net). We thank J. Albuixech for his help in the field. JJC acknowledges the support of ARAID.

References

Aspelmeier S, Leuschner Ch (2006) Genotypic variation in drought response of silver birch (*Betula pendula* Roth): leaf and root morphology and carbon partitioning. Trees 20:42–52.

Barnola P, Crabbé J (1993). L'activite cambiale, composante active ou passive dans les reactions de croissance de l'arbre ? Acta Botanica Gallica 140:403–412.

Barthélémy D, Caraglio Y (2007) Plant architecture: A dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. Ann Bot 99:375–407.

Bentler PM (1995) EQS Structural Equations Program Manual. Multivariate Software. Encino.

Bollen KA (1989) Structural Equations with Latent Variables. Wiley, New York.

Brouat C, Gibernau M, Amsellem L, McKey D (1998) Corner's rules revisited: ontogenetic and interspecific patterns in leaf-stem allometry. New Phytol 139:459–470.

Brouat C, McKey D (2001) Leaf–stem allometry, hollow stems, and the evolution of caulinary domatia in myrmecophytes. New Phytol 151:391–406.

Castro-Díez P, Villar-Salvador P, Pérez-Rontomé C, Maestro-Martínez M, Montserrat-Martí G (1997) Leaf morphology and leaf chemical composition in three *Quercus* (Fagaceae) species along a rainfall gradient in NE Spain. Trees 11:127–134.

Cochard H, Coste S, Chanson B, Guehl JM, Nicolini E (2005) Hydraulic architecture correlates with bud organogenesis and primary shoot growth in beech (*Fagus sylvatica*). Tree Physiol 25:1545–1552.

Esteso-Martínez J, Valladares F, Camarero JJ, Gil-Pelegrín E (2006) Crown architecture and leaf habit are associated with intrinsically different light-harvesting efficiencies in *Quercus* seedlings from contrasting environments. Ann For Sci 63:511–518.

Falster DS, Warton DI, Wright IJ (2006) SMATR: Standardised major axis tests and routines, version 2.0. http://www.bio.mq.edu.au/ecology/SMATR

Harmer R (1991) The effect of bud position on branch growth and bud abscission in *Quercus petraea* (Matt.) Liebl. Ann Bot 67:463–468.

Harvey PJ, Pagel MD (1991). The comparative method in evolutionary biology. Oxford University Press, Oxford.

Himrane H, Camarero JJ, Gil-Pelegrín E (2004) Morphological and ecophysiological variation of the hybrid oak *Quercus subpyrenaica* (*Q. faginea* × *Q. pubescens*). Trees 18:566–575.

Jöreskog KG (1993) Testing structural equation models. In: Bollen K, Long JS (eds) Testing Structural Equation Models. Sage, Newbury Park, 294–316.

Lauri PE, Bourdel G, Trottier C, Cochard H (2008) Apple shoot architecture: evidence for strong variability of bud size and composition and hydraulics within a branching zone. New Phytol 178:798–807.

Lauri PE, Kelner JJ, Trottier C, Costes E (2010) Insights into secondary growth in perennial plants: its unequal spatial and temporal dynamics in the apple (*Malus domestica*) is driven by architectural position and fruit load. Ann Bot 105: 607–616.

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

Montserrat-Martí G, Camarero JJ, Palacio S, Pérez-Rontomé C, Milla R, Albuixech J, Maestro M (2009) Summer-drought constrains the phenology and growth of two co-existing Mediterranean oaks with contrasting leaf habit: implications for their persistence and reproduction. Trees 23:787–799.

Niklas KJ (1994) Plant Allometry: the Scaling of Form and Process. University of Chicago Press, Chicago.

Normand F, Bissery C, Damour G, Lauri PÉ (2008) Hydraulic and mechanical stem properties affect leaf–stem allometry in mango cultivars. New Phytol 178:590–602.

Preston KA, Ackerly DD (2003) The evolution of allometry in modular organisms. In: Pigliucci M, Preston KA (eds) Phenotypic Integration: Studying the Ecology and Evolution of Complex Phenotypes, Oxford University Press, Oxford, 80–103.

Rasband WS (1997-2009) ImageJ. N.I.H., Bethesda, USA, http://rsb.info.nih.gov/ij/

Shipley B (2004) Analysing the allometry of multiple interacting traits. Persp Plant Ecol Evol Syst 6:235–241.

Sokal RR, Rohlf FJ (1995) Biometry: the Principles and Practice of Statistics in Biological Research. Freeman, New York.

Sperry JS, Hacke UG, Pittermann J (2006) Size and function in conifer tracheids and angiosperm vessels. Am J Bot 93:1490–1500.

Sun S, Jin D, Shi P (2006) The leaf size-twig size spectrum of temperate woody species along an altitudinal gradient: an invariant allometric scaling relationship. Ann Bot 97:97–107.

Suzuki M, Hiura T (2000) Allometric differences between current-year shoots and large branches of deciduous broad-leaved tree species. Tree Physiol 20:203–209.

Vesk PA, Westoby M (2004) Funding the bud bank: a review of the costs of buds. Oikos 106:200–208.

Warton DI, Weber NC (2002) Common slope tests for bivariate structural relationships. Biom J 44:161–174.

Warton DI, Wright IJ, Falster DS, Westoby M (2006) Bivariate line-fitting methods for allometry. Biol Rev Camb Philos Soc 81:259–291.

Westoby M, Wright IJ (2003) The leaf size-twig size spectrum and its relationship to other important spectra of variation among species. Oecologia 135:621–628.

Wilson BF, Kelty MJ (1994) Shoot growth from the bud bank in black oak. Can J For Res 24:149–154.

Supplementary Information

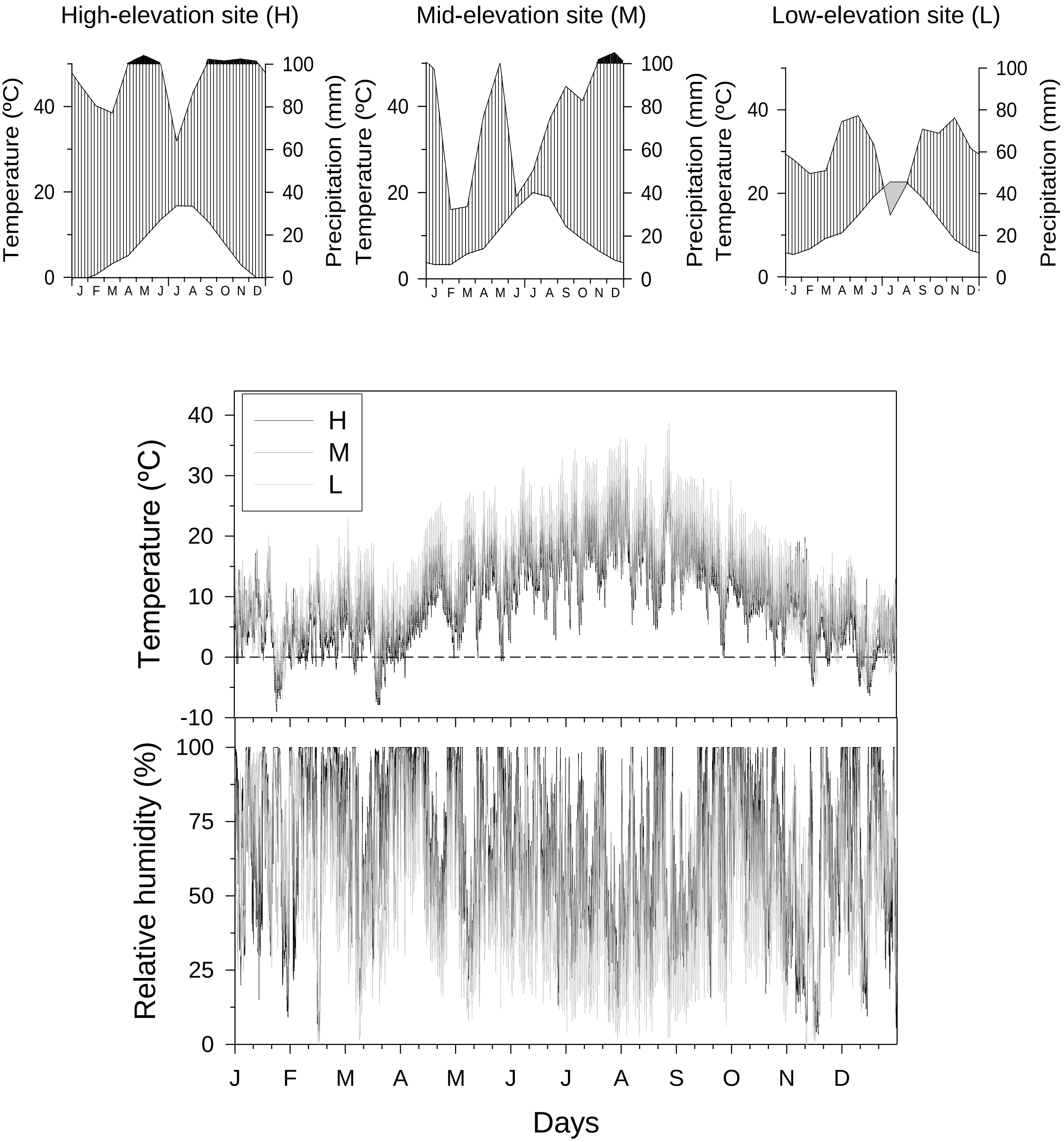


Figure S1. Climatic diagrams of the three study sites (period 1970-2007) and daily climatic data (air temperature and relative humidity recorded) during the study year (2007) in the three study sites (H, high elevation site; M, mid elevation site; L, low elevation site).

Table S1. Meteorological stations used to describe the climatic conditions (T, monthly mean temperature; P, monthly total precipitation P) in the three study sites (H, high elevation site; M, mid elevation site; L, low elevation site).

Site	Station	Latitude (N)	Longitude (W)	Elevation (m)	Variable	Period (T / P)	Missing data T/P (%)	T (ºC)	P (mm)
L	Embalse de la Sotonera	42º 06’	0º 40’	413	T / P	1939-2007 / 1919-2007	0.0 / 0.0	13.4	469
L	Huesca-Monflorite	42º 05’	0º 20’	541	T / P	1944-2007 / 1951-2007	0.0 / 0.0	13.5	549
L	Apies	42º 14’	0º 24’	680	T / P	1970-2007 / 1968-2004	4.0 / 3.6	13.5	686
L	Nueno	42º 16’	0º 26’	726	T / P	1973-2007 / 1962-2007	6.8 / 0.0	12.4	723
M	Hostal de Ipiés	42º 26’	0º 24’	780	T / P	1973-2007 / 1949-2007	11.9 / 6.8	11.2	728
M-H	Arguis “DGA”	42º 19’	0º 26’	1039	T / P	1992-2007 / 1992-2007	47.2 / 9.9	10.3	899
H	Nocito	42º 19’	0º 15’	930	T / P	1973-2007 / 1951-2007	0.0 / 0.0	10.6	868

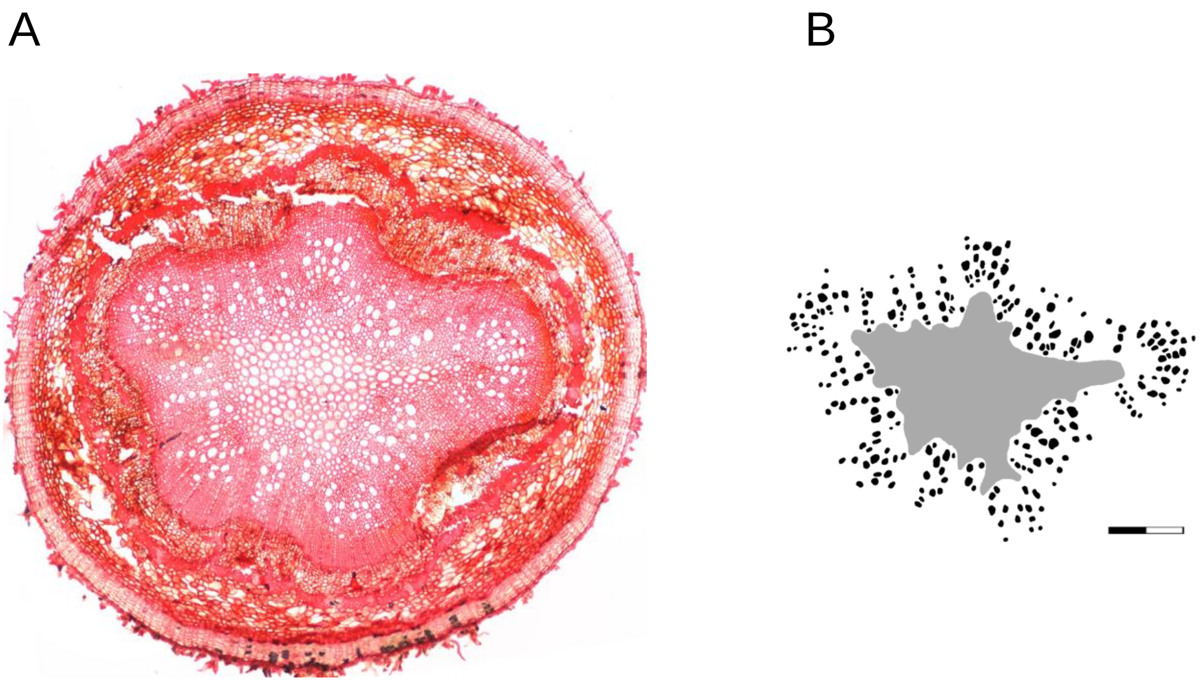


Figure S2. Cross-section of a current-year shoot of a *Q. faginea* tree from the high elevation site (A) and corresponding analysed image (B) with the pith (grey area) and the vessels (black polygons). The scale bar measures 0.2 mm.

Table S2. Correlation coefficients between the current-year shoot variables (mean values per tree) measured in summer and winter in the three sites (H, high elevation site; M, mid elevation site; L, low elevation site). Variables' abbreviations: SL, stem length; SCA, stem cross sectional area; SM, stem mass; LM, leaf mass; AM, acorn mass; Ln, leaf number; An, acorn number; ABM, apical bud mass. Note that R^2 values are much higher than in Table 4 which was calculated using individual shoot data.

Summer	SL			SCA			SM			LM			AM			Ln		
	H	M	L	H	M	L	H	M	L	H	M	L	H	M	L	H	M	L
SCA	0.40	0.49	0.49															
SM	0.91	0.88	0.88	0.52	0.67	0.69												
LM	0.76	0.72	0.67	0.55	0.61	0.74	0.85	0.81	0.82									
AM	0.26	0.50	0.17	0.15	–	0.40	0.36	0.65	0.26	–	0.48	0.28						
Ln	0.68	0.69	0.64	0.39	0.34	0.35	0.67	0.66	0.56	0.73	0.67	0.46	–	–	–			
An	–	–	0.20	0.13	–	0.39	–	–	0.20	–	–	–	0.49	0.60	0.77	–	–	0.17

Winter	SL			SCA			ABM		
	H	M	L	H	M	L	H	M	L
SCA	0.79	0.70	0.62						
ABM	0.39	0.54	0.13	0.45	0.63	0.29			
No. buds	0.60	0.60	0.41	0.59	0.45	0.34	0.19	0.35	0.26

All correlation coefficients are significant ($P < 0.05$). All relationships were assessed using the Pearson correlation coefficient excepting those involving the number of leaves, acorns and buds which were based on the Spearman correlation coefficient.

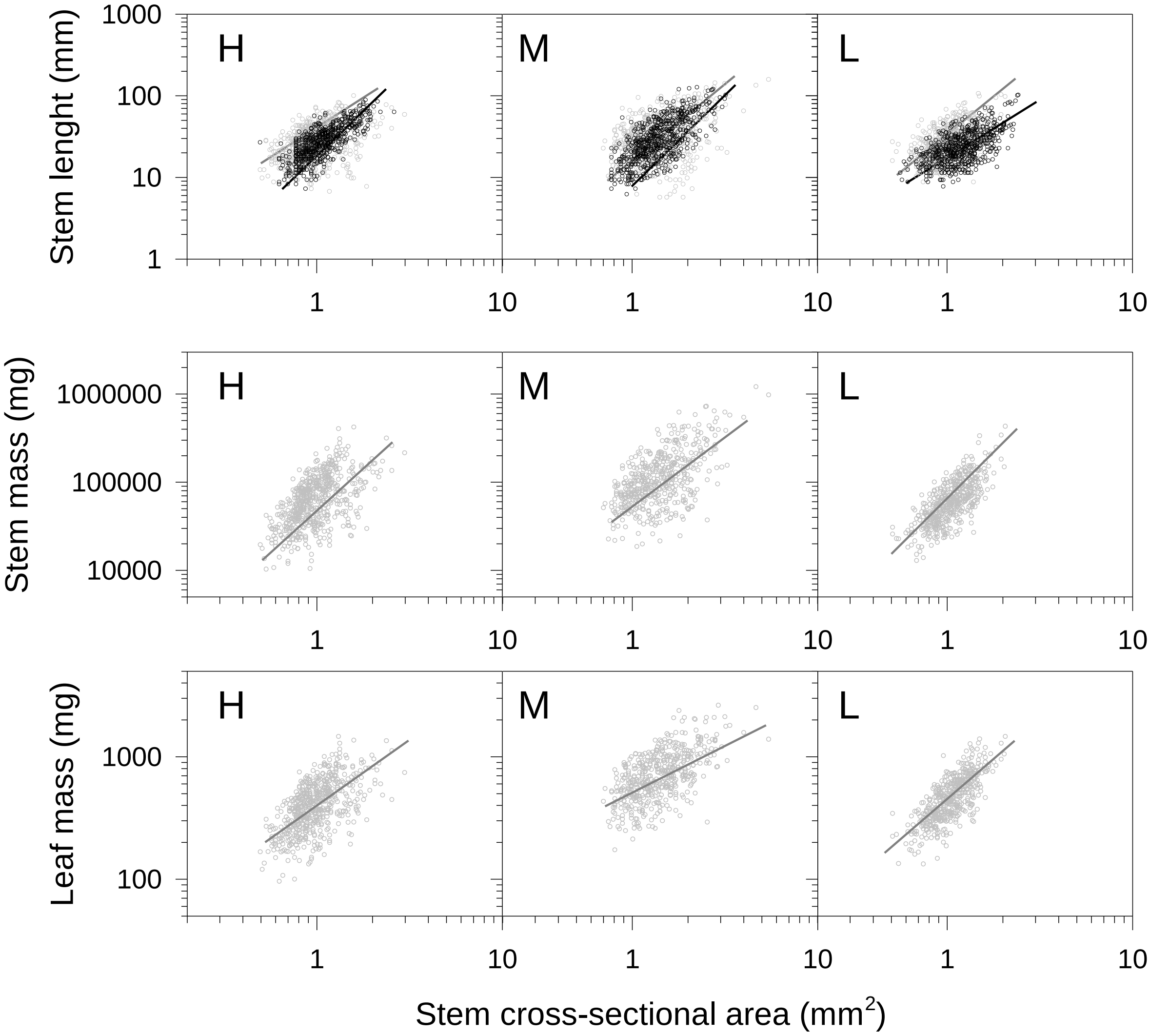


Figure S3. Allometric relationships among selected shoot variables for the three study sites (H, high elevation site; M, mid elevation site; L, low elevation site). Black lines and symbols correspond to winter samples and grey lines and symbols indicate summer samples.

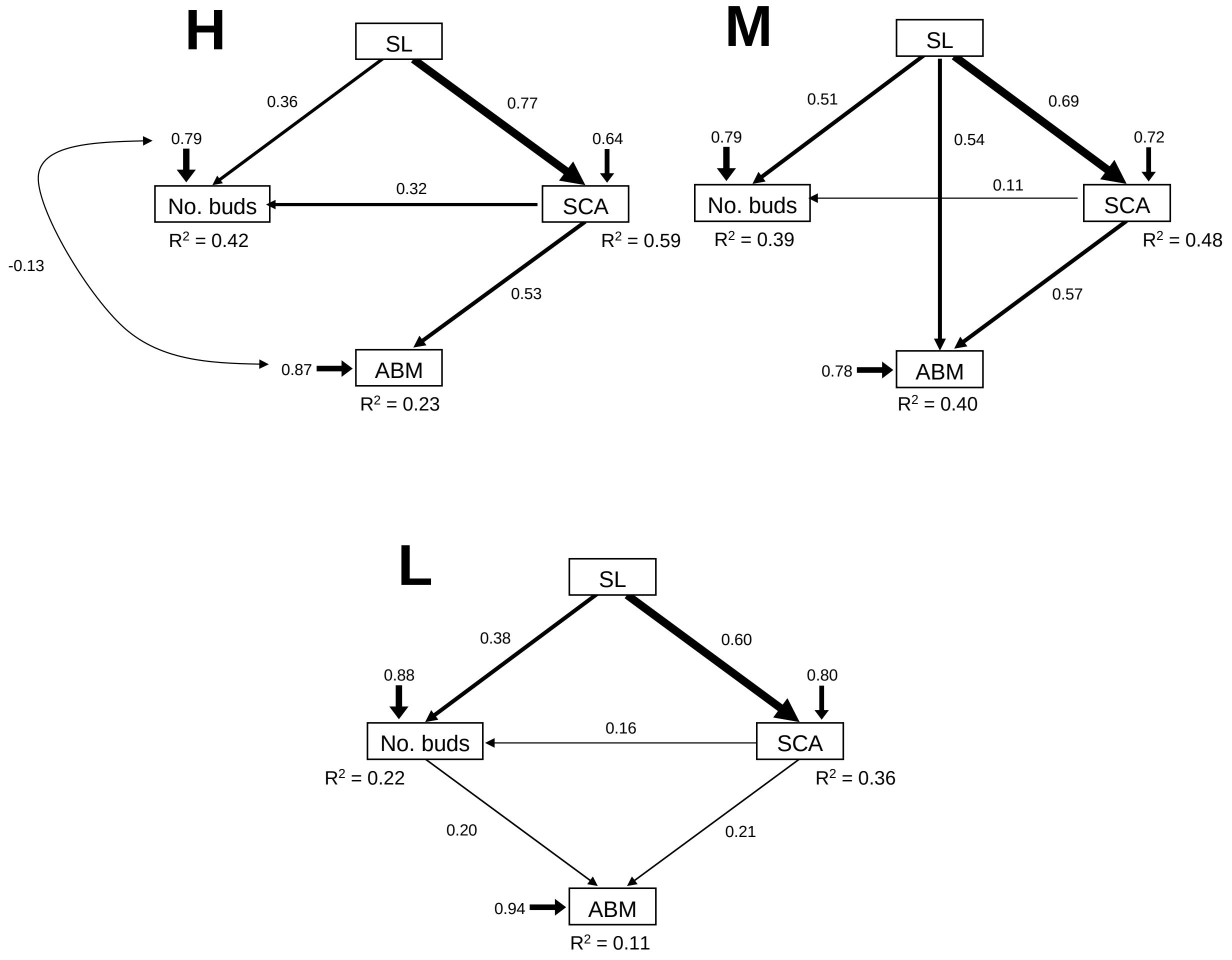


Figure S4. Selected structural equation models of primary and secondary-growth variables for each study site. The bidirectional arrow represents an undirected correlation in the model of the site H. Sites' codes are: H, high-elevation site; M, mid-elevation site; L, low-elevation site. Boxes correspond to measured variables and directed arrows (paths) represent a causal influence. Path coefficients, corresponding to the estimated strength of one variable's influence on another, appear near arrows, and the arrow width is scaled proportionately. The arrows pointing measured variables indicate the error terms associated with their measurement, i.e. unexplained variance. The proportion of explained variance (R^2) is interpreted similarly to a regression analysis. Variables' abbreviations: SL, stem length; SCA, stem cross sectional area; No. buds, number of buds per shoot; ABM, apical bud dry mass. Significant (*P* < 0.10) path standardized coefficients, indicating the strength of one variable's influence on another appear next to arrows, and the arrow width is scaled proportionately.

CHAPTER III

Acorn production is linked to secondary growth but not to declining carbohydrate concentrations in current-year shoots of two oak species

III. Acorn production is linked to secondary growth but not to declining carbohydrate concentrations in current-year shoots of two oak species

Abstract In trees, reproduction constitutes an important resource investment which may compete with growth for resources. However, detailed analyses on how growth and fruit production interact at the shoot level are scarce. Primary canopy growth depends on the development of current-year shoots and their secondary growth might also influence the number and size of fruits supported by them. We hypothesise that an enhanced thickening of current-year shoots is linked positively to acorn production in oaks. We analysed the effect of acorn production on shoot growth of two co-occurring Mediterranean oak species with contrasting leaf habit (*Quercus ilex*, *Quercus faginea*). Length and cross-sectional area of current-year shoots, apical bud mass, number of leaves and acorns, xylem and conductive area, number of vessels of acorn-bearing and non-bearing shoots were measured in summer and autumn. Nitrogen and carbohydrates analyses were also performed in stems and leaves of both shoot types. Stem cross-sectional area increased in acorn-bearing shoots when compared with non-bearing shoots for both species and such surplus secondary growth was observed since summer. In bearing shoots, the total transversal area occupied by vessels decreased significantly from basal to apical positions along the stem as did the xylem area and the number of vessels. Leaves of bearing shoots showed lower nitrogen concentration than those of non-bearing shoots. Carbohydrate concentrations did not differ in stems and leaves as a function of the presence of acorns. Such results suggest that carbohydrates may preferentially be allocated towards reproductive shoots, possibly through enhanced secondary growth, satisfying all their carbon demands for growth and reproduction. Our findings indicate that acorn production in the two studied oaks depends on shoot secondary growth.

Keywords Acorns, Mediterranean climate, *Quercus ilex* subsp. *Ballota*, *Quercus faginea*, Stem diameter, Xylem, Nitrogen, Non-structural carbohydrates.

Introduction

Reproduction represents an important resource sink for trees (Roff 1992). This resource investment can compete with other growth processes in trees leading to different trade-offs, for instance between fruit production and vegetative growth (Tuomi et al. 1983; Koenig and Knops 1998; Obeso 2002; Monks and Kelly 2006; Hirayama et al. 2007). These trade-offs are thought to act hierarchically in trees (Obeso 1997), as they might be more apparent at low modular levels, i.e. among shoots within a branch, than at high modular levels, i.e. among branches within a tree, because the overall costs of reproductive modules could be compensated by the growth of vegetative ones (Watson and Casper 1984).

The crowns of trees are formed by populations of shoots of various ages and sizes (Hallé et al. 1978). Current-year shoots hold buds and leaves (Kozlowski 1971) and they become net exporters of carbohydrates soon after bud break (Johnson and Lakso 1986; Hasegawa et al. 2003; Keel and Schädel 2010; Landhäusser 2010). Consequently, the development of flowers and fruits depends on photosynthates provided by the reproductive shoot and the neighbouring ones (Lauri et al. 1996; Hoch 2005). In fact, the number of leaves held by reproductive shoots may have a stronger influence on the fruit set than the number of flowers (Watson 1986; Lauri et al. 1996).

Stem secondary growth accounts for most of the biomass gain of shoots and, in trees with shoot growth limited to a restricted period as in most oak species, it occurs generally after the cessation of the main peak of shoot extension (Barnola and Crabbé 1993). Such thickening constitutes a strong sink for carbohydrates, which can affect positively the fruit set of the shoot (Forshey and Elfving 1989). The interactions between secondary shoot growth and fruit production have received little attention in trees, in spite of the need to find mechanistic links between growth and reproduction at the shoot level. However, the studies show contrasting results for the relationship between growth and fruiting. For instance, fruit development is affected differently by primary and secondary growth in current-year shoots of fruit-trees (Barnola and Crabbé 1993; Farina et al. 2006; Solar et al. 2006; Lauri et al. 2010). According to George et al. (1996) and Porter et al. (2002), shoot diameter is related positively to fruit set and fruit size.

In this study, we analysed the effect of acorn production on the current-year shoot growth and chemical composition of two co-occurring Mediterranean oak species with contrasting leaf habits: the evergreen *Quercus ilex* and the winter deciduous *Quercus faginea*. We hypothesised that the presence of fruits leads to an enhanced thickening of current-year stems, i.e. stems bearing acorns should be thicker than non-bearing stems. To test this hypothesis, we analysed the influence of fruit production on growth variables (stem length and cross-sectional area, xylem anatomy, number of leaves and acorns, bud size) and stores (nitrogen and carbohydrates concentrations in stems and leaves) in current-year shoots bearing and not bearing acorns in two study species.

Materials and methods

Study area

The study site was located in Agüero, Aragón, north eastern Spain (42° 18′ N, 0° 47′ W, 750 m a.s.l.). Climate is Mediterranean and continental being characterised by dry summers and cold winters with 635 mm and 13.8 ºC of total annual precipitation and mean annual temperature, respectively. Soil in this area is a Calcisol (FAO 1998), formed on Miocene clays with bedrock of calcareous sandstone. Vegetation is an open tall scrub with scattered low trees, dominated by *Q. ilex* L. subsp. *ballota* (Desf.) Samp. (hereafter abbreviated as *Q. ilex*), *Q. faginea* Lam., *Arbutus unedo* L. and *Pinus halepensis* Mill., approximately with similar dominance, and other less abundant woody species (Montserrat-Martí et al. 2009).

Study species

Quercus ilex is an evergreen oak tree with semi-ring porous wood (Campelo et al. 2007), which in Iberian Peninsula grows preferentially inland and in many continental areas (Amaral Franco 1990). *Quercus faginea* is a deciduous oak tree with ring-porous wood (Corcuera et al. 2004) and with a wide distribution in sub-Mediterranean areas of the Iberian Peninsula (Amaral Franco 1990). Both oak species frequently coexist in the study area forming multi-stemmed trees, although *Q. faginea* usually grows in moister areas than *Q. ilex*. Montserrat-Martí et al. (2009) reported that the period of fruit development was quite similar at both species and acorns performed most of their development from July to October in the study site. Meanwhile secondary stem growth occurs mostly from June to September (Alla, unpublished data).

Field sampling and measurements

To evaluate the period when acorn-bearing may affect stem secondary growth, two samplings were carried out in late June 2010 (summer sampling) when current-year shoots have fully extended and in mid-October 2010 (autumn sampling) when secondary growth and acorn development were almost finished. In each sampling ten individuals of each species with similar size and with abundant acorn production (>90% crown covered by shoots bearing acorns) were selected (Table 1). Since percentage estimates of crown covered by acorns vary among observers and localities, all estimates were performed by the first two authors. We randomly selected five trees per species with maximum number of shoots bearing acorns which were considered fully covered by acorns (100%).

Table 1. Morphological features of studied trees (mean values ± SE).

Species	Number of trees	Diameter at 1.3 m (cm)	Height (m)	No. stems per tree
Q. ilex	10	10.6 ± 0.6b	4.1 ± 0.1b	9 ± 1a
Q. faginea	10	13.8 ± 0.9a	5.5 ± 0.3a	4 ± 1b

Different letters indicate significant differences between species ($P < 0.05$, ANOVA).

These trees were used as reference trees with ‘‘maximum-fruit load’’ to correct for observer bias in estimates of fruit load of similar sized trees in the study area (Graves 1980).

In each sampled tree, 25 current-year non-bearing shoots (hereafter abbreviated as S-) and 25 bearing shoots (hereafter abbreviated as S+) were randomly collected on the south light-exposed side of the crown, yielding a total of 500 current-year shoots per species and sampling time. These shoots were kept in separate bags at 4 ºC until measured in the laboratory. Stem length and diameter (the average of two perpendicular measures taken in the stem after converted to stem cross-sectional area), number of leaves, acorns and stalks were measured in each sampled shoot. All sampled shoots were fractionated (stem, leaves and acorns) and samples were oven dried at 60 ºC to a constant weight. The dry weight of each fraction was obtained with a precision scale (stem mass, leaf mass, acorn mass). In autumn, additionally, the apical bud of each shoot was removed. Buds were oven dried at 60 ºC to a constant weight before being individually weighted to the nearest 0.001 g to obtain the apical bud mass.

Wood anatomy of current-year shoots

In October 2010, we collected five bearing shoots with two acorn stalks (S+ shoots) and five non-bearing shoots (S- shoots) for each species. To evaluate the effects of acorn production on xylem anatomy of shoots avoiding the possible effect of shoot size on xylem development, we selected S- and S+ shoots whose mean length did not differ significantly ($P > 0.05$, ANOVA). In the case of S+ shoots, cross sections of 10–20 lm thick were taken consecutively at low (L), middle (M) and high (H) positions with respect to the two scars left by acorn stalks in the stem using a sliding microtome (Leica SM2010R, Germany) (Fig. 1). We followed the same procedure with S- shoots taking cross-sectional cuts in three positions similar to those considered for S+ shoots. Stems were fixed in formaldehyde–ethanol–acetic acid solution and stored in 50% ethanol. Cross sections were stained with safranin (0.5 g in 100 ml 96% ethanol) solution, mounted using Eukitt® (Merck, Germany), and photographed by Olympus BH2 light microscope at 100× magnification attached to a digital camera (Leica DFC290). Photographs were processed and converted into black-and-white images using Photoshop (Adobe Systems Incorporated, USA). The xylem and pith area, the number of vessels and their total vessel-area were analysed using the ImageJ software (Rasband and Ferreira 2011). Only vessels whose area was greater than 0.0001 mm^2 were considered. Finally, we calculated the relative conductive area (CA) as the ratio between the total lumen area of vessels and the total analysed area, as well as the predicted hydraulic conductance which was calculated as the sum of the fourth power diameters of all the vessels from each section assuming that the vessel area had a circular shape (Sperry et al. 2006).

Chemical analyses

Nitrogen, soluble sugars and starch concentrations were measured in the stems and leaves of current-year shoots of the two species sampled in autumn 2010. Samples were oven dried and milled to a fine powder (IKA MF10, IKAWerke, Staufen, Denmark). Nitrogen mass-based concentrations were measured with an elemental analyser (Elementar VarioMAX N/CM, Hanau, Germany). Soluble sugars were extracted with 80% (v/v) ethanol and concentrations were determined colorimetrically using the phenol–sulphuric method of Dubois et al. (1956) as modified by Buysse and Merckx (1993). Starch and complex sugars remaining in the undissolved pellet after ethanol extractions were enzymatically reduced to glucose and analysed as described in Palacio et al. (2007). The sum of soluble sugars and starch measured in glucose equivalents are referred to as total non-structural carbohydrates.

Statistical analyses

All data were checked for normality and homoscedasticity prior to statistical analyses. The variables stem length and cross sectional area were log-transformed to reach normality. To evaluate differences in stem length and cross-sectional area, number of leaves, stem mass, leaf mass and apical bud mass between acorn-bearing and non-bearing shoots for the different samplings and to compare mean values of these variables among sampling seasons (summer and autumn) we used linear mixed models with ‘‘shoot type’’ and ‘‘seasons’’ as fixed factors and ‘‘trees’’ as random factors (Littell et al. 2006). We used the Restricted Maximum Likelihood method (REML) and Type III sum of squares within the MIXED procedure (SAS 9.0, Institute Inc., Cary, USA). Mean values of shoot types and cross-sectional cuts were compared using Bonferroni tests when variances were equal or Dunnett’s T3 tests otherwise. We also assessed differences in the distributions of vessels according to their transversal areas using the *G* test (Sokal and Rohlf 1995).

Results

Effect of shoot variables

Most of the measured shoot variables showed significant differences between bearing (S+) and non-bearing (S-) shoots in the summer and autumn samples (Table 2). In *Q. ilex*, S+ presented higher values than S- for most variables (stem length and cross-sectional area, number of leaves, stem and leaf mass) in both studied seasons. In *Q. faginea*, only stem cross-sectional area of S+ showed higher value than S- in both seasons and stem mass in summer. In this species, S- showed significantly higher number of leaves in both

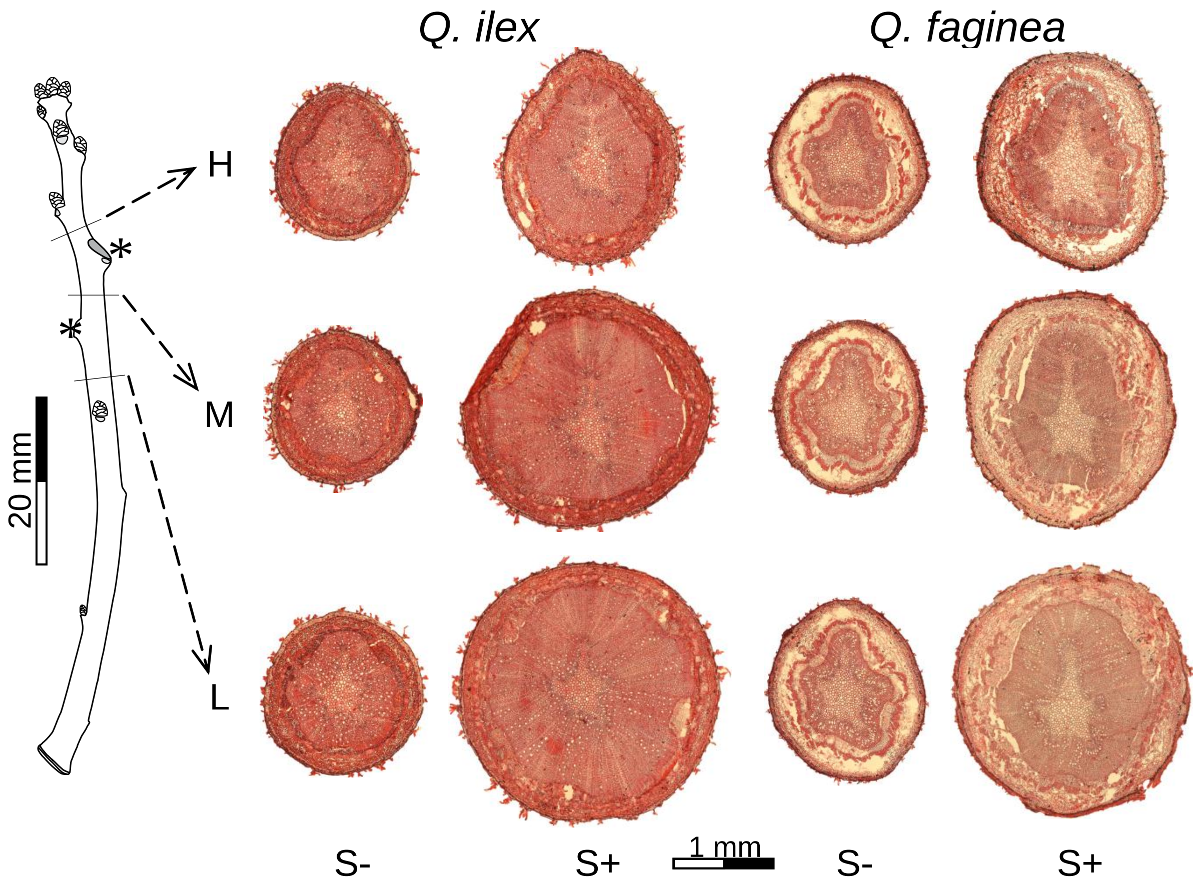


Figure 1. Cross-sectional images of four current-year non-bearing (S-) and bearing (S+) shoots of *Q.ilex* and *Q. faginea* trees sampled in 2010. The photographs correspond to three consecutive sections done below (*L*), between (*M*) and on top of (*H*) two consecutive scars (*asterisks*) of acorns stalks located along the stem.

seasons and leaf mass in autumn than S+. In addition, all variables except leaf mass in *Q. ilex* differed significantly between seasons. No significant differences in the apical bud mass were observed between S+ and S-. Finally, the mean number of acorns per stem decreased from summer to autumn in both species.

Differences in wood-anatomical features

Overall, S+ showed a larger xylem area and a higher number of vessels than S- in both species (P < 0.0001), being these differences more marked in *Q. ilex* than in *Q. faginea* (Figs. 1, 2). However, the percentage of conductive area increased in S- as compared with S+ (P < 0.0001) because of the disproportionate production of more xylem area as compared with the total lumen area of vessels. The total transversal area occupied by vessels decreased significantly (P < 0.0001) from basal to apical positions along the stem in the S+, as did the xylem area and the number of vessels (P < 0.0001) (Figs. 1, 2). For instance, the total transversal vessel area above the acorn scars (H) decreased by -55% and -27% in *Q. ilex* and *Q. faginea* S+ shoots,

Table 2 Mean values (±SE) of the variables measured in different types of shoots sampled in summer and autumn and related statistics (*F*, *P* in parenthesis) of mixed models.

Species	Season	Shoot type	Stem length (mm)	Stem cross-sectional area (mm^2)	Number of leaves	Number of acorns	Stem mass (mg)	Leaf mass (mg)	Apical bud mass (mg)
Q. ilex	Summer	S-	47.3 ± 0.9	1.18 ± 0.02	7.74 ± 0.11	–	56.2 ± 1.6	384.6 ± 8.4	–
		S+	54.9 ± 0.9	1.62 ± 0.02	8.49 ± 0.14	2.00 ± 0.07	81.5 ± 2.0	457.0 ± 9.5	–
		F (*P*)	**44.18 (<0.0001)**	**444.63 (<0.0001)**	**23.73 (<0.0001)**	–	**110.62 (<0.0001)**	**38.87 (<0.0001)**	–
	Autumn	S-	45.0 ± 0.9	1.53 ± 0.03	7.51 ± 0.13	–	76.8 ± 3.1	382.7 ± 10.3	2.78 ± 0.36
		S+	50.7 ± 1.3	2.88 ± 0.06	7.64 ± 0.18	1.40 ± 0.04	129.5 ± 5.6	456.3 ± 13.0	2.88 ± 0.32
		F (*P*)	**16.85 (<0.0001)**	**271.09 (<0.0001)**	0.34 (0.56)	–	**85.26 (<0.0001)**	**18.99 (<0.0001)**	0.06 (0.82)
		F_{Season} (*P*)	**12.13 (<0.0005)**	**298.26 (<0.0001)**	**11.31 (<0.0008)**	**53.67 (<0.0001)**	**117.85 (<0.0001)**	0.47 (0.49)	–
Q. faginea	Summer	S-	35.4 ± 0.9	1.17 ± 0.01	6.38 ± 0.09	–	58.7 ± 2.0	393.6 ± 8.7	–
		S+	34.9 ± 0.8	1.48 ± 0.02	6.08 ± 0.08	2.23 ± 0.07	67.1 ± 2.1	404.0 ± 9.0	–
		F (*P*)	0.03 (0.86)	**318.99 (<0.0001)**	**7.64 (0.006)**	–	**11.88 (<0.0001)**	0.93 (0.33)	–
	Autumn	S-	32.2 ± 1.1	1.38 ± 0.02	5.82 ± 0.10	–	84.9 ± 3.7	372.4 ± 10.3	6.67 ± 0.78
		S+	30.9 ± 1.0	2.08 ± 0.04	5.37 ± 0.10	1.44 ± 0.05	89.1 ± 4.3	344.7 ± 9.7	5.48 ± 0.75
		F (*P*)	0.98 (0.32)	**347.87 (<0.0001)**	**10.80 (0.001)**	–	0.70 (0.40)	**4.50 (0.034)**	1.19 (0.29)
		F_{Season} (*P*)	**16.94 (<0.0001)**	**375.15 (<0.0001)**	**53.46 (<0.0001)**	**83.26 (<0.0001)**	**73.06 (<0.0001)**	**22.36 (<0.0001)**	–

S-, non-bearing shoots; S+, bearing shoots. Significant ($P < 0.05$) effects between shoot types and seasons based on Restricted Maximum Likelihood methods are in bold.

respectively, when compared with the vessel area measured below (L) the acorn scars (Fig. 1). This reduction in vessel area was always greater in S+ than in S- and in *Q. ilex* than in *Q. faginea* (Figs. 1, 2, 3). Finally, no significant differences were found in pith area among cross-sectional cuts of both shoot types (data not shown). Note that the wood of current-year stems in *Q. faginea* is semi-ring porous.

In *Q. ilex*, the total predicted hydraulic conductance was three times higher in S+ than S- shoots in the basal L position, whereas the ratio of conductances between these two shoot types was 0.3 in the apical H position. In *Q. faginea*, the conductance ratios between S+ and S- shoots were 1.2 and 0.08 in the L and H positions, respectively. Referring to *G* tests, the distributions of vessels according to their transversal area differed significantly among S+ and S- shoots in both oak species and for the three stem positions (Fig. 3). The greatest difference in vessel distributions between shoot types appeared for the apical sections taken above acorn scars because of the higher frequency of vessels with smallest transversal area in S+ than in S-, particularly in *Q. faginea*. This caused that in the apical sections of the shoot most of the total predicted hydraulic conductance was accounted for by the smallest vessels in S+ shoots (Fig. 4).

Chemical variables

From the chemical analyses of autumn sampling, we only detected a significant higher nitrogen concentration in S- leaves as compared to S+ leaves in both species (Table 3). No significant differences were found for carbohydrate data but some trends were observed. First, in *Q. ilex* the starch concentration in stems increased in S+ as compared with S-. Second, in *Q. faginea* the concentration of soluble sugars in leaves was higher in S+ than in S-.

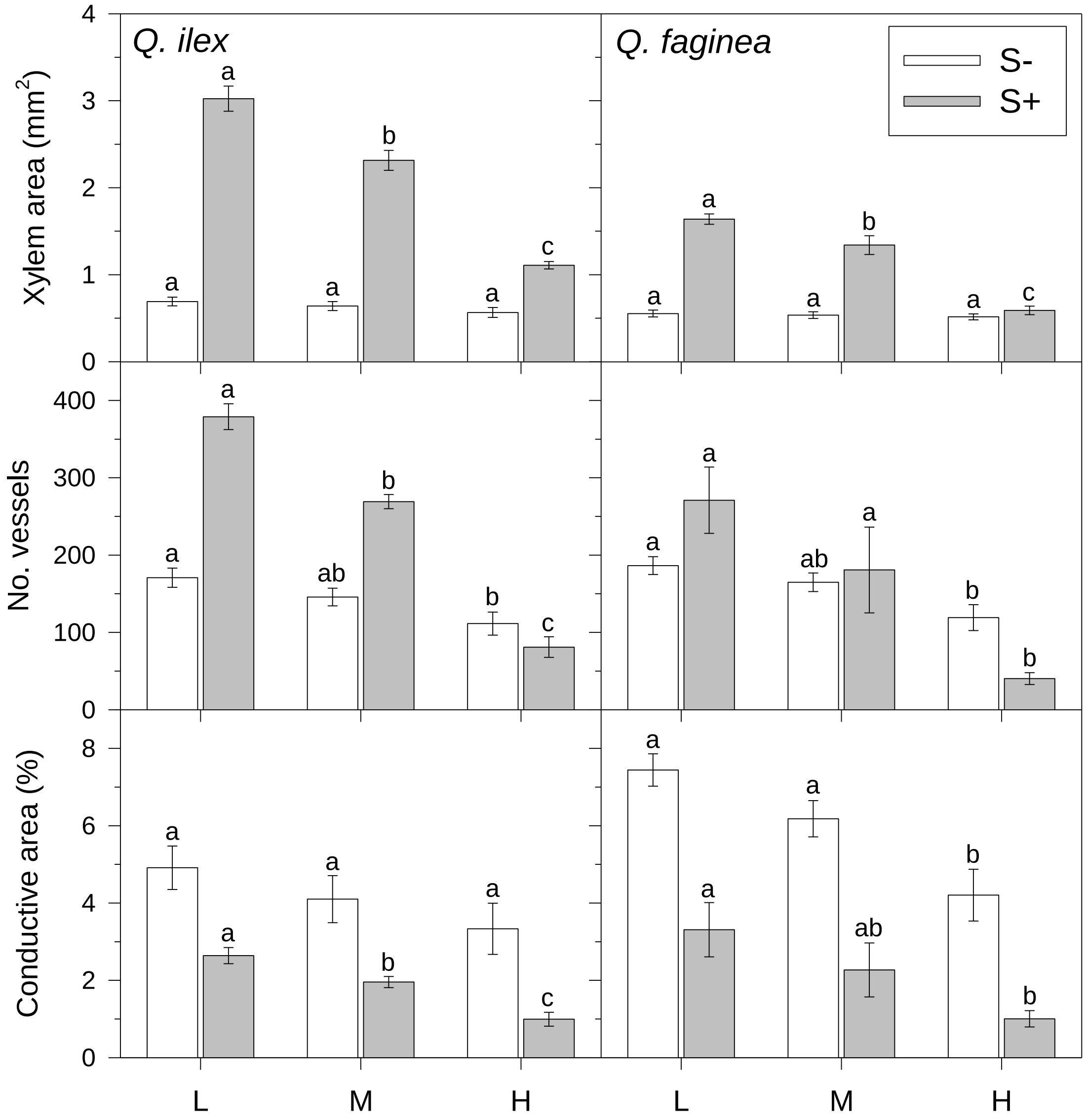
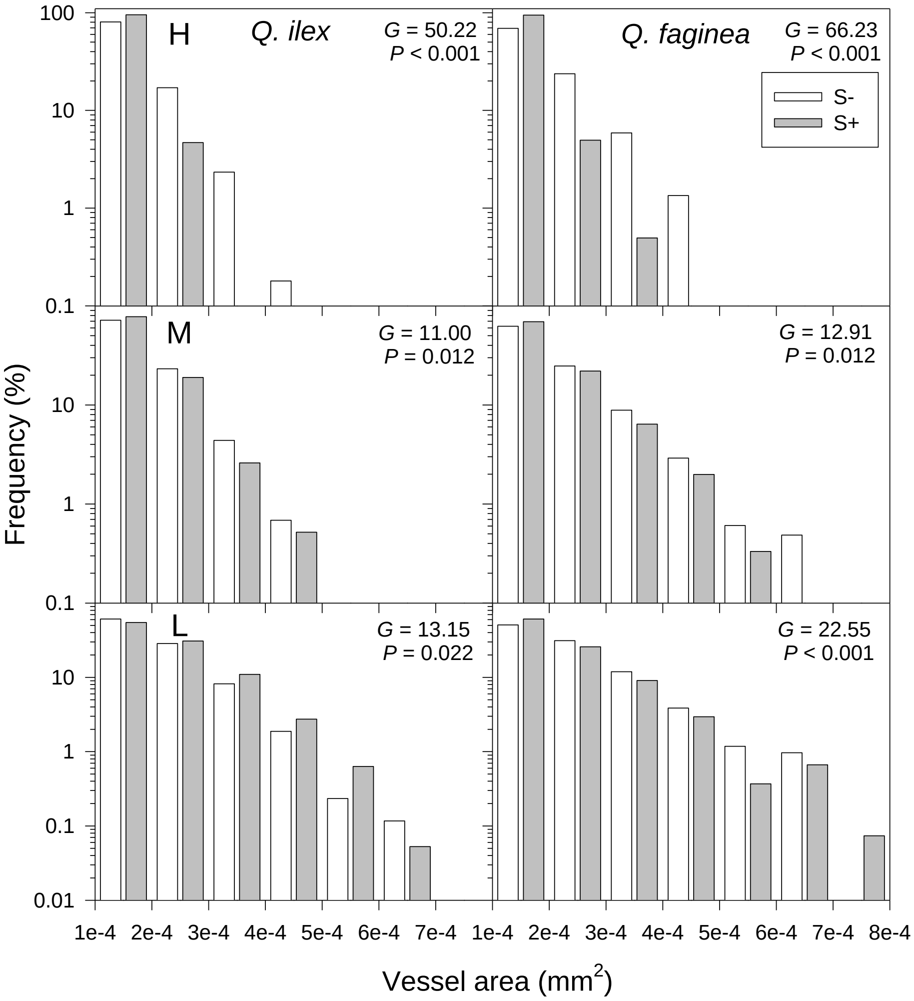


Figure 2. Comparisons of several wood anatomical variables measured at different positions along the stem (*L*, *M*, and *H* correspond to locations below, between and above scars of acorns stalks, respectively; see Fig. 1) of non-bearing (S-) and bearing (S+) shoots in both studied species for trees sampled in 2010. Different letters correspond to significant ($P < 0.05$) differences among positions for each shoot type.

Figure 3. Distribution of vessels according to their transversal area for the three cross-sectional cuts (*L*, *M*, and *H* corresponding to locations below, between and above scars of acorns stalks, respectively (see Fig. 1), in non-bearing (S-) and bearing (S+) shoots in both studied species. The distributions of vessels according to their transversal areas were compared among shoot types using *G* tests whose significance levels (*P*) are also displayed in each graph. Note the logarithmic scales.



Discussion

In agreement with the posed hypothesis, our results demonstrate that the stem cross-sectional areas of bearing shoots (S+) were thicker than those of non-bearing shoots (S-) in both studied oak species. The performed comparisons among variables measured in shoots differing in acorn production showed diverse specific patterns. In *Q. ilex* all measured variables tended to be higher in S+ than those in S-, whereas in *Q. faginea* the stem length, number of leaves, the apical bud and leaf mass in autumn decreased in S+ as compared to S-. However, the stem cross-sectional area was the variable that increased more consistently in S+ of both species. Thick stems may provide a greater structural support and higher total water conductivity than thin stems (Villar-Salvador et al. 1997). S+ of both species did not show any decrease in the relative conductive area as compared with S-, which suggests that the acorn production was linked to an increase of both secondary growth and the absolute hydraulic conductivity. Indeed, the acorn water content of *Q. ilex* and *Q. faginea* is highest in September and October (55–60%, unpublished data), i.e.

when the maximum rates of acorn development occur (Montserrat-Martí et al. 2009). Cochard et al. (2005) proposed a similar positive relation between bud development and the stem hydraulic conductivity.

In the studied species, thickened stems with increased hydraulic conductivity seem to be necessary for developing acorns in summer, when most acorn development is performed concurring with the period of maximum water deficit (Montserrat-Martí et al. 2009). Sánchez-Humanes et al. (2010) also found in *Quercus lobata* that the stems of bearing branches had greater diameters than those of nonbearing ones. According to our results, S+ were always thicker than S-, particularly in the case of *Q. ilex*. On one side, this might be due to the contrasting phenology of each species since *Q. faginea* starts shoot growth and complete most of the phenophases earlier than *Q. ilex* (Montserrat-Martí et al. 2009). On the other side, the evergreen *Q. ilex* might accumulate and allocate carbohydrates and nutrients stored in old leaves to develop current-year shoots, while *Q. faginea* should preferentially allocate these resources in wood.

Stem length in *Q. faginea* did not vary between bearing and non-bearing shoots while in *Q. ilex* shoots were always longer when acorns were present. Several reasons may explain this result. First, reproductive costs for growth may be allocated hierarchically within the tree crown and change across modular levels (Obeso 2004). In other studies it has been observed that non-bearing shoots from reproductive branches are few and short as compared with similar shoots from non-reproductive branches (Kawamura and Takeda 2006; Sánchez-Humanes et al. 2010). Second, the relations between growth and reproduction are not necessarily negative, as has been reported for *Pinus radiata* (Cremer 1992) or *Eurya japonica* (Suzuki 2000). For instance, pruning enhances shoot growth, photosynthesis rates and fruit production in apple trees (Forshey and Elfving 1989). Also, the existence of negative correlations between variables related to growth and reproduction does not necessarily imply a trade-off between them as Knops et al. (2007) suggested for Californian oaks.

The increment of current-year stem cross-sectional areas may allow shoots, branches or tree canopies to support more leaf area (Al Afas et al. 2005). Previous studies have reported that fruiting reduces leaf area and decreases nitrogen concentration in the leaves of reproductive shoots (Fujii and Kennedy 1985; Urban et al. 2004). In *Fagus sylvatica*, Han et al. (2011) also found that shoot nitrogen concentration is decreased significantly by the presence of fruits. Accordingly, our results showed lower nitrogen concentrations in the leaves of S+ than in those of S-, although in *Q. ilex* leaf number and leaf mass of S+ were also larger.

Figure 4. Distribution of vessels according to their relative contribution to the total predicted hydraulic conductance (*Kh*) for the three cross-sectional cuts (*L*, *M*, and *H* corresponding to locations below, between and above scars of acorns stalks, respectively (see Fig. 1), in non-bearing (S-) and bearing (S+) shoots in both studied species.

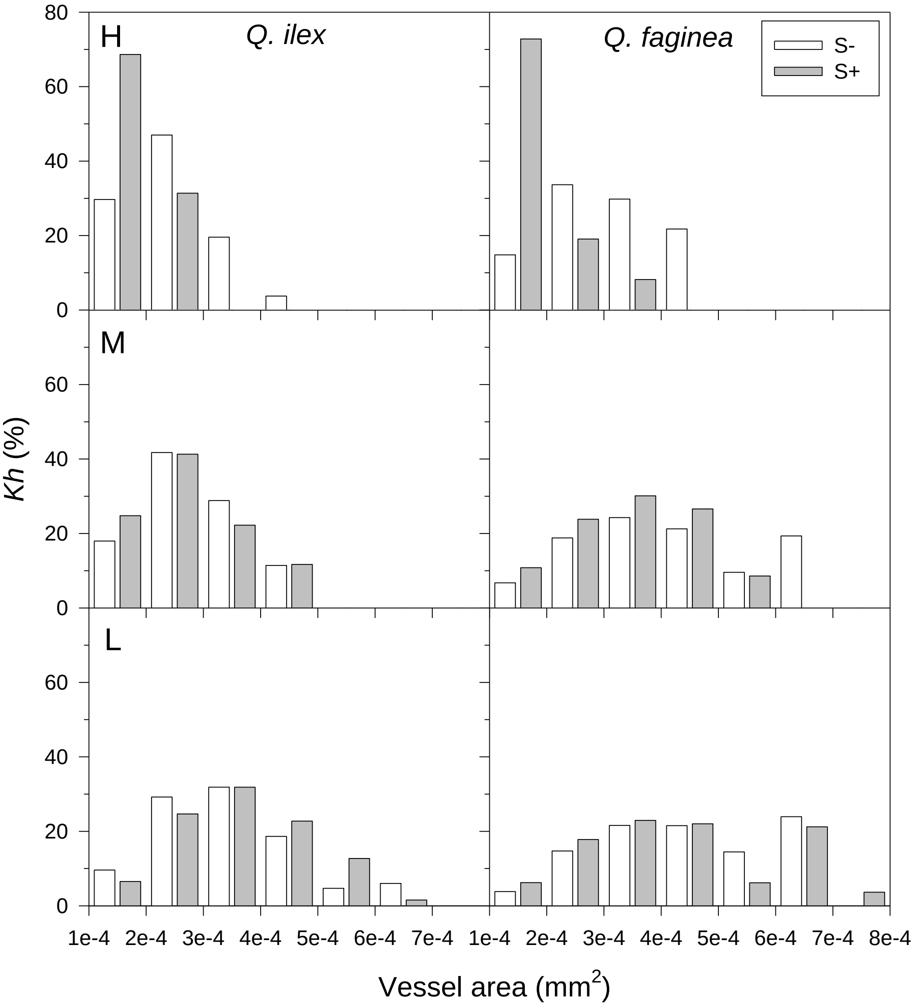


Table 3. Mean annual concentrations (±SE) of nitrogen (N), soluble sugars (SS), starch and total non-structural carbohydrates (NSC) in stems and leaves of shoots with and without acorns in *Q. ilex* and *Q. faginea* trees sampled in autumn 2010.

Species	Variable	Acorn	N (%)	SS (%)	Starch (%)	NSC (%)
Q. ilex	Stem	S-	0.68 ± 0.02	2.50 ± 0.21	2.32 ± 0.11	4.83 ± 0.23
		S+	0.74 ± 0.03	2.25 ± 0.20	2.65 ± 0.19	4.90 ± 0.16
		F (*P*)	3.32 (0.09)	0.48 (0.50)	2.11 (0.17)	0.13 (0.72)
	Leaves	S-	1.22 ± 0.03	3.24 ± 0.18	3.41 ± 0.07	6.65 ± 0.20
		S+	1.11 ± 0.04	3.10 ± 0.30	3.58 ± 0.26	6.69 ± 0.41
		F (*P*)	**5.08 (0.04)**	0.13 (0.73)	0.47 (0.50)	0.01 (0.91)
Q. faginea	Stem	S-	0.69 ± 0.02	2.78 ± 0.08	3.97 ± 0.21	6.75 ± 0.20
		S+	0.68 ± 0.02	2.69 ± 0.12	4.11 ± 0.19	6.80 ± 0.22
		F (*P*)	0.06 (0.81)	0.40 (0.54)	0.25 (0.62)	0.03 (0.86)
	Leaves	S-	1.48 ± 0.07	4.59 ± 0.11	4.84 ± 0.18	9.43 ± 0.25
		S+	1.23 ± 0.07	4.84 ± 0.11	4.84 ± 0.32	9.68 ± 0.35
		F (*P*)	**6.72 (0.02)**	2.38 (0.14)	0 (0.99)	0.34 (0.56)

Significant ($P < 0.05$) differences between shoot types are in bold. Abbreviations are as in Table 3.

Throughout the fruiting season, nitrogen concentration of leaves does not decrease in the first stages of fruit development (Ichie et al. 2005). Our results suggest that the translocation of nitrogen from leaves to fruits occurs during the late processes of fruit development as has been found by Rufat and DeJong (2001). When growing fruits increase their demand of nitrogen as they enlarge, leaf senescence and leaf shedding may be enhanced, as has been reported for several oak species after years of large acorn crops and masting events (Singh et al. 1990; Camarero et al. 2010). This might explain the significant decrease of leaf number from summer to autumn in both species. In addition, the stem length measured in both shoot types and species decreased in the autumn sampling as compared with the summer one, which may be caused by the selective acorn-induced shedding of long shoots between both seasons (Alla et al. 2011).

We did not find differences in the carbohydrate concentrations of stems and leaves as a function of the presence of acorns in shoots. Therefore, our data do not support resource theories based on the accumulation of carbohydrates or on the preferential allocation of carbon resources to developing fruits. Several studies highlight the ability of vegetative and reproductive organs to assimilate substantial amounts of carbon evidencing the carbon autonomy of fruit-bearing shoots (Aschan and Pfanz 2003; Hasegawa et al. 2003; Hoch 2005; Hoch and Keel 2006). Starch concentration may decline in reproductive shoots as a response to mast fruiting (Miyazaki et al. 2002), but we did not detect this effect in the shoots of trees with abundant acorn production. This might be explained if reproductive shoots were preferred in carbon allocation satisfying all their demands for growth and reproduction, but this should be tested in further studies. Such idea also concurs with studies which indicate that reproductive shoots obtain photosynthates from nearby non-reproductive shoots (Forshey and Elfving 1989; Obeso 2004; Miyazaki et al. 2007; Sánchez-Humanes et al. 2010).

The presence of acorns influenced drastically the transversal vessel area in current-year shoot stems. In these stems, the conductive area and the number of vessels diminished towards the stem apex, i.e. near the apical buds, whereas in non-bearing shoots the differences in wood anatomy along the stem were negligible. Enhanced secondary shoot growth is linked to the production of wide vessels in the xylem and an increased hydraulic conductivity, which may lead to the production of bigger apical buds and to a greater primary growth in the next spring (Cochard et al. 2005; Alla et al. 2011). According to our results, these processes do not seem to operate in bearing shoots, possibly because acorns compete with buds for water and nutrients. In fact, Camarero et al. (2010)

suggested that growth processes which overlap in time with acorn development, such as bud formation, might be negatively affected when trees produce large fruit crops. The postulated within-shoot competition between acorns and buds might also affect the total number of buds produced per unit of shoot length. However, we only observed this in *Q. faginea* in which the ratio of bud number per shoot length was lower in S+ than in S- (data not shown). Gross (1972) also reported that abundant crops in birch led to a reduction in the amount of buds, which caused a decrease of the primary crown growth in the next spring. Overall, our findings indicate that there may be trade-offs between acorn production and bud growth in current-year shoots mediated by secondary growth as Han et al. (2008) also reported for *Fagus crenata*.

An interesting question arising from our results refers to the moment when the thickening of reproductive stems is produced, as stems begin their thickening in early summer, i.e. much before acorn enlargement starts (Montserrat-Martí et al. 2009). This seems to indicate that there is a clear predetermination for fruit production before summer. Moreover, many acorns may be shed between early stages of fruit development and maturation (ca. 30% according to Montserrat-Martí et al. 2009), thus reducing the investment in water and carbon. It could well be that acorns shed in summer corresponded to current-year shoots whose thickening failed. What would the consequences be for the tree if many reproductive shoots with thick stems lose their fruits during the period of active acorn enlargement? We hypothesise that these trees might develop bigger buds which would determine a larger crown growth in the next spring than that of trees with reduced acorn loss. Further studies are required to fully understand how the secondary growth interacts with acorn development in oaks.

In conclusion, the presence of acorns is linked to enhanced thickening of current-year stems and this overgrowth starts in early summer much before than acorns start their major phase of enlargement. Bearing stems have a higher vessel number and a larger total area and total conducting area than non-bearing stems in the basal position of the stem. The number of vessels and their total area decreased to the stem apex, particularly from the upper acorn stalk towards the apical bud of stems bearing acorns. Our findings suggest potential trade-offs between acorn production and bud development in current-year shoots mediated by secondary growth. Bearing and non-bearing stems did not show differences of carbohydrate concentrations on stems and leaves in both studied oak species, and only the leaves of bearing stems presented lower nitrogen concentrations than those of non-bearing ones. The absence of differences in carbohydrate concentrations between bearing and non-bearing shoots suggests that acorn production is mostly dependent on recently synthesised carbohydrates, whereas the decline in nitrogen concentrations found in shoots bearing acorns indicates that nitrogen is allocated towards fruits.

Acknowledgements

A.Q.A. and J.J.C. acknowledge the support of MAEC-AECID and ARAID, respectively. This work was supported by the Spanish Ministerio de Ciencia e Innovación and FEDER (grant numbers CGL2007-66066-C04-02/BOS, CGL2008-04847-C02-01, RTA2005-00100-CO2-00, SUM2006-00025-00-00). We thank the collaborative support within the Globimed network. We acknowledge Sara Palacio for revising a previous version of this work. We thank Elena Lahoz for performing the chemical analyses and Pedro Sánchez Navarrete for his help in field sampling.

References

Al Afas N, Pellis A, Niinemets U, Ceulemans R (2005) Growth and production of a short rotation coppice culture of poplar. II. Clonal and year-to-year differences in leaf and petiole characteristics and stand leaf area index. Biomass Bioenerg 28:536–547. DOI: 10.1016/j.biombioe.2004.11.010

Alla AQ, Camarero JJ, Rivera P, Montserrat-Martí G (2011) Variant allometric scaling relationships between bud size and secondary shoot growth in *Quercus faginea*: implications for the climatic modulation of canopy growth. Ann For Sci 68: 1245–1254. DOI: 10.1007/s13595-011-0093-z

Amaral Franco J (1990) *Quercus*. In: Castroviejo S, Laínz M, López González G, Montserrat P, Muñoz Garmendia F, Paiva J, Villa L (ed) Flora Ibérica. Real Jardín Botánico, C S I C, pp 15–36

Aschan G, Pfanz H (2003) Non-foliar photosynthesis - a strategy of additional carbon acquisition. Flora 198:81–97. DOI: 78/0367-2530-00080

Barnola P, Crabbé J (1993) L'activite cambiale, composante active ou passive dans les reactions de croissance de l'arbre ? Acta Botanica Gallica 140:403–412

Buysse J, Merckx R (1993) An improved colorimetric method to quantify sugar content of plant tissue. J Exp Bot 44:1627–1629. DOI: 10.1093/jxb/44.10.1627

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. DOI: 10.1007/s00468-010-0462-5

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. DOI: 10.1139/X07-050

Cochard H, Coste S, Chanson B, Guehl JM, Nicolini E (2005) Hydraulic architecture correlates with bud organogenesis and primary shoot growth in beech (*Fagus sylvatica*). Tree Physiol 25:1545–1552. DOI: 10.1093/treephys/25.12.1545

Corcuera L, Camarero JJ, Gil-Pelegrin E (2004) Effects of a severe drought on growth and wood anatomical properties of *Quercus faginea*. IAWA J 25:185–204

Cremer KW (1992) Relations between reproductive growth and vegetative growth of *Pinus radiata*. For Ecol Manag 52:179–199

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356. DOI: 10.1021/ac60111a017

FAO (1998) World reference base for soil resources. ISRIC and ISSS, Rome

Farina V, Lo Bianco R, Inglese P (2006) Shoot growth, crop load, and fruit quality within vase-shaped canopies of "*Fairtime*" peach trees. Eur J Hortic Sci 71:227–230

Forshey CG, Elfving DC (1989) The relationship between vegetative growth and fruiting in apple trees. Hortic Rev 11:229–287

Fujii JA, Kennedy RA (1985) Seasonal changes in the photosynthetic rate in apple trees: a comparison between fruiting and non-fruiting trees. Plant Physiol 78:519–524

George AP, Nissen RJ, Collins RJ, Rasmussen TS (1996) Effects of shoot variables and canopy position on fruit set, fruit quality and starch reserves of persimmon (*Diospyros kaki* L) in subtropical Australia. J Hortic Sci 71:217–226

Graves WC (1980) Annual oak mast yields from visual estimates. In: Plumb TR (ed) Symposium on the ecology, management, and utilization of California oaks. USDA Forest Service, Claremont, pp 270–274

Gross HL (1972) Crown deterioration and reduced growth associated with excessive seed production by birch. Can J Bot 50:2431–2437. DOI: 10.1139/b72-312

Hallé F, Oldeman RAA, Tomlinson PB (1978) Tropical trees and forests: an architectural analysis. Springer, New York

Han Q, Kabeya D, Iio A, Kakubari Y (2008) Masting in *Fagus crenata* and its influence on the nitrogen content and dry mass of winter buds. Tree Physiol 28:1269–1276

Han Q, Kabeya D, Hoch G (2011) Leaf traits, shoot growth and seed production in mature *Fagus sylvatica* trees after 8 years of CO_2 enrichment. Ann Bot 107:1405–1411

Hasegawa S, Koba K, Tayasu I, Takeda H, Haga H (2003) Carbon autonomy of reproductive shoots of Siberian alder (*Alnus hirsuta var. sibirica*). J Plant Res 116:183–188. DOI: 10.1007/s10265-003-0085-7

Hirayama D, Nanami S, Itoh A, Yamakura T (2007) Individual resource allocation to vegetative growth and reproduction in subgenus Cyclobalanopsis (*Quercus*, *Fagaceae*) trees. Ecol Res 23:451–458. DOI: 10.1007/s11284-007-0398-4

Hoch G (2005) Fruit-bearing branchlets are carbon autonomous in mature broad-leaved temperate forest trees. Plant Cell Environ 28:651–659. DOI: 10.1111/j.1365-3040.2004.01311.x

Hoch G, Keel SG (2006) 13C labelling reveals different contributions of photoassimilates from infructescences for fruiting in two temperate forest tree species. Plant Biol 8:606–614. DOI: 10.1055/s-2006-924279

Ichie T, Kenzo T, Kitahashi Y, Koike T, Nakashizuka T (2005) How does *Dryobalanops aromatica* supply carbohydrate resources for reproduction in a masting year? Trees 19:704–711. DOI: 10.1007/s00468-005-0434-3

Johnson RS, Lakso AN (1986) Carbon balance model of a growing apple shoot. I: development of the model. J Am Soc Hortic Sci 111:160–164

Kawamura K, Takeda H (2006) Cost and probability of flowering at the shoot level in relation to variability in shoot size within the crown of *Vaccinium hirtum* (*Ericaceae*). New Phytol 171:69–80. DOI: 10.1111/j.1469-8137.2006.01737.x

Keel SG, Schädel C (2010) Expanding leaves of mature deciduous forest trees rapidly become autotrophic. Tree Physiol 30:1253–1259. DOI: 10.1093/treephys/tpq071

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

Koenig WD, Knops JMH (1998) Scale of mast-seeding and tree-ring growth. Nature 396:225–226. DOI: 10.1038/24293

Kozlowski TT (1971) Growth and development of trees. Academic Press, New York

Landhäusser SM (2010) Aspen shoots are carbon autonomous during bud break. Trees 25:531–536. DOI: 10.1007/s00468-010-0532-8

Lauri PÉ, Térouanne É, Lespinasse JM (1996) Quantitative analysis of relationships between inflorescence size, bearing-axis size and fruit-set: an apple tree case study. Ann Bot 77:277–286. DOI: 10.1006/anbo.1996.0031

Lauri PÉ, Kelner JJ, Trottier C, Costes E (2010) Insights into secondary growth in perennial plants: its unequal spatial and temporal dynamics in the apple (*Malus domestica*) is driven by architectural position and fruit load. Ann Bot 105:607–616

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

Miyazaki, Y, T. Hiura, E. Kato, and R. Funada (2002) Allocation of resources to reproduction in *Styrax obassia* in a masting year. Ann Bot 89:767–772. DOI: 10.1093/aob/mcf107.

Miyazaki Y, Hiura T, Funada R (2007) Allocation of photo-assimilated ^{13}C from reproductive and non-reproductive shoots to fruits in *Styrax obassia*. Plant Sp Biol 22:53–57

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

Montserrat-Martí G, Camarero JJ, Palacio S, Pérez-Rontomé C, Milla R, Albuixech J, Maestro M (2009) Summer-drought constrains the phenology and growth of two coexisting Mediterranean oaks with contrasting leaf habit: implications for their persistence and reproduction. Trees 23:787–799. DOI: 10.1007/s00468-009-0320-5

Obeso JR (2004) A hierarchical perspective in allocation to reproduction from whole plant to fruit and seed level. Persp Plant Ecol Evol Syst 6:217–225. DOI: 10.1078/1433-8319-00080

Obeso JR (1997) Costs of reproduction in *Ilex aquifolium*: effects at tree, branch and leaf levels. J Ecol 85:159–166. DOI: 10.2307/2960648

Obeso JR (2002) The costs of reproduction in plants. New Phytol 155:321–348

Palacio S, Maestro M, Montserrat-Marti G (2007) Seasonal dynamics of non-structural carbohydrates in two species of Mediterranean sub-shrubs with different leaf phenology. Environ Exp Bot 59:34–42

Porter GW, Sherman WB, Beckman TG, Krewer GW (2002) Fruit weight and shoot diameter relationship in early ripening peaches. J Am Pomol Soc 56(1):30–33

Rasband WS, Ferreira T (2011) ImageJ. NIH, Bethesda.

Roff DA (1992) Cost of reproduction. In: The evolution of life histories: Theory and analysis. Chapman and Hall, New York, pp 145–178

Rufat J, DeJong TM (2001) Estimating seasonal nitrogen dynamics in peach trees in response to nitrogen availability. Tree Physiol 21:1133–1140. DOI: 10.1093/treephys/21.15.1133

Sánchez-Humanes B, Sork VL, Espelta JM (2010) 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. DOI: 10.1007/s00442-010-1819-6

Singh SP, Rawat YS, Rana BS, Negi GCS (1990) Effects of unusually large seed crop on litterfall and nitrogen retranslocation in Himalayan oaks. For Ecol Manag 32:79–86. DOI: 16/0378-1127(90)90162-5

Sokal RR, Rohlf FJ (1995) Biometry: the principles and practices of statistics in biological research. W H Freeman, New York

Solar A, Solar M, Štampar F (2006) Stability of the annual shoot diameter in Persian walnut: a case study of different morphotypes and years. Trees 20:449–459. DOI: 10.1007/s00468-006-0060-8

Sperry JS, Hacke UG, Pittermann J (2006) Size and function in conifer tracheids and angiosperm vessels. Am J Bot 93:1490-1500

Suzuki A (2000) Patterns of vegetative growth and reproduction in relation to branch orders: the plant as a spatially structured population. Trees 14:329–333. DOI: 10.1007/s004680050226

Tuomi J, Hakala T, Haukioja E (1983) Alternative concepts of reproductive effort, costs of reproduction, and selection in life-history evolution. Am Zool 23:25–34. DOI: 10.1093/icb/23.1.25

Urban L, Lu P, Thibaud R (2004) Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiol 24:387–399. DOI: 10.1093/treephys/24.4.387

Villar-Salvador P, Castro-Díez P, Pérez-Rontomé C, Montserrat-Martí G (1997) Stem xylem features in three *Quercus* (*Fagaceae*) species along a climatic gradient in NE Spain. Trees 12:90–96. DOI: 10.1007/PL00009701

Watson MA (1986) Integrated physiological units in plants. Trends Ecol Evol 1:119–123. DOI: 10.1016/0169-5347(86)90005-4

Watson MA, Casper BB (1984) Morphogenetic constraints on patterns of carbon distribution in plants. Ann Rev Ecol Syst 15:233–258. DOI: 10.1146/annurev.es.15.110184.001313

CHAPTER IV

Seasonal patterns of bud development and secondary growth as related to climate in two co-existing Mediterranean Quercus species

IV. Seasonal patterns of bud development and secondary growth as related to climate in two co-existing Mediterranean *Quercus* species

Abstract In trees, bud development is driven by endogenous and exogenous factors such as shoot growth and climate, respectively. However, there is scarce knowledge on how these drivers affect bud size and development across different time scales. We describe the seasonal patterns of apical bud development as related to primary and secondary growth in two coexisting Mediterranean oaks with contrasting leaf habit (*Quercus ilex*, evergreen; *Quercus faginea*, deciduous) over three years with different climatic conditions. Furthermore, we determine the climatic factors driving the patterns in shoot length, secondary growth of main and current-year stems and bud size of both species by correlating these data with climatic variables at different time scales (5 to 30 days) over a 15-year period. We found that the maximum growth rate of buds is reached between late July and mid-August in both species, but the peak of bud enlargement occurred earlier in the deciduous than in evergreen species. Moreover, the apical bud size increased as minimum air temperatures did during the period of maximum bud growth rates. The forecasted rising minimum air temperatures predicted by climatic models will lead to increasing bud size and consequently may alter crown architecture differentially in sympatric Mediterranean oaks.

Keywords Bud size, Current-year stem, *Quercus faginea*, *Quercus ilex* subsp. *ballota*, Temperature, Summer growth.

Introduction

Many temperate *Quercus* species present preformed growth of their shoots *(Fontaine et al.*, 1999; Barthélémy and Caraglio, 2007). In such species, shoot primordia are initiated inside the buds in a growing season but remain un-extended during the autumn-winter resting period until the following spring (Puntieri *et al.*, 2002). Meristems and preformed primordia generally are enclosed and protected by specialized elements, which determine the type of bud (Puntieri *et al.*, 2007). *Quercus* species have typical scaled buds (Keator, 1998) which show different protective elements (hypsophylls, scales and cataphylls) and present a regular pattern of development along the year (Nitta and Ohsawa, 1998). In spring, once shoot extension is accomplished, the shoot presents a small hypsophyllary bud at the apex. This bud develops many scales in the following weeks, switching to a small scaled bud. Scaled buds grow until early or mid-autumn, when they enter dormancy (Arora *et al.*, 2003; Horvath *et al.*, 2003). This resting stage is broken in late winter or early spring when buds start swelling (Marks, 1975;

Montserrat-Martí *et al*., 2009). Indeed, the adaptive value of scaled buds seems to be the protection of the shoot primordia against climatic stressors such as low temperatures during the cold winter season (Nitta and Ohsawa, 1998).

The buds formed within an individual tree show different sizes (Gill, 1971). This suggests that bud development is modulated by several factors including the species, the individual tree size and vigour, and the position of the bud in the shoot (Remphrey and Powell, 1984; Sabatier and Barthélémy, 2001; Puntieri *et al*., 2002; Ishihara and Kikuzawa, 2009; Alla *et al*., 2011*b*). Among the endogenous factors that drive bud size development, secondary growth has been found to be a critical driver since changes in current-year stem cross-sectional area are linked to the stem hydraulic conductivity and the size of buds (Cochard et al., 2005; Alla *et al*., 2011*b*). However, it remains unclear when the secondary growth of trunks and current-year stems occur in relation to the pattern of bud development, whether these processes overlap in time, and if climate indirectly affects the development of buds through the control of secondary growth. Climate and species are two of the main exogenous factors affecting bud size in trees (Kozlowski *et al*., 1973; Salminen and Jalkanen, 2004; Meier and Leuschner, 2008; Sanz-Pérez and Castro-Díez, 2010).

Despite their relevance, the driving factors controlling the seasonal pattern of bud development have received little attention excepting in the case of conifers (Kozlowski *et al*., 1973; Lanner, 1985; Chen *et al*., 1996). In a previous study, Montserrat-Martí *et al*., (2009) analysed the seasonal patterns of bud development in two co-existing Mediterranean *Quercus* species with contrasting leaf habit, namely *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. (evergreen; hereafter abbreviated as *Q. ilex*) and *Q. faginea* Lam. (winter deciduous). They showed that the buds of both species reached the maximum growth rate in summer, although their data were population means and lacked the temporal resolution to determine the precise period of maximum growth rate. In addition, they showed that these two species present a reduced growth activity in response to summer drought. In this season shoot and secondary growth are almost arrested and acorns enlarge at a very low rate. Their study also showed that budburst and shoot extension occurred earlier in *Q. faginea* than in *Q. ilex*, so the buds of *Q. faginea* grow during more time than those of *Q. ilex*, assuming that cessation of bud growth is triggered by low winter temperatures in both species.

Montserrat-Martí *et al*. (2009) also reported an alternating yearly pattern of bud and shoot size in *Q. ilex* (years of large buds and small shoots alternate with years of small buds and large shoots), which was not observed in *Q. faginea*. Such interesting pattern may be related to the different seasonal patterns of bud development in the two studied species. Here, we hypothesize that this pattern might be caused in *Q. ilex* by the short time available to complete its bud development after shoot development is accomplished as compared with *Q. faginea*.

In this study we aimed: (*i*) to describe the seasonal patterns of bud development and secondary growth of main (trunk) and current-year stems of *Q. ilex* and *Q. faginea* over three years with contrasting climatic conditions, (*ii*) to determine if these co-existing oak species present different bud developmental patterns that could explain differences in crown growth, and (*iii*) to identify the climatic factors which most influence bud size of both species over a 15-year period. Our hypotheses were: (*i*) buds will attain their maximum growth rate when the climatic conditions are harsh enough for cessation of other aboveground growth types (primary, secondary and reproductive), usually occurring in mid-summer, (*ii*) the peak of bud enlargement will occur later in the evergreen *Q. ilex* than in the deciduous *Q. faginea*, as the second species presents a longer period of bud development than the first one, and (*iii*) the main climatic drivers of bud development will be those more important for growth in summer, namely water availability. In this study, other growth types happening in summer (lammas growth and acorn development) were discarded as they tend to occur in a small percentage of branches (Alla *et al.*, unpubl. res.). Furthermore, the size of apical buds did not differ between shoots bearing and not bearing acorns (Alla *et al.* 2011*a*).

Material and methods

Study area and species

The study site is located in Agüero, Aragón, north eastern Spain (42° 18′ N, 0° 47′ W, 750 m a.s.l.). Climate is Mediterranean and continental being characterized by dry summers and cold winters, with 544 mm and 13.8 ºC of total annual precipitation and mean annual temperature, respectively. Soil in this area is a Calcisol (FAO, 1998), formed on Miocene clays with bedrock of calcareous sandstone. Vegetation is a scrubland dominated by *Q. ilex*, *Q. faginea*, *Arbutus unedo* L. and *Pinus halepensis* Mill., approximately with similar dominance, and other less abundant woody species (see Montserrat-Martí *et al.*, 2009 for further details on the study site).

Quercus ilex is an evergreen oak tree with semi-ring to diffuse porous wood (Campelo *et al.*, 2007), which grows preferentially inland under continental conditions in the Iberian Peninsula (Amaral Franco, 1990). *Q. faginea* is a deciduous oak tree with ring-porous wood (Corcuera *et al.*, 2004*a*) and with a wide distribution in sub-Mediterranean areas of the Iberian Peninsula (Amaral Franco, 1990). Both oak species frequently coexist in the study area forming multi-stemmed trees (Table 1).

Table 1. Morphological and structural features of the studied species and trees (means ± SE).

Species	Diameter at 1.3 m (cm)	Height (m)	No. stems per tree	Shoot length (mm)		
				2006	2007	2008
Q. ilex	10.2 ± 1.2b	3.4 ± 0.4b	6.5 ± 1.0a	29.4 ± 2.0C	43.2 ± 2.2A	35.7 ± 2.0B
Q. faginea	14.5± 1.8a	5.7 ± 0.9a	4.0 ± 1.2b	17.7 ± 1.8B	24.7 ± 2.6A	23.4 ± 1.9A

Small letters correspond to significant differences between species and capital letters indicate significant differences among years in the case of shoot length ($P < 0.05$, Mann-Whitney *U* tests).

Field sampling and variables measured

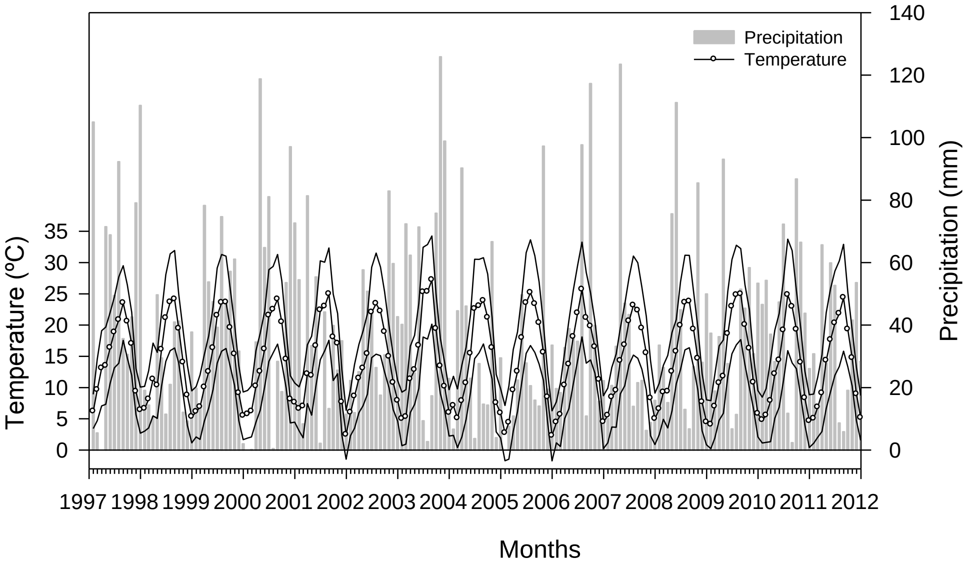
Two different approaches were undertaken: the seasonal development measurements were made over three years (2006-2008) and the inter-annual measurements were taken over fifteen years (1997-2011). Climate varied markedly among the studied years. During the monitoring period of seasonal development higher summer temperature and lower precipitation in 2007 as compared with 2006 and 2008 were detected, whereas during the long-term period 1998, 2005 and 2011 were dry years while 2003 was very warm (Fig. 1).

Seasonal development

In spring 2006 before budburst, four mature individuals per species were randomly selected, tagged and their size (diameter at 1.3 m, height) and number of stems per tree were measured (Table 1). Within each tree, ten current-year shoots were randomly selected and collected monthly from the southern side and upper third of the crown during three consecutive years (2006, 2007 and 2008). Current-year shoots were frozen until measures were taken in the laboratory. To minimize shoot length variability, the average length of the shoots selected for analyses was similar among sampled trees for each species. Shoot length (SL) and diameter (the average of two perpendicular measures taken in the middle of the stem without nodes) were measured in each shoot with a centesimal calliper (Mitutoyo, Kawasaki, Japan). Diameter was transformed to stem cross-sectional area (hereafter abbreviated as SCA) assuming a circular shape. The apical bud of each shoot was removed at 10x magnification under a stereomicroscope. Buds were oven-dried at 60 °C to a constant weight before being individually weighted to the nearest 0.001 g to obtain the apical bud mass (hereafter abbreviated as ABM).

To quantify changes in stem secondary growth the radial increment of those trees sampled for bud development was measured using manual band dendrometers (Agriculture Electronics Corporation, Tucson, USA) during the three years of study. In March 2006, dendrometers were placed at 1.3 m on the thickest stem of the selected individuals. Bark was carefully brushed off before installing the dendrometers,

Figure 1. Monthly climatic data (mean temperatures, total precipitation) during the study period (1997-2011).



which were read at 24-day intervals on average. Displacement measurements were taken with a precision of 0.1 mm and readings after rainy days were discarded. Radial growth was transformed to basal area (hereafter abbreviated as BA) considering the initial diameter of the trees.

Inter-annual measurements

Samplings for the inter-annual assessment of bud size and shoot growth were done at mid-October from 1997 to 2011, i.e. during 15 years. Each year 20 mature trees were randomly selected for each species. Annually, we collected 15 shoots per tree from the southern side and upper third of the crown (*n* = 300 shoots). Then, we randomly selected 30 shoots per species, excluding very big and very small shoots and also lammas shoots, to get the annual mean values of shoot length and SCA and bud size. October was selected as the most adequate time for annual sampling because shoot development is completed and buds have almost reached their final size by that time of the year. Shoot length and SCA were measured and apical buds were removed. After being oven-dried the apical buds were individually weighted as previously described. Detailed bud dissection was not performed due to the elevated number of sampled buds, and also because of the difficulty of dissection as each bud contains numerous scales, cataphylls and stipules (Nitta and Ohsawa, 1998; Fontaine *et al.* 1998; Heuret *et al.* 2003). Furthermore, in both species buds are too small to facilitate bud dissection, particularly in the case of *Q. ilex*.

To estimate the radial growth patterns of the sampled trees during the period 1997-2011, the main trunk was cored at 1.3 m using an increment Pressler borer. All wood samples were prepared following standard dendrochronological methods, they were air dried, sanded and visually cross-dated (Cook and Kairiukstis, 1990). Tree-ring widths were then measured at a precision of 0.001 mm using a LINTAB measuring system (Rinntech, Heidelberg, Germany). Lastly, they were standardized and transformed into tree-ring width indices (RWI) by fitting a horizontal line to raw width data and retaining the residuals, i.e. the differences between observed and fitted values.

Climatic data and water balance

Daily climate data (maximum temperature, Tmax; minimum temperature, Tmin; and total precipitation, P) for the period 1997-2011 were obtained from the weather station of Luna (42º 10′ N, 0º 56′ W, 409 m a.s.l.), situated at ca. 18 km from the study site. Daily air temperature were obtained from January 2007 up to December 2011 at the study site using a Hobo® data logger which recorded temperatures every 30 minutes (Onset Computer Co., Bourne, USA) (Fig. 1). To estimate local temperatures for the previous years (1997-2006), a linear regression between local data (y) and data from the weather station (x) was calculated ($y = 0.2131 + 0.9067x$; $r = 0.97$, $P < 0.0001$). Daily cumulative water balance (hereafter abbreviated as WB) was calculated as explained in Montserrat-Martí *et al*. (2009).

Data analyses

To estimate the growth rates of measured variables (BA, SCA, ABM) we fitted the following logistic model to cumulative growth data (y) as a function of time (x) using the CurveExpert software (Hyams, 2010):

$$y = a \,/\, (1 + e^{(b - cx)}) \qquad (1)$$

where a is the maximum or asymptotic value, b is the maximum daily rate and c is the Julian day when the maximum growth rate was attained.

Correlation analyses were performed using the Pearson correlation coefficient to evaluate the relationships between growth (RWI, SL, SCA, ABM) and climatic variables (Tmax, Tmin, P, WB). Correlations were calculated for the 15-year (1997-2011) study period considering the growing season from March to early October. To detect potential lags or cumulative effects between climatic and growth variables, climatic data were averaged (Tmax, Tmin) or summed (P, WB) for periods of increasing time span (5, 10, 15 and 30 days). To simplify, we only present the maximum correlations, in absolute terms, and the period when it was observed.

Results

Seasonal dynamics of basal area, stem cross-sectional area and bud size

Growth of all measured variables, except the ending of ABM development, started and finished earlier in *Q. faginea* than in *Q. ilex* during the three years of study (Figs. 2 and 3; Table 2). *Q. faginea* showed higher BA and larger ABM than *Q. ilex*, while SCA did not differ between species. BA presented a larger

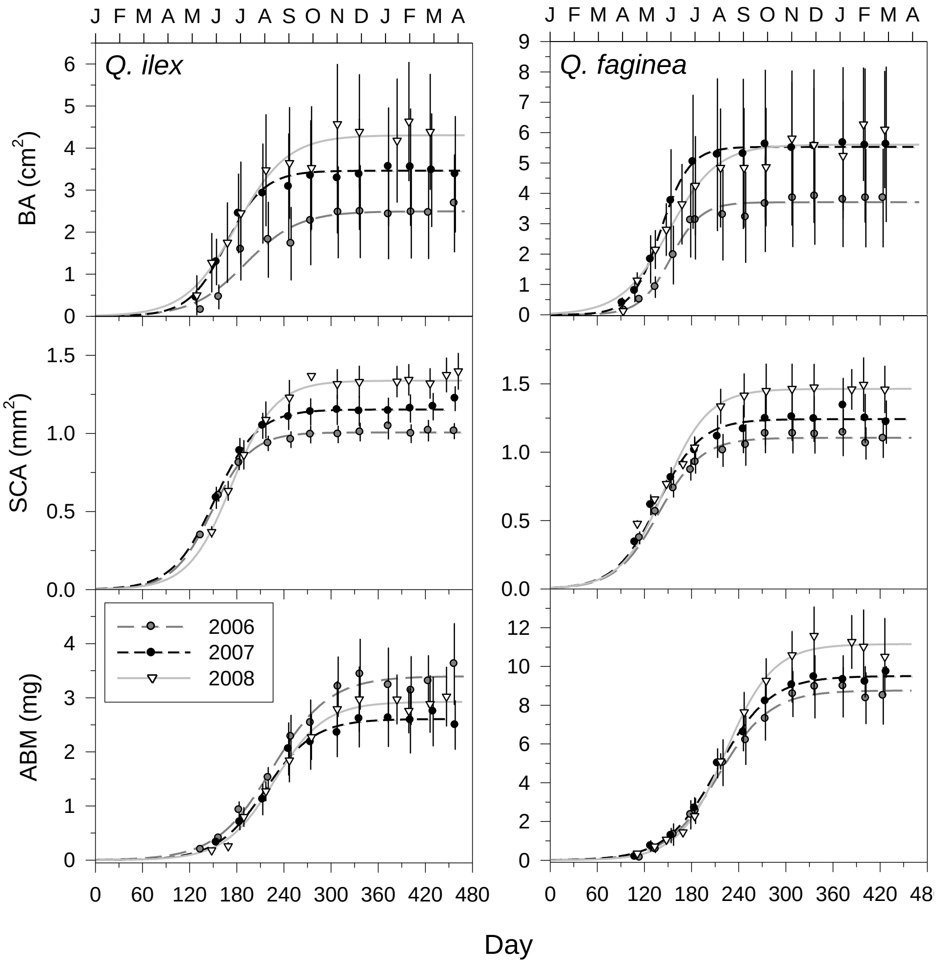


Figure 2. Cumulative stem basal area (BA) and current-year stem cross-sectional area (SCA) as compared with apical bud mass (ABM) in *Q. ilex* and *Q. faginea*. The different symbols and lines correspond to data (means ± SE) and fitted logistic models, respectively, for the three years of study.

Table 2. Statistical analyses based in the logistic model [$y = a / (1 + e^{(b - cx)})$] as a function of time (*x*, in Julian days) for cumulative values of stem basal area (BA), current-year stem cross-sectional area (SCA) and apical bud mass (ABM) of *Q. ilex* (*Qi*) and *Q. faginea* (*Qf*). Models were highly significant ($P < 0.001$) in all cases.

		a		*b*		*c*		R^2	
		Qi	*Qf*	*Qi*	*Qf*	*Qi*	*Qf*	*Qi*	*Qf*
BA	2006	2.50	3.71	0.0185	0.0477	11 July	4 June	0.968	0.990
	2007	3.46	5.53	0.0359	0.0751	17 June	22 May	0.982	0.999
	2008	4.31	5.60	0.0333	0.0458	29 June	1 June	0.983	0.979
SCA	2006	1.01	1.12	0.0101	0.0101	30 May	20 May	0.997	0.990
	2007	1.15	1.26	0.0106	0.0114	3 June	18 May	0.999	0.987
	2008	1.34	1.49	0.0121	0.0131	23 June	26 May	0.995	0.995
ABM	2006	3.40	8.76	0.0234	0.0668	15 August	4 August	0.987	0.999
	2007	2.61	9.51	0.0210	0.0718	6 August	3 August	0.986	0.999
	2008	2.93	11.15	0.0230	0.0923	16 August	12 August	0.991	0.999

Abbreviations: *a*, maximum value (units: BA, cm^2; SCA, mm^2; ABM, mg), *b*, maximum rate (units: BA, cm^2 d^{-1}; SCA, mm^2 d^{-1}; ABM, mg d^{-1}), *c*, day of maximum rate.

variation among years in *Q. ilex* than in *Q. faginea*, with lowest values in 2006 and highest ones in 2008. In the case of SCA both species showed increasing values from 2006 to 2008. In *Q. ilex*, ABM showed an alternate yearly pattern with the highest values in 2006 and the lowest ones in 2007, whereas in *Q. faginea* ABM behaved as the SCA did, i.e. showing minimum and maximum mean values in 2006 and 2008, respectively ($P < 0.05$, ANOVA) (Table 2).

Considering all analysed variables and years, maximum growth rates were always higher for *Q. faginea* than for *Q. ilex*. In the case of BA the maximum growth rates were higher in 2007 than in other years, while in the case of SCA and ABM the maximum rates were highest in 2008 (Fig. 3). The day of the maximum growth rate was always observed earlier in *Q. faginea* than in *Q. ilex* for all variables, and such date showed a higher year-to-year variability in *Q. ilex* than in *Q. faginea*. The SCA of *Q. faginea* was the variable showing the earliest day of maximum growth rate, which was on average the 21st May, whereas the ABM of *Q. ilex* was the variable with the corresponding latest day of maximum growth rate being on average the 12th August (Table 2, Fig. 3). Maximum growth rate of ABM was attained in the first two weeks of August in both species and for the three years of study (Table 2).

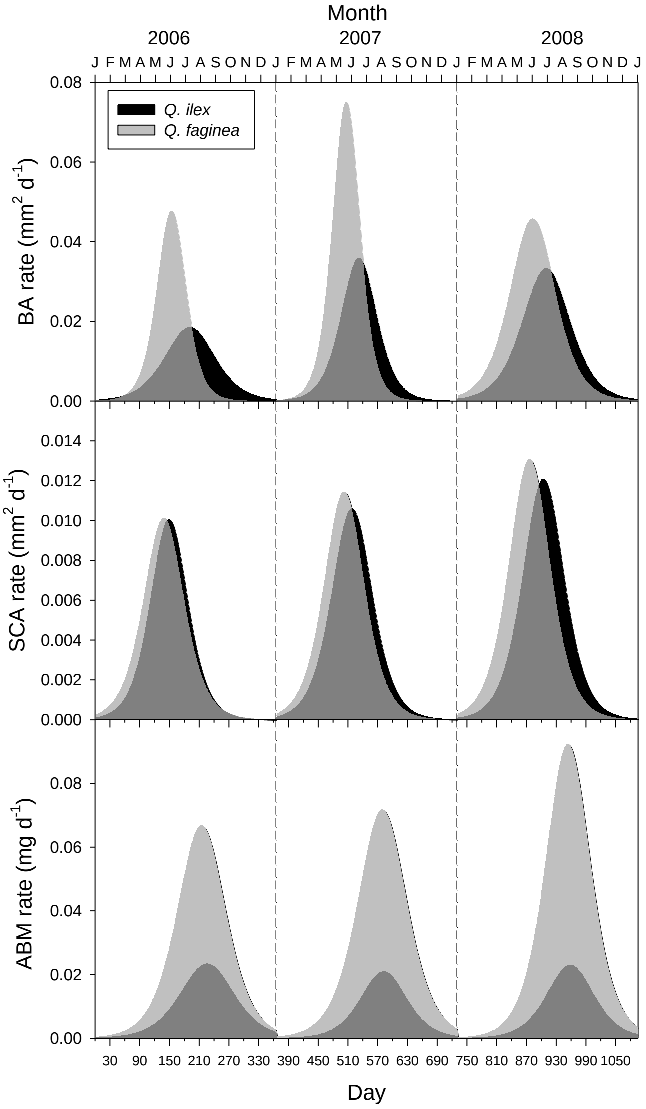
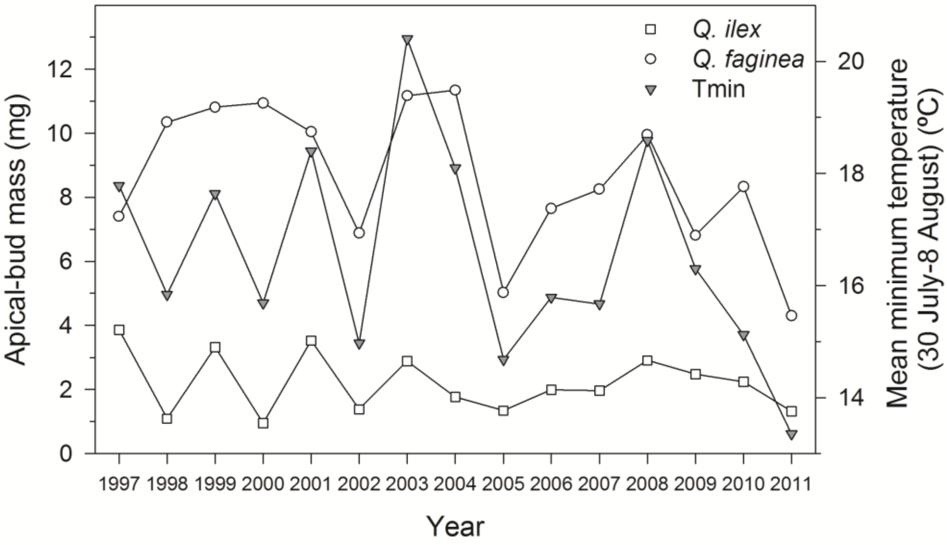


Figure 3. Growth rates of basal area, current-year stem cross-sectional area and apical bud mass during the three years of seasonal study based in fitted logistic models (see Fig. 2). Black areas correspond to *Q. ilex* rates, grey areas represent *Q. faginea* rates and dark grey areas indicate the intersections between both species.

Climatic drivers of growth and bud development

Most significant correlations between climate and growth parameters were observed for time spans ranging between 5 and 15 days (Table 3). Maximum (Tmax) and minimum (Tmin)

Figure 4. Inter-annual variations of the apical bud mass of *Q. ilex* and *Q. faginea* as related to minimum temperatures of the ten-day interval 30 July-8 August along the 15 years of study (1997-2011).



spring and early summer temperatures correlated negatively with RWI, SL and SCA, whereas late July to early August temperatures, particularly Tmin, showed positive relationships with ABM for both species. Spring precipitation and cumulative water balance were positively correlated with all growth variables, except in the case of ABM of *Q. ilex* and *Q. faginea*, which were more related to early summer water availability and water balance in late summer, respectively. ABM was mainly and positively related to early August Tmin considering 5- to 15-day spans, but also to 5-day Tmax (Table 3, Fig. 4). Furthermore, ABM responded positively to early July and negatively to late August precipitation and water availability in *Q. ilex* and *Q. faginea*, respectively (Table 3).

Discussion

The maximum growth rate of the buds of both oak species is reached in mid-summer, between the 3rd and the 16th August, although the peak growth rate of *Q. faginea* was 3-11 days earlier than that of *Q. ilex*. Despite the moderate temporal lag in maximum bud growth rates, both oak species present very similar patterns of apical bud development. This brief period in early August probably corresponds to the most unfavourable period for the vegetative growth in Mediterranean areas such as the study site. During summer, low precipitation amounts and elevated air temperatures, causing high evapotranspiration rates, lead to soil water deficit (Mitrakos, 1980; Alla *et al*. 2011*a*). However, despite both studied species present a very low growth activity in this period their buds enlarge intensively as previously described by Montserrat-Martí *et al*. (2009). Contrastingly, the mid-summer water shortage experienced by plants under Mediterranean climatic conditions limits the hydration levels required by some meristematic tissues to grow (Palacio

Table 3. Significant ($P < 0.05$) Pearson correlation coefficients obtained between growth (tree-ring width index, current-year shoot length, current-year stem cross-sectional area and apical bud mass) of *Q. ilex* (*Qi*) and *Q. faginea* (*Qf*) and related climatic variables (Tmax, Tmin, mean maximum and minimum temperatures, respectively; P, precipitation; WB, cumulative water balance). The dates of the displayed correlations are indicated between parentheses. Correlations were calculated for the period 1997-2011 considering the growing season from March to early October. Climatic data were averaged (Tmax, Tmin) or summed (P, WB) for periods of increasing time span (5, 10, 15 and 30 days).

Variable	Time span (days)	Tree-ring width index (RWI)		Shoot length (SL)		Stem cross-sectional area (SCA)		Apical bud mass (ABM)	
		Qi	*Qf*	*Qi*	*Qf*	*Qi*	*Qf*	*Qi*	*Qf*
Tmax	5	-0.55 (11-15 May)	-0.55 (11-15 May)	-0.62 (11-15 April)	-0.81 (1-5 May)	-0.60 (26-30 March)	-0.67 (26-30 March)	0.65 (30 July-3 August)	0.61 (30 July-3 August)
	10	-0.57 (20-29 June)	-0.57 (10-19 June)	-0.51 (1-10 April)	-0.70 (1-10 May)	–	-0.71 (21-30 May)	–	–
	15	-0.60 (15-29 June)	-0.62 (15-29 June)	-0.61 (1-15 April)	-0.70 (1-15 May)	-0.63 (16-30 March)	-0.64 (16-30 March)	–	–
	30	-0.69 (June)	-0.61 (June)	-0.63 (April)	-0.63 (April)	-0.52 (May)	-0.66 (May)	–	–
Tmin	5	-0.51 (25-29 June)	-0.77 (20-24 June)	-0.64 (10-14 June)	-0.70 (1-5 May)	-0.61 (26-30 March)	–	0.66 (30 July-3 August)	0.57 (30 July-3 August)
	10	-0.54 (10-19 July)	-0.72 (20-29 June)	–	-0.63 (1-10 May)	-0.55 (21-30 March)	–	0.70 (30 July-8 August)	0.72 (30 July-8 August)
	15	-0.81 (15-29 June)	-0.52 (15-29 June)	–	–	-0.72 (16-30 March)	–	0.50 (30 July-13 August)	0.74 (30 July-13 August)
	30	–	-0.65 (June)	–	–	–	–	–	–
P	5	–	–	–	0.70 (26-30 April)	0.60 (20-24 May)	0.53 (26-30 March)	0.74 (30 June-4 July)	-0.59 (24-28 August)
	10	–	0.50 (21-30 May)	–	0.71 (21-30 April)	–	–	0.68 (30 June-9 July)	–
	15	0.68 (16-30 April)	–	–	–	–	–	0.70 (1-15 July)	–
	30	–	–	–	–	–	–	–	–
WB	5	–	–	–	0.70 (26-30 April)	–	0.57 (26-30 March)	0.69 (30 June-4 July)	-0.65 (24-28 August)
	10	–	–	–	0.67 (21-30 April)	–	–	0.64 (30 June-9 July)	–
	15	0.72 (16-30 April)	–	–	–	–	–	–	–
	30	–	–	–	–	–	–	–	–

et al., 2008). Our findings suggest that conditions suitable for growth may be provided inside enclosed structures which are highly protected by an envelope of scale-like structures, namely bud scales and cataphylls protecting shoot primordia and scaly cupules enclosing developing acorns. Contrastingly, primary and secondary growths are mostly arrested at mid-summer in the studied species (Montserrat-Martí *et al*., 2009; Gutiérrez *et al*., 2011).

The variables of primary growth were influenced by climate when growth started and when it was fully performed. Similarly, the radial growth on both studied species were negatively influenced by temperature and positively by water availability in spring and early summer, when secondary growth attain its maximum rate, in agreement with other published results (Corcuera *et al*., 2004*a*; Corcuera *et al*., 2004*b*; Gutiérrez *et al*., 2011). Moreover, a higher availability of water in early summer seems to favour the earlier accomplishment of vegetative shoot growth and the start of bud development in *Q. ilex*. The same correlation was not found in *Q. faginea*, as it finishes its shoot development at mid spring, 4-7 weeks earlier than *Q. ilex*. On the other hand, precipitation at the end of August probably favour acorn growth over bud growth in *Q. faginea* but not in *Q. ilex*, reflecting the early acorn development of the former species (Montserrat-Martí *et al*., 2009).

The most unexpected and interesting result was the positive association between apical bud mass and temperature in summer, particularly the minimum, coinciding with the period of maximum bud growth rates between late July and mid-August. High air temperatures in summer restrict primary and secondary growth through enhanced evapotranspiration rates since they preclude tissue hydration during the driest period of the growing season (Montserrat-Martí *et al*. 2009). Nevertheless, the growth of scale-protected structures such as buds seems to be enhanced by warm summer nights, i.e. high minimum temperatures, as growing tissues are actively enlarging at night (Boyer, 1968; Ortega, 2010). Our results suggest that buds are more responsive to higher night temperatures during summer than other meristematic tissues such as the cambium which seem to be more controlled by the tree water status (Gutiérrez *et al*., 2011). Despite the studied oak species have developed relatively profound roots and may access deep soil water reserves (Castro-Díez *et al*., 1997; Villar-Salvador *et al*., 1997), during summer only buds responded positively to warm night temperatures by enlarging.

Our results challenge the hypothesis posed by Montserrat-Martí *et al*. (2009) to explain the alternating year-to-year pattern of long shoots and small buds with short shoots and big buds formed the following year observed in *Q. ilex*. The idea that the production of numerous long shoots would constrain bud development, does not seem to fully agree with our results or, at least, must be complemented by the effect of the early to mid-August minimum temperatures on bud growth. In the studied climatic series we observed a regular alternating pattern of minimum summer temperatures during the period 1997-2004 (Fig. 4). In this period, the minimum temperatures from late July to mid-August strongly correlated with bud size in *Q. ilex*, explaining the pattern described by Montserrat-Martí *et al.* (2009). However, we did not observe the same effect in *Q. faginea*, despite showing a similar temperature-bud correlation or even stronger than in the case of *Q. ilex*. This could be explained by the longer period of bud development of *Q. faginea* as compared with *Q. ilex*, which would mitigate the effects of temperature in this short summer period on the final bud size.

The alternating pattern of high and low minimum temperatures from late July to mid-August observed between 1995 and 2004 was not detected in any other period of the available climatic records in the study area. We guess that it probably occurred by chance. On the other hand, during the last 60 years the minimum temperatures of the considered summer period have significantly increased at a mean rate of +0.03 ºC yr^{-1} (r = 0.30, P = 0.017). Such observation is consistent with the increase of air temperatures associated to ongoing climate warming (IPCC, 2007). This trend might result in the increase of the apical bud size in the forthcoming years, which could have consequences for the development of tree crowns. However, we do not know how plastic bud growth would be and to what extent would it allow trees adjusting their phenological patterns to the forecasted warmer conditions. For example, the severe summer droughts recorded in 2005 and 2011 led to the formation of very small buds, probably because plants were not able to adequately hydrate bud tissues at mid-summer (Alla *et al.*, unpubl. res.). The involvement of more than one driving factor on the final size of buds makes difficult to predict the variations of bud size associated to climate change.

We consider this study a relevant example showing the usefulness of the functional analysis of phenological patterns of tree species as a tool to interpret their responses to environmental factors and to facilitate the understanding of their ecological adaptations. The study of the temporal arrangement of different phenological events including the developmental pattern of buds allows analysing how these events overlap with each other, and how they are affected by different climatic stressors. Taking into account that growth in seasonally dry and cold climates is limited by temperature and water availability (Chapin *et al.*, 1987), and that different plant structures show distinct climatic thresholds, the accurate knowledge of the temporal arrangement of phenophases is key for interpreting the functional responses of plants.

This is especially important under Mediterranean continental conditions with two seasons of strong climatic stress (dry summer, cold wither) whose duration varies among years (Mitrakos, 1980).

To conclude, *Q. faginea* started bud development earlier than *Q. ilex*, but both species reached maximum bud growth rates in the first two weeks of August, with a few days of advanced peak in the deciduous species. Warm night temperatures in this short summer period enhance bud enlargement which suggests the existence of a high minimum air temperature threshold for the bud growth as compared with secondary and reproductive growth. These findings suggest that the rise in air temperature forecasted by climatic models will enhanced bud size in both studied Mediterranean oak species. If bud size is also related to the size of shoots produced, as is frequently indicated (Little, 1970; Garrett and Zahner, 1973; Kozlowski et al., 1973; Alla et al., unpubl. res.), the effects of a potential warming-induced bud enlargement on crown architecture could be relevant for understanding tree growth.

Acknowledgements

A.Q.A. and J.J.C. acknowledge the support of MAEC-AECID and ARAID, respectively. This work was supported by the Spanish Ministerio de Ciencia e Innovación and FEDER (grant numbers CGL2008-04847-C02-01, CGL2010-16880 BOS, CGL2011-26654 BOS). We thank the support of the Globimed network. We thank the “Oficina del Regante de Aragón” and “Agencia Estatal de Meterorología (AEMET)” for providing climatic data. We acknowledge Sara Palacio for revising a previous version of this work. We thank J. Albuixech and E. Lahoz for their help in the field and the laboratory, respectively.

References

Alla AQ, Camarero JJ, Maestro-Martínez M, Montserrat-Martí G (2011a) Acorn production is linked to secondary growth but not to declining carbohydrate concentrations in current-year shoots of two oak species. Trees, in press. DOI: 10.1007/s00468-011-0658-3

Alla AQ, Camarero JJ, Rivera P, Montserrat-Martí G (2011b) Variant allometric scaling relationships between bud size and secondary shoot growth in *Quercus faginea*: implications for the climatic modulation of canopy growth. Ann For Sci 68: 1245–1254. DOI: 10.1007/s13595-011-0093-z

Amaral Franco J (1990) *Quercus*. In: Castroviejo S, Laínz M, López González G, Montserrat P, Muñoz Garmendia F, Paiva J, Villa L, eds. *Flora Ibérica*. Real Jardín Botánico, CSIC, 15–36

Arora R, Rowland LJ, Tanino K (2003) Induction and release of bud dormancy in woody perennials: a science comes of age. HortScience 38: 911–921

Barthélémy D, Caraglio Y (2007) Plant architecture: A dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. Ann Bot 99: 375–407

Boyer JS (1968) Relationship of water potential to growth of leaves. Plant Physiol 43: 1056–1062.

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

Castro-Díez P, Villar-Salvador P, Pérez-Rontomé C, Maestro-Martínez M, Montserrat-Martí G (1997) Leaf morphology and leaf chemical composition in three *Quercus* (Fagaceae) species along a rainfall gradient in NE Spain. Trees 11: 127–134.

Chapin FS, Bloom AJ, Field CB, Waring RH (1987) Plant responses to multiple environmental factors. Bioscience 37: 49–57

Chen HJ, Bollmark M, Eliasson L (1996) Evidence that cytokinin controls bud size and branch form in Norway spruce. Physiol Plant 98: 612–618

Cochard H, Coste S, Chanson B, Guehl JM, Nicolini E (2005) Hydraulic architecture correlates with bud organogenesis and primary shoot growth in beech (*Fagus sylvatica*). Tree Physiol 25: 1545–1552

Cook ER, Kairiukstis LA (1990) *Methods of dendrochronology: applications in the environmental sciences*. Boston, MA: Kluwer Academic Publishers.

Corcuera L, Camarero JJ, Gil-Pelegrin E (2004a) Effects of a severe drought on growth and wood anatomical properties of *Quercus faginea*. IAWA J 25: 185–204

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

FAO (1998) *World reference base for soil resources*. Rome: ISRIC and ISSS.

Fontaine F, Druelle JL, Clément C, Burrus M, Audran JC (1998) Ontogeny of proventitious epicormic buds in *Quercus petraea*. I. In the 5 years following initiation. Trees 13: 56–62.

Fontaine F, Chaar H, Colin F, Clément C, Burrus M, Druelle JL (1999) Preformation and neoformation of growth units on 3-year-old seedlings of *Quercus petraea*. Can J Bot 77: 1623–1631

Garrett PW, Zahner R (1973) Fascicle density and needle growth responses of red pine to water supply over two seasons. Ecology 54: 1328–1334

Gill AM (1971) The formation, growth and fate of buds of *Fraxinus americana* L. in central Masachusetts. Harvard For Pap 20: 1–16

Gutiérrez E, Campelo F, Camarero JJ, *et al.* (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

Heuret P, Guédon Y, Guérard N, Barthélémy D (2003) Analysing branching pattern in plantations of young red oak trees (*Quercus rubra* L., Fagaceae). Ann Bot 91: 479–492

Horvath DP, Anderson JV, Chao WS, Foley ME (2003) Knowing when to grow: signals regulating bud dormancy. Trends Plant Sci 8: 534–540

Hyams DG (2010) *CurveExpert*. USA: The University of Tennessee, Chattanooga.

IPCC (2007) Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL, eds. *Climate change 2007: the physical science basis. Contribution of Working Group I to the fourth assessment report of the Intergovernmental Panel on Climate Change.* Cambridge, UK and New York, NY, USA: Cambridge University Press.

Ishihara MI, Kikuzawa K (2009) Annual and spatial variation in shoot demography associated with masting in *Betula grossa*, comparison between mature trees and saplings. Ann Bot 104: 1195–1205

Keator G (1998) *The life of an oak: an intimate portrait*. California, USA: Heyday.

Kozlowski TT, Torrie JH, Marshall PE (1973) Predictability of shoot length from bud size in *Pinus resinosa* Ait. Can J For Res 3: 34–38

Lanner RM (1985) On the insensitivity of height growth to spacing. For Ecol Manage 13: 143–148

Little CHA (1970) Apical dominance in long shoots of white pine (*Pinus strobus*). Can J Bot 48. 239–253

Marks PL (1975) On the relation between extension growth and successional status of deciduous trees of the North-eastern United States. Bull Torrey Bot Club 102: 172–177

Meier IC, Leuschner C (2008) Leaf size and leaf area index in *Fagus sylvatica* forests: competing effects of precipitation, temperature, and nitrogen availability. Ecosystems 11: 655–669

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

Montserrat-Martí G, Camarero JJ, Palacio S, *et al.* (2009) Summer-drought constrains the phenology and growth of two coexisting Mediterranean oaks with contrasting leaf habit: implications for their persistence and reproduction. Trees 23: 787–799

Nitta I, Ohsawa M (1998) Bud structure and shoot architecture of canopy and understory evergreen broad-leaved trees at their northern limit in East Asia. Ann Bot 81: 115–129

Ortega JKE (2010) Plant cell growth in tissue. Plant Physiol 154: 1244–1253

Palacio S, Hester AJ, Maestro M, Millard P (2008) Browsed *Betula pubescens* trees are not carbon-limited. Funct Ecol 22: 808–815

Puntieri JG, Grosfeld JE, Stecconi M, Brion C, Barthélémy D (2007) Bud and growth-unit structure in seedlings and saplings of *Nothofagus alpina* (Nothofagaceae). Am J Bot 94: 1382–1390

Puntieri JG, Stecconi M, Barthélémy D (2002) Preformation and neoformation in shoots of *Nothofagus antarctica* (G. Forster) Oerst. (Nothofagaceae) shrubs from northern Patagonia. Ann Bot 89: 665–673

Remphrey WR, Powell GR (1984) Crown architecture of *Larix laricina* saplings: shoot preformation and neoformation and their relationships to shoot vigour. Can J Bot 62: 2181–2192

Sabatier S, Barthélémy D (2001) Bud structure in relation to shoot morphology and position on the vegetative annual shoots of *Juglans regia* L. (Juglandaceae). Ann Bot 87: 117–123

Salminen H, Jalkanen R (2004) Does current summer temperature contribute to the final shoot length on *Pinus sylvestris*? A case study at the northern conifer timberline. Dendrochronologia 21: 79–84

Sanz-Pérez V, Castro-Díez P (2010) Summer water stress and shade alter bud size and budburst date in three Mediterranean *Quercus* species. Trees 24: 89–97

Villar-Salvador P, Castro-Díez P, Pérez-Rontomé C, Montserrat-Martí G (1997) Stem xylem features in three *Quercus* (Fagaceae) species along a climatic gradient in NE Spain. Trees 12: 90–96

CHAPTER V

The fate of buds revisited: bud mortality and bursting depend on bud size

V. The fate of buds revisited: bud mortality and bursting depend on bud size

Abstract The bud demography of two coexisting Mediterranean oak species with contrasting leaf habit (*Quercus ilex*, evergreen; *Q. faginea*, deciduous) was studied over four years. The main objective was to analyse the effect of bud size on the fate of buds with different positions along the shoot (apical, leaf axillary and scale axillary buds). The number, length and position of all buds, stems and leaves (only in the evergreen) were recorded every year. The two coexisting oak species presented different strategies in bud production and survival since the evergreen species showed a higher production of buds per shoot and higher bud mortality but smaller buds than the deciduous one. Bud size and position were highly related since apical buds where longer than the axillary ones and bud length declined basipetally along the stem. The longer buds presented higher probability of bursting than the shorter ones, although no absolute size threshold was found below which bud bursting was impaired. Moreover, apical buds showed a higher probability of bursting than axillary ones, being this probability higher in *Q. faginea* branches than in *Q. ilex*. Buds were able to persist and burst up to four years in the evergreen species, whereas most bursting buds were one-year-old in *Q. faginea*.

Keywords Bud demography, Bud size, Bud position, Budburst, *Quercus ilex* subsp. *ballota*, *Quercus faginea*, Plant architecture.

Introduction

Bud production and survival within the crown determine tree architecture (Maillette 1982a; Harmer 1991, 1992). After overwintering, buds may burst giving rise to either vegetative or reproductive shoots, but some buds may also die or remain dormant, becoming part of the bud bank (Maillette 1982a). Accordingly, except in the very occasional buds that produce shoots in the same year of their formation (lammas shoots), most buds grow into new shoots in the next spring whereas the rest die and abscise or become dormant persisting in older shoots (Ward 1964; Harmer 1991). For example, in many tree species, leafless buds at the base of the shoot remain small at the end of the growing season, they do not usually expand in the next spring and may persist for many years. These buds enlarge as they grow outwards, and may eventually become embedded in the bark, forming a source of epicormic shoots (Gill 1971; Harmer 1991; Wilson and Kelty 1994).

It is well established that bud position within the crown and the shoot and age of branches are important determinants of bud fate (Maillette 1982a; Macdonald et al. 1984; Jones and Harper 1987; Sabatier and Barthélémy 2001). For example, in birch, buds within the crown may produce long or short shoots (Maillette 1982a) but young branches produce more buds and have lower bud mortality than older ones, thus leading to a variable amount of living buds between years (Jones and Harper 1987). In tree species with preformed shoots, such as *Quercus* ones (Fontaine et al. 1999; Barthélémy and Caraglio 2007), the number of buds in a shoot is closely related to the length of the supporting shoot (Ward 1964; Harmer 1991, 1992). In these species, bud size may also determine bud fate and explain the different patterns in bud production (Maillette 1982b). However, as far as it is known, the potential effects of bud size on the bud fate of broadleaf trees remain unexplored (Maillette 1982a, 1987; Jones and Harper 1987; Lehtilä et al. 1994; Tolvanen et al. 2002; Negi 2006).

Quercus species show a large year-to-year variability in bud size related to different factors like climate, tree vigour and size, and the position of buds within the crown (Alla et al. submitted). Such variability in bud size may be also determined by the position of buds along the parent shoot. For example, it is well established that apical buds exert a strong dominance over the other buds and display higher vigour and have greater probabilities to produce shoots than the rest of buds in the shoot (Buck-Sorlin and Bell 2000; Sabatier and Barthélémy 2001; Puntieri et al. 2002). However, no previous study has assessed the relationship between bud size and bud fate on the following years, how such relationship depends on bud position within the shoot, and the inter-annual variability of bud size-fate relationships in broadleaf tree species.

The aim of this study was to analyse the effect of bud size on the fate of buds with different positions within the shoot (apical, leaf axillary and scale axillary buds) in two coexisting Mediterranean oak species with contrasting leaf habit: *Q. ilex* L. subsp. *ballota* (Desf.) Samp. (evergreen; hereafter abbreviated as *Q. ilex*) and *Q. faginea* Lam. (winter deciduous). Specifically, we tested the following hypotheses: (*i*) larger buds will have higher chances to burst on the following years than smaller buds irrespective of their position along the shoot, and (*ii*) there will be a threshold of bud size below which bud bursting will be impaired, such size threshold will be larger in apical than in the rest of buds.

Material and methods

Study site and species

The study site is located in Agüero, Aragón, north eastern Spain (42° 18′ N, 0° 47′ W, 750 m a.s.l.). Climate is Mediterranean and continental being characterized by a dry summer and a cold winter with 631 mm and 13.8 ºC of total annual precipitation and mean annual temperature, respectively. Soil in this area is a Calcisol (FAO 1998), formed on Miocene clays with bedrock of calcareous sandstone. Vegetation is an open scrubland with scattered low trees, dominated by *Q. ilex*, *Q. faginea*, *Arbutus unedo* L. and *Pinus halepensis* Mill., approximately with similar dominance, and other less abundant woody species .For further details on the study site see Montserrat-Martí et al. (2009).

Quercus ilex is an evergreen oak tree with semi-ring to diffuse porous wood (Campelo et al. 2007), which in the Iberian Peninsula grows preferentially inland under continental conditions (Amaral Franco 1990). *Q. faginea* is a deciduous oak tree with ring-porous wood (Corcuera et al. 2004) and with a wide distribution in sub-Mediterranean areas of the Iberian Peninsula (Amaral Franco 1990). Both oak species present scaled buds with preformed growth. According to Montserrat-Martí et al. (2009), *Q. ilex* seems to display great variations in bud and shoot size among years with years of large buds producing many long shoots and those of small buds producing less short shoots, while *Q. faginea* produces comparatively larger buds and longer shoots every year. The study species frequently coexist in the study area forming multi-stemmed trees of similar age but *Q. faginea* individuals are taller than *Q. ilex* ones (Table 1).

Table 1. Morphological features of the study trees (means ± SE) and number of sampled trees and branches, and measured shoots and buds for different years.

Species	Diameter at 1.3 m (cm)	Height (m)	No. stems per tree	Age at 1.3 m* (years)	No. trees (branches)				No. shoots				No. buds			
					2005	2006	2007	2008	2005	2006	2007	2008	2005	2006	2007	2008
Q. ilex	10.3 ± 0.9b	4.9 ± 0.3b	5.2 ± 0.9	29 ± 4	10 (20)	10 (20)	10 (10)	7 (7)	131	205	86	52	987	1367	571	262
Q. faginea	13.7 ± 0.8a	7.4 ± 0.4a	3.8 ± 0.7	33 ± 2	10 (20)	10 (20)	8 (8)	7 (7)	135	310	174	155	845	1725	1140	774

Different letters correspond to significant ($P < 0.05$, ANOVA) differences between species.
*Age was estimated by taking radial cores at 1.3 m using a Pressler increment borer.

Branch demography

In winter 2006, 10 mature individuals per species were randomly selected, tagged and their size (diameter at 1.3 m, height) and number of stems per tree were measured (Table 1). At this time of the year shoot development has been completed and trees are in winter quiescence. Within each tree, two three-year-old branches were selected on the southern side and upper third of the crown. Branches were marked, drawn in synthetic diagrams and the numbers and length of their different cohorts of shoots, buds and leaves (in *Q. ilex*) recorded. Number and position of dead buds at the beginning of the census were also recorded. The length and fate (survival, abscission or burst – just for buds) of the different cohorts of buds, shoots and leaves (just for *Q. ilex*) of each branch was measured annually during three consecutive years (January 2006, 2007 and 2008) up to January 2009. The study period started after the second most severe drought (2005) affecting the study area since 1950, reaching a water deficit 66% higher than the 1950-2008 average (Supplementary Information, Fig. S1).

On every sampling date, the fate of the different elements of the branch was recorded, and the following variables were measured on every one-year-old shoot: length of living buds, number of living and dead buds and bud scars (the sum of these three numbers render the total number of buds), and number of living and dead leaves in the case of *Q. ilex*. Living buds were categorized according to their position within the shoot as: apical, leaf axillary (buds arranged in leaf axes) and scale axillary (buds in the axil of scales). Dead buds were distinguished by their shrivelled typical appearance and branches without living buds were considered dead. Shoot, leaf (in the case of the evergreen *Q. ilex*) and bud fates were compared with previous censuses. Shoots and leaves may remain or die and disappear whereas buds may burst, die or remain dormant as part of the bud bank of the next winter. Schematic diagrams of the complete branches showing the position of shoots, leaves and buds within the branch were drawn on each sampling date to study and summarize the datasets (Fig. 1). Data of all branches for the first two years were obtained non-destructively, but by the second and third year of sampling several branches were cut down to reduce the number of studied shoots and buds. In January 2009 all remaining branches were also harvested and the different measurements taken in the laboratory. All elements within the branch were numbered from the apex to the base of the branch to facilitate their monitoring on each annual survey. Shoot and bud length were measured in the field using a measuring magnifier (Befort Wetzlar 10x, Germany) and a centesimal calliper (Mitutoyo, Kawasaki, Japan) whereas in the laboratory buds were measured to the nearest 0.1 mm (10x magnification) under a stereomicroscope (MZ12.5 Leica Microsystems, Heerbrugg, Switzerland).

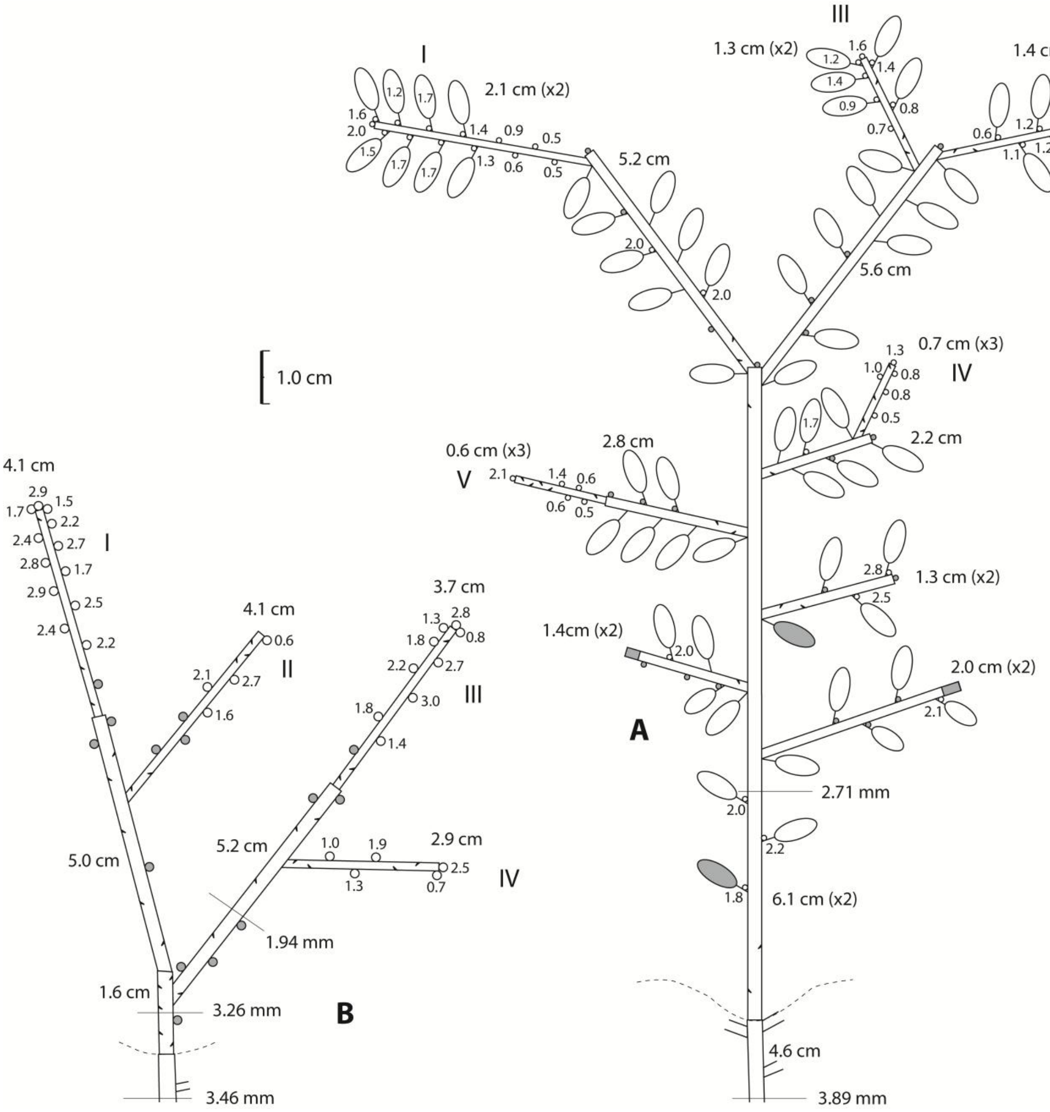


Figure 1. Schematic representation of representative 3-year-old branches with all their components of *Q. ilex* (A) and *Q. faginea* (B) measured in January. Dashed lines mark the base of the 3-year-old branch, while straight continuous lines indicate stem diameters. The real stem-unit-growth length is displayed in *Q. faginea*, whereas the shoot lengths of *Q. ilex* were multiplied (in parentheses) to improve the visual clarity of the drawings. Grey apical parts of stems are dead. Current-year shoots were numbered from the apex to the base using Roman numbers. Leaves are present in the branches of the evergreen, *Q. ilex*. White circles represent living buds and grey ones dead buds. The length of buds (in mm), is presented near the living ones. Scars left by fallen buds are shown by ticks.

Data analyses

Bud mortality (m) was calculated using the formula proposed by Sheil et al. (1995):

$$m = 1 - (N_1/N_0)^{1/t} \quad (1)$$

where N_0 and N_1 are bud counts at the beginning and end of the measurement interval, and t is the time between two measurements (in our case t = 1 year).

Differences in the distributions of apical and longest axillary buds according to their size were assessed using the *G*-test (Sokal and Rohlf 1995). To estimate the probability of bud bursting in the next growing season as a function of bud length we fitted binary logistic regressions. Since these data do not follow normal distributions, the differences in length between bursting and non-bursting buds were assessed using Mann-Whitney *U* tests.

The effect of bud size on the fate of buds was explored by Generalized Linear Mixed Models (GLMM) taking into account the position of buds and the size of the parent shoot. Since there seems to be a relationship between bud position and bud length, we first explored the relationship between both variables, evaluating if there were any differences in the size of buds with different positions along the parent shoot. Leaf and scale axillary buds were grouped as "axillary buds", since scale axillary buds produced very few shoots and could not be analysed as a separate group. The variable "bud length" was square-root transformed to reach normality. Differences in size between apical and axillary buds were evaluated by considering "year" and "bud position" (apical / axillary) as fixed factors, "individual tree" and "branch" as random factors and the "length of the bearing shoot" as a covariate. The full model was explored by fitting a Gaussian distribution to the response variable "bud size". This analysis showed that bud position was a strong determinant of bud size (F = 695.1, $P < 0.001$ and $F = 849.4$, $P < 0.001$, for *Q. ilex* and *Q. faginea*, respectively). Indeed, both variables were strongly correlated in both species ($r > 0.85$), when included as separate factors in the analysis. For this reason, and to avoid collinearity issues (Zuur et al. 2010), we explored the effect of bud size on bud fate by considering the interaction between "bud position" and "bud length" as a fixed factor, while disregarding individual effects of each factor separately. The model was completed as above by including "year" as a fixed factor, "individual tree" and "branch" as random factors and the "shoot length of the bearing shoot" as a covariate. Factor effects were tested by fitting a bimodal distribution to the response variable "bud fate". All variables were introduced using a stepwise procedure to obtain the most parsimonious model and to avoid collinearity. The stepwise analysis showed that the interaction between "year" and "bud position x bud size" led to collinearity issues, and was hence not considered in our analysis (Zuur et al. 2010). Finally, the individual effect of bud position on bud fate (irrespective of bud size) was explored by bimodal GLMMs with "bud position" and "year" as fixed factors, "individual tree" and "branch" as random factors and the "shoot length of the bearing shoot" as a covariate. Variables were introduced following a stepwise procedure to avoid collinearity issues. Statistical analyses were performed using the "lattice" and "lme4" packages of the R program (R Development Core Team 2011).

We calculated the annual production rate ($\lambda(t)$) of buds and current-year shoots to evaluate if the amount of both annually formed components are in a net equilibrium or whether there is a deficit or a surplus of formed buds or shoots (Maillette 1982a). The rate $\lambda(t)$ was calculated following Ishihara and Kikuzawa (2009) as:

$$\lambda(t) = N(t)/N(t-1) \qquad (2)$$

where N is the total number of living buds or shoots in years t and t-1.

Results

Bud and shoot size variability across positions and time

Shoot length varied among species and years being usually longer in *Q. ilex* than in *Q. faginea* (Supplementary Information, Fig. S2). However, the distributions of current-year shoot lengths were skewed towards short length values in both species. The shortest (largest) shoots were produced in 2008 (2006) and 2006 (2007) by *Q. ilex* and *Q. faginea*, respectively. The total number of buds produced per shoot varied as a function of the shoot length (Table 2; Supplementary Information, Figs. S3, S4). However, the mean number of living buds produced per shoot varied from 6 to 7 and from 5 to 6 in *Q. ilex* and *Q. faginea*, respectively (Table 2).

Bud length varied from 0.2 to 4.7 mm in *Q. ilex* and from 0.2 to 7.8 mm in *Q. faginea*, with the largest buds being formed in 2006 in both species (Figs. 2, 3 and 4). The minimum bud length did not significantly differ between study years ($P > 0.05$) whereas the maximum bud length varied across years in both species. Furthermore, bud length varied significantly within shoot positions ($F = 695.1$, $P < 0.001$ and $F = 849.4$, $P < 0.001$, for *Q. ilex* and *Q. faginea*, respectively), declining basipetally along the shoot, i.e. from the largest apical buds to the smallest axillary scale buds (Table 2).

Relationship between bud size, bud position and fate

Bud mortality during the year of bud formation oscillated between 40% and 50% and from 30% to 40% in *Q. ilex* and *Q. faginea*, respectively. Buds of *Q. ilex* showed the highest lifespan keeping the ability to burst for up to four years. On the contrary, very few *Q. faginea* buds lived up to one (1 bud in 2004) or two (2 buds in 2005) years but normally they died after the first spring. The percentage of buds living one year after their formation varied among years from 9% (2005) to 16% (2006) in *Q. ilex*, being 10% to 17% of them apical buds. Contrastingly, in *Q. faginea* the percentage of buds surviving after the year of formation was less than 1% over all studied years and all of them were axillary (Table 2). Living buds that persisted in old shoots enlarged in all shoot positions. Lammas shoots were very scarce accounting for less than 1% of all shoots and being more frequent in *Q. faginea* than in *Q. ilex*, where they were present only in the last sampling year (*data not shown*).

Table 2. Mean (standard error) values of bud numbers per shoot, apical, leaf axillary and scale axillary buds and stem length and the percentage of the evolution according to each bud position for the two study species and the four monitored years.

Variables (units) (*year*)	*Q. ilex*				*Q. faginea*			
	2005	2006	2007	2008	2005	2006	2007	2008
Bud number per shoot	7.5	6.7	6.6	5.0	6.3	5.6	6.6	5.0
ABL (mm) (*t*)	1.79 (0.06)	2.13 (0.06)	1.49 (0.06)	1.87 (0.08)	2.46 (0.06)	3.92 (0.06)	3.46 (0.07)	3.52 (0.07)
ABBL (mm) (*t*)	1.91 (0.07)	2.44 (0.14)	2.19 (0.13)	–	2.50 (0.05)	4.01 (0.15)	3.45 (0.10)	–
ASL (cm) (*t*+1)	3.20 (0.23)	3.03 (0.35)	1.89 (0.31)	–	2.47 (0.15)	2.17 (0.24)	2.69 (0.05)	–
ABB (%) (*t*+1)	57.14	34.15	39.39	–	61.70	54.22	57.25	–
LABL (mm) (*t*)	1.68 (0.15)	1.46 (0.15)	1.66 (0.15)	–	–	–	–	–
LABL (mm) (*t*+1)	1.90 (0.19)	1.51 (0.12)	1.93 (0.19)	–	–	–	–	–
LAB (%) (*t*+1)	9.53	34.14	12.13	–	0.00	0.00	0.00	–
DABL (mm) (*t*)	1.62 (0.11)	2.14 (0.07)	1.32 (0.06)	–	2.41 (0.11)	3.66 (0.15)	3.58 (0.16)	–
DAB (%) (*t*+1)	33.33	31.71	48.48	–	38.30	45.78	42.75	–
LBL (mm) (*t*)	1.34 (0.02)	1.75 (0.03)	1.10 (0.09)	1.61 (0.05)	2.14 (0.03)	3.07 (0.04)	2.69 (0.05)	2.91 (0.05)
LBBL (mm) (*t*)	1.71 (0.04)	2.39 (0.08)	1.40 (0.55)	–	2.59 (0.05)	4.07 (0.12)	3.25 (0.13)	–
LSL (cm) (*t*+1)	2.88 (0.11)	2.47 (0.14)	1.93 (0.50)	–	1.76 (0.06)	2.18 (0.15)	1.87 (0.13)	–
LBB (%) (*t*+1)	18.75	18.45	18.65	–	30.24	30.00	19.13	–
LLBL (mm) (*t*)	1.08 (0.04)	1.20 (0.05)	0.40[1]	–	0.97 (0.19)	1.64 (0.43)	2.07 (0.62)	–
LLBL (mm) (*t*+1)	1.37 (0.06)	1.48 (0.07)	1.10[1]	–	1.73 (0.70)	1.42 (0.17)	2.17 (0.64)	–
LLB (%) (*t*+1)	22.32	22.33	10.37	–	0.44	0.42	0.37	–
DLBL (mm) (*t*)	1.20 (0.03)	1.74 (0.03)	1.10 (0.08)	–	1.91 (0.04)	2.96 (0.05)	2.62 (0.05)	–
DLB (%) (*t*+1)	58.93	59.22	70.98	–	69.32	69.58	80.50	–
SBL (mm) (*t*)	0.60 (0.02)	0.69 (0.03)	0.68 (0.05)	0.66 (0.04)	0.79 (0.05)	0.67 (0.03)	0.59 (0.02)	0.91 (0.06)
SBBL (mm) (*t*)	0.85 (0.05)	–	–	–	1.00[1]	0.70[1]	–	–
SSL (cm) (*t*+1)	1.95 (1.35)	–	–	–	1.70[1]	1.70[1]	–	–
SBB (%) (*t*+1)	1.63	0.00	0.00	–	3.13	0.63	0.00	–
LSBL (mm) (*t*)	0.70 (0.06)	0.88 (0.13)	–	–	0.92 (0.18)	1.20 (0.06)	1.65 (1.05)	–
LSBL (mm) (*t*+1)	1.15 (0.25)	1.43 (0.33)	–	–	1.26 (0.21)	1.75 (0.95)	2.45 (0.65)	–
LSB (%) (*t*+1)	3.25	2.69	0.00	–	14.71	1.39	1.82	–
DSBL (mm) (*t*)	0.59 (0.02)	0.68 (0.03)	0.68 (0.05)	–	0.78 (0.06)	0.64 (0.05)	0.58 (0.01)	–
DSB (%) (*t*+1)	95.12	97.31	100	–	82.16	97.98	98.18	–

[1] n = 1. Abbreviations: ABL-apical bud length; ABBL, apical bursting bud length; ASL, apical stem length; ABB, apical bursting bud; LABL, living apical bud length; LAB, living apical bud; DABL, dead apical bud length (measured in year *t* and dead in year *t*+1); DAB, dead apical buds; LBL, leaf axillary bud length; LBBL, leaf axillary bursting bud length; LSL, leaf axillary stem length; LBB, leaf axillary bursting bud; LLBL, living leaf axillary bud length; LLB, living leaf axillary bud; DLBL, dead leaf axillary bud length (measured in year *t* and dead in year *t*+1); DLB, dead leaf axillary buds; SBL, scale axillary bud length; SBBL, scale axillary bursting bud length; SSL, scale axillary stem length; SBB, scale axillary bursting bud; LSBL, living scale axillary bud length; LSB, living scale axillary bud; DSBL, dead scale axillary bud length (measured in year *t* and dead in year *t*+1); DSB, dead scale axillary buds.

GLMMs showed that bud size had a strong impact on the fate of buds in different positions (*Z*-value =17.1, $P < 0.001$), larger buds having a higher probability to burst than smaller ones across bud positions (Figs. 3 and 4). The only exceptions to this finding were the apical buds of *Q. faginea* formed in 2006, whose length was significantly lower ($P < 0.05$) than that of non-bursting buds (Table 2, Fig. 4). Apical buds presented a higher probability of bursting than axillary buds and this difference was more marked in *Q. faginea* (54-62% *vs*. 17-29%) than in *Q. ilex* (34-57% *vs*. 15-17%) since the last species showed a huge variability in bursting probability among years (Table 2). Moreover, apical buds presented a lower mortality, varying from 30% to 50% in both species, than the largest axillary buds, which presented mortality rates between 50% and 80%. However, apical buds were significantly larger than the largest axillary buds only in the driest year (2005) for *Q. ilex* and in more humid years (2006 and 2008) for *Q. faginea* (Fig. 2).The relative frequency of bursting buds decreased as branches aged being 17-25% and 25-40% in *Q. ilex* and *Q. faginea*, respectively.

The size of living buds with a 50% probability to burst varied among years from 2.1 to 2.9 mm in *Q. ilex* and from 2.7 to 4.8 mm in *Q. faginea*, being the largest bursting buds formed in 2006 for both species (Fig. 3). However, no size threshold was found below which bud bursting was impaired, since even the shortest buds could burst and produce shoots on the following spring (Figs. 3 and 4).

Annual balances between bud and shoot numbers

The total number of current-year buds produced per branch alternated between years of high and low bud production in *Q. ilex*, whereas in *Q. faginea* it increased over time (Fig. 5). These data represent all available current-year buds and shoots excluding older dormant buds or shoots produced by them. The number of living buds per branch showed a huge variability among years since they varied from 28 to 43 in *Q. ilex* and from 36 to 110 in *Q. faginea*. The production rates per branch from 2006 to 2008 presented an altering pattern among years in *Q. ilex* (1.5, 0.8, 1.1), whereas these rates decreased as branches aged in *Q. faginea* (1.7, 1.6, 1.1). In *Q. ilex*, the highest percentage of living buds in the next year after their formation (in 2006) coincided with the lowest bud production rate per branch (in 2007). Moreover, the number of shoots showed a similar pattern that the number of buds per branch for both species but with the lowest production rates found in 2005 in *Q. ilex* (Fig. 5).

Discussion

Our results showed that larger buds presented a higher probability to burst than smaller ones in the two oak species, independently of their

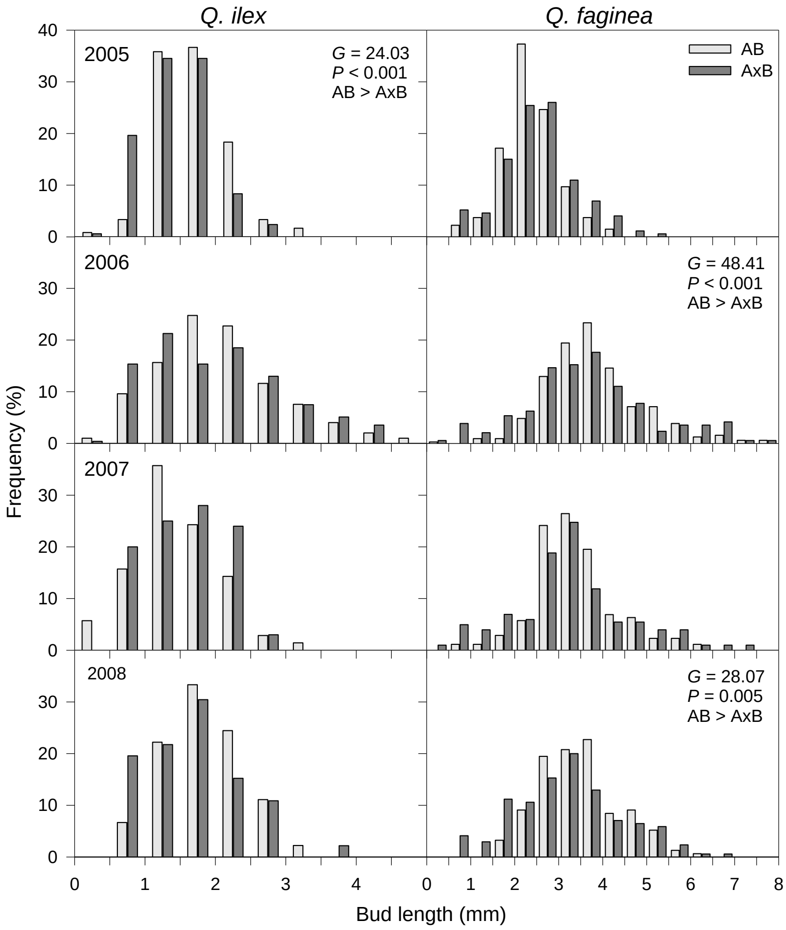


Figure 2. Distribution of apical (AB) and axillary largest (AxB) buds according to their length. The distributions of lengths were compared among the two bud types using *G*-tests whose values and related significance levels (*P*) are presented in the case of significant differences ($P < 0.05$). In all those cases the mean apical bud length was higher than that of axillary buds (AB > AxB).

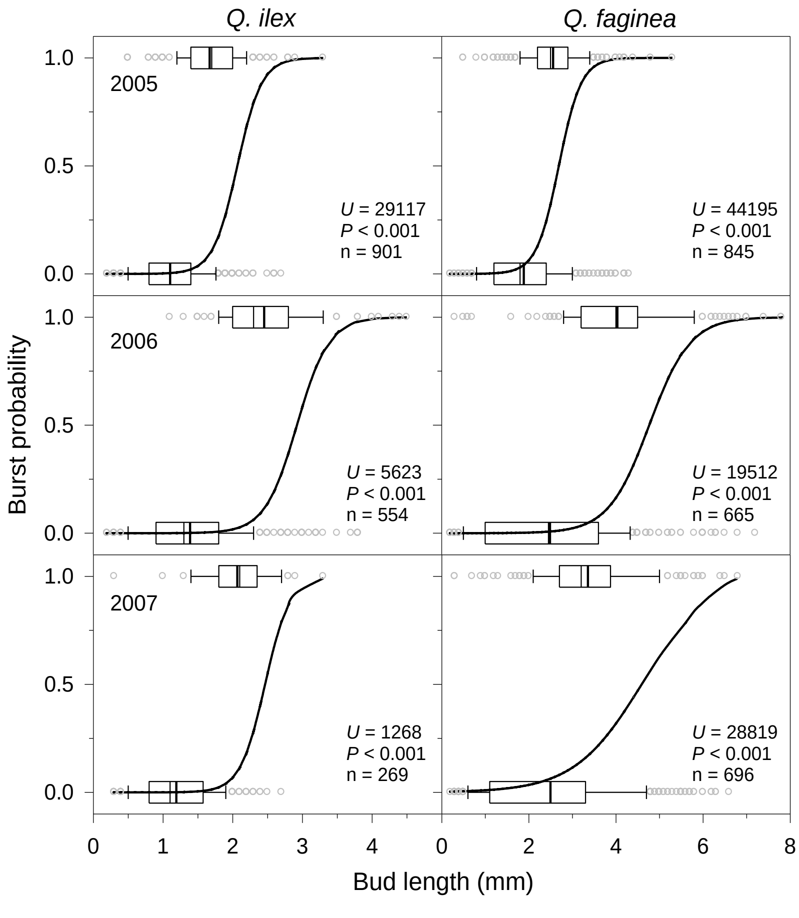
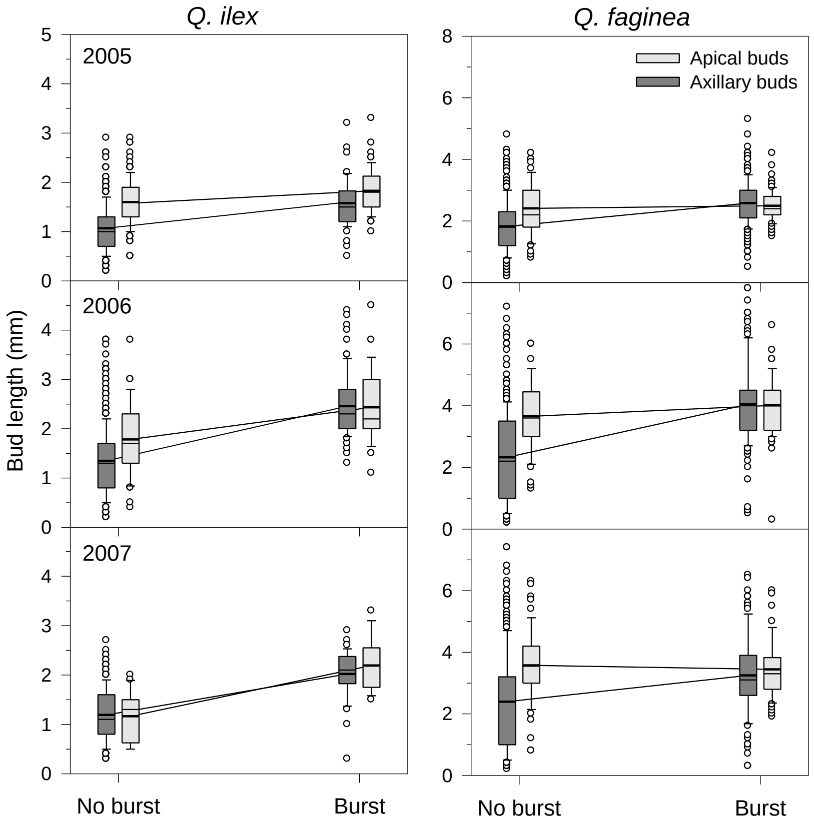


Figure 3. Probability of burst for all living buds according to their length. Curves corresponds to fitted logistic models and boxes (showing median values as thin lines and outliers) to buds that die or remain dormant (0, non-bursting buds) or that develop a new shoot (1, bursting buds) in the following year *t*+1, respectively. In the box plots, the thin and thick lines correspond to the median and the mean, respectively. We found highly significant differences in length between bursting and non-bursting buds for each year and species. The length of bursting and non-bursting buds was compared using Mann-Whitney *U* tests.

Figure 4. Bud length of bursting and non-bursting buds of apical and the axillary buds (leaf and scale axillary buds). In the box plots, the thin and thick lines correspond to the median and the mean, respectively.



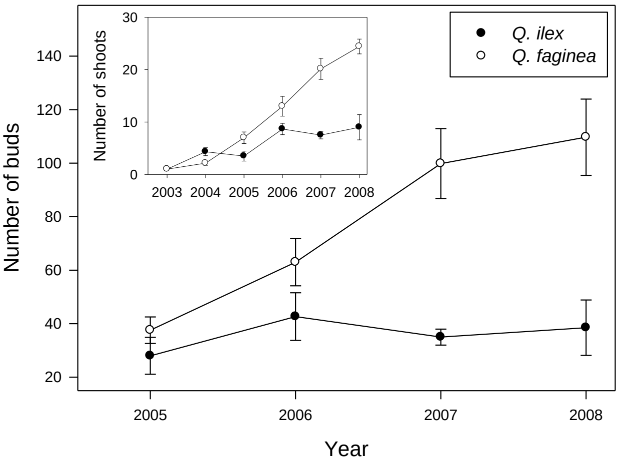


Figure 5. Number of current-year buds and shoots per branch measured for each study year. Displayed data correspond to branches sampled at the end of the study in 2009.

position along the bearing shoot. However, apical buds were larger and displayed a higher probability to burst than axillary ones. Such burst probability was quantified using statistical models which confirmed that, within each position, larger buds were more prone to burst than smaller ones. Nevertheless, no minimum threshold of bud length existed below which bursting was prevented. Bud survival to the next spring appeared to be favoured in the evergreen species (*Q. ilex*), since a significant percentage of living buds persisted during several years in *Q. ilex* whereas in *Q. faginea* most of them only persisted for one year. On the other side, bud and shoot production increased throughout the studied years in *Q. faginea* while, in *Q. ilex*, it alternated between years of high and low production, as previously reported by Montserrat-Martí et al. (2009).

Size and position effects on bud fate

The two oak species studied presented different strategies for bud production and survival that could be related to their contrasting leaf habit. The evergreen species showed higher bud production per shoot and bud mortality, but it had smaller buds than the deciduous species. However, in both species larger buds displayed higher chances of bursting than smaller ones. The possibility for apical buds, which display higher vigour than axillary ones, to burst or persist to the next year (the latter observed only in the case of *Q. ilex*), agrees with previous studies on bud demography (Maillette 1982a; Jones and Harper 1987). The size of apical and axillary buds depends on the nutrient supply they require during the period of bud development (Little 1970) and nutrients move preferentially to regions of high auxin concentrations, (Phillips 1975). Consequently, the observed gradient in bud size along the parent shoot seems to be ultimately caused by auxin, which is synthesized in young expanding leaves at the shoot apex, and transported basipetally along the stem, inhibiting the outgrowth of axillary buds (Cline 1997; Ljung et al. 2001; Mina Tanaka et al. 2006; Shimizu-Sato et al. 2009).

We do not found an absolute threshold of bud size below which budburst could be impaired, since even the shortest buds could produce new shoots. However, we observed a relative threshold probability of bud burst since larger buds had higher possibilities to burst than shorter ones. This relative threshold varied over years as the maximum bud length did depending on environmental conditions. The fate and size of buds appear to be more conditioned by the local environment in which they have developed than by their position on the tree (Jones and Harper 1987). Due to the apical dominance, we could expect that in the very dry years apical buds are relatively larger than axillary ones (as in the case of *Q. ilex*), since plants give higher priority to them than to axillary ones and resources are limited. On the other side, in wetter years axillary buds may reach their maximum development while apical buds continue to grow. In this context, apical buds of *Q. faginea*, which in the study area is close to its xeric limit of distribution, would reach their maximum development during moist years.

Implications for tree architecture

The evergreen species produced viable buds up to four years old whereas most bursting buds were one-year-old in the deciduous one. Therefore, *Q. ilex* is able to form a bud bank with buds of different ages which are able to burst over the next years. This may explain the alternating year-to-year pattern of bud production in *Q. ilex*, whereas in *Q. faginea* the development of the crown depends entirely on the production and survival of current buds, whose amount in the studied branches increased through time. Jones and Harper (1987) found that the total number of buds can never decline, whereas the number of living buds can increase or decrease with time. Such contrasting patterns in bud production allow describing two different architectural strategies in both species. *Q. ilex* may store viable buds during several years to produce many shoots in those years with favourable climatic conditions (e.g., wet and mild springs) or to replace damaged shoots due to biotic or abiotic factors (Nitta and Ohsawa 1998). Contrastingly, *Q. faginea* relies on the abundant production of current buds which allows this species to grow vigorously in response to favourable climate. However, its crown development seems to be more vulnerable to the dry Mediterranean conditions than that of *Q. ilex* (Montserrat-Martí et al. 2009; Sanz-Pérez and Castro-Díez 2010). A significant percentage of apical buds survived over years in *Q. ilex*, which seems to be typical of evergreen species, whereas in the deciduous one most of them burst in the following spring and the rest die off as previously described for other deciduous species (L. Maillette 1982a). These results may explain the differences in the crown architecture between both species. For example, in our studied area the *Q. faginea* trees trend to be taller than similar-aged *Q. ilex* ones due to the numerous shoots derived of apical buds, while *Q. ilex* develops its crown more horizontally, gaining a more “shrubby” development.

In conclusion, bud size and position are important determinants of the fate of buds, i.e. bursting and surviving, in both studied species. Apical buds have higher chances of bursting into new shoots, since more than 50% of new shoots came from apical buds. Also, larger buds (irrespective of the position along the parent shoot) have higher probability to produce new shoots than smaller ones. However, all buds, irrespective of their position and length, may burst into new shoots.

Acknowledgements

A.Q.A., J.J.C. and S.P. acknowledge the support of MAEC-AECID, ARAID and the Spanish Ministerio de Ciencia e Innovación (Juan de la Cierva subprogram), respectively. This work was supported by the Spanish Ministerio de Ciencia e Innovación and FEDER (grant numbers CGL2008-04847-C02-01, CGL2010-16880 BOS, CGL2011-26654 BOS). We thank the support of the Globimed network. We thank the “Agencia Estatal de Meterorología” for providing climatic data. We thank Guillermo Bueno for statistical advice and J. Albuixech for help during fieldwork.

References

Alla AQ, Camarero JJ, Montserrat-Marti G (2012) Seasonal patterns of bud development and secondary growth as related to climate in two co-existing Mediterranean *Quercus* species. Ann Bot (Submitted)

Amaral Franco J (1990) *Quercus*. In: Castroviejo S, Laínz M, López González G, Montserrat P, Muñoz Garmendia F, Paiva J, Villa L (eds) Flora Ibérica. Real Jardín Botánico, CSIC, pp. 15–36

Barthélémy D, Caraglio Y (2007) Plant architecture: A dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. Ann Bot 99:375–407. DOI: 10.1093/aob/mcl260

Buck-Sorlin GH, Bell AD (2000) Crown architecture in *Quercus petraea* and *Q. robur*: the fate of buds and shoots in relation to age, position and environmental perturbation. Forestry 73:331–349. DOI: 10.1093/forestry/73.4.331

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. DOI: 10.1139/X07-050

Cline MG (1997) Concepts and terminology of apical dominance. Am J Bot 84:1064–1064

Corcuera L, Camarero JJ, Gil-Pelegrin E (2004) Effects of a severe drought on growth and wood anatomical properties of *Quercus faginea*. IAWA J 25:185–204

FAO (1998) World reference base for soil resources ISRIC and ISSS, Rome

Fontaine F, Chaar H, Colin F, Clément C, Burrus M, Druelle JL (1999) Preformation and neoformation of growth units on 3-year-old seedlings of *Quercus petraea*. Can J Bot 77:1623–1631. DOI: 10.1139/b99-138

Gill AM (1971) The formation, growth and fate of buds of *Fraxinus americana* L. in central Massachusetts. Harvard For Pap 20:1–16

Harmer R (1992) Relationships between shoot length, bud number and branch production in *Quercus petraea* (Matt.) Liebl. Forestry 65:61–72. DOI: 10.1093/forestry/65.1.61

Harmer R (1991) The effect of bud position on branch growth and bud abscission in *Quercus petraea* (Matt.) Liebl. Ann Bot 67:463–468

Ishihara MI, Kikuzawa K (2009) Annual and spatial variation in shoot demography associated with masting in *Betula grossa*: comparison between mature trees and saplings. Ann Bot 104:1195–1205. DOI: 10.1093/aob/mcp217

Jones M, Harper JL (1987) The influence of neighbours on the growth of trees I: The demography of buds in *Betula pendula*. Proc R Soc Lond 232:1–18

Kozlowski TT, Torrie JH, Marshall PE (1973) Predictability of shoot length from bud size in *Pinus resinosa* Ait. Can J For Res 3:34–38. DOI: 10.1139/x73-005

Lehtilä K, Tuomi J, Sulkinoja M (1994) Bud demography of the mountain birch *Betula pubescens* Ssp. *tortuosa* near tree line. Ecology 75:945–955. DOI: 10.2307/1939418

Little CHA (1970) Apical dominance in long shoots of white pine (*Pinus strobus*). Can J Bot 48:239–253. DOI: 10.1139/b70-036

Ljung K, Bhalerao RP, Sandberg G (2001) Sites and homeostatic control of auxin biosynthesis in Arabidopsis during vegetative growth. Plant J 28:465–474. DOI: 10.1046/j.1365-313X.2001.01173.x

Macdonald AD, Mothersill DH, Caesar JC (1984) Shoot development in *Betula papyrifera*. III. Long-shoot organogenesis. Can J Bot 62:437–445. DOI: 10.1139/b84-066

Maillette L (1987) Effects of bud demography and elongation patterns on *Betula cordifolia* near the tree line. Ecology 68:1251–1261. DOI: 10.2307/1939209

Maillette L. (1982a) Structural dynamics of silver birch. I. The fates of buds. J Appl Ecol 19:203–218. DOI: 10.2307/2403005

Maillette L (1982b) Structural dynamics of silver birch. II. A matrix model of the bud population. J Appl Ecol 19:219–238. DOI: 10.2307/2403006

Montserrat-Martí G, Camarero JJ, Palacio S, Pérez-Rontomé C, Milla R, Albuixech J, Maestro M (2009) Summer-drought constrains the phenology and growth of two coexisting Mediterranean oaks with contrasting leaf habit: implications for their persistence and reproduction. Trees 23:787–799. DOI: 10.1007/s00468-009-0320-5

Negi GCS (2006) Leaf and bud demography and shoot growth in evergreen and deciduous trees of central Himalaya, India. Trees 20:416–429. DOI: 10.1007/s00468-006-0056-4

Nitta I, Ohsawa M (1998) Bud structure and shoot architecture of canopy and understory evergreen broad-leaved trees at their northern limit in East Asia. Ann Bot 81:115–129. DOI: 10.1006/anbo.1997.0545

Phillips IDJ (1975) Apical dominance. Ann Rev Plant Physiol 26:341–367. DOI: 10.1146/annurev.pp.26.060175.002013

Puntieri JG, Stecconi M, Barthélémy D (2002) Preformation and neoformation in shoots of *Nothofagus antarctica* (G. Forster) Oerst. (Nothofagaceae) shrubs from northern Patagonia. Ann Bot 89:665–673. DOI: 10.1093/aob/mcf108

Sabatier S, Barthélémy D (2001) Bud structure in relation to shoot morphology and position on the vegetative annual shoots of *Juglans regia* L. (Juglandaceae). Ann Bot 87:117–123. DOI: 10.1006/anbo.2000.1312

Sanz-Pérez V, Castro-Díez P (2010) Summer water stress and shade alter bud size and budburst date in three Mediterranean *Quercus* species. Trees 24:89–97. DOI: 10.1007/s00468-009-0381-5

Sheil D, Burslem DFRP, Alder D (1995) The interpretation and misinterpretation of mortality rate measures. J Ecol 83:331–333. DOI: 10.2307/2261571

Shimizu-Sato S, Tanaka M., Mori H. (2009) Auxin–cytokinin interactions in the control of shoot branching. Plant Mol Biol 69:429–435. DOI: 10.1007/s11103-008-9416-3

Sokal RR, Rohlf FJ (1995) Biometry: the principles and practices of statistics in biological research. W. H. Freeman, New York

Tanaka Mina, Takei K, Kojima M, Sakakibara H, Mori Hitoshi (2006) Auxin controls local cytokinin biosynthesis in the nodal stem in apical dominance. Plant J 45:1028–1036. DOI: 10.1111/j.1365-313X.2006.02656.x

Tolvanen A, Schroderus J, Henry GHR (2002) Age- and stage-based bud demography of *Salix arctica* under contrasting muskox grazing pressure in the High Arctic. Evol Ecol 15:443–462. DOI: 10.1023/A:1016049301905

Ward WW (1964) Bud distribution and branching in red oak. Bot Gaz 125:217–220

Wilson BF, Kelty MJ (1994) Characteristics of shoots from the bud bank in black oak. Bull Torrey Bot Club 121:62–68. DOI: 10.2307/2996884

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1:3–14. DOI: 10.1111/j.2041-210X.2009.00001.x

Supplementary Information

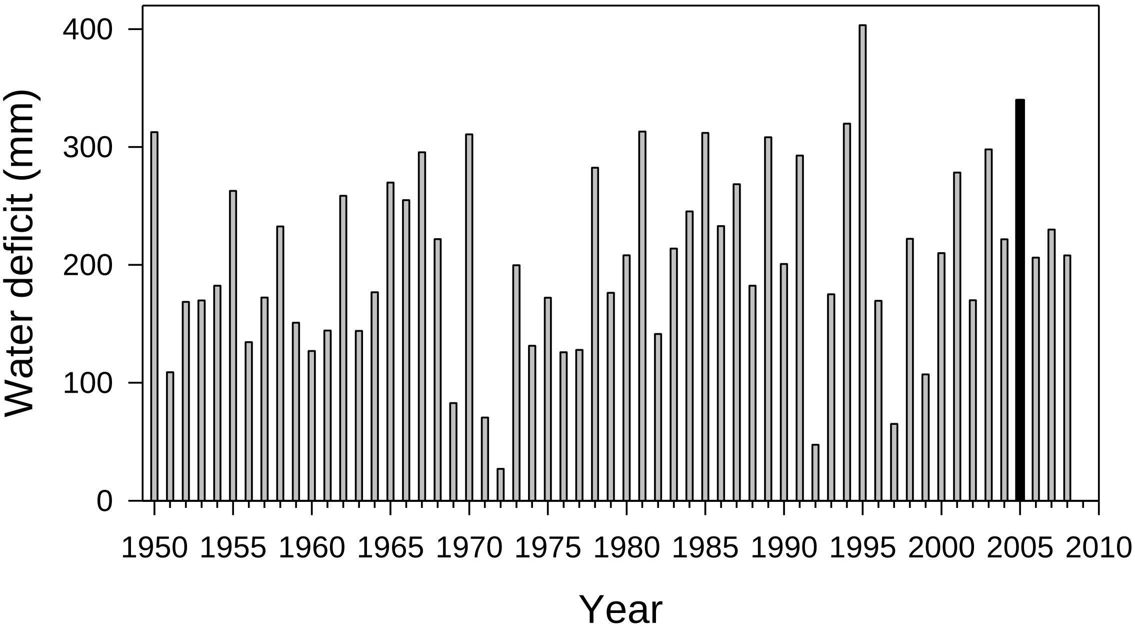


Figure S1. Water deficit calculated for the study site for the period 1950-2008. Note the year 2005 (black bar) which is the second one with highest deficit since 1950 after 1995, which was the driest one. Water deficit was calculated as explained by Montserrat-Martí *et al*. (2009) using regional annual data of mean temperature and total precipitation for the period 1950-2008.

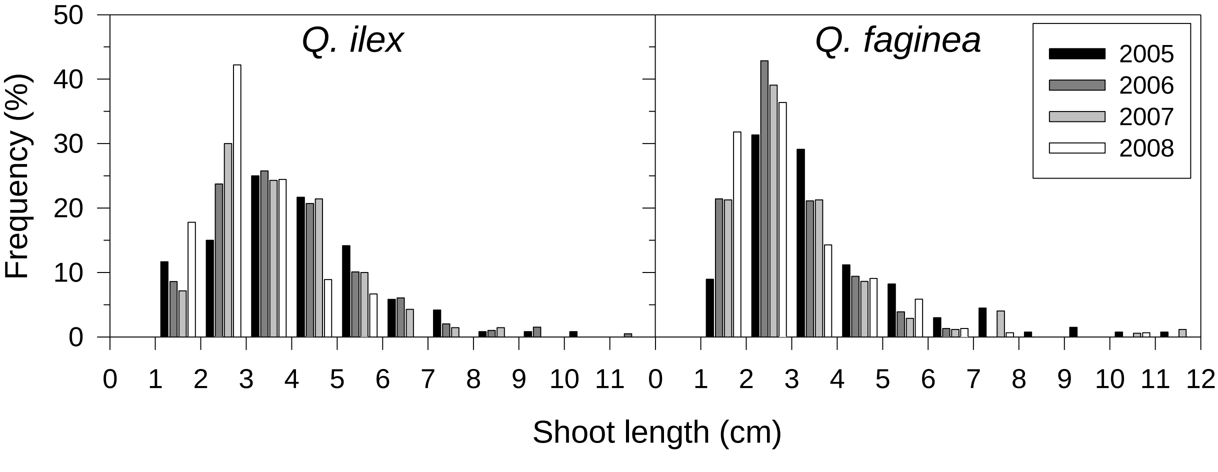


Figure S2. Distribution of shoots according to their length for the two species and the four study years.

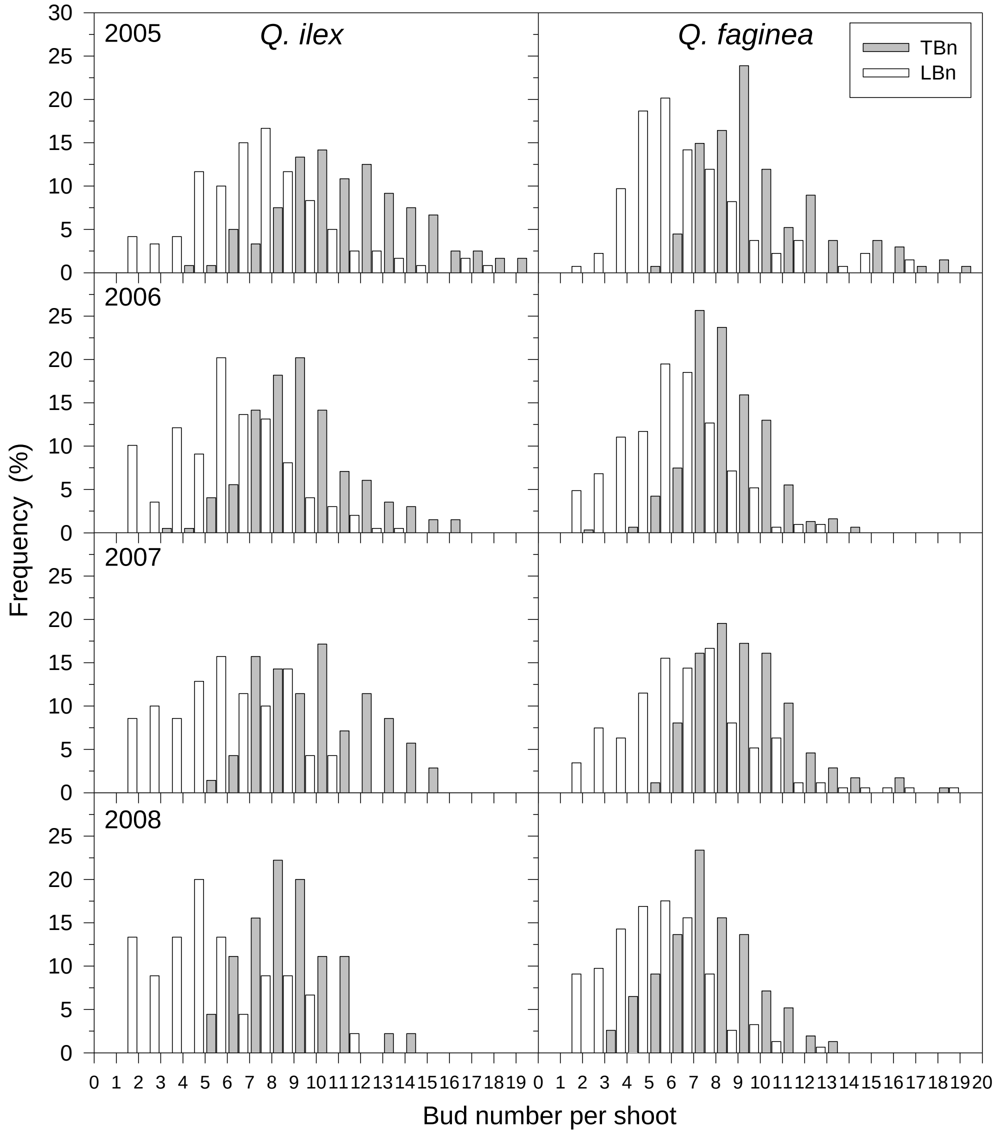
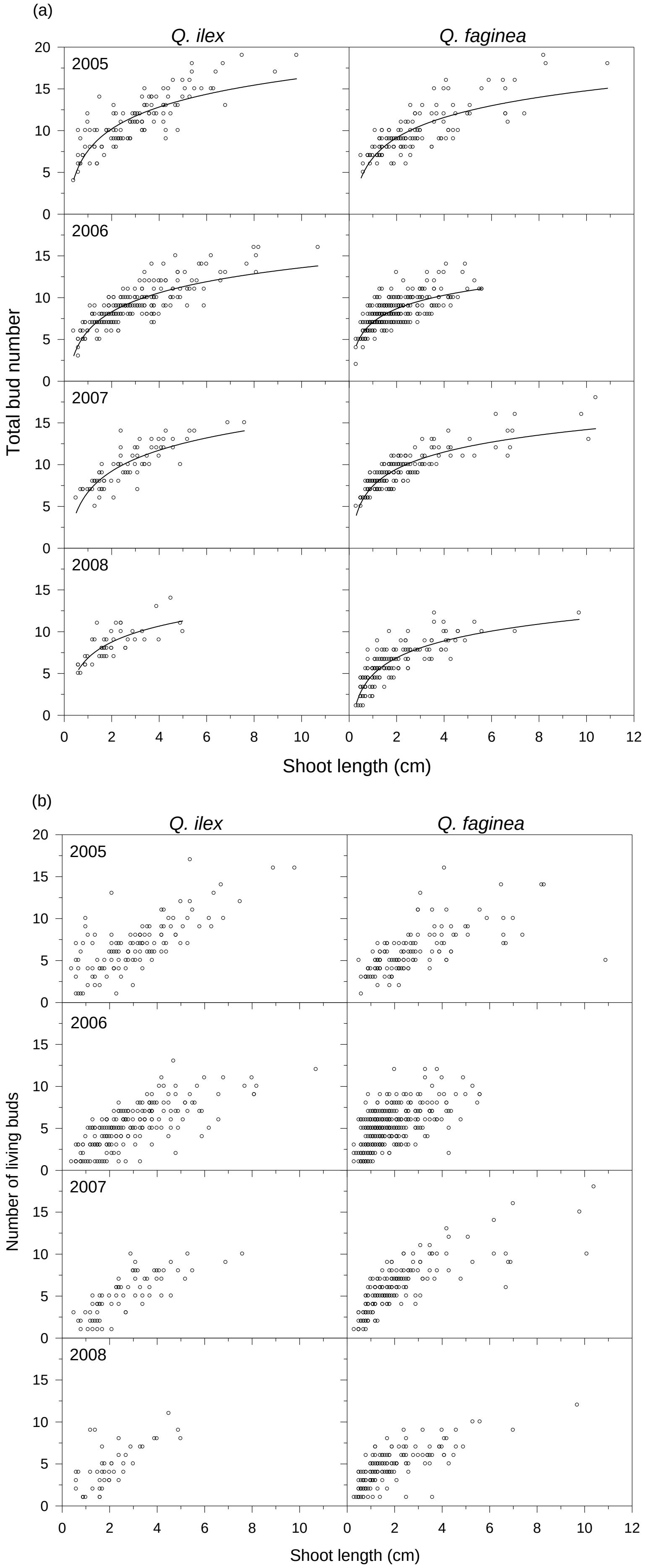


Figure S3. Frequency distribution of bud number per shoot according to total bud number (TBn, living and dead buds and bud scars, grey bars) and living bud number (LBn, white bars) for the four study years and both oak species.

Figure S4. Relationship between the total (living and dead buds and bud scars) number of buds (a) and the number of living buds (b) *vs*. shoot length. Note the logarithmic scale of the relationship in figure (a). All relationships were highly significant (*P*<0.01).



4. GENERAL DISCUSSION

This study provided evidence on the interactions between primary and secondary growth in two Mediterranean oak species with contrasting leaf habits, namely the evergreen *Quercus ilex vs*. the deciduous *Q. faginea*. These relationships were observed for instance between stem cross-sectional area of current year shoots and apical bud mass and also between the former variable and acorn mass indicating a strong dependency of bud size and fruit production on stem secondary growth. The most reasonable explanation for the above relationships is that thicker stems improve vascular supply which would support bigger buds and acorns (Cochard et al. 2005).

In general, the tree-ring witdth and wood-anatomical features showed a high climatic signal, mostly in response to late-winter, spring and early-summer rainfall amounts. This is particularly the case for earlywood vessel area, which means that water availability in the aforementioned periods is among the more critical factors for earlywood growth. On the other hand, the negative association between earlywood vessel area and late-winter temperature suggests that low temperatures in this period influence the physiological processes (e.g., carbohydrate storage and movilisation), favouring the formation of wider earlywood vessel, thus enhancing so the hydraulic conductivity and inducing a higher primary growth (i.e. shoot and leaf growth) in spring (Fonti et al. 2007; Čufar et al. 2008). As a matter of fact, the close association between earlywood vessel area and climatic variables (temperature and precipitation), indicates that earlywood anatomical variables could be considered as proxies of xylem plasticity in response to climatic variability.

The strong association between primary and secondary growth was clearly manifested in the production of wide earlywood vessels which allows ring-porous species to rapidly transport water for leaf development during the early growing season. This statement is in agreement with previous studies which showed that the first earlywood vessels are formed earlier or at the same time than budburst (Frankenstein et al. 2005; Zweifel et al. 2006).

An interesting finding was that bud size increased as stem cross-sectional area did suggesting that buds enlarge allometrically as a function of vascular area if climatic stress decreases. This also indicates that bud development is driven by the shoot hydraulic architecture since thicker stems with wider vessels favoured the development of bigger buds. The influence of stem cross-sectional area also on the number of buds formed per stem indicates a great control of shoot structure by secondary growth. The variability in bud size and number as well as in bud-shoot relationships is related to the different climatic stress the trees withstand. Indeed, bud development at sites situated in the xeric and cold locations were respectively influenced by the water deficit and short growing season determined by low temperatures as found in a previous study considering leaf area (Sun et al. 2006).

The finding that shoots bearing acorns were thicker than those not bearing shoots suggests that the earlier may provide a higher structure support and total water conductivity compared to the later. The high hydraulic conductivity may favour the development of acorns during summer, the period with maximum water deficit (Montserrat-Martí et al. 2009). The positive association between transversal vessel area and secondary shoot growth is related to the production of wide vessels in the xylem and therefore an enhanced hydraulic conductivity. This positive relationship may lead to the production of bigger apical buds and higher primary growth in the following spring (Cochard et al. 2005). According to our findings, these processes do not operate in shoots bearing acorns most likely because the competition for water and nutrients between acorns and buds. Considering that both growth processes greatly overlapped in time, bud development could be negatively influenced when trees produce large fruit crops (Camarero et al. 2010). Moreover, the findings that stems begin thickening in late spring indicates that there is a clear predetermination for fruit production before summer.

The maximum growth rates of buds observed in mid-summer in the studied oak species, which corresponds to the most unfavourable period for vegetative growth, can be explained by high water use efficiency of buds, enabled by the protection provided by scales and cataphylls against water loss. Additionally, the high growth rates of buds in mid-summer coincide with the arrest of most primary and secondary growth in this period, making the water reserves available for bud development (Montserrat-Martí et al. 2009; Gutiérrez et al. 2011). This finding provides insight to the adaptation abilities of the studied species and facilitates the interpretation of their responses to the environmental stressors.

The longer buds showed a higher probability to burst than shorter ones, which was more evident in the case of apical buds than in axillary ones. This can be explained by the movement of nutrients to the regions of high auxin concentration which is crucial for apical dominance (Phillips 1975). Auxin, produced in young leaves at the shoot apex and transported basipetally to the stem, constrains the development of axillary buds (Shimizu-Sato et al. 2009). However, no minimum threshold of bud length existed below which bursting was hampered but there was found a variable threshold depending on the bud size. This link was strongly influenced by the climatic conditions as demonstrated the fact that in the driest studied years the apical buds were longer than the axillary ones. On the other hand, in wet years the axillary buds reached their maximum growth while apical buds continue to growth favoured by higher stem cross-sectional area and hydraulic conductivity.

The research of the temporal arrangement of the different phenological events including bud development features makes possible to analyse how these events interact with each other and the way they are influenced by various climatic drivers. Considering the fact that in dry and cold climates growth is limited by temperature and water availability and that variable plant structures demonstrate distinct climatic thresholds, the information concerning the temporal arrangement of phenophases is crucial for the interpretation of plant functional responses.

Future research lines

The fulfilment of the present PhD Memory has left many unsolved questions. Several of them are important to improve our knowledge on the relationships between primary and secondary growth. However, the most immediately required are the following ones:

It is necessary to better understand how the secondary growth interacts with acorn development in oaks, i.e. to investigate in detail what would be the consequences for the tree if many reproductive shoots with thick stems lose their fruits during the early period of active acorn enlargement. It is hypothesised that trees could develop bigger buds which would determine a larger crown growth in the next spring than that of trees with reduced acorn loss.

With regard to the relationships between bud size and secondary shoot growth more research is needed to clarify the interactions of the above growth variables and climatic stress in contrasted climatic conditions. I hypothesise a higher hydraulic efficiency of the vascular system of current-year shoots in the trees affected by low climatic stresses than elsewhere.

Concerning bud development relationships with climate, additional investigation is required to understand the influence of predicted air temperature rise on bud size. It is hypothesised that the influence of a potential warming-induced bud enlargement on crown architecture would be relevant to understand tree growth and function.

5. GENERAL CONCLUSIONS

1. The predicted warmer and drier conditions in the Mediterranean area will not significantly affect the growth of the Iberian *Q. faginea* populations near their xeric distribution limit considering the high plastic response of this species to climatic stress (Chapter I).

2. The positive relationship between stem cross-sectional area and apical bud mass in current-year shoots were found to be allometric. These associations appeared to be stronger in the mid-elevation site as compared to the other two studied sites. Therefore, trees growing in the mid-elevation site produced bigger buds with greater potential for primary shoot growth in the following season than trees from the other sites (Chapter II).

3. The presence of acorns is linked to an enhanced thickening of the current-year stems bearing them. This overgrowth starts in late spring or early summer much before acorns start their major phase of enlargement. Bearing stems have a higher vessel number and a larger total area and total conducting area than non-bearing stems in the basal position of the stem. The number of vessels and their total area decreased to the stem apex, particularly from the upper acorn stalk towards the apical bud of the bearing stems (Chapter III).
4. The maximum rates of bud growth were achieved in the first two weeks of August in both species, favoured by the warm night temperatures (Chapter IV).

5. The larger buds showed a higher probability to burst than smaller ones, which was more emphasised in the apical buds than in the axillary ones. However, there was no minimum threshold of bud length which could impair bud burst (Chapter V).

6. REFERENCES

Aloni R (1991) Wood formation in deciduous hardwood trees. In: Raghavendra AS (ed) Physiology of trees Wiley, New York, pp. 75–197

Amaral Franco J (1990) *Quercus*. In: Castroviejo S, Laínz M, López González G, Montserrat P, Muñoz Garmendia F, Paiva J, Villa L (eds) Flora Ibérica. Real Jardín Botánico, CSIC, pp. 15–36

Arora R, Rowland LJ, Tanino K (2003) Induction and release of bud dormancy in woody perennials: a science comes of age. HortScience 38:911–921

Baas P, Schweingruber FH (1987) Ecological trends in the wood anatomy of trees, shrubs and climbers from Europe. IAWA Bull 8:245–274

Barthélémy D, Caraglio Y (2007) Plant architecture: A dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. Ann Bot 99:375–407. DOI: 10.1093/aob/mcl260

Blanco-Castro E, Casado-Gonzáles MA, Costa-Tenorio M, et al. (1997) Los bosques ibéricos : una interpretación geobotánica Planeta, Barcelona, España

Bret-Harte MS, Shaver GR, Chapin III FS (2002) Primary and secondary stem growth in arctic shrubs: implications for community response to environmental change. J Ecol 90:251–267. DOI: 10.1046/j.1365-2745.2001.00657.x

Buck-Sorlin GH, Bell AD (2000) Crown architecture in *Quercus petraea* and *Q. robur*: the fate of buds and shoots in relation to age, position and environmental perturbation. Forestry 73:331–349. DOI: 10.1093/forestry/73.4.331

Caesar JC, Macdonald AD (1984) Shoot development in Betula papyrifera. IV. Comparisons between growth characteristics and expression of vegetative long and short shoots. Can J Bot 62:446–453. DOI: 10.1139/b84-067

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. DOI: 10.1007/s00468-010-0462-5

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. DOI: 10.1139/X07-050

Carlquist S (1975) Ecological Strategies of Xylem Evolution University of California Press, Berkeley, USA

Cochard H, Coste S, Chanson B, Guehl JM, Nicolini E (2005) Hydraulic architecture correlates with bud organogenesis and primary shoot growth in beech (*Fagus sylvatica*). Tree Physiol 25:1545–1552. DOI: 10.1093/treephys/25.12.1545

Corcuera L, Camarero JJ, Gil-Pelegrin E (2004) Effects of a severe drought on growth and wood anatomical properties of *Quercus faginea*. IAWA J 25:185–204

Čufar K, Prislan P, Luis M, Gričar J (2008) Tree-ring variation, wood formation and phenology of beech (*Fagus sylvatica*) from a representative site in Slovenia, SE Central Europe. Trees 22:749–758. DOI: 10.1007/s00468-008-0235-6

Ellmore GS, Ewers FW (1986) Fluid flow in the outermost xylem increment of a ring-porous tree, *Ulmus americana*. Am J Bot 73:1771–1774. DOI: 10.2307/2444244

Fonti P, Solomonoff N, García-González I (2007) Earlywood vessels of *Castanea sativa* record temperature before their formation. New Phytol 173:562–570. DOI: 10.1111/j.1469-8137.2006.01945.x

Frankenstein C, Eckstein D, Schmitt U (2005) The onset of cambium activity - A matter of agreement? Dendrochronologia 23:57–62. DOI: 16/j.dendro.2005.07.007

George AP, Nissen RJ, Collins RJ, Rasmussen TS (1996) Effects of shoot variables and canopy position on fruit set, fruit quality and starch reserves of persimmon (*Diospyros kaki* L) in subtropical Australia. J Hortic Sci 71:217–226

Gill AM (1971) The formation, growth and fate of buds of *Fraxinus americana* L. in central Massachusetts. Harvard For Pap 20:1–16

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. DOI: 10.1007/s00468-011-0540-3

Hacke UG, Sperry JS (2001) Functional and ecological xylem anatomy. Persp Plant Ecol Evol Syst 4:97–115. DOI: 10.1078/1433-8319-00017

Harmer R (1992) Relationships between shoot length, bud number and branch production in *Quercus petraea* (Matt.) Liebl. Forestry 65:61–72. DOI: 10.1093/forestry/65.1.61

Harmer R (1989) Some aspects of bud activity and branch formation in young oak. Ann For Sci 46:3. DOI: 10.1051/forest:19890551

Harmer R (1991) The effect of bud position on branch growth and bud abscission in *Quercus petraea* (Matt.) Liebl. Ann Bot 67:463–468

Hasegawa S, Koba K, Tayasu I, Takeda H, Haga H (2003) Carbon autonomy of reproductive shoots of Siberian alder (*Alnus hirsuta* var. *sibirica*). J Plant Res 116:183–188. DOI: 10.1007/s10265-003-0085-7

Hirayama D, Nanami S, Itoh A, Yamakura T (2007) Individual resource allocation to vegetative growth and reproduction in subgenus *Cyclobalanopsis* (*Quercus*, Fagaceae) trees. Ecol Res 23:451–458. DOI: 10.1007/s11284-007-0398-4

Hoch G (2005) Fruit-bearing branchlets are carbon autonomous in mature broad-leaved temperate forest trees. Plant Cell Environ 28:651–659. DOI: 10.1111/j.1365-3040.2004.01311.x

Horvath DP, Anderson JV, Chao WS, Foley ME (2003) Knowing when to grow: signals regulating bud dormancy. Trends Plant Sci 8:534–540. DOI: 10.1016/j.tplants.2003.09.013

Ishihara MI, Kikuzawa K (2009) Annual and spatial variation in shoot demography associated with masting in *Betula grossa*: comparison between mature trees and saplings. Ann Bot 104:1195–1205. DOI: 10.1093/aob/mcp217

Johnson R. S, Lakso AN (1986) Carbon balance model of a growing apple shoot. I: development of the model. J Am Soc Hortic Sci 111:160–164

Keel SG, Schädel C (2010) Expanding leaves of mature deciduous forest trees rapidly become autotrophic. Tree Physiol 30:1253–1259. DOI: 10.1093/treephys/tpq071

Kikuzawa K (1983) Leaf survival of woody plants in deciduous broad-leaved forests. 1. Tall trees. Can J Bot 61:2133–2139. DOI: 10.1139/b83-230

Koenig WD, Knops JMH (1998) Scale of mast-seeding and tree-ring growth. Nature 396:225–226. DOI: 10.1038/24293

Kozlowski TT (1971) Growth and development of trees Academic Press, New York, USA

Kozlowski TT, Torrie JH, Marshall PE (1973) Predictability of shoot length from bud size in *Pinus resinosa* Ait. Can J For Res 3:34–38. DOI: 10.1139/x73-005

Landhäusser SM (2010) Aspen shoots are carbon autonomous during bud break. Trees 25:531–536. DOI: 10.1007/s00468-010-0532-8

Lauri PÉ, Kelner JJ, Trottier C, Costes E (2010) Insights into secondary growth in perennial plants: its unequal spatial and temporal dynamics in the apple (*Malus domestica*) is driven by architectural position and fruit load. Ann Bot 105:607–616

Lauri PÉ, Térouanne É, Lespinasse JM (1996) Quantitative analysis of relationships between inflorescence size, bearing-axis size and fruit-set: an apple tree case study. Ann Bot 77:277–286. DOI: 10.1006/anbo.1996.0031

Limousin JM, Rambal S, Ourcival JM, Rodríguez-Calcerrada J, Pérez-Ramos IM, Rodríguez-Cortina R, Misson L, Joffre R (2011) Morphological and phenological shoot plasticity in a Mediterranean evergreen oak facing long-term increased drought. Oecologia. DOI: 10.1007/s00442-011-2221-8

Maillette L (1982) Structural dynamics of silver birch. I. The fates of buds. J Appl Ecol 19:203–218. DOI: 10.2307/2403005

Meier IC, Leuschner C (2008) Leaf size and leaf area index in *Fagus sylvatica* forests: competing effects of precipitation, temperature, and nitrogen availability. Ecosystems 11:655–669. DOI: 10.1007/s10021-008-9135-2

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

Montserrat-Martí G, Camarero JJ, Palacio S, Pérez-Rontomé C, Milla R, Albuixech J, Maestro M (2009) Summer-drought constrains the phenology and growth of two coexisting Mediterranean oaks with contrasting leaf habit: implications for their persistence and reproduction. Trees 23:787–799. DOI: 10.1007/s00468-009-0320-5

Nitta I, Ohsawa M (1998) Bud structure and shoot architecture of canopy and understory evergreen broad-leaved trees at their northern limit in East Asia. Ann Bot 81:115–129. DOI: 10.1006/anbo.1997.0545

Obeso JR (2002) The costs of reproduction in plants. New Phytol 155:321–348

Orshan G ed. (1989) Plant pheno-morphological studies in Mediterranean type ecosystems Kluwer Academic Publishers

Phillips IDJ (1975) Apical dominance. Ann Rev Plant Physiol 26:341–367. DOI: 10.1146/annurev.pp.26.060175.002013

Porter GW, Sherman WB, Beckman TG, Krewer GW (2002) Fruit weight and shoot diameter relationship in early ripening peaches. J Am Pomol Soc 56(1):30–33

Preston KA, Ackerly DD (2003) Hydraulic architecture and the evolution of shoot allometry in contrasting climates. Am J Bot 90:1502–1512. DOI: 10.3732/ajb.90.10.1502

Puntieri JG, Stecconi M, Barthélémy D (2002) Preformation and neoformation in shoots of *Nothofagus antarctica* (G. Forster) Oerst. (Nothofagaceae) shrubs from northern Patagonia. Ann Bot 89:665–673. DOI: 10.1093/aob/mcf108

Remphrey WR, Bartlett GA, Davidson CG (2002) Shoot morphology and fate of buds in relation to crown location in young *Fraxinus pennsylvanica* var. *subintegerrima*. Can J Bot 80:1274–1282. DOI: 10.1139/b02-120

Remphrey WR, Powell GR (1984) Crown architecture of *Larix laricina* saplings: shoot preformation and neoformation and their relationships to shoot vigour. Can J Bot 62:2181–2192. DOI: 10.1139/b84-298

Roff DA (1992) Cost of reproduction. In: The evolution of life histories: Theory and analysis. Chapman and Hall, New York, pp. 145–178

Sabatier S, Barthélémy D (2001) Bud structure in relation to shoot morphology and position on the vegetative annual shoots of *Juglans regia* L. (Juglandaceae). Ann Bot 87:117–123. DOI: 10.1006/anbo.2000.1312

Salminen H, Jalkanen R (2004) Does current summer temperature contribute to the final shoot length on *Pinus sylvestris*? A case study at the northern conifer timberline. Dendrochronologia 21:79–84. DOI: 10.1078/1125-7865-00039

Sanz-Pérez V, Castro-Díez P (2010) Summer water stress and shade alter bud size and budburst date in three Mediterranean *Quercus* species. Trees 24:89–97. DOI: 10.1007/s00468-009-0381-5

Shimizu-Sato S, Tanaka M, Mori H (2009) Auxin–cytokinin interactions in the control of shoot branching. Plant Mol Biol 69:429–435. DOI: 10.1007/s11103-008-9416-3

Sun S, Jin D, Shi P (2006) The leaf size–twig size spectrum of temperate woody species along an altitudinal gradient: An invariant allometric scaling relationship. Ann Bot 97:97–107. DOI: 10.1093/aob/mcj004

Suzuki M, Hiura T (2000) Allometric differences between current-year shoots and large branches of deciduous broad-leaved tree species. Tree Physiol 20:203–209. DOI: 10.1093/treephys/20.3.203

Suzuki M, Yoda K, Suzuki H (1996) Phenological comparison of the onset of vessel formation between ring-porous and diffuse-porous deciduous trees in a Japanese temperate forest. IAWA J 17:431–444

Takenaka A (1997) Structural variation in current-year shoots of broad-leaved evergreen tree saplings under forest canopies in warm temperate Japan. Tree Physiol 17:205–210. DOI: 10.1093/treephys/17.3.205

Tardif JC, Conciatori F (2006) Influence of climate on tree rings and vessel features in red oak and white oak growing near their northern distribution limit, southwestern Quebec, Canada. Can J For Res 36:2317–2330

Tessier L, Nola P, Serre-Bachet F (1994) Deciduous *Quercus* in the Mediterranean region: tree-ring/climate relationships. New Phytol 126:355–367. DOI: 10.1111/j.1469-8137.1994.tb03955.x

Tyree MT, Davis SD, Cochard H (1994) Biophysical perspectives of xylem evolution: is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction? IAWA J 15:335–360

Vesk PA, Westoby M (2004) Funding the bud bank: a review of the costs of buds. Oikos 106:200–208. DOI: 10.1111/j.0030-1299.2004.13204.x

Westoby M, Wright IJ (2003) The leaf size-twig size spectrum and its relationship to other important spectra of variation among species. Oecologia 135:621–628. DOI: 10.1007/s00442-003-1231-6

Wilson BF, Kelty MJ (1994) Characteristics of shoots from the bud bank in black oak. Bull Torrey Bot Club 121:62–68. DOI: 10.2307/2996884

Yagi T (2000) Morphology and biomass allocation of current-year shoots of ten tall tree species in cool temperate Japan. J Plant Res 113:171–183. DOI: 10.1007/PL00013928

Yagi T, Kikuzawa K (1999) Patterns in size-related variations in current-year shoot structure in eight deciduous tree species. J Plant Res 112:343–352. DOI: 10.1007/PL00013862

Yáñez-Espinosa L, Terrazas T, López-Mata L (2006) Integrated analysis of tropical trees growth: A multivariate approach. Ann Bot 98:637–645. DOI: 10.1093/aob/mcl142

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. DOI: 10.1093/jxb/erj125

7. APPENDICES

Chapter I

Alla AQ, Camarero JJ. 2012. Contrasting responses of radial growth and wood anatomy to climate in a Mediterranean ring-porous oak: implications for its future persistence or why the variance matters more than the mean. *European Journal of Forest Research*. In press. DOI: 10.1007/s10342-012-0621-x

Chapter II

Alla AQ, Camarero JJ, Rivera P, Montserrat-Martí G. 2011. Variant allometric scaling relationships between bud size and secondary shoot growth in *Quercus faginea*: implications for the climatic modulation of canopy growth. *Annals of Forest Science* **68**: 1245–1254. DOI: 10.1007/s13595-011-0093-z

Chapter II

Alla AQ, Camarero JJ, Maestro-Martínez M, Montserrat-Martí G. 2012. Acorn production is linked to secondary growth but not to declining carbohydrate concentrations in current-year shoots of two oak species. *Trees* **26**:841–850. DOI: 10.1007/s00468-011-0658-3

Zaragoza 2012