

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

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Abstract

• **Introduction and statement of the research questions** 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.

• **Methods** 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.

• **Results** 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.

• **Conclusions** 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.

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1 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 Kelly 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.

2 Materials and methods

2.1 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.

2.2 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 percen-

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 (°C)	Minimum and maximum air temperatures in 2007 (°C)	Number of days with air temperatures below 0°C in 2007	Mean relative air humidity in 2007 (%)	Total annual precipitation (mm)	Estimated water deficit (mm)/period ^a
H	42° 19' 01"	0° 24' 46"	1,470	9.1±0.1 c	−8.9/30.7	33	67.6±0.2 a	1,215	0
M	42° 19' 30"	0° 25' 31"	1,135	10.8±0.1 b	−6.8/32.3	14	65.4±0.2 b	899	0
L	42° 15' 50"	0° 25' 59"	700	13.1±0.1 a	−6.3/38.8	12	51.8±0.2 c	670	270/July–August

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)

tages 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 (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 midcrown 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 60°C 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 ($r=0.97$, $n=290$, $P<0.001$).

2.3 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 analyzed using the ImageJ software (Rasband 1997–2009) considering all vessels whose area was greater than 0.0001 mm² (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).

2.4 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 (r_s). Means are reported with their standard errors.

2.5 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 ($a \neq 1$). 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).

2.6 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 R-CFI 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).

3 Results

3.1 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

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

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

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

^aNumber of rings counted in wood cores sampled at 1.3 m

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).

3.2 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).

3.3 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 ($G=124.31$, $P<0.001$).

3.4 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

Table 3 Mean (\pm 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)
Summer	H	29.0±0.6 b	1.0±0.1 c	73.8±2.1 b	432.1±8.4 c	–	–	2.41±0.02 b
	M	35.0±1.0 a	1.5±0.1 a	135.4±5.5 a	757.7±16.7 a	–	–	2.52±0.02 a
	L	29.5±0.7 b	1.1±0.1 b	67.1±1.9 b	487.1±9.5 b	–	–	2.23±0.02 c
	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±0.6 b	1.3±0.1 b	–	–	8.9±0.1 b	6.0±0.1 a	–
	M	32.6±0.8 a	1.5±0.1 a	–	–	15.4±0.3 a	5.4±0.1 b	–
	L	26.8±0.5 c	1.1±0.1 c	–	–	6.8±0.1 c	4.0±0.1 c	–
	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

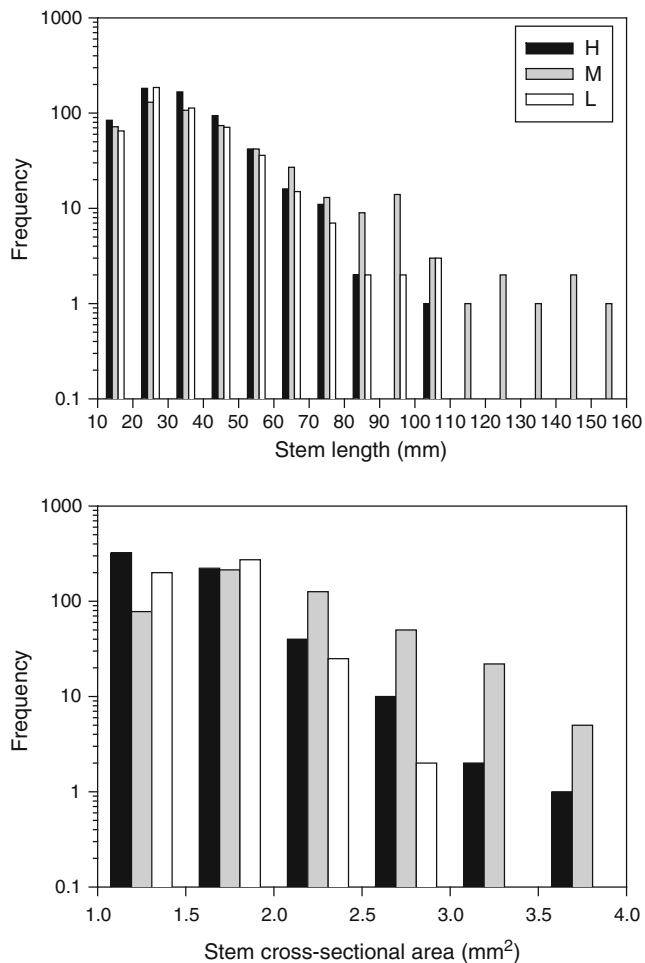


Fig. 1 Distributions of stem length and stem cross-sectional area according to their size in the three study sites (*H* high-elevation site, *M* mid-elevation site, *L* low-elevation site). Note the logarithmic scale in both graphs

(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 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.

4 Discussion

4.1 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

Table 4 Summary of allometric analyses

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

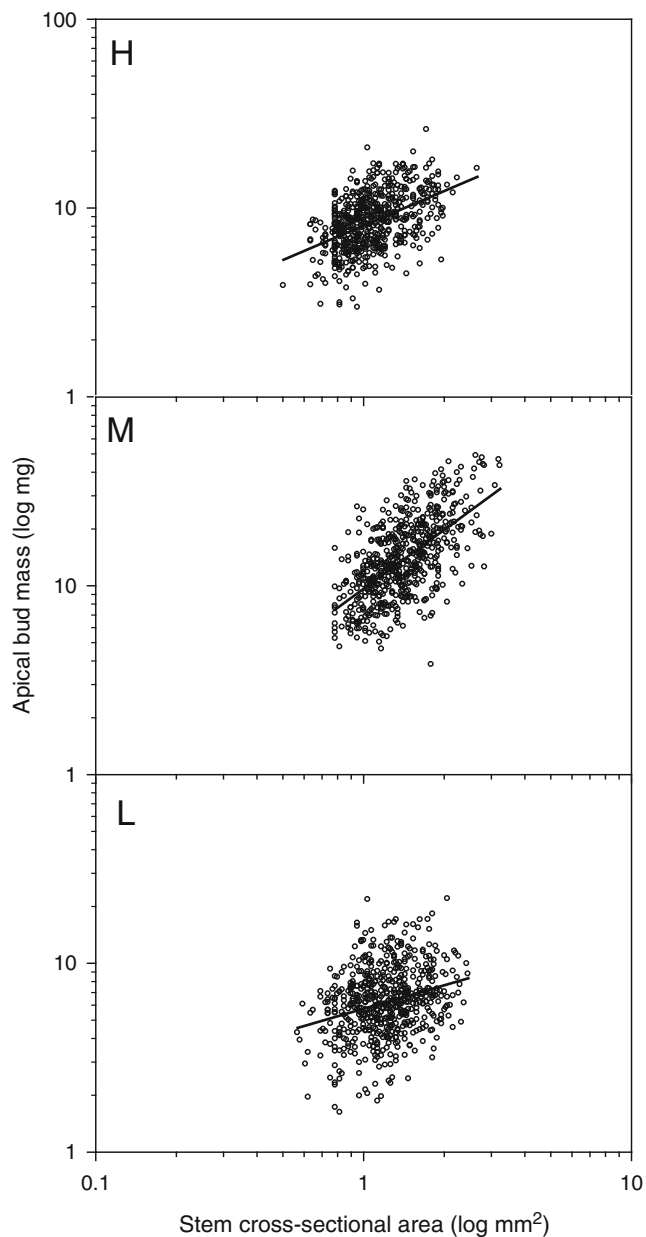


Fig. 2 Allometric relationships between stem cross-sectional area (SCA) and apical bud mass (ABM) in the three study sites (*H* high-elevation site, *M* mid-elevation site, *L* low-elevation site). Note the logarithmic scales

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 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 through 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 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.

4.2 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

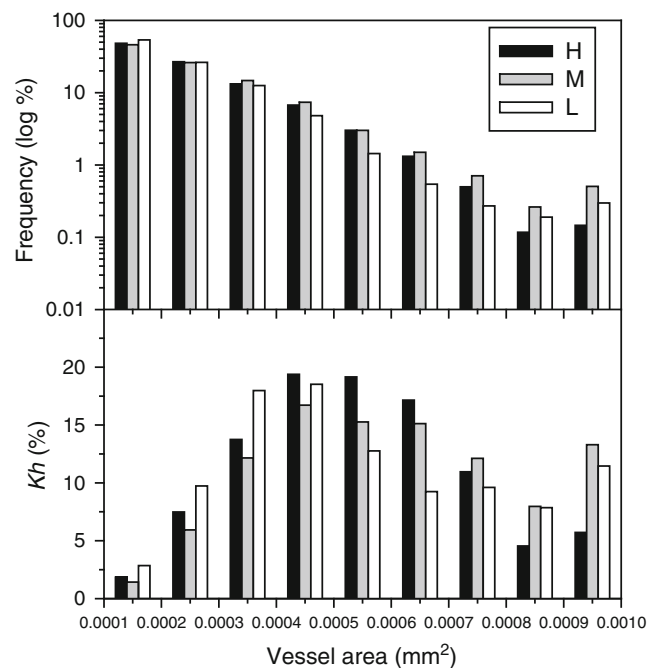


Fig. 3 Distribution of vessels according to their area and to their relative (%) contribution to the total predicted hydraulic conductance (K_h) for the three study sites (sites' codes are as in Fig. 1). Note the logarithmic scale in the upper graph

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

Model	<i>df</i>	<i>P</i>	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: χ^2 S-B Satorra–Bentler robust chi square, R-RMSEA robust root mean square error of approximation, SRMR standardized root mean square residual, GFI goodness-of-fit index, AGFI adjusted goodness-of-fit index, and R-CFI robust comparative fit index

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

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 in that site (Castro-Díez et al. 1997). In the mid-elevation site, for a given increase in shoot cross-sectional 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 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.

4.3 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

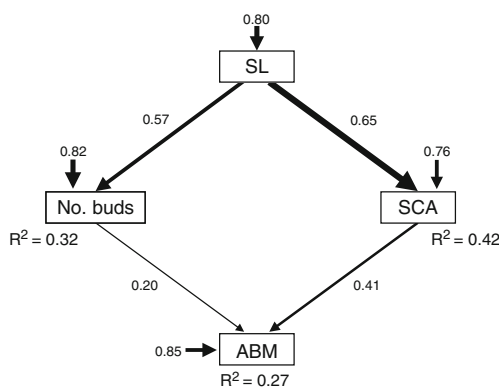


Fig. 4 Selected structural equation model of primary and secondary growth variables for the global dataset. Boxes correspond to measured variables and directed arrows (paths) represent a causal influence. Path coefficients, corresponding to the estimated strength of one variable's influence on another, 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

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.

5 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.

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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'activité cambiale, composante active ou passive dans les réactions 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, McKey D (2001) Leaf–stem allometry, hollow stems, and the evolution of caulinary domatia in myrmecophytes. *New Phytol* 151:391–406
- 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
- Castro-Díez P, Villar-Salvador P, Pérez-Rantomé 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. Available from: <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, pp 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, pp 80–103
- Rasband WS, 1997–2009. ImageJ. N.I.H., Bethesda, USA. Available from: <http://rsb.info.nih.gov/ij/>. Accessed 14 January 2008
- Shiple 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