

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

Arben Q. Alla · J. Julio Camarero ·  
Melchor Maestro-Martínez · Gabriel Montserrat-Martí

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**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 (Kozłowski 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,

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A. Q. Alla (✉) · M. Maestro-Martínez · G. Montserrat-Martí  
Instituto Pirenaico de Ecología (CSIC),  
Avenida Montañana 1005, 50080 Zaragoza, Spain  
e-mail: beniialla@ipe.csic.es

J. J. Camarero  
ARAID-Instituto Pirenaico de Ecología (CSIC), Avda.  
Montañana 1005, 50080 Zaragoza, Spain

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°C 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%). 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).

**Table 1** Morphological features of studied trees (mean values  $\pm$  SE)

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

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

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°C 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°C 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°C 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  $\mu\text{m}$  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<sup>®</sup> (Merck, Germany), and photographed by Olympus BH2 light microscope at 100 $\times$  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  $\text{mm}^2$  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, IKA-Werke, 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

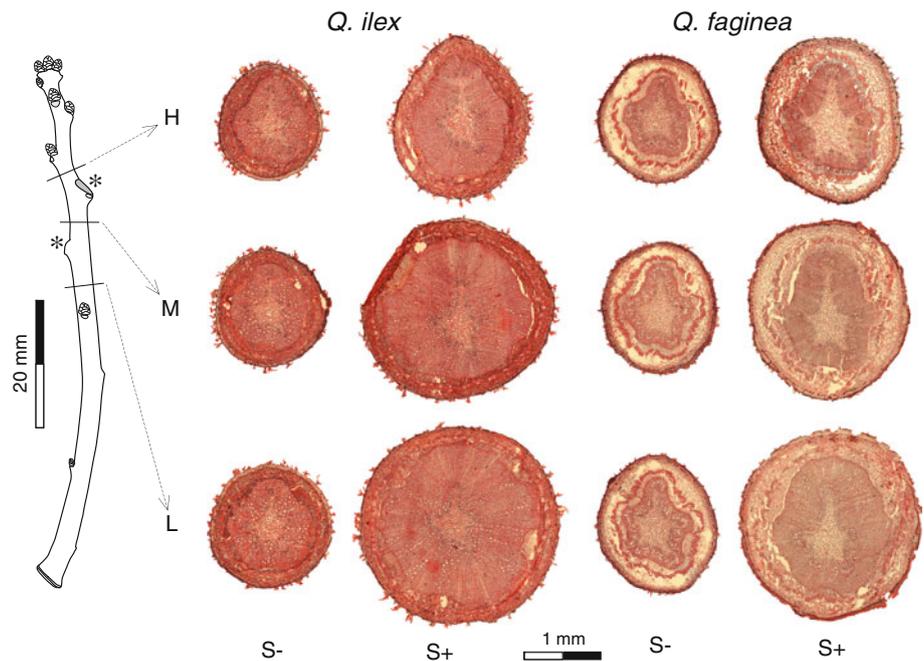
All data were checked for normality and homoscedasticity prior to statistical analyses. The variables stem length and cross sectional area were log-transformed to reach normality. To evaluate differences in stem length and cross-sectional area, number of leaves, stem mass, leaf mass and apical bud mass 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

**Fig. 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



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

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

higher value than S– in both seasons and stem mass in summer. In this species, S– showed significantly higher number of leaves in both 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.

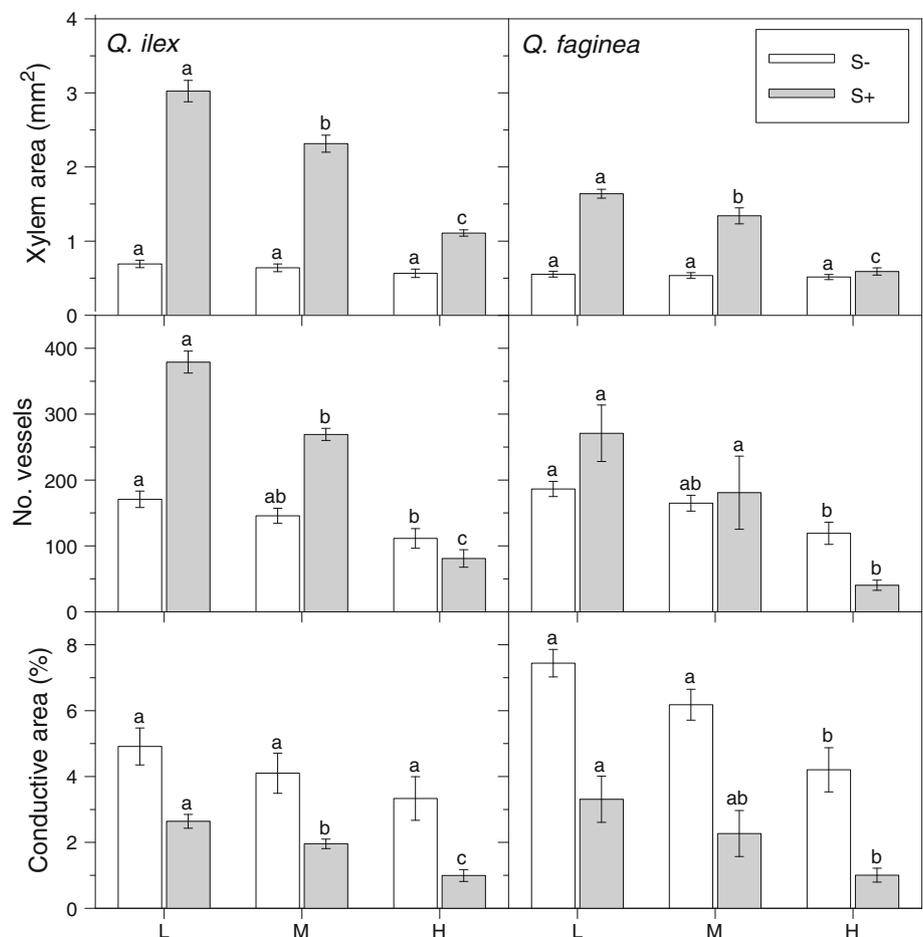
Differences in wood-anatomical features

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, 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

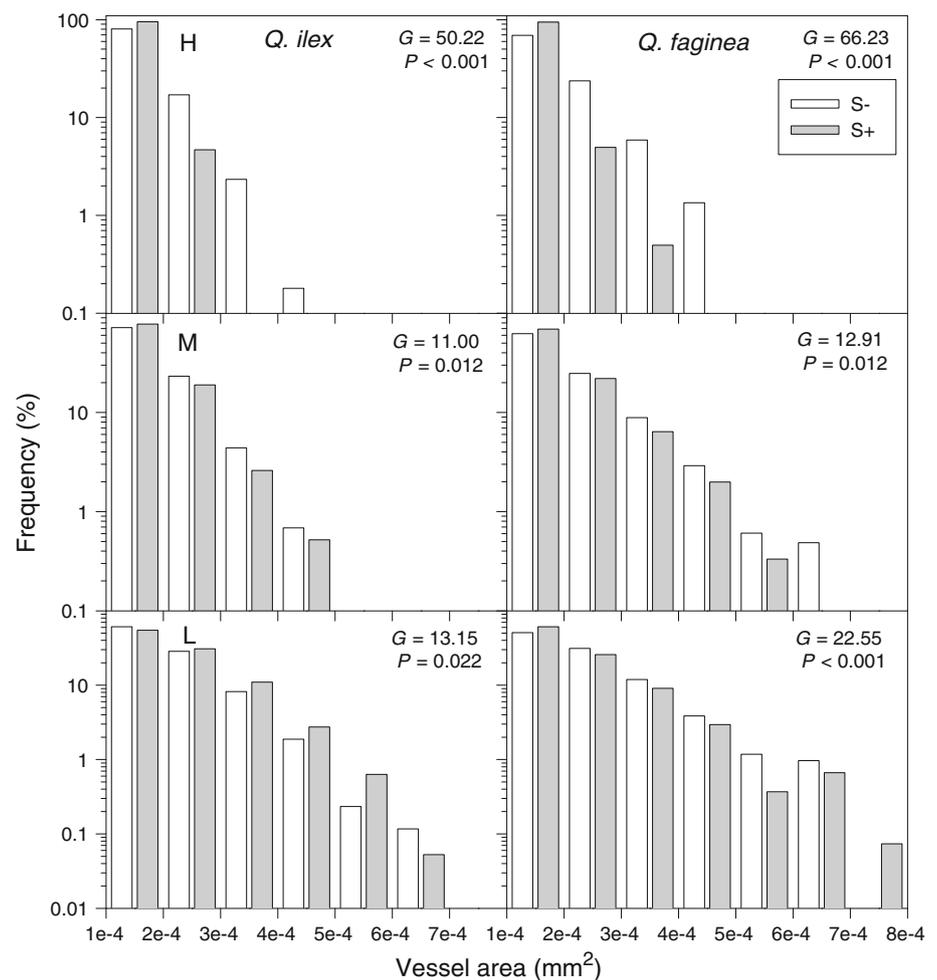
differences were found in pith area among cross-sectional cuts of both shoot types (data not shown). Note that the wood of current-year stems in *Q. faginea* is semi-ring porous.

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).

**Fig. 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



**Fig. 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



## 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*−.

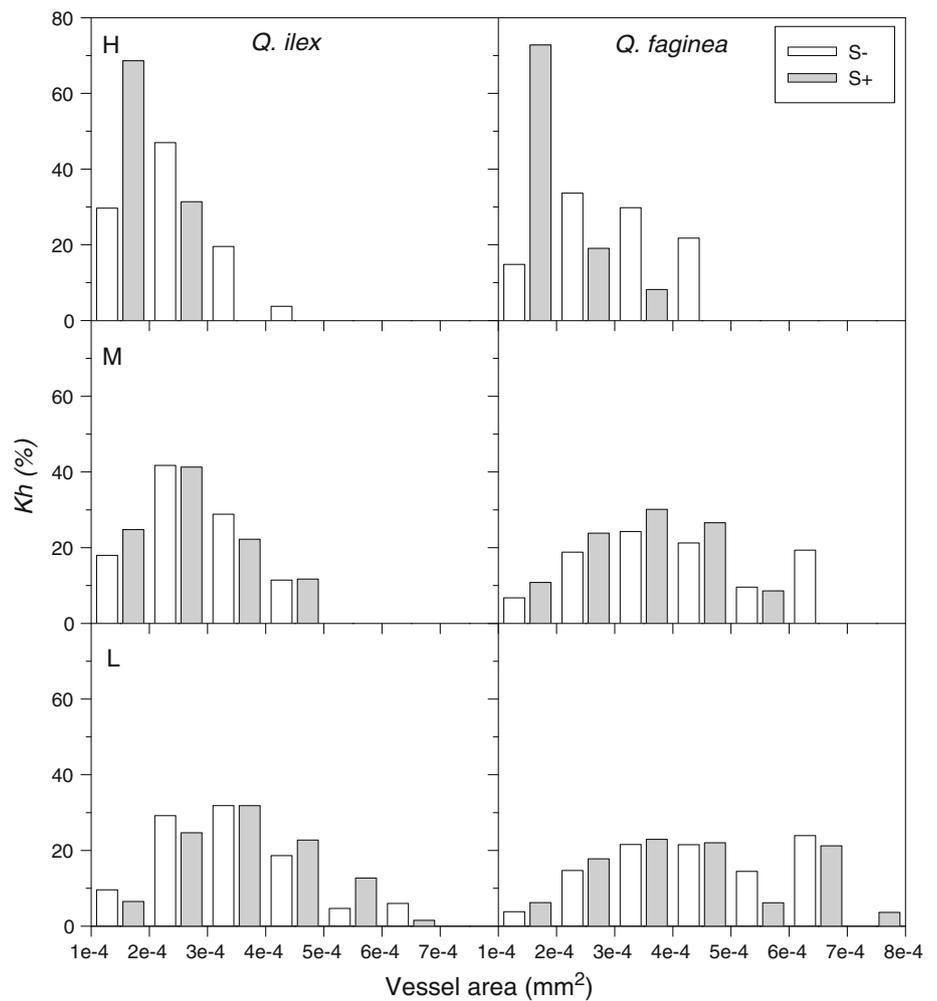
## Discussion

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

**Fig. 4** Distribution of vessels according to their relative contribution to the total predicted hydraulic conductance ( $K_h$ ) 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 ( $\pm$ 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 (%)
<i>Q. ilex</i>	Stem	S-	0.68 $\pm$ 0.02	2.50 $\pm$ 0.21	2.32 $\pm$ 0.11	4.83 $\pm$ 0.23
		S+	0.74 $\pm$ 0.03	2.25 $\pm$ 0.20	2.65 $\pm$ 0.19	4.90 $\pm$ 0.16
		<i>F</i> ( <i>P</i> )	3.32 (0.09)	0.48 (0.50)	2.11 (0.17)	0.13 (0.72)
	Leaves	S-	1.22 $\pm$ 0.03	3.24 $\pm$ 0.18	3.41 $\pm$ 0.07	6.65 $\pm$ 0.20
		S+	1.11 $\pm$ 0.04	3.10 $\pm$ 0.30	3.58 $\pm$ 0.26	6.69 $\pm$ 0.41
		<i>F</i> ( <i>P</i> )	<b>5.08 (0.04)</b>	0.13 (0.73)	0.47 (0.50)	0.01 (0.91)
<i>Q. faginea</i>	Stem	S-	0.69 $\pm$ 0.02	2.78 $\pm$ 0.08	3.97 $\pm$ 0.21	6.75 $\pm$ 0.20
		S+	0.68 $\pm$ 0.02	2.69 $\pm$ 0.12	4.11 $\pm$ 0.19	6.80 $\pm$ 0.22
		<i>F</i> ( <i>P</i> )	0.06 (0.81)	0.40 (0.54)	0.25 (0.62)	0.03 (0.86)
	Leaves	S-	1.48 $\pm$ 0.07	4.59 $\pm$ 0.11	4.84 $\pm$ 0.18	9.43 $\pm$ 0.25
		S+	1.23 $\pm$ 0.07	4.84 $\pm$ 0.11	4.84 $\pm$ 0.32	9.68 $\pm$ 0.35
		<i>F</i> ( <i>P</i> )	<b>6.72 (0.02)</b>	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

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 non-bearing 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. 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.

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## 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 (eds) *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
- Buyse 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<sub>2</sub> 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
- Kozłowski 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, Hiura T, Kato E, Funada R (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 <sup>13</sup>C from reproductive and non-reproductive shoots to fruits in *Styrax obassia*. *Plant Species 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 (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
- Obeso JR (2004) A hierarchical perspective in allocation to reproduction from whole plant to fruit and seed level. *Perspective Plant Ecol Evol Syst* 6:217–225. doi:10.1078/1433-8319-00080
- Palacio S, Maestro M, Montserrat-Martí 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:10.1016/0378-1127(90)90162-5
- Sokal RR, Rohlf FJ (1995) Biometry: the principles and practices of statistical research 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