



Limited plasticity of shoot preformation in response to light by understorey saplings of common walnut (*Juglans regia*)

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Abstract

Background and aims

Analysis of the variability of organogenesis and extension (two basic processes of primary growth) is needed to improve our understanding of architectural plasticity in trees. An example of such plasticity is the difference in size between fully grown shoots found at different positions on the tree. The contribution of preformation processes to these differences was explored by determining the intraspecific variability of shoot preformation of main stems of understorey saplings of *Juglans regia*.

Methodology

In 2007, two samples of annual shoots that differentiated in 2006 were taken from saplings growing in a Mediterranean mixed forest in the south of France. At the first sampling, the terminal winter bud of each parent shoot was dissected and the nodes of these buds were counted. For the second sampling, annual shoot growth was measured after the 2008 growing season. The percentage of light transmitted through the understorey was determined by hemispherical photographs.

Principal results

The effect of plant development (ontogeny) on the number of preformed leaves and the annual shoot extension was strong. Light availability also contributed to explaining differences in the length of annual shoots. The impact of light on the number of preformed leaves was minimal.

Conclusions

Ontogeny (onset of branching) and reduced irradiance both affected leaf preformation in buds of annual shoots, with ontogeny being the more influential. The results suggest that the shoot preformation did not contribute to the plasticity of morphology of annual shoots. Light availability was more influential and promoted annual shoot extension.

Introduction

The composition and dynamics of tree canopies cause spatial and temporal heterogeneity of light availability for understorey vegetation (Valladares and Niinemets 2008). During establishment in the forest, small trees must necessarily cope with shade and wait for an

opportunity, such as that created by a forest gap, to reach the forest canopy (Wright *et al.* 2000). During this early phase, the plant makes physiological compromises to deal with low irradiance levels, but must also retain the ability to respond quickly to any increase in light availability (Henry and Aarssen 2001). The ability of individuals to respond to environmental changes by

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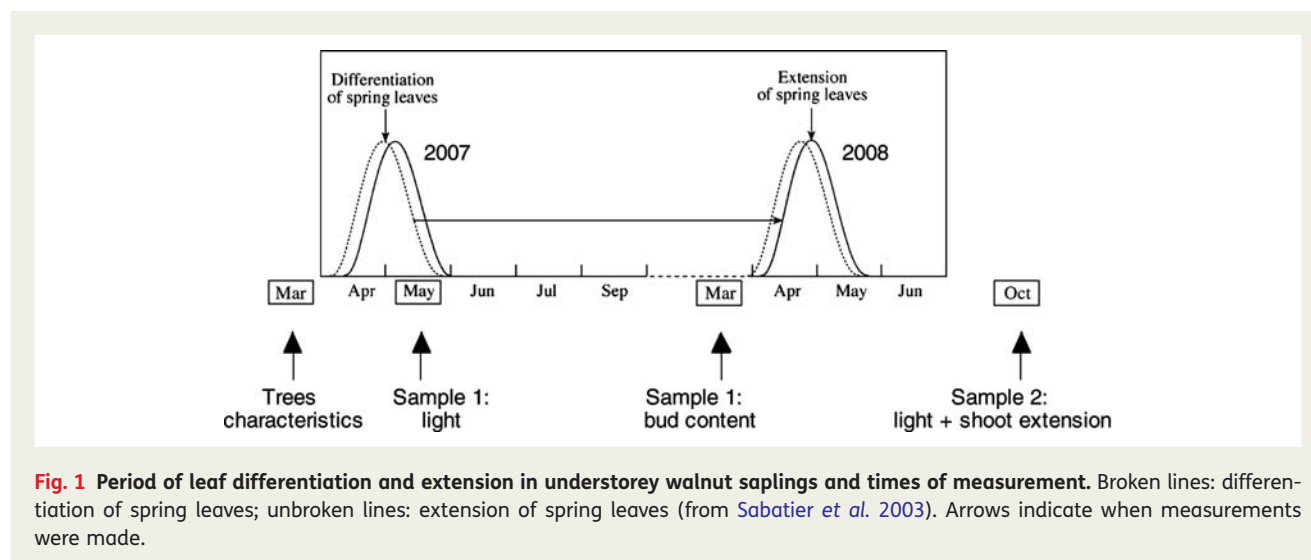


Table 1 Analysis of height, basal diameter, age, number of branches and number of embryonic green leaves in understorey walnut saplings at two separate sampling times. Measurements were made on shoots that emerged in 2008 on saplings that were either branched or unbranched. Sample 1 included terminal buds. Sample 2 included fully extended terminal shoots derived from buds similar to those analysed in Sample 1

	Sample 1		Sample 2	
	Branched (n = 7)	Unbranched (n = 36)	Branched (n = 7)	Unbranched (n = 32)
Height (cm)	269.7 (60.3)b	97.7 (57.5)a	211.1 (66.6)b	83.1 (48.4)a
Diameter (mm)	22.5 (5.4)b	9.8 (4.4)a	19.7 (5.4)b	8.7 (3.6)a
Age (year)	17.6 (5.0)a	10.9 (7.1)a	16.3 (3.7)a	10.9 (6.8)a
Number of branches per tree	4.3 (5.6)a		3.1 (2.3)a	
Number of embryonic green leaves per bud/number of leaves per shoot	9.3 (1.1)b	6.7 (2.0)a	6.9 (1.1)a	5.9 (1.5)a

Means are shown with standard deviations in parentheses. Means sharing a letter within a row are not statistically different (Tukey's test at $P = 0.05$).

adjustments to phenotype (i.e. phenotypic plasticity; Novoplansky 2002) has consequences for plant survival (Sanchez-Gomez *et al.* 2006).

The relationship between shade tolerance and leaf anatomy and morphology is well known (Rozendaal *et al.* 2006). At a higher scale, Delagrangue *et al.* (2006) have shown that crown structure, viewed as the spatial arrangement of leaves, has a large effect on light interception efficiency. The importance of crown architecture in responses to shade is well recognized (Valladares and Niinemets 2008).

To study crown architecture, we used a method that allows identification of the endogenous components of development. These include growth in length and girth, branching, sexuality and reiteration (Barthélémy and Caraglio 2007). Growth by stems is the result of two

complementary processes: node organogenesis and extension (Champagnat *et al.* 1986). For a particular organ, differentiation and extension may take place simultaneously or at different times. Organs that differentiate during one growing flush but extend only after a resting period are described as preformed organs, while organs that differentiate and extend in the same growing flush are described as neoformed organs (Barthélémy and Caraglio 2007). Neoformation is uncommon in temperate species (Kozłowski, 1971) and linked with favourable growth conditions and some architectural constraints due to bud position in the plants (Guédon *et al.* 2006; Puntieri *et al.* 2007). Known variability of shoot preformation is related to the period of bud differentiation (Sabatier *et al.* 2003), the architectural position of the bud (Sabatier and

Barthélémy 2001b; Puntieri *et al.* 2007), the architectural stage of tree development (Puntieri *et al.* 2007) and hydraulic architecture (Cochard *et al.* 2005). The involvement of shoot preformation in the plastic responses of trees is little understood. Gordon *et al.* (2006) reported that, in orchard conditions, light availability within the crown has no effect on the number of leaves preformed each year. It is well known that in the forest understorey, shade changes leaf morphology, anatomy and physiology, and tree architecture in ways that optimize light capture (Valladares and Niinemets 2008; Barthélémy *et al.* 2009). However, few studies have investigated shoot preformation of annual shoots in shaded conditions. Studies of common walnut (*Juglans regia*) indicate that most annual shoots are exclusively preformed in winter buds (Sabatier and Barthélémy 2001b). Visible shoots consist of leaves that became differentiated the previous spring at the time the previous shoot was extending and remain enclosed in buds from summer to early spring the following year (Sabatier *et al.* 2003).

The present study assesses relationships between shoot preformation, tree ontogeny and the light environment in walnut saplings under a mixed forest canopy. We allocated the total variability in size and growth either to differences in light availability or to ontogeny. The work tested effects on bud composition and annual shoot extension.

Materials and methods

Study site

The studied stand is located in a mixed forest near Montpellier (43°40'33"N; 3°51'53"E) in the south of France. Mean stand elevation is 57 m and the climate is typical Mediterranean (e.g. dry summer, rainfall mainly in autumn and spring), with a mean annual cumulative rainfall of 732 mm, an annual mean daily maximum temperature of 20.2 °C and an annual mean daily minimum temperature of 9.4 °C for the period 1977–2006. The forest canopy mainly comprised *Quercus ilex*, *Quercus pubescens*, *Pinus halepensis* and *Tilia platyphyllos*. The understorey consisted of saplings of *Q. ilex*, *Q. pubescens*, *T. platyphyllos*, *Celtis australis*, *Prunus avium*, *J. regia*, *Hedera helix* and *Ruscus aculeatus*. Light transmitted down to the understorey ranged from 5 to 35 % of full sunlight.

Data set

To evaluate shoot preformation in *J. regia* saplings of the understorey, 82 saplings, originally from seedlings, were identified and labelled in March 2007 (Fig. 1). These young trees belonged to the regenerating cohort

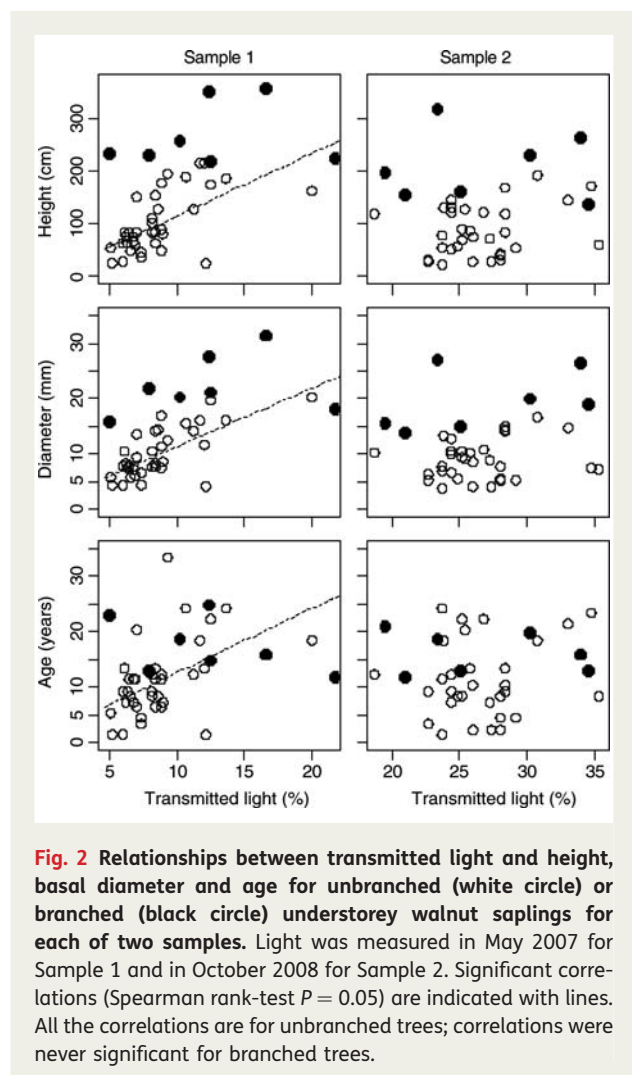
originating from a single mother tree located on the edge of the forest. Individuals were selected at two different architectural stages of development: (i) 'unbranched tree', i.e. without any side shoots, and (ii) 'branched tree' with at least one 1-year-delayed side shoot (defined in Sabatier and Barthélémy 2001a). Tree height and number of branches per sapling were recorded. The basal diameter of the main stem was measured with digital callipers at the level of the cotyledonary node. In walnuts, the limit between two successive annual shoots is marked by a set of short internodes and bud scale leaves or cataphylls (Sabatier and Barthélémy 2001a; Barthélémy and Caraglio 2007). Counting such marks along the main stem indicated the age of the trees. Two samples of trees were randomly selected at the end of winter 2007. The first sampling (43 labelled trees) was destructive and used for the analysis of bud content. The second sampling (39 labelled trees) was used to determine shoot extension.

Sample 1 In March 2008 (Fig. 1), before spring budburst, the terminal bud of each main stem was fixed in 70 % ethanol and dissected using an Olympus SZx9 stereomicroscope at up to $\times 57$ magnification. Bud content and structure were described following Sabatier *et al.* (2003). This extended from bud periphery to the meristematic dome and comprised cataphylls, embryonic green leaves and primordia. Embryonic green leaves were distinguished from cataphylls by the presence of well-differentiated leaflets. Primordia were distinguished from embryonic green leaves by the absence of leaflets. The total number of nodes per bud was obtained by adding together the numbers of cataphylls, embryonic green leaves and primordia.

Sample 2 In October 2008, when shoot growth had ceased (Fig. 1), the length and number of leaves of the annual shoot of the main stem were recorded for each tree.

Light measurements

Light availability in the understorey was quantified by hemispherical photography, a widely used technique for exploring forest structure and understorey light conditions (Kobe and Hogarth 2007). Photographs were taken above the terminal bud of the main stem of each sampled tree using a horizontally levelled digital camera (Coolpix 8400, Nikon) mounted on a tripod and oriented towards the zenith, using a fish-eye lens of 180° field of view (Coolpix FC-E9, Nikon). Photographs were analysed for the percentage of transmitted light from April to October using the program GLA2 of Frazer *et al.* (1999). For Sample 1, photographs of each tree were taken in May 2007 (Fig. 1), at the end of growth unit extension.



The percentage of transmitted light in the understorey forest was estimated during the period when those buds that would generate shoots the following year (2008) were forming. For Sample 2, photographs of each tree were taken in October 2008 (Fig. 1), before leaf fall. The percentage of transmitted light was estimated during the period in 2008 when shoots were extending.

Data analysis

A simple linear model was chosen that describes three quantitative variables (number of embryonic green leaves per bud, number of nodes per bud and length of annual shoots) using the following quantitative dendrometric variables: height, diameter, age and architectural stage of development (i.e. unbranched tree vs. branched tree). The model we selected limited the extent of over-fitting and used analysis of deviance incorporating chi-square testing. Our selection of the most suitable model began

with the most complete model that incorporated height, diameter, age of trees, architectural stage of tree, and interactions between the architectural stage of tree and the three quantitative variables. However, interactions between quantitative variables rendered the model overly complex and generated inconveniently large data sets. Of the three models we compared, the most appropriate one incorporated tree height, architectural stage of development and their interaction.

To assess the effect of percentage of transmitted light on annual growth, the percentage of transmitted light was added as a covariable. In selected models to avoid collinearity the effects were assessed through type I analysis of variance (ANOVA). The tested hypothesis was: does transmitted light have an effect on the quantitative variables of interest after assessing any effect of tree development? Data were analysed with software R (R Development Core Team 2008).

Results

Tree characteristics

Both Sample 1 and Sample 2 had similar values for tree height, stem diameter and age. Branched trees in both samples had similar numbers of branches (Table 1). Branched trees were taller and had thicker stems than unbranched trees. The number of embryonic green leaves per bud and the number of annual-shoot leaves were similar for all samples except for branched trees of Sample 1.

For Sample 1, there were positive correlations between the percentage of transmitted light for unbranched trees and tree height, basal diameter and age (Fig. 2). In Sample 2, there was no clear relationship between tree size characteristics and the percentage of transmitted light. This may be a consequence of the time of year when light measurements were made, this being later in the growing season compared with Sample 1.

Relationships between bud content and size, age of tree or available light

The number of primordia within buds was constant. For unbranched trees, the numbers of embryonic green leaves, cataphylls and nodes per bud were positively correlated with tree height, diameter and age, as well as with the percentage of transmitted light (Fig. 3). The number of nodes in each bud was higher for branched trees than for unbranched trees. For branched trees, no correlation was found between the number of embryonic green leaves or total nodes within the buds and tree height, tree diameter, tree age or the percentage of transmitted light (Fig. 3).

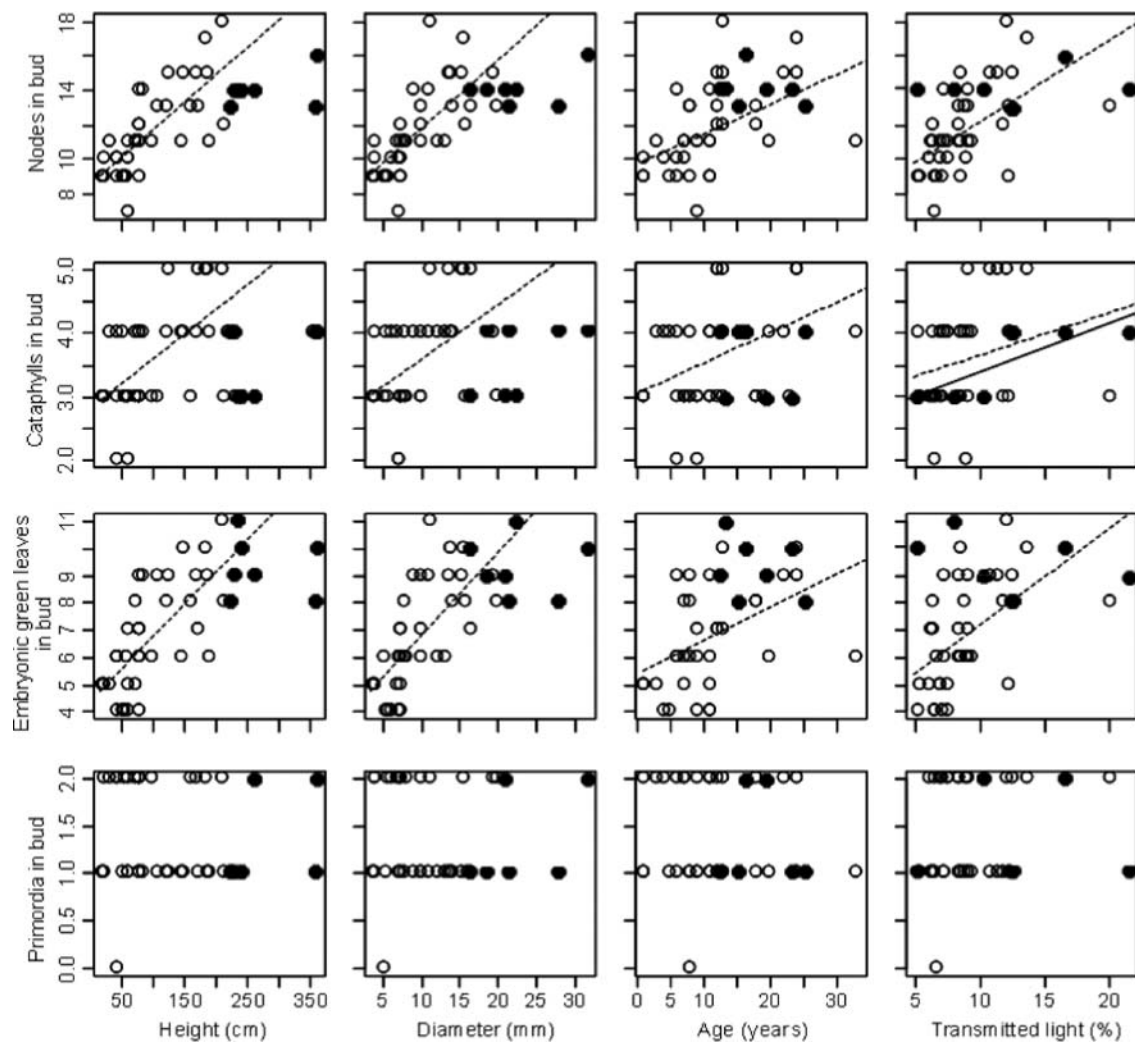


Fig. 3 Relationships between light availability and bud content in understory walnut saplings. Relationships are shown between (A) stem height, basal diameter, age of saplings, transmitted light and (B) number of nodes, cataphylls, embryonic green leaves or primordia in terminal buds of unbranched (open circles) and branched (closed circles) saplings. Dashed lines for unbranched trees and bold lines for branched trees are inserted where significant correlations were detected (Spearman rank-test $P = 0.05$).

Relationships between growth unit length and size, age of trees or available light

Positive correlations were found between the length of the annual shoot derived from 2007-produced buds and height, diameter or age only in unbranched trees. Only in unbranched trees was a positive correlation found between the length of the annual shoot derived from 2007-produced buds and the percentage of transmitted light (Fig. 4).

Light effect on primary growth components

The percentage of transmitted light has no significant effect on the number of nodes per bud ($P = 0.32$) or on

the number of embryonic green leaves per bud ($P = 0.74$). The effect of transmitted light was significantly positive on the length of the annual shoot ($P = 0.03$, Table 2, coefficient estimated = +0.256). Multivariate models (Table 2) highlight the absence of any direct correlation between bud content and transmitted light.

Discussion

Tree ontogeny

The number of leaves on annual shoots was similar to the number of embryonic green leaves found in winter buds. This result confirms that the leaves of annual shoots of

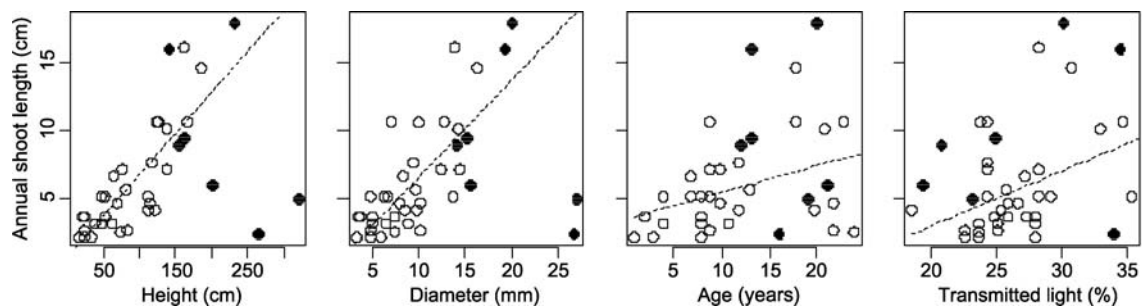


Fig. 4 Relationships between the length of annual shoots and the height, basal diameter, age or percentage of transmitted light for unbranched (open circles) and branched (closed circles) saplings of understorey walnut. Significant correlations (Spearman rank-test $P = 0.05$) are indicated with lines. Only unbranched saplings gave significant correlations.

Table 2 Analysis of variance relating height and architectural stage of development (branched or unbranched) of understorey walnut saplings to number of nodes within buds, number of embryonic leaves within buds and annual shoot length

Source of variation	No. of nodes in bud			No. of embryonic leaves in bud			Annual shoot length		
	Df	F value	P	Df	F value	P	Df	F value	P
Tree height	1	45.51	5.4e-08***	1	42.51	1.1e-07***	1	25.77	1.4e-05***
Architectural stage	1	5.80	0.021*	1	0.84	0.36 ns	1	0.07	0.79 ns
Tree height \times architectural stage	1	4.38	0.043*	1	6.06	0.019*	1	27.33	8.7e-06***
Transmitted light	1	1.01	0.32 ns	1	0.11	0.74 ns	1	5.14	0.030*
Residuals	38			38			34		

Type I ANOVA tested the effects of transmitted light (bold-valued line), knowing the effects of tree height and architectural stage. Degrees of freedom (Df), F-values and level of significance (P) are shown. Significance levels: ns: $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

J. regia trees are all preformed in buds that developed the previous year (Sabatier and Barthélémy 2001b).

For unbranched trees, the number of embryonic green leaves within buds, and to a lesser extent the number of cataphylls, increased with tree height and stem diameter. The number of embryonic green leaves per bud also increased if trees had branched. The results confirm that meristem characteristics change as the plant grows larger and it develops branches (Barthélémy and Caraglio 2007). Thus, during early development, the main stem of the walnut produces more and more leaves each year as the plants increase in size. This effect is linked with increasing capacity to transport assimilates as the amount of sapwood expands stem diameter (Cochard et al. 2005). The basal diameter gives information not only on the conduction capacity of the main stem but also on integrated branch conduction. When the tree becomes branched, the relationship between leaf number and basal diameter of the main

stem changes. The results suggest that the architectural stage of development should be taken into account in the analyses of dimension allometries in trees.

Shoot preformation and shoot length responses to light availability

We found no effect of light availability during the organogenesis period on the number of embryonic green leaves or on the number of nodes per bud. As in *Prunus persica* (Gordon et al. 2006), the extent of preformation in buds of *J. regia* was independent of the proportion of total sunlight reaching shoots during bud organogenesis. Nevertheless, available light may have an indirect and delaying effect on shoot preformation. For example, in *Fagus sylvatica* the light environment has a 1-year-delayed effect on secondary growth (Collet et al. 2001). This, in turn, affects shoot preformation in the following year (Cochard et al. 2005). The delayed effect of the response places an important

limit to the adaptive value of trait plasticity (Valladares *et al.* 2007).

Otherwise, light availability had a positive and immediate effect on annual shoot length. This relation was also found by Osada *et al.* (2004). Annual shoot length is the sum of extension growth by each internode of the shoot. As light has no effect on the number of nodes, only extension seems to contribute to the plastic response of primary growth of walnut to light availability. These results suggested that the walnut saplings respond to an increase of available light with an increased annual shoot length. This can be interpreted as a mechanism for the effective exploitation of recently formed gaps in the forest canopy. It is also the case that leaf morpho-anatomical and physiological properties may also respond strongly to changes in light availability (Portsmuth and Niinemets 2007). This highlights the need to take a multiscale and integrative perspective when studying plant plasticity.

Conclusions and forward look

A positive response of walnut saplings to increasing availability of light (i.e. less shading) strongly affected the amount of annual shoot extension. The number of preformed leaves was not changed by the light regime and therefore was not involved in this phenotypic plastic response. The onset of branching on young saplings markedly increased the number of embryonic green leaves per bud. Analysis of the causality of branching expression will now be needed to understand better the effect of variability of annual shoot morphology on whole tree architecture.

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Contributions by the authors

O.T. carried out the tree growth and light measurement, data analysis and statistical analyses, drafted the manuscript and participated in the sampling protocol. S.A.S. conceived the study and the sampling and measurement protocols, participated in tree growth measurement and helped to draft the manuscript. Both authors read and approved the final manuscript.

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Conflict of interest statement

None declared.

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