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Original article

Effects of relative irradiance on the leaf structure of *Fagus sylvatica* L. seedlings planted in the understory of a *Pinus sylvestris* L. stand after thinning

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Abstract – Beech seedlings were established in the understory of a *Pinus sylvestris* plantation close to one of the southernmost populations of beech in Europe, the beech-oak forest of Montejo de la Sierra. Four years later, the overstory was partially reduced by removing pine trees. Solar radiation in the understory was evaluated by hemispherical canopy photographic technique and the effects of relative irradiance increment on the leaf anatomy of beech seedlings were analyzed during the two years after opening the stand. The increase in specific leaf mass (SLM) in seedlings during both years runs in parallel with the increase in relative irradiance estimated by the global light factor (GLF) which expresses the proportion of global radiation relative to that in the open. There were significant relationships between the light index as a surrogate of light environment and the morphological and anatomical characteristics of the leaves. In the first year, SLM increase was more related to total blade thickness. In the second year, thickness of palisade parenchyma (PP) appears more relevant than that of spongy tissue (SP) as indicated by the absence of significance in the relationship between SP and SLM. Moreover, stomatal density was also higher according to increasing relative irradiance. The shift response of beech seedlings to the overstory opening makes evident their capability of acclimatization to light increase through changes in leaf anatomy.

***Fagus sylvatica* / morphology leaf / hemispherical photography / regeneration / shelterwood**

Résumé – Effets, après éclaircie, de l'irradiation relative sur la structure de la feuille de semis de *Fagus sylvatica* L. plantés sous couvert d'un peuplement de *Pinus sylvestris* L. Des plants de hêtre ont été mis en place sous le couvert d'une plantation de Pin sylvestre localisée près d'une des populations de hêtre la plus méridionale, la hêtraie chênaie de Montejo de la Sierra. Quatre ans après, l'étage dominant a été partiellement réduit au cours d'une éclaircie des pins. La radiation solaire dans le sous étage a été évaluée par la technique de la photographie hémisphérique de la canopée. Les effets de l'accroissement de l'irradiation relative sur l'anatomie de la feuille des plants de hêtre ont été analysés pendant les deux années suivant l'éclaircie. L'accroissement de la masse spécifique de la feuille (SLM) des plants durant les deux années est directement lié à l'augmentation de l'irradiation relative estimée par le coefficient global de lumière (GLF) lequel exprime la proportion d'irradiation relative globale par rapport à la mesure hors couvert. Il y a des relations significatives entre l'indice de lumière pris comme estimateur de l'environnement lumineux et les caractéristiques de la morphologie et de l'anatomie des feuilles. Au cours de la première année, l'augmentation de la SLM était la mieux corrélée avec l'épaisseur totale du limbe. Au cours de la seconde année, l'épaisseur du parenchyme palissadique (PP) apparaît plus pertinente que celle des tissus spongieux (SP) comme l'indique l'absence de signification statistique dans la relation entre SP et SLM. Cependant, la densité des stomates est aussi plus élevée en raison d'une augmentation de l'irradiation relative. Le décalage, dans la réponse des plants de hêtre, à l'ouverture de la canopée démontre la capacité d'acclimatation à une augmentation de lumière par des modifications de l'anatomie de la feuille.

***Fagus sylvatica* / morphologie de la feuille / photographie hémisphérique / régénération / coupe d'abri**

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1. INTRODUCTION

The use of shelterwoods in late successional species regeneration is a necessary requirement in the countries of the Mediterranean basin. This use involves a high plasticity of the species in response to light environment changes, shade to sun acclimatizations allowing seedling recruitment in the understory and a fast response to sudden increase in light as a consequence of the opening of the overstory.

The capability of trees to adapt to environmental variation lies both in their genotypic [4, 24, 40] and phenotypic plasticity [1, 14, 29]. This is expressed in terms of physiological and morphological changes that allow the plant acclimatization to the new conditions [5]. In the case of increase in irradiance as a result of opening the stand, seedlings which would have grown under the trees canopy usually exhibit an increased growth rate [11]. So, in the long term, in forest ecosystems unaffected by great disturbances, shade-tolerant species are favored [2]. Moreover, the condition of beech as a shade-tolerant species [13] is tightly linked to its successional status and to the possibility of recruitment under the shadow of other tree species.

In temperate species with determinate growth and single-flushing, the light environment of the previous year is considered a determining factor in the structural characteristics of the leaf [16]. So, once a bud is formed, any short-term change in leaf anatomy by current-year light conditions should be very restricted. This implies a limitation in the capability for acclimatization in the face of a sudden shift of the daily photonic photosynthetic flux density (PPFD), which may lead to photoinhibition and loss of photosynthetic capability [20, 43]. However, beech seems to show a high acclimatization potential when irradiance increases in the long term, due to its physiological [18, 42] and morphological [41, 45] plasticity. This is particularly effective in forest openings [26].

Changes in leaf anatomy in response to light under controlled conditions have been studied extensively [9, 12], but not so much in natural conditions [17]. In contrast to the anatomical leaf alterations derived from some type of stress (e.g. water stress), morphological changes, as radiation increases, in seedlings previously grown under shadow, are interpreted as an acclimatization process to the new light conditions [14, 21].

Higher specific leaf mass (SLM) is one of the main consequences of increasing irradiance [15, 25] and involves an increase of the photosynthetic rate expressed on a leaf area. The relationship between net photosynthesis and SLM has been shown elsewhere [19, 31, 38]. The SLM increase is a consequence of thickness and density of the leaf lamina [48].

The effect of overstory type on the physiological traits of beech seedlings growing underneath two pine and oak canopies were studied previously (Aranda, unpublished data). Seedling responses were influenced by the interaction of irradiance transmitted by the overstory and water availability. In the present study we investigated the changes produced on leaf anatomy and SLM in underplanted beech seedlings in response to overstory thinning of a *Pinus sylvestris* stand and the subsequent increase in the relative irradiance. A different response to relative irradiance was expected for the two years as leaf primordia experienced different relative irradiances at the stage of bud formation. This may indicate a limited ability in leaf morphological acclimatization potential subject to a change in current-year light environment. This work is part of a broader research project on morphological and physiological changes occurring in beech seedlings induced by the increase of relative irradiance.

2. MATERIALS AND METHODS

In 1994 beech seedlings were planted (2.5 m × 2.5 m) in the understory of a forty-year old plantation of *Pinus sylvestris* L., having 1 015 trees per ha, 55 m^2 ha^{-1} basal area and 18 m dominant height. The plantation was located at Montejo de la Sierra (41º7' N 3º30' W), in the middle of the Iberian Peninsula, at 1 300 m altitude and 15% slope, S-SE orientation. A beech forest, one of the southernmost of the species, was nearby.

At the beginning of 1998, a felling was carried out in a strip of the pinewood. Trees in alternate rows following level lines were cut down, so 50% of the pines were kept. Four situations were considered: C (control), where the original density of trees was maintained; T1, T2 and T3 where beech seedlings could be expected were differently affected in terms of radiation and water availability. *Figures 1a* and *1b* show sectional and ground plan views of beech and pine distribution after the felling. Ten beech seedlings were randomly selected in each situation and used for measurements.

The light environment of every seedling was assessed with the hemispherical canopy photographic technique [6, 39]. A Nikon® FM camera supplied with a Sigma® 8 mm fisheye lens was mounted on a self-levelling

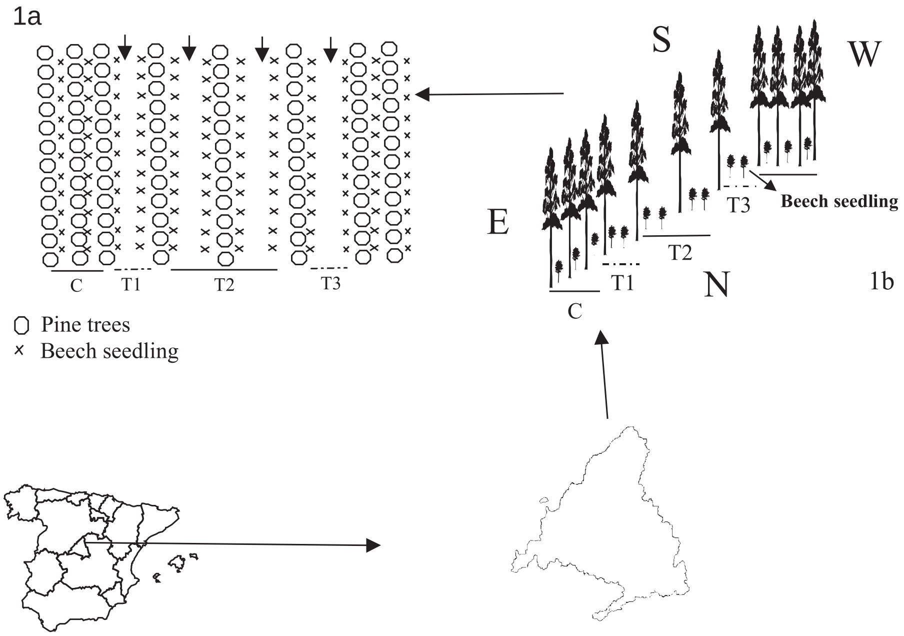

Figure 1. Beech seedling distribution and pine trees left after the cutting: in ground plant (1a) and sectional view (1b). Suppressed pine rows are marked with an arrow. Strips C, T1, T2 and T3 concern to the four situations considered (see text).

camera mount which facilitated photograph acquisition. Photographs were taken in the first hours of the morning, avoiding direct radiation. Afterwards they were digitalized with a scanner (Olympus ES-10, Olympus Optical Co. Europe GMBH) and analysed with the commercial HemiView software (Hemiview 2.1, Canopy Analysis Software, Delta-T Devices Ltd). The parameters calculated were indirect light factor (ILF), direct light factor (DLF) and global light factor (GLF), which express the proportion of indirect, direct and global radiation relative to that in the open. A uniformly overcast sky distribution model was assumed to calculate parameters, with a proportion of 0.1 for the total PPFD above the canopy that is diffuse and an atmosphere transmitivity of 0.8.

Three times in 1998 and 1999, in the evening, leaf discs were taken out from leaves belonging to the first flushing cycle in the middle of the crown. The samples were carried to the laboratory and oven-dried for 48 hours at 70 ºC. The SLM was calculated as a quotient of dry weight to area.

Both years, at the end of July, additional samples from the same seedlings and leaves close to the aforementioned, were taken out, fixed in formaldehyde:acetic acid:water (FAA, 5:5:90) and kept in 70% ethanol until use. Free-hand cross sections (20 μ) were made in the middle of the blade, halfway between midrib and margin. Three sections per leaf were stained with green iodine and Congo red and examined at × 600 with an optical microscope. Total blade thickness, upper and lower epidermis, palisade and spongy parenchyma, were measured using an eye piece micrometer. Epidermal acetate impressions [35] of abaxial surface of the leaves were made and stomata counted in six random fields per sample using a calibrated grid in 1999.

A nested analysis of variance was applied to the study of specific leaf mass with year and treatment as main factors and date nested within year. Anatomical data were analysed with a factorial ANOVA taking year and treatment as main factors. When main factors were significant, a Duncan test ($P < 0.05$) was used to test differences between mean values of treatments (BMDP statistical package, BMDP Statistical Software, Cork Ireland, 1990). The relationship between SLM and anatomical traits was investigated with linear regression models preceded by data transformation when necessary. Because the different index light factors were highly correlated, only the relationship between SLM and global light factor (GLF) is presented.

3. RESULTS

3.1. Light environment

The thinning of the stand led to higher values of global light factor in T2 and T3 (*figure 2*) with respect to C; the value of GLF increased from 0.301 ± 0.017 in C to 0.387 ± 0.016 and 0.372 ± 0.023 in T2 and T3 respectively.

3.2. SLM and leaf anatomy

Both years, differences in SLM were highly significant between treatments (*table I*). SLM was higher for T2 and T3 than for C and T1 in most dates (*table II*). There were no significant differences between years ($P = 0.3555$) and only differences among dates within each year were marginally significant ($P = 0.0951$ in nested ANOVA). This was because on 11 June 1999, SLM was slightly lower than values measured at the end of June and July (*table II*). At the end of July 1999, SLM values for C and T2 were respectively 4.00 ± 0.11 and 5.27 ± 0.16 mg cm^{-2}; for the same date in 1998 they reached respectively 4.00 ± 0.13 and 4.97 ± 0.15 mg cm^{-2}.

Both years, the increase in SLM was positively correlated with the increase in GLF (*figure 3*). When slopes and intercepts of the fitted regression lines for every year were compared, differences were only significant for intercepts ($P = 0.03$). After assuming equality between slopes, intercepts of SLM-GLF relationship were 2.72 and 2.98 in 1998 and 1999 respectively.

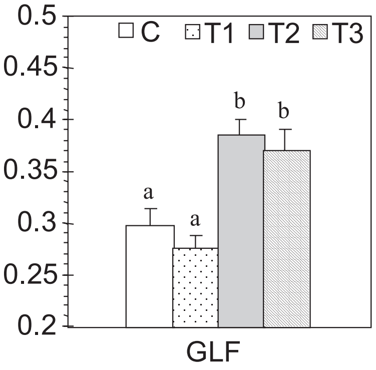

Figure 2. Global light factor as surrogate of irradiance levels for the different treatments after opening the pine plantation in 1998. Statistical differences between situations are marked with different letters ($P < 0.05$).

Table I. Nested anova of SLM, year and treatment taken as main factors.

	d.f.	M.S.	*P*-value
Year	1	0.1818	0.3555
Date (Y)	4	0.4248	0.0951
Treatment	3	13.1492	0.0000
T × Y	3	0.3671	0.1616
T × D(Y)	12	0.1374	0.7998
Residual	230	0.2122	

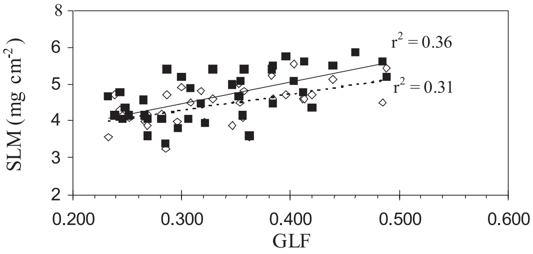

Figure 3. Relationships between SLM (mg cm^{-2}) and GLF (%) in 1999 (continuous line) and 1998 (dotted line). Determination coefficients are marked in the figure. Regressions in both years were established taken all data from the end of July in both years.

Table II. Specific leaf mass (SLM – mg cm^{-2}) for the four treatments and three dates each year (1998 and 1999). Stomatal density is also shown for 1999. Mean values (± s.e.) of ten plants (one leaf each plant).

	1998			1999			
SLM	10 June	11 July	30 July	11 June	30 June	29 July	Stomatal density
Control	4.04 ± 0.14 a	4.05 ± 0.10 a	4.00 ± 0.13 a	4.05 ± 0.14 a	3.86 ± 0.11 a	4.00 ± 0.11 a	217 ± 8 a
T1	4.11 ± 0.21 a	4.29 ± 0.14 a	4.10 ± 0.10 a	4.15 ± 0.13 a	4.35 ± 0.12 b	4.42 ± 0.15 a	231 ± 13 ab
T2	4.86 ± 0.17 b	4.86 ± 0.13 b	4.97 ± 0.15 b	4.92 ± 0.18 b	5.17 ± 0.16 c	5.27 ± 0.16 b	282 ± 14 b
T3	4.78 ± 0.14 b	4.71 ± 0.12 b	4.75 ± 0.14 b	4.36 ± 0.14 a	4.70 ± 0.11 b	4.90 ± 0.18 b	230 ± 10 ab

In 1999 seedlings exhibited a higher stomatal density for T2 than for C, with intermediate values for T1 and T3 (*table II*). No significant differences were found between situations regarding the stomata size, whose mean value was 21 µm.

3.3. Morphology

As a whole, the range of variation for leaf blade thickness was 96.3 ± 2.8 – 115.9 ± 2.57 µm (*figure 4*). In spite of the short range of variation, both years the leaf blade thickness was significantly ($P < 0.05$) higher in T2 and T3 than in C. In 1998 it had an intermediate value for T1 seedlings. There were no significant differences between years (*table III*). Concerning palisade parenchyma (PP), differences were only significant between treatments and an interaction year × treatment was found ($P = 0.0275$). Differences between situations on PP were higher in 1999 than in 1998 (*figure 5*). Differences in spongy parenchyma (SP) were significant as much for the year as for the treatment (*table III*). Nevertheless, differences between treatments were small in 1998 and no significant in 1999. In no year there were statistically significant differences between situations in the lower and upper epidermis thickness ($P > 0.05$).

Both years, there was a positive relationship between SLM and PP ($P = 0.007$ and $P = 0.0008$ for 1998 and 1999 respectively). The relationship SLM and SP was only significant for 1998 (*figure 6*).

In 1999 blade thickness exhibited a positive correlation with GLF, being taken all measurements as a group (*figure 7*). In 1998, only trend of increasing blade thickness with GLF was found ($P > 0.05$).

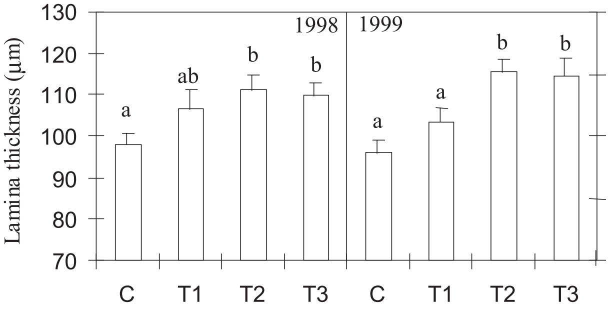

Figure 4. Blade leaf thickness (µm) measured in 1998 and 1999 in samples taken at the end of July ($n = 10$). Significant statistically differences are marked with different letters ($P < 0.05$). Bars denoted average values ± s.e.

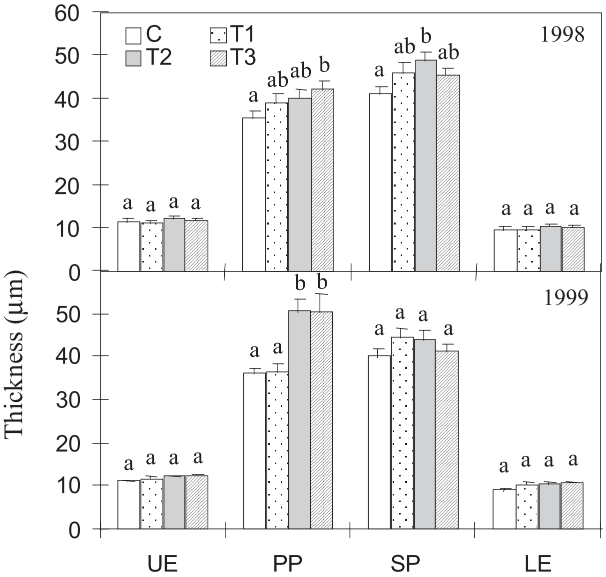

Figure 5. Thickness of the different blade leaf tissues at the four situations in 1998 (upper panel) and 1999 (lower panel); UE – upper epidermis, PP – palisade parenchyma, SP – spongy parenchyma and LE – lower epidermis. Bars (average values ± s.e., $n = 10$) with the same letter were not significantly different.

Table III. Summarised results of two-way ANOVA testing the effect of year and treatment on anatomical parameters. Year and treatment were taken as main factors.

	Lamina thickness			Palisade parenchyma*			Spongy parenchyma*		
	d.f.	M.S.	*P*-value	d.f.	M.S.	P-value	d.f.	M.S.	*P*-value
Year	1	0.0447	0.9820 ns	1	0.00003	0.1307 ns	1	0.000067	0.0047 **
Treatment	3	722.46	0.0001 ***	3	0.00001	0.0001 ***	3	0.000028	0.0170 *
T × Y	3	123.043	0.2498 ns	3	0.00004	0.0275 *	3	0.000044	0.6381 ns
Residual									

* Data of both parenchyma types were transformed before analysis.

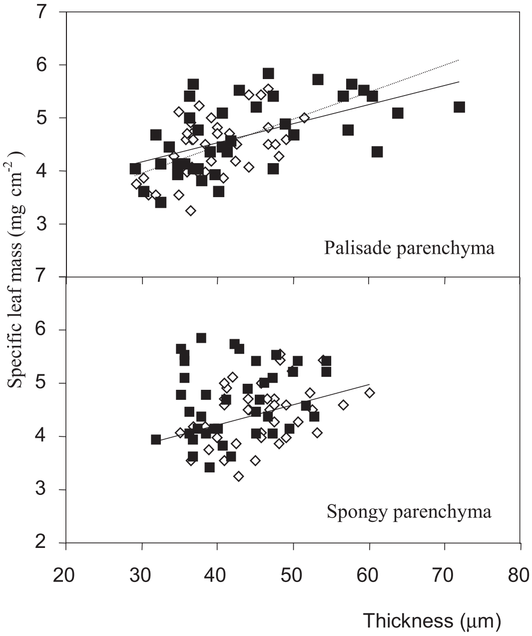

Figure 6. Regression between specific leaf mass (SLM) and palisade (PP) or spongy (SP) parenchyma thickness in 1998 (white points) and 1999 (black points). For 1998 (continuous line) and 1999 (dashed line) the regression equations between SLM and PP were respectively: SLM = 3.10 + 0.036 PP (r^2 = 0.31) and SLM = 2.42 + 0.051 PP (r^2 = 0.25). The relationship between SLM and SP was significant only in 1999: SLM = 2.71 + 0.038 SP (r^2 =0.29).

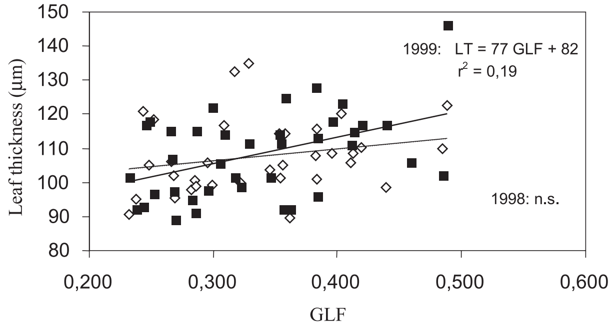

Figure 7. Regression between leaf thickness and GLF. This was only significant (P < 0.05) in 1999.

4. DISCUSSION

Irradiance level, estimated from light index increased in the understory in the two years after the thinning of pine trees. The increase in stomatal density, blade thickness, leaf density and specific leaf mass of beech seedlings revealed a positive correlation with the light environment calculated from GLF [15, 33, 34]. The increase in SLM involved a functional advantage that enabled the plant to acclimatize to the new environment [3, 22, 49]. Moreover, the relation between SLM and photosynthetic capability has been shown elsewhere [38]; it indicates the importance of SLM in the CO_2 assimilation capacity for seedling [22, 36], tree [28] and canopy [15, 37]. Understory beech seedlings at Montejo also experienced photosynthetic rate changes both years after clearing the pine trees (Aranda, unpublished data). The fast acclimatization of beech seedlings to the new light situation after the overstory opening proves the plasticity of the species to irradiance changes. Acclimatization to increasing light in terms of changes in morphological [27] and physiological leaf traits [18] has a direct consequence in survival [30, 32] and growth of beech seedlings [45, 46, 47].

In the present study the SLM differences among light environments were shown within the same year of pine felling. Although the year factor was not significant for SLM, the differences found among treatments were brought about by different anatomical adjustments according to the year. Changes in SLM may be linked to blade thickness and density changes, or to both [48]. In the second year, a higher SLM under the two situations under the highest irradiances was linked to the increase in the thickness of palisade parenchyma, as only the PP-SLM relationship was significant. This involves an increase in leaf density for T3 and T4 seedlings, as cells are more densely packed. Furthermore, some leaf samples in T2 and T3 showed two palisade layers in the second year. In some instances, fully differentiated leaves can acclimatize to new light environment through reorganization of leaf anatomy [7, 20]. However, a significant "carry over" effect on leaf morphology from previous light environment has been described [10, 36, 44]. Data reported in the present study show lower anatomical response to the new light environment in the first year after overstory felling. A higher intercept in the relationship SLM-GLF in 1999, and more significantly higher PP development in 1999 than in 1998 for seedlings growing in the highest light environment, may be interpreted as if there was a better adjustment to the new environmental conditions the second year after pine thinning This would be in accordance with the "carry over" effect, presumably as a consequence of the determinism of leaf differentiation in the year of bud formation [23].

Eschrich et al. (1989) showed that in *Fagus sylvatica* the differentiation of sun versus shade leaves takes place at the end of July and the capability for any further structural change is very limited. The number of palisade parenchyma layers is determined in the winter buds. Further, Thiébaut et al. (1990) showed that the light environment previous to leaf development was a determinant of leaf anatomy and observed changes in leaf morphology depending on light intensity and flush cycle. In contrast, for Kimura et al. (1998) leaf properties in *Fagus japonica* were determined by current-year PPFD, suggesting a trade-off between differentiation of shade and sun leaves and plasticity of the palisade parenchyma. Nevertheless, it should be recognized that at present, between-year differences being only in anatomical traits, these lead to misinterpretation of results.

In summary, results make evident that beech seedlings are able to acclimate to new light conditions generated by opening the overstory canopy. This acclimatization is acquired through changes in the morphology (present results), and also in the physiology (Aranda, unpublished data). It makes possible to plan the use of early successional species (e.g. pines) as protective cover for planting late successional species in forest restoration (e.g. beech) and generation of mixed species stands. Further silvicultural practices will enable the manipulation of beech seedlings in the understory and shorten the time in the ecological succession [8]. As a whole, this kind of approach will benefit forest management improving stand modelling in accordance with the temperament of species.

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