Fernando Valladares · José Manuel Chico
Ismael Aranda · Luis Balaguer · Pierre Dizengremel
Esteban Manrique · Erwin Dreyer

The greater seedling high-light tolerance of *Quercus robur* over *Fagus sylvatica* is linked to a greater physiological plasticity

Abstract The responses of *Quercus robur* (oak) and *Fagus sylvatica* (beech) seedlings to four different light environments (full, 50%, 40% and 15% sunlight) and to a rapid increase in irradiance were explored during the summer, after 2 years of growth in a forest nursery at Nancy (France). Significant differences between the two species were found for most variables. Phenotypic plasticity for morphological variables (root-shoot ratio, leaf size, leaf weight ratio) was higher in beech than in oak, while the reverse was true for anatomical (stomatal density, epidermis thickness, exchange surface area of the palisade parenchyma) and physiological (maximum photosynthetic rate, stomatal conductance, Rubisco activity) variables. Predawn photochemical efficiency (F_v/F_m) was higher in oak than in beech in all light environments except in 15% sunlight. F_v/F_m was significantly lower in 100% sunlight than in the other light environments in beech but not in oak. Maximum photosynthetic rates (A_{max}) increased with increasing light availability in the two species but they were always higher in oak than in beech. Oak exhibited higher Rubisco activity than beech in full sunlight. The transfer of shade-adapted seedlings to the open caused a decrease of F_v/F_m, which was larger for beech than for oak. Transferred oak but not beech plants recovered gradually to the control F_v/F_m values. The decreased chlorophyll content and the increased non-photochemical quenching observed in high-light beech seedlings were not enough to avoid photoinhibition. The results suggest that a greater tolerance of strong irradiance is linked to an enhanced physiological plasticity (variables related to photosynthesis), while shade tolerance relies on an enhanced plasticity in light-harvesting variables (crown morphology and chlorophyll content).

Keywords Light acclimation · Phenotypic plasticity · Photoinhibition · Photosynthesis · Sun and shade

F. Valladares (✉) · E. Manrique
Centro de Ciencias Medioambientales,
CSIC, Serrano 115 dpdo, 28006 Madrid, Spain
e-mail: valladares@ccma.csic.es
Tel.: +34-91-7452500 ext 1287, Fax: +34-91-5640800

I. Aranda
Unidad de Anatomía, Fisiología y Genética Forestal,
Escuela Técnica Superior de Ingenieros de Montes,
Universidad Politécnica, 28040 Madrid, Spain

L. Balaguer
Departamento de Biología Vegetal I. Facultad de Biología,
Universidad Complutense, 28040 Madrid, Spain

P. Dizengremel
Unité Mixte de Recherches INRA-UHP,
Ecologie et Ecophysiologie Forestières,
54506 Vandoeuvre les Nancy Cedex, France

J.M. Chico · E. Dreyer
Unité Mixte de Recherches INRA-UHP,
Ecologie et Ecophysiologie Forestières,
54280 Champenoux, France

Introduction

Full sunlight can be detrimental for photosynthesis but deep shade also limits plant growth and survival. Since mechanisms to cope with full sunlight significantly differ from those to cope with shade, plants must either specialise to one end of this environmental gradient or enhance their capacity to accommodate their phenotypes to the light currently available at each particular site (Valladares 1999, 2000). Plant response to the light environment ranges from an immediate, primarily physiological acclimation to a long-term adjustment that renders a differentiated phenotype (Schlichting 1986; Valladares and Pearcy 1997; Silvertown 1998). Light in forests is very dynamic and forest species segregate not only according to their different light requirements but also according to their different acclimation capacity (Brzeziecki and Kienast 1994 and references therein). A comparative study of the responses of oaks to shade showed that variation in shade acclimation could partially explain interspecific differences in interactions with other species, patterns of recruitment and successional roles (Callaway 1992). Early successional, high-light species were shown to be more plastic than late successional, shade-tolerant species (Strauss-Debenedetti and

Bazzaz 1991, 1996; Bazzaz 1996), although exceptions to this rule have also been found (e.g. Chazdon 1992; Abrams and Mostoller 1995).

Most studies of plant response to light have been aimed at unveiling the morphological and physiological mechanisms and the ecological implications of the tolerance to the extremes, i.e. tolerance to either sun or shade, but noticeably less effort has been invested in the exploration of trends in the plastic response to light (Valladares 2000; Valladares et al. 2000a, b). Pedunculate oak (*Quercus robur*) and beech (*Fagus sylvatica*), two important European forest trees, are a case in point. Even though numerous studies have addressed their tolerance and response to shade and/or intense light (Tognetti et al. 1994, 1997, 1998; Minotta and Pinzauti 1996; Johnson et al. 1997; Cochard et al. 1999), according to our knowledge their relative plasticity has only been compared in one study of the morphology and growth of seedlings (van Hees 1997). Despite the fact that at the seedling stage beech is one of the most shade-tolerant trees in Europe, undoubtedly more shade-tolerant than pedunculate oak (Brzeziecki and Kienast 1994; Welander and Ottosson 1998), the comparison of van Hees (1997) showed a rather similar plastic response to light for the morphological traits studied. Was this lack of differences in the plasticity of these two species an exception to the trend that shade tolerance is linked to low plasticity? Could the plastic response to light differ between the two species for other traits not compared in this study?

Light response of oak and beech is of particular interest because regeneration of these species, either natural or via silvicultural practices, is influenced by their shade tolerance and by inter- and intraspecific competition for light (Newbold and Goldsmith 1990; Emborg 1998; von Lüpke 1998; Welander and Ottosson 1998, 2000). However, some studies contradict expectations. Despite the higher shade tolerance of beech, first-year seedlings of oak and beech seem to be equally capable of survival and growth under very low light conditions and both could regenerate under a dense canopy (Welander and Ottosson 1998). Shade-grown leaves and plants of beech were found to acclimate effectively to full sunlight conditions, showing few symptoms of photoinhibition or damage by excessive light (Johnson et al. 1997; Tognetti et al. 1997, 1998). These somewhat unexpected results can be due to a number of reasons: first, the response of a species to the light environment is a complex function of a large number of traits, which in turn vary in importance throughout the ontogeny (Henry and Aarssen 1997; Welander and Ottosson 1998); second, plant performance can be influenced not only by current but also by previous light conditions (Ziegenhagen and Kausch 1995; Tognetti et al. 1998; Welander and Ottosson 1998; Aranda et al. 2002); third, comparative studies of the two species under the very same environmental conditions and experimental settings are scarce, which makes comparisons less accurate (Newbold and Goldsmith 1990; Welander and Ottosson 1998).

In the present work, we explored the response to four different light levels of seedlings of pedunculate oak and beech grown under the same environmental conditions. In addition, shade-grown plants of the two species were exposed to a sudden increase in available light to explore short-term responses of an already developed phenotype. Seedlings were allowed to grow for 2 years to avoid interference with the resources of the acorn, which have been shown to enhance growth under low-light especially in oak seedlings (Grime and Jeffrey 1965; Sonesson 1994). Special emphasis was placed on the plasticity of physiological traits (gas exchange, in vivo chlorophyll fluorescence of PSII, Rubisco activity), since plasticity of morphological and growth features was already addressed in previous work (van Hees 1997). According to our comparative studies of other woody plants (Valladares et al. 2000a, b; Balaguer et al. 2001), we expected lower plasticity in the speciesw of deep shade (beech) than in the more light-demanding one (pedunculate oak). However, an alternative hypothesis, i.e. that shade tolerance can be achieved by enhanced plasticity, was also feasible, as argued previously for the two species studied here (van Hees 1997). Exploring the plasticity for a wide range of morphological and physiological traits could not only validate emerging trends in the phenotypic plasticity of plants but also clarify the mechanisms involved in the survival at the extremes of environmental gradients.

Materials and methods

Plant material and experimental design

Seeds of pedunculate oak (*Quercus robur* L.) and European beech (*Fagus sylvatica* L.) were collected in autumn 1992 and transplanted to 40-l containers in spring 1993. Seeds were collected in the Plateau of Lorraine, Eastern France. Seedlings were grown outdoors for 2 years in the nursery of INRA, Nancy, France. Growth medium was a mixture of peat-sand (1:1 v/v) supplied with slow-release fertiliser (Nutricote) containing N:P:K (13:13:13) and oligoelements at 4 g l^{-1} substrate (40 g per seedling). Eighty plants per species were distributed at random in four different light environments produced using increasing layers of neutral shade cloth. One group of plants was grown in the open and the other three groups were grown under 50, 40 and 15% of full sunlight (Table 1). Air temperature did not differ significantly among the different light environments (Table 1). Plants were watered every other day to full soil capacity. At the beginning of the summer of 1994, half of the plants from each light environment were transferred to the open to elicit short-term responses to a sudden increase in light availability.

Plant morphology, leaf anatomy and chlorophyll content

A minimum of ten plants of each species and treatment were harvested at the end of the experiment and separated into leaves, stems and roots for leaf surface area and dry weight determinations. With these data, root-shoot ratio, leaf size, leaf weight ratio (LWR), leaf area ratio (LAR) and specific leaf area (SLA) were calculated. The thickness of the leaf blade and its tissues (epidermis, spongy and palisade parenchyma), the relative exchange surface area of the palisade parenchyma (surface area of the cell walls of this layer divided by the surface area of one side of the

leaf), and the stomatal density and length were measured in fresh material with a light microscope. Total chlorophyll content was determined spectrophotometrically by incubating leaf fragments in dimethyl sulfoxide for 2 h in the dark at 65°C following the protocols of Barnes et al. (1992).

Chlorophyll fluorescence and gas exchange measurements

In vivo Chl a fluorescence signals of ten plants per species and treatment were measured at predawn and at midday with a portable fluorimeter (PAM-2000, Walz, Effeltrich, Germany) equipped with a leaf-clip holder which monitored incident photosynthetic photon flux density (PFD) and leaf temperature. Measurements were carried out on fully developed leaves from the upper third part of the crown. Maximal (F_m) and minimal (F_o) fluorescence were measured at predawn to calculate the maximal photochemical efficiency of PSII (F_v/F_m; $F_v=F_m-F_o$). For quenching analysis, minimal fluorescence yield of a pre-illuminated sample (F_o') was assessed in leaves darkened immediately after every saturation pulse and subsequently exposed to far-red light for 5.5 s. Non-photochemical quenching (NPQ=$[F_m-F_m']/F_m'$; F_m', the maximal fluorescence under ambient light conditions), quantum yield of noncyclic electron transport ($\Phi_{PSII}=[F_m'-F_t]/F_m'$, where F_t is the fluorescence under ambient light), and photochemical efficiency of the open reaction centres of PSII (F_v'/F_m'; $F_v'=F_m'-F_o'$) were measured at midday ± 1 h. Fluorescence parameters were estimated according to standard protocols (Genty et al. 1989; Maxwell and Johnson 2000). Leaf gas exchange parameters, namely maximum net photosynthetic rate (A_{max}) and stomatal conductance to water vapour (g_{H2O}), were measured during the morning hours with a portable IRGA (Li-6200, Li-Cor). Incident PFD and leaf temperature were recorded simultaneously. PFD values at the time of the measurements were always saturating, and leaf temperature inside the cuvette never exceeded the external air temperature by >3°C.

Rubisco extraction and activity

The activity of ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) was determined for eight full-sunlight plants and eight 15% sunlight plants of each species at two times during the day (dawn and midday). Leaf material (ca. 5 g) was cleaned and homogenised. Proteins were extracted in 100 mM HEPES-KOH pH 7.5, 6.6% (w/w) polyvinyl pyrrolidone (PVP 25), 7% (w/w) polyethylene glycol (PEG 20), 2 mM dithiotreitol (DTT), 10% (v/v) glycerol, 5 mM $MgCl_2$, 5 mM EGTA, 1 mM phenyl-methylsulphonyfluoride (PMSF), 20 μM amidinophenylmethanesulfonylfluoride (APMSF), 1 μM leupeptin and 1 μM pepstatin. The homogenate was centrifuged for 25 min at 30,000 g and 4°C. The supernatant was used for determination of enzyme activities following Pelloux et al. (2001). Enzyme purification was carried out according to Afif et al. (1993), and protein concentration was estimated spectrophotometrically after Paulsen and Lane (1966). Initial and final carboxylase activity were measured as described in Ward and Keys (1989) and Pelloux et al. (2001). The state of activation of Rubisco was estimated from initial activity and expressed as a percentage of total activity.

Table 1 Maximum and minimum photosynthetic photon flux density (*PFD*, μmol m^{-2} s^{-1}) and air temperature (°C) during the measurements (midday ± 1 h, summer 1994) in the four different light environments (% of full sunlight) for the two species studied. Data are the means ± SD of 7 days

Light environment	Quercus robur		Fagus sylvatica	
	PFD (max–min)	Temperature (max–min)	PFD (max–min)	Temperature (max–min)
Open (full sunlight)	1536±35–1263±67	33.1±0.3–23.5±0.2	1437±92–724±29	34.2±0.4–28.4±0.8
50%	772±15–537±79	34.7±0.2–24.2±0.1	812±112–457±24	33.3±0.3–28.6±0.4
40%	630±22–524±71	34.1±0.1–23.9±0.1	740±15–240±21	33.1±0.8–30.2±0.1
15%	219±33–151±29	33.8±0.1–23.9±0.1	234±15–80±5	33.8±0.1–29.7±0.1

Table 2 Morphological variables for the two species studied: root-shoot ratio, leaf size (cm²), leaf weight ratio (g g⁻¹), leaf area ratio (m² kg⁻¹), and specific leaf area (m² kg⁻¹). Data are the means ± SD of ten plants. Letter code indicates significant differences (ANOVA, $P<0.05$) among treatments within each species; an asterisk in the light environment (% of full sunlight) indicates significant differences between the two species

Variable	Light environment	Species	
		Quercus robur	Fagus sylvatica
Root-shoot ratio	Open (full sunlight)	1.14±0.03 a	1.28±0.08 a
	50%*	1.04±0.05 a, b	0.84±0.07 b
	40%*	0.98±0.05 b	0.79±0.07 b
	15%*	1.11±0.05 a	0.69±0.03 c
Leaf size	Open (full sunlight)*	15.0±2.7 a	9.6±3.0 a
	50%*	16.8±3.8 a, b	8.9±5.3 a
	40%*	20.2±6.1 b	8.0±3.3 a
	15%*	15.6±3.6 a	4.1±1.4 b
Leaf weight ratio	Open (full sunlight)*	0.159±0.016 a	0.073±0.015 a
	50%*	0.148±0.026 a	0.093±0.022 b
	40%*	0.157±0.025 a	0.083±0.014 a, b
	15%*	0.162±0.027 a	0.072±0.014 a
Leaf area ratio	Open (full sunlight)*	2.09±0.49 a	1.26±0.46 a
	50%*	2.14±0.59 a	1.52±0.59 a
	40%	2.37±1.14 a	2.04±1.00 b
	15%*	3.22±1.37 b	2.10±0.90 b
Specific leaf area	Open (full sunlight)*	13.24±1.63 a	17.25±1.79 a
	50%*	14.77±1.02 a, b	18.41±1.89 a
	40%*	15.52±0.90 b	23.98±2.52 b
	15%*	20.12±2.66 c	28.80±2.43 c

Fig. 1 Time course of the photochemical efficiency (F_v/F_m) of pedunculate oak and beech plants under four different light environments during the summer of 1994. *Closed symbols* indicate plants kept all the time in the corresponding light environment, *open symbols* indicate plants transferred on day 0 to the open (full sunlight). Data points are the means ± SD of 10 plants
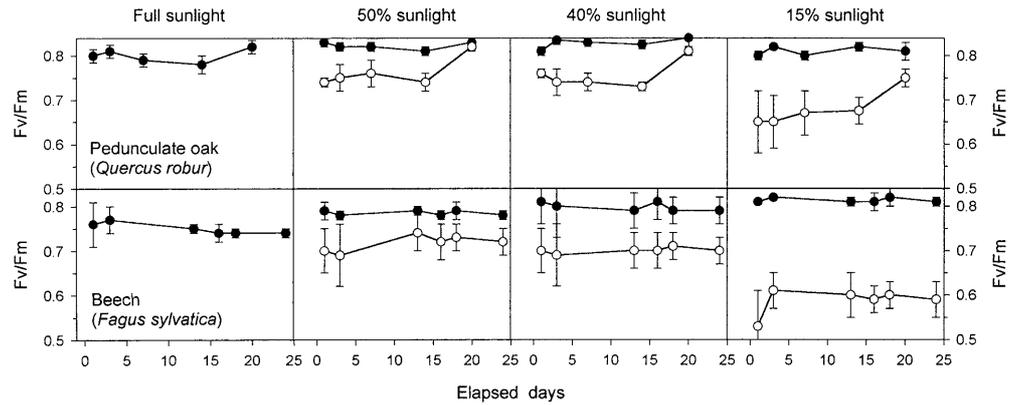
Table 3 Leaf anatomy variables for the two species studied: stomatal density (mm^{-2}), stomatal length (µm), leaf blade thickness (mm), epidermis thickness (% of leaf thickness), palisade parenchyma thickness (% of leaf thickness), and exchange surface area of the palisade parenchyma (m^2 per m^2 of one side of the leaf). Data are the means ± SD of ten plants. Letter code indicates significant differences (ANOVA, $P<0.05$) among treatments within each species; an asterisk in the light environment (% of full sunlight) indicates significant differences between the two species

Variable	Light environment	Species	
		Quercus robur	*Fagus sylvatica*
Stomatal density	Open (full sunlight)*	596.1±100.3 a	434.7±48.0 a
	50%*	612.7±147.7 a	413.6±87.9 a, b
	40%*	481.0±121.6 b	381.0±117.3 b
	15%	367.5±82.7 c	310.5±81.9 c
Stomatal length	Open (full sunlight)*	24.8±1.3 a, b	21.8±1.1 a
	50%*	23.8±0.8 a	21.6±2.0 a
	40%*	25.7±1.6 b	20.1±0.6 b
	15%*	22.8±1.5 c	20.8±1.1 a
Leaf thickness	Open (full sunlight)*	142.6±13.6 a	94.8±9.5 a
	50%*	139.3±23.4 a	90.6±11.4 a
	40%*	129.1±15.7 a	75.6±13.6 b
	15%*	92.2±11.6 b	72.5±8.7 b
Epidermis thickness	Open (full sunlight)	17.1±2.0 a	19.8±2.3 a
	50%*	15.6±2.4 a	20.6±2.7 a
	40%	18.7±1.7 a	21.9±4.8 a
	15%	22.6±6.5 a	22.9±3.5 a
Palisade parenchyma	Open (full sunlight)*	38.2±6.1 a	34.9±4.0 a, b
	50%*	38.1±5.4 a	38.0±3.0 a
	40%	39.0±4.6 a	32.8±3.6 b
	15%*	37.9±5.4 a	35.5±4.0 a, b
Exchange surface area of palisade parenchyma	Open (full sunlight)*	21.7±2.1 a	17.2±0.9 a
	50%*	21.6±3.9 a	16.6±1.3 a, b
	40%*	17.8±3.2 b	15.0±2.6 b
	15%	12.8±1.9 c	12.0±1.4 c

Plasticity index and statistical analyses

An index of phenotypic plasticity ranging from 0 to 1 was calculated for each variable and species as the difference between the minimum and the maximum mean values among the four light treatments divided by the maximum mean value, as in previous studies (Valladares et al. 2000a, b; Balaguer et al. 2001). This index was calculated for gas exchange, fluorescence and Rubisco responses to PFD and it has the advantage that changes in variables expressed in different units can be compared. Two-way ANOVA was used to test for differences between the two species, light treatments and interactions with Systat (SPSS Inc. Chicago).

Results

The tallest plants (1.5–2 m) of the two species studied were found in 50% of sunlight, followed by those in full sunlight (1.5 m), 40% sunlight (1 m) and 15% sunlight (<1 m). Root-shoot ratio increased with increasing light availability and was higher in oak than in beech

(Table 2). LWR and LAR were also higher in oak than in beech, while the reverse was true for SLA. LAR and SLA increased with decreasing light availability in the two species while LWR remained constant across the treatments (Table 2). Stomatal density and leaf thickness were higher in oak than in beech and decreased with decreasing light availability in the two species (Table 3). The relative exchange surface area of the palisade parenchyma also decreased with decreasing light availability and was higher in oak than in beech for most light treatments (Table 3).

Predawn photochemical efficiency (F_v/F_m) was higher in oak than in beech in all light environments (Fig. 1, Table 4). While F_v/F_m did not vary across light environments in oak, it was significantly lower in full sunlight than in the other light environments in the case of beech. Midday quantum yield under ambient PFD ($\Phi_{PS\ II}$) decreased with increasing PFD in both species; $\Phi_{PS\ II}$ was significantly higher in oak than in beech in full sunlight

Fig. 2 Time course of the maximum net photosynthetic rate (A_{max}) of pedunculate oak and beech plants under four different light environments during the summer of 1994. *Closed symbols* indicate plants kept all the time in the corresponding light environment, *open symbols* indicate plants transferred on day 0 to the open (full sunlight). Data points are the means ± SD of ten plants
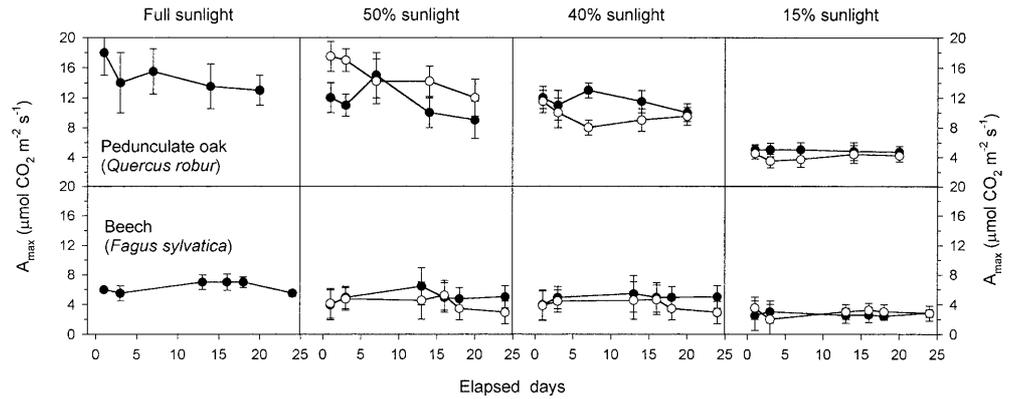
Table 4 Fluorescence and gas exchange variables in the four different light environments (% of full sunlight) for the two species studied during the early summer of 1994. The variables are: photochemical efficiency (F_v/F_m), quantum yield under ambient light ($\Phi_{PS\ II}$), photochemical efficiency of the open reaction centres (F_v'/F_m'), photochemical quenching (qP), non-photochemical quenching (NPQ), and maximum photosynthetic rate (A_{max}, μmol CO_2 m^{-2} s^{-1}). Data are the means ± SD of ten plants for the different days throughout the month of measurements. Letter code indicates significant differences (ANOVA, Tukey test, $P<0.05$) among treatments within each species. An asterisk in the light environment indicates significant differences (ANOVA, Tukey test, $P<0.05$) between the two species

Variable	Light environment	Species	
		Quercus robur	*Fagus sylvatica*
F_v/F_m	Open (full sunlight)*	0.80±0.03 a	0.75±0.03 a
	50%*	0.82±0.01 a	0.78±0.01 b
	40%*	0.82±0.02 a	0.78±0.01 b
	15%	0.81±0.02 a	0.79±0.01 b
$\Phi_{PS\ II}$	Open (full sunlight)*	0.27±0.12 a	0.19±0.09 a
	50%*	0.64±0.10 b	0.34±0.13 b
	40%	0.61±0.11 b	0.55±0.11 c
	15%	0.68±0.05 b	0.62±0.07 c
F_v'/F_m'	Open (full sunlight)*	0.44±0.05 a	0.28±0.13 a
	50%*	0.71±0.06 b	0.49±0.05 b
	40%*	0.71±0.06 b	0.63±0.04 c
	15%	0.74±0.009 b	0.69±0.04 c
qP	Open (full sunlight)*	0.63±0.21 a	0.50±0.17 a
	50%*	0.89±0.11 b	0.61±0.22 a
	40%	0.86±0.12 b	0.84±0.11 b
	15%	0.93±0.08 b	0.88±0.08 b
NPQ	Open (full sunlight)	2.74±1.50 a	3.29±1.33 a
	50%	1.11±0.77 b	1.73±0.90 b
	40%	1.10±0.57 b	0.94±0.43 b
	15%	0.60±0.18 b	0.70±0.19 b
A_{max}	Open (full sunlight)*	14.30±2.45 a	6.67±0.76 a
	50%*	11.29±3.62 b	6.19±0.74 a
	40%*	11.19±2.51 b	6.93±0.67 a
	15%*	5.23±1.10 c	3.20±0.51 b

the open reaction centres (F_v'/F_m') and for the photochemical quenching (qP): higher values were found in oak than in beech in full sunlight and 50% sunlight, and there were no significant differences among species in 40% and 15% sunlight (Table 4). As expected, NPQ exhibited the reversed pattern, with the highest values at the highest PFD. No significant differences among species were found for NPQ. All the observations from chlorophyll fluorescence were an index of lower photosynthetic activity with increasing light in beech than in oaks, and of an earlier light saturation in the former species. This was confirmed by the fact that A_{max} increased with increasing PFD in the two species, but the increase was greater in oak than in beech and A_{max} was significantly higher in the former than in the latter in all the light environments (Fig. 2, Table 4).

Transfer of plants from the shade to the sun caused an immediate decrease of F_v/F_m (Fig. 1). The decrease in F_v/F_m was larger for plants grown at the lowest PFD, and it was always greater for beech than for oak. By the end of the period of measurements, oak plants transferred to full sunlight from 50 and 40% sunlight recovered the F_v/F_m values exhibited by the control plants in their respective light environments, but this was not the case for transferred beech plants (Fig. 1). Only oak plants grown at 15% sunlight transferred to full sunlight still exhibited an F_v/F_m lower than their non-transferred counterparts at the end of the experiment, but the trend towards a complete recovery was clear (Fig. 1). $\Phi_{PS\ II}$ was lower in full sunlight than in the shade and also lower in oak than in beech for all pair-wise comparisons among transferred and non-transferred plants; the same trend was observed in F_v'/F_m' and in qP (data not shown). NPQ increased in all plants transferred to full sunlight. Although in general oak exhibited higher NPQ values than beech, by the end of the experiment the latter exhibited higher values than the former in full sunlight. A_{max} was not significantly different between transferred and non-transferred plants revealing the lack of acclimation of photosynthetic capacity to the changes in irradiance climate (Fig. 2).

Total chlorophyll content increased with decreasing light availability, but the increase was more pronounced in beech than in oak (Fig. 3). Chlorophyll content of the

and in 50% sunlight (Table 4). Decrease of $\Phi_{PS\ II}$ with PFD was more accentuated in beech than in oak: $\Phi_{PS\ II}$ decreased by 45% from 15% sunlight to 50% sunlight in the former, while it decreased only by 6% in the latter. The same trend with PFD and the same difference between the two species was observed for the efficiency of

Fig. 3 Time course of the chlorophyll concentration of the leaves of pedunculate oak and beech plants under four different light environments during the summer of 1994. *Closed symbols* indicate plants kept all the time in the corresponding light environment, *open symbols* indicate plants transferred on day 0 to the open (full sunlight). Data points are the means ± SD of ten plants
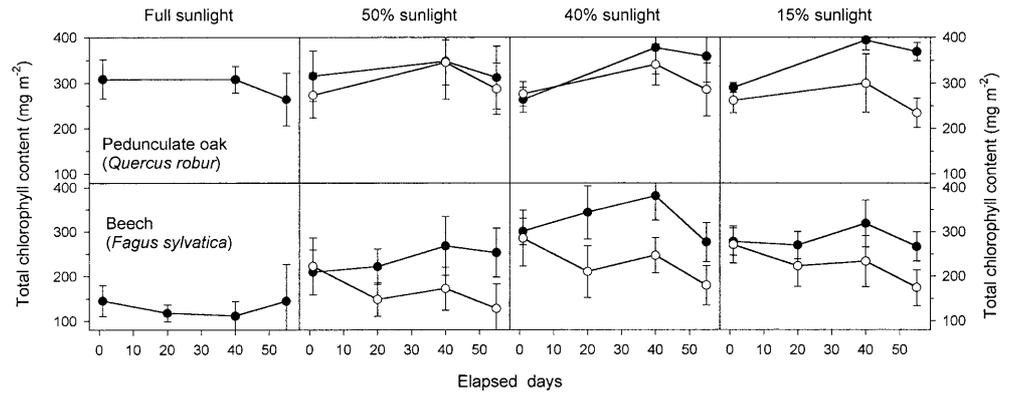
Table 5 Plasticity index [(max-min)/max] in response to light of *Quercus robur* and *Fagus sylvatica* calculated as in Valladares et al. (2000b) for the variables of Tables 2–5 plus total chlorophyll concentration and stomatal conductance to water (g_{H2O}). Variables are arranged by categories and within each category they are arranged by increasing *Q. robur* to *F. sylvatica* difference in the plasticity index ($\Delta_{Q.\ robur–F.\ sylvatica}$)

Category	Variable	Plasticity index		$\Delta_{Q.\ robur–F.\ sylvatica}$
		Quercus robur	*Fagus sylvatica*	
Leaf pigments	Chlorophyll concentration	0.17	0.57	−0.40
Morphology	Root-shoot	0.14	0.78	−0.64
	Leaf size	0.25	0.58	−0.33
	LWR	0.10	0.22	−0.12
	LAR	0.35	0.39	−0.04
	SLA	0.38	0.35	0.03
Morphology mean		0.24	0.46	−0.22
Leaf anatomy	Stomatal length	0.12	0.05	0.07
	Exchange surface area (palisade parenchyma)	0.43	0.32	0.11
	Stomatal density	0.40	0.29	0.11
	Palisade parenchyma thickness	0.15	0.01	0.14
	Leaf blade thickness	0.36	0.21	0.15
	Epidermis thickness	0.32	0.13	0.19
Leaf anatomy mean		0.30	0.17	0.13
Leaf physiology	NPQ	0.77	0.75	0.02
	qP	0.50	0.44	0.06
	A_{max}	0.80	0.60	0.20
	g_{H2O}	0.43	0.05	0.38
	Rubisco activity	0.51	0.12	0.39
Leaf physiology mean		0.60	0.39	0.21
Total mean		0.35	0.34	0.01

shade plants transferred to the open decreased gradually to approach the values of the sun plants and, again, this trend was more evident in beech than in oak (Fig. 3).

Oak exhibited higher initial and total Rubisco activity than beech in full sunlight, although differences vanished at 15% sunlight (Fig. 4). Rubisco activation was higher in oak than in beech both in full sunlight and in 15% sunlight in the early morning but not in the afternoon, when the two species exhibited a similarly high Rubisco activation (Fig. 4).

Phenotypic plasticity in response to light differed significantly between species and among variables (Table 5). Plastic phenotypic response to light was higher in beech than in oak for chlorophyll content and leaf morphology, while the reverse was true for variables related to leaf anatomy and physiology. Averaging the phenotypic plasticity index for all variables rendered a similar value for the two species (Table 5).

Discussion

Higher physiological plasticity in pedunculate oak than in beech

For the physiological traits explored here, pedunculate oak exhibited a higher phenotypic plasticity in response to light than beech (Table 5). This finding agrees with previous studies where high-light species exhibited greater photosynthetic plasticity than shade-tolerant species (Bazzaz and Carlson 1982; Strauss-Debenedetti and Bazzaz 1996; Valladares 2000; Valladares et al. 2000b). It also agrees with the trade-off between tolerance of shade and growth rate in high irradiance (Pacala et al. 1994) and with the negative correlation between responsiveness to light and survival in deep shade (Grubb et al. 1996). The relatively low physiological plasticity of beech agrees with the trend discussed in previous studies
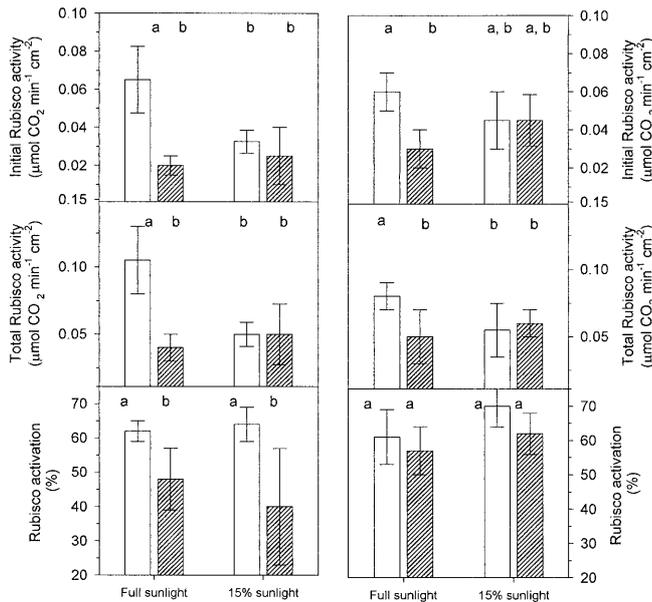
Fig. 4 Initial and total Rubisco activity and level of Rubisco activation at 08:00 am (*left graphs*) and at 14:00 (*right graphs*) for pedunculate oak (*open bars*) and beech (*shaded bars*) in full sunlight and in 15% sunlight. Bars indicate mean ± SD of eight samples. Letter code indicates significant differences (ANOVA, Tukey test, $P<0.05$)

that specialisation to adverse environments is linked to phenotypic stability and a conservative resource-use strategy even when resources are temporarily abundant (Lortie and Aarssen 1996; Valladares et al. 2000a, b; Balaguer et al. 2001). However, mean phenotypic plasticity was similar for beech and pedunculate oak (Table 5). This was due to the fact that while oak was more plastic in physiological features, beech was more plastic for morphological variables, one group of variables counteracting the effect of the other on the mean phenotypic plasticity index. Morphological plasticity of beech was found to be similar to that of oak in a previous study (van Hees 1997). This discrepancy can be explained by the fact that the study of van Hees (1997) dealt only with 1-year-old seedlings while 2-year-old seedlings were studied here. Since the resources of the acorn can significantly influence the performance of seedlings during their first year of growth (Welander and Ottosson 1998), the morphological plasticity of beech at the seedling stage is likely to have been underestimated by van Hees (1997).

Beech is known to grow and develop under both full sunlight and heavily shaded conditions and to maintain living branches in a wide range of light environments, including the deep shade of the under-canopy (Ellenberg 1988; Tognetti et al. 1997). These observations have led to expect a high phenotypic plasticity in this species (van Hees 1997; Cochard et al. 1999), which is in contrast with the expectations for a shade-tolerant species as discussed above. High light in gaps is associated with increased transpiration and water deficits and the capacity

of beech to cope with the enhanced risk of cavitation associated with high light has been shown to rely not only on a noticeable stomatal control (Aranda et al. 2000) but also on a remarkable plasticity of xylem conductance and vulnerability to cavitation in response to light (Cochard et al. 1999). However, how plasticity of beech related to this feature compares with that of pedunculate oak remains unknown. This emphasises the need for comparative studies under the same experimental conditions in order to understand the functional role of the phenotypic plasticity and to fully unveil the underlying morphological and physiological mechanisms of their response to light.

The relatively high plasticity in traits associated with light harvesting (leaf and crown morphology, and chlorophyll content) found in beech, the more shade-tolerant species, suggests that selection for plasticity is not only different under different levels of adversity, as suggested by Lortie and Aarssen (1996), Valladares et al. (2000a, b) and Balaguer et al. (2001), but also different for each set of traits.

Moderate tolerance of high light of beech seedlings

Strong irradiance can lead to photoinhibition and leaf overheating, especially in shade-tolerant species and shade-acclimated individuals (Valladares and Pearcy 1997; Kitao et al. 2000). Susceptibility to photoinhibition seems to be linked to the successional status of the species in temperate forests (Kitao et al. 2000), although the data to support this trend are still far from sufficient. Photoinhibition is ubiquitous in nature, especially when photosynthetic capacity is low (Valladares and Pearcy 1999; Martínez-Ferri et al. 2000; Ort 2001). Beech has been shown to exhibit a significant photoprotective response to high light, both at the physiological level, involving changes in pigment composition and antioxidant systems (Garcia-Plazaola and Becerril 2000), and at the structural level, involving increased leaf angles (Tognetti et al. 1994). In fact, we found a significant enhancement of non-photochemical quenching and a decrease in chlorophyll content in beech seedlings exposed to high light (Table 4, Fig. 3). However, these mechanisms proved insufficient for avoiding photoinhibition in shade-grown seedlings of beech, which agrees with previous studies (Tognetti et al. 1997, 1998). Beech seedlings studied here exhibited higher photoinhibition, lower Rubisco activity, and lower photosynthetic rates in full sunlight than pedunculate oak. The short-term response of beech to increased light, which was associated with a remarkable non-recoverable reduction in F_v/F_m, was especially poor when compared to that of pedunculate oak (Figures 1 and 2). qP and Φ_{PSII} were also lower in beech seedlings exposed to full sunlight than in their pedunculate oak seedlings counterpart. This relatively low photochemical capacity to utilise full sunlight of beech seedlings was associated with a non-photochemical quenching of absorbed radiation that was found to be

significantly higher than that of pedunculate oak seedlings by the end of the period of measurements. However, this enhanced thermal dissipation was not enough to protect PSII against excessive light as mentioned above. Shade-grown plants are known for their reduced photoprotective systems (e.g. xanthophyll pigments) compared with sun-grown plants (Demmig-Adams et al. 1995; Demmig-Adams and Adams 2000). But when energy dissipation in the antenna system of PSII is low, a more active photosynthetic electron transport may help to mitigate the effect of photodamage in shade-grown plants (Kitao et al. 2000). The significant reduction in chlorophyll content of beech seedlings exposed to high light can be interpreted as a photoprotective mechanism, since it reduces light absorption by PSII and alleviates overexcitation (Kyparissis et al. 2000). However, the light eventually absorbed by the leaves of beech seedlings in the open was still excessive. The photosynthetic rates of beech, inherently lower than those of pedunculate oak, seem to have overimposed an enhanced risk of photodamage in shade-grown individuals of this species. High risk of photoinhibition under strong irradiance even in sun-grown individuals could result from the well-established trade-off between shade tolerance and low growth rate in high irradiance (e.g. Grubb et al. 1996).

Tolerance to strong irradiance can be explored in either long- or short-term experiments which mimic performance in the open or acclimation to a sudden forest gap formation, respectively. The limited capacity of beech seedlings to acclimate to a sudden increase in light agrees with the short-term response to light of late successional species (Lovelock et al. 1994; Strauss-Debenedetti and Bazzaz 1996; Naidu and DeLucia 1997). In the long term, both species exhibit a positive response to a moderate increase in radiation, as indicated by our results and previous studies (Welander and Ottosson 1998). Our observations provide further support for the results of Tognetti et al. (1997, 1998) that small gaps may represent the most favourable environment for photosynthesis and growth of beech seedlings due to its limited capacity to cope with strong irradiance. This is in contrast to pedunculate oak seedlings, which not only tolerate full sunlight as found here but also require increased irradiance after the first year of emergence for successful regeneration (Welander and Ottosson 1998). These species differences in their physiological response to light (tolerance to strong irradiance and physiological plasticity) reinforce the view of a different regeneration niche for each of the two species studied (understorey and small gaps for beech versus big gaps and clearings for pedunculate oak).

In conclusion, our study suggests that while shade tolerance relies on an enhanced plasticity in light-harvesting variables (crown morphology and chlorophyll content), a greater tolerance to strong irradiance is linked to an enhanced physiological plasticity, specifically to an enhanced plasticity in variables influencing photosynthetic carbon gain (from the exchange surface area of the palisade parenchyma to the stomatal conductance and the carboxylation capacity of the leaves). This enhanced plasticity translates into a relatively large photosynthetic capacity of sun-grown individuals to use full sunlight, which was more efficient in avoiding photoinhibition than the photoprotective mechanisms triggered in the species exhibiting low photosynthetic rates.

References

Abrams MD, Mostoller SA (1995) Gas exchange, leaf structure and nitrogen in contrasting successional tree species growing in open and understory sites during a drought. Tree Physiol 15:361–370

Afif D, Gérant D, Cavalié G, Dizengremel P (1993) Physical, immunological and kinetic properties of ribulose-1,5-bisphosphate carboxylase/oxygenase from fir (*Abies alba*) and spruce (*Picea abies*). Physiol Plant 88:113–122

Aranda I, Gil L, Pardos JA (2000) Water relations and gas exchange in *Fagus sylvatica* L. and *Quercus petraea* (Mattuschka) Liebl. in a mixed stand at their southern limit of distribution in Europe. Trees 14:344–352

Aranda I, Gil L, Pardos JA (2002) Effects of thinning in a *Pinus sylvestris* L. stand on foliar water relations of *Fagus sylvatica* L. seedlings planted within the pinewood. Trees (in press)

Balaguer L, Martínez-Ferri E, Valladares F, Pérez-Corona ME, Baquedano FJ, Castillo FJ, Manrique E (2001) Population divergence in the plasticity of the response of *Quercus coccifera* to the light environment. Funct Ecol 15:124–135

Barnes JD, Balaguer L, Manrique E, Elvira S, Davison AW (1992) A reappraisal of the use of DMSO for the extraction and determination of chlorophyll a and b in lichens and higher plants. Environ Exp Bot 32:85–100

Bazzaz FA (1996) Plants in changing environments: linking physiological, population, and community ecology. Cambridge University Press, Cambridge

Bazzaz FA, Carlson RW (1982) Photosynthetic acclimation to variability in the light environment of early and late successional plants. Oecologia 54:313–316

Brzeziecki B, Kienast F (1994) Classifying the life-history strategies of trees on the basis of the Grimian model. For Ecol Manage 69:167–187

Callaway RM (1992) Morphological and physiological responses of three California oak species to shade. Int J Plant Sci 153:434–441

Chazdon RL (1992) Photosynthetic plasticity of two rain forest shrubs across natural gap transects. Oecologia 92:586–595

Cochard H, Lemoine D, Dreyer E (1999) The effects of acclimation to sunlight on the xylem vulnerability to embolism in *Fagus sylvatica* L. Plant Cell Environ 22:101–108

Demmig-Adams B, Adams WW (2000) Photosynthesis – harvesting sunlight safely. Nature 403:373–374

Demmig-Adams B, Adams III WW, Logan BA, Verhoeven AS (1995) Xanthophyll cycle-dependent energy dissipation and flexible photosystem II efficiency in plants acclimated to light stress. Aust J Plant Physiol 22:249–260

Ellenberg H (1988) Vegetation ecology of Central Europe. Cambridge University Press, Cambridge

Emborg J (1998) Understorey light conditions and regeneration with respect to the structural dynamics of a near-natural temperate deciduous forest in Denmark. For Ecol Manage 106:83–95

Garcia-Plazaola JI, Becerril JM (2000) Photoprotection mechanisms in European beech (*Fagus sylvatica* L.) seedlings from diverse climatic origins. Trees 14:339–343

Genty B, Briantais J-M, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990:87–92

Grime JP, Jeffrey DW (1965) Seedling establishment in vertical gradients of sunlight. J Ecol 53:621–642

Grubb PJ, Lee WG, Kollmann J, Wilson JB (1996) Interaction of irradiance and soil nutrient supply on growth of seedlings of ten European tall-shrub species and *Fagus sylvatica*. J Ecol 84:827–840

Henry HAL, Aarssen LW (1997) On the relationship between shade tolerance and shade avoidance strategies in woodland plants. Oikos 80:575–582

Johnson JD, Tognetti R, Michelozzi M, Pinzauti S, Minotta G, Borghetti M (1997) Ecophysiological responses of *Fagus sylvatica* seedlings to changing light conditions.2. The interaction of light environment and soil fertility on seedling physiology. Physiol Plant 101:124–134

Kitao M, Lei TT, Koike T, Tobita H, Maruyama Y (2000) Susceptibility to photoinhibition of three deciduous broadleaf tree species with different successional traits raised under various light regimes. Plant Cell Environ 23:81–89

Kyparissis A, Drilias P, Manetas Y (2000) Seasonal fluctuations in photoprotective (xanthophyll cycle) and photoselective (chlorophylls) capacity in eight Mediterranean plant species belonging to two different growth forms. Austr J Plant Physiol 27:265–272

Lortie CJ, Aarssen LW (1996) The specialization hypothesis for phenotypic plasticity in plants. Int J Plant Sci 157:484–487

Lovelock CE, Jebb M, Osmond CB (1994) Photoinhibition and recovery in tropical plant species: response to disturbance. Oecologia 97:297–307

Martínez-Ferri E, Balaguer L, Valladares F, Chico JM, Manrique E (2000) Energy dissipation in drought-avoiding and drought-tolerant tree species at midday during the Mediterranean summer. Tree Physiol 20:131–138

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence – a practical guide. J Exp Bot 51:659–668

Minotta G, Pinzauti S (1996) Effects of light and soil fertility on growth, leaf chlorophyll content and nutrient use efficiency of beech (*Fagus sylvatica* L) seedlings. For Ecol Manage 86:61–71

Naidu SL, DeLucia EH (1997) Acclimation of shade-developed leaves on saplings exposed to late-season canopy gaps. Tree Physiol 17:367–376

Newbold AJ, Goldsmith FB (1990) The regeneration of oak and beech: a literature review. Discussion papers in conservation, no. 33. University College, London

Ort DR (2001) When there is too much light. Plant Physiol 125:29–32

Pacala SW, Canham CD, Silander JA, Kobe RK (1994) Sapling growth as a function of resources in a north temperate forest. Can J For Res 24:2172–2183

Paulsen JM, Lane MD (1966) Spinach ribulose diphosphate carboxylase. Purification and properties of the enzyme. Biochemistry 5:2350–2357

Pelloux J, Jolivet Y, Fontaine V, Banvoy J, Dizengremel P (2001) Changes in Rubisco and Rubisco activase gene expression and polypeptide content in *Pinus halepensis* M. subjected to ozone and drought. Plant Cell Environ 24:123–131

Schlichting CD (1986) The evolution of phenotypic plasticity in plants. Annu Rev Ecol Syst 17:667–693

Silvertown J (1998) Plant phenotypic plasticity and non-cognitive behaviour. Trends Ecol Evol 13:255–256

Sonesson LK (1994) Growth and survival after cotyledon removal in *Quercus robur* seedlings, grown in different natural soil types. Oikos 69:65–70

Strauss-Debenedetti S, Bazzaz FA (1991) Plasticity and acclimation to light in tropical moraceae of different successional positions. Oecologia 87:377–387

Strauss-Debenedetti S, Bazzaz F (1996) Photosynthetic characteristics of tropical trees along successional gradients. In: Mulkey SS, Chazdon RL, Smith AP (eds) Tropical forest plant ecophysiology. Chapman and Hall, New York, pp 162–186

Tognetti R, Michelozzi M, Borghetti M (1994) Response to light of shade-grown beech seedlings subjected to different watering regimes. Tree Physiol 14:751–758

Tognetti R, Johnson JD, Michelozzi M (1997) Ecophysiological responses of *Fagus sylvatica* seedlings to changing light conditions.1. Interactions between photosynthetic acclimation and photoinhibition during simulated canopy gap formation. Physiol Plant 101:115–123

Tognetti R, Minotta G, Pinzauti S, Michelozzi M, Borghetti M (1998) Acclimation to changing light conditions of long-term shade-grown beech (*Fagus sylvatica* L.) seedlings of different geographic origins. Trees 12:326–333

Valladares F (1999) Architecture, ecology and evolution of plant crowns. In: Pugnaire FI, Valladares F (eds) Handbook of functional plant ecology. Dekker, New York, pp 121–194

Valladares F (2000) Light and plant evolution: adaptation to the extremes versus phenotypic plasticity. In: Greppin H (ed) Advanced studies in plant biology. University of Geneva, Geneva, pp 341–355

Valladares F, Pearcy RW (1997) Interactions between water stress, sun-shade acclimation, heat tolerance and photoinhibition in the sclerophyll *Heteromeles arbutifolia*. Plant Cell Environ 20:25–36

Valladares F, Pearcy RW (1999) The geometry of light interception by shoots of *Heteromeles arbutifolia*: morphological and physiological consequences for individual leaves. Oecologia 121:171–182

Valladares F, Martinez-Ferri E, Balaguer L, Perez-Corona E, Manrique E (2000a) Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy? New Phytol 148:79–91

Valladares F, Wright SJ, Lasso E, Kitajima K, Pearcy RW (2000b) Plastic phenotypic response to light of 16 congeneric shrubs from a Panamanian rainforest. Ecology 81:1925–1936

Van Hees AFM (1997) Growth and morphology of pedunculate oak (*Quercus robur* L.) and beech (*Fagus slvatica* L.) seedlings in relation to shading and drought. Ann For Sci 54:9–18

Von Lüpke B (1998) Silvicultural methods of oak regeneration with special respect to shade tolerant mixed species. For Ecol Manage 106:19–26

Ward DA, Keys AJ (1989) A comparison between the coupled spectrophotometric and uncoupled radiometric assays for RuBP carboxylase. Photosynth Res 22:167–171

Welander NT, Ottosson B (1998) The influence of shading on growth and morphology in seedlings of *Quercus robur* L. and *Fagus sylvatica* L. For Ecol Manage 107:117–126

Welander NT, Ottosson B (2000) The influence of low light, drought and fertilization on transpiration and growth in young seedlings of *Quercus robur* L. For Ecol Manage 127:139–151

Ziegenhagen B, Kausch W (1995) Productivity of young shaded oaks (*Quercus robur* L) as corresponding to shoot morphology and leaf anatomy. For Ecol Manage 72:97–108