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Changes in total leaf nitrogen and partitioning of leaf nitrogen drive photosynthetic acclimation to light in fully developed walnut leaves

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ABSTRACT

Comprehensive studies on the processes involved in photosynthetic acclimation after a sudden change in light regime are scarce, particularly for trees. We tested (i) the ability of photosynthetic acclimation in the foliage of walnut trees growing outdoors after low-to-high and high-to-low light transfers made early or late in the vegetation cycle, and (ii) the relative importance of changes in total leaf nitrogen versus changes in the partitioning of leaf nitrogen between the different photosynthetic functions during a 2 month period after transfer. Changes in maximum carboxylation rate, light-saturated electron transport rate, respiration rate, total leaf nitrogen, ribulose 1-5-bisphosphate carboxylase/oxygenase (Rubisco) and total chlorophylls were surveyed before and after the change in light regime. Respiration rate acclimated fully within 1 week of transfer, and full acclimation was observed 1 month after transfer for the amount of Rubisco. In contrast, total nitrogen and photosynthetic capacity acclimated only partially during the 2 month period. Changes in photosynthetic capacity were driven by changes in both total leaf nitrogen and leaf nitrogen partitioning. The extent of acclimation also depended strongly on leaf age at the time of the change in light regime.

Key-words: Chlorophyll; electron transport capacity; maximum carboxylation rate; Rubisco.

INTRODUCTION

Leaf photosynthetic characteristics exhibit remarkable adaptability to light levels (Field & Mooney 1986; Evans 1989). During expansion and growth, single leaves usually acclimate to the vertical gradient of mean irradiance encountered within plant stands (Hirose & Werger 1987; Anten, Hernandez & Medina 1996; Anten & Werger 1996; Kull & Niinemets 1998) or to the heterogeneous light

regime observed within individual tree crowns (DeJong & Doyle 1985; Le Roux, Sinoquet & Vandame 1999a; Le Roux *et al.* 2001). Generally, leaves developed at high irradiance exhibit higher nitrogen (N) content and photosynthetic capacity per unit leaf area as compared with shade leaves (Boardman 1977; Givnish 1988; Sims & Pearcy 1989; Niinemets, Kull & Tenhunen 1998). Many studies have analysed the changes in leaf anatomical and biochemical characteristics that drive photosynthetic acclimation to light (Kappel & Flore 1983; Seemann *et al.* 1987; Seemann 1989; Chazdon & Kaufmann 1993; Hikosaka 1996; Rôças, Barros & Scarano 1997; Niinemets *et al.* 1998). Because more than 50% of total leaf N is allocated to the photosynthetic apparatus (Evans & Seemann 1989), photosynthetic acclimation to light has been described largely in terms of changes in total leaf N and/or in the partitioning of total leaf N between the different pools of the photosynthetic apparatus (Evans 1987; Chow *et al.* 1988; Hikosaka & Terashima 1995).

In contrast, the processes involved in the photosynthetic acclimation of mature leaves following a sudden change in light regime (as observed after green pruning in tree orchards or gap formation in forests, for instance) is documented only poorly. Some studies have measured changes in the light-saturated photosynthetic rate and respiration rate of leaves after a change in light regime without analysing the concurrent changes in the main photosynthetic functions (i.e. light harvesting, electron transport and carboxylation) and in the key biochemical pools of the photosynthetic apparatus (von Caemmerer & Farquhar 1984; Bauer & Thoni 1988; Turnbull, Doley & Yates 1993; Brooks, Hinckley & Sprugel 1994; Naidu & DeLucia 1997). In contrast, other studies have accurately quantified changes in a given photosynthetic function and/or a few biochemical characteristics of the leaves, such as the thylakoid compounds or Calvin cycle enzymes, generally under controlled conditions (Prioul, Brangeon & Reyss 1980; Besford 1986; Davies *et al.* 1986; De la Torre & Burkey 1990; Burkey & Wells 1991; Evans 1993). To our knowledge, very few studies have surveyed the relative importance of concurrent changes in total leaf N versus changes in the partitioning of leaf N between light harvesting, electron transport and carboxylation after a sudden change in light regime. Evans (1989) studied changes in photosynthetic capacity, N con-

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tent and N partitioning in fully developed leaves of *Cucumis sativus* subjected to changes in an irradiance regime. In *Glycine max* plants grown in a climate chamber, Pons & Pearcy (1994) showed that after a high ($400 \mu\text{mol m}^{-2} \text{s}^{-1}$) to low ($50 \mu\text{mol m}^{-2} \text{s}^{-1}$) light transfer, photosynthetic acclimation resulted both from changes in total leaf N and from changes in the partitioning of leaf N within the photosynthetic apparatus. Such a comprehensive analysis of photosynthetic acclimation has never been made, either for plants growing outdoors or for trees.

The objectives of this study were to test (i) the ability of photosynthetic acclimation in hybrid walnut (*Juglans nigra x regia*) growing outdoors after low-to-high and high-to-low light transfers made early or late in the vegetation cycle, and (ii) the relative importance of changes in total leaf N versus changes in the partitioning of N between the different photosynthetic functions during a 2 month period after transfer. We determined changes in maximum carboxylation rate, light-saturated electron transport rate, respiration rate, non-structural carbohydrates, total leaf N, ribulose 1.5-bisphosphate carboxylase/oxygenase (Rubisco) and total chlorophyll before and after a change in light regime. The results are discussed in terms of the mechanisms involved in photosynthetic acclimation after a change in light regime.

MATERIALS AND METHODS

Plant material and growth conditions

One-year-old hybrid walnut trees (*Juglans nigra x regia*) were grown outdoors in 35 dm^3 pots filled with a soil/peat mixture (1/2 v/v). One lot of 16 1-year-old plants was grown during 1999; another lot of 14 1-year-old plants was grown during 2000. To avoid complete light or shade exposure of the whole plant, each tree had only two opposite and erected branches. This was obtained by removing the apical and axillary buds before bud burst, with the exception of two vigorous axillary buds. Immediately after bud burst, the lower branch was placed in a tunnel made with altuglass screens. Two types of screen, mimicking high-light and deep-shade conditions, were used. The first (neutral) screen transmitted 90% of incident radiation without modification of the light spectrum (red/far red = 1.25), as measured with a spectroradiometer (LI-1800; Li-Cor Inc., Lincoln, NE, USA). The other (green) screen transmitted 10% of incident radiation (red/far red = 0.22). Plants were watered automatically once or twice a day according to evaporative demand.

The lower branches of eight and seven plants were grown in each screen tunnel during 1999 and 2000, respectively. Four plants were transferred from high- to low-light conditions and four were transferred from low- to high-light conditions on 29 July (91 d after bud burst) in 1999 and on 30 June (58 d after bud burst) in 2000. Four treatments were thus identified: constant low light (LL), constant high light (HH), low-to-high light transfer (LH) and high-to-low light transfer (HL). All physiological and biochemical mea-

surements were subsequently made on single leaflets from fully developed leaves of the treated branch. The oldest and youngest leaves were excluded from these measurements. Air temperature and incident global radiation were recorded during the experiment period for both years.

Leaf mass : area ratio and non-structural carbohydrates

Leaflets were sampled on eight dates in 1999 and seven in 2000. One leaflet was harvested at random from each treated branch on each date. The leaflets' areas were measured with an area meter (LI-3100; Li-Cor), before they were freeze-dried and their dry mass measured. Samples were milled, and total non-structural carbohydrates (TNCs) [i.e. glucose fructose sucrose (GFS) and starch] were extracted prior to quantification. A hot ethanol : water buffer (80 : 20, v/v) was used for GFS, then extracts were purified on ion-exchange resins (Bio-Rad AG 1-X8; Bio-Rad Lab., Hercules, CA, USA in the carbonate form and Dowex 50 W (Supelco, Belle Fonte, PA, USA) in the H^+ form. The amount of GFS was determined spectrophotometrically after a hexokinase, glucose-6-phosphate linked assay (Boehringer 1984). Starch was quantified by enzymatic assay after hydrolysis with amyloglucosidase (Boehringer 1984). The TNC-free leaf mass : area ratio (LMA) was computed as (total leaf dry mass - TNC mass) / fresh leaf area.

Total leaf N, Rubisco and chlorophyll

The amount of total leaf N per unit dry mass (N_m , %) was measured on the same dry samples with an elemental analyser (Carlo ERBA-1108; Carlo, Milan, Italy). Total leaf N was expressed per unit area (N_a) as ($N_m \times \text{leaf dry mass}$) / (fresh leaf area $\times 100$).

Total chlorophyll was estimated with a portable chlorophyll meter (SPAD-502; Minolta Camera Co., Osaka, Japan) on the leaflets used for leaf gas exchange measurements. SPAD data were converted into the total amount of chlorophyll per unit leaf area using a calibration curve ($y = 0.0606 e^{0.0502x}$, $N = 64$, $r^2 = 0.87$). This curve was established from destructive leaflet sampling, chlorophyll extraction in acetone and spectrophotometrical measurements according to Porra, Thompson & Kriedemann (1989).

One 5 cm^2 disc sampled on a fresh leaflet was used to quantify Rubisco on each date and for each branch. Discs were frozen immediately in liquid N_2 and stored at -80°C . Sampled discs were ground in liquid N_2 until complete evaporation, then 5 cm^3 of extraction buffer (pH 7.6) containing 100 mol m^{-3} Tris and 200 mol m^{-3} sodium borate was added. Three protease inhibitors [10 mm^3 of each per cm^3 of extraction buffer: 2.3 mol m^{-3} Benzamidine, 2 mol m^{-3} ϵ -aminocarpic acid, 40 mol m^{-3} phenylmethylsulfonyl fluoride (PMSF)] and 500 mg polyvinylpyrrolidone (PVPP) were also added. Rubisco extracts were centrifuged for 2 min at $14\,000 \text{ rpm}$ and diluted 1 : 2000. Elisa assays were performed as described by Catt & Millard (1988) by the

double antibody technique, using a 1 : 1000 dilution of primary polyclonal antibody raised in sheep against wheat Rubisco, and a 1 : 300 dilution of secondary antibody against sheep conjugated to alkaline phosphatase (Sigma A-5187). Purified Rubisco from wheat was used as a standard. The second antibody reacted with p-nitrophenol phosphate (Sigma 104-0) by developing yellow coloration. When sufficient colour had developed (5–10 min), the reaction was stopped by the addition of 10 mm³ 5 mol m⁻³ NaOH and the optical density was read by a plate reader (Titertek Multiscan MCC1; Biolog Inc., Hayward, CA, USA).

Gas exchange measurements

Leaf gas exchange was measured with an infrared gas analyser–leaf chamber system (LI-6400, Li-Cor). Measurements were made on four dates in 1999 and seven in 2000. Leaf gas exchange was measured on the same leaflet of each plant throughout the experiment. Photosynthesis response curves to internal CO₂ partial pressure ($A-p_i$) were established at a leaf temperature of 25 °C and under high irradiance (1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and different CO₂ partial pressures (190, 150, 110, 90, 70, 50, 35, 25, 20, 15 and 10 Pa CO₂). Respiration rate was measured after 20 min of acclimation in darkness at 25 °C and 35 Pa CO₂. A version of the Farquhar & von Caemmerer (1982) photosynthesis model proposed by Harley *et al.* (1992) was used to analyse the $A-p_i$ curves. A fitting procedure (SAS software for Solaris version 6.12, SAS Institute Inc., Cary, NC, USA) was used to estimate the two key model parameters, i.e. maximum carboxylation rate (V_{cmax}) and the light-saturated electron transport rate (J_{max}) (see Le Roux *et al.* 1999b).

Calculations of leaf N partitioning between carboxylation, bioenergetics and light capture

The model proposed by Niinemets & Tenhunen (1997) was used to determine the coefficients for leaf N partitioning between carboxylation (mainly Rubisco) (P_r), bioenergetics (P_b) and thylakoid light-harvesting components (P_l) based on measured N_a values and estimated values of V_{cmax} , J_{max} and chlorophyll concentration. In this model, P_r is the foliar N investment in carboxylation capacity (i.e. influencing V_{cmax}), P_b is the N investment for the capacity of electron transport (i.e. influencing J_{max}) and P_l is the N investment in light harvesting. P_r [g N in Rubisco (g total leaf N)⁻¹] and P_b [g N in cyt *f*, ferredoxin NADP reductase and coupling factor (g total leaf N)⁻¹] are given as follows:

$$P_r = V_{\text{cmax}} / (6.25 \times V_{\text{cr}} N_a) \quad (1)$$

$$P_b = J_{\text{max}} / (8.06 \times J_{\text{mc}} N_a) \quad (2)$$

where V_{cr} is the specific activity of Rubisco [i.e. the maximum rate of RuBP carboxylation per unit Rubisco protein in $\mu\text{mol CO}_2$ (g Rubisco)⁻¹ s⁻¹], J_{mc} is the potential rate of photosynthetic electron transport per unit cyt *f* [mol electrons (mol cyt *f*)⁻¹ s⁻¹], 6.25 [g Rubisco (g N in Rubisco)⁻¹

converts N content to protein content, and 8.06 [$\mu\text{mol cyt } f$ (g N in bioenergetics)⁻¹] is used assuming a constant 1 : 1 : 1.2 molar ratio for cyt *f* : ferredoxin NADP reductase : coupling factor (Niinemets & Tenhunen 1997). According to Niinemets & Tenhunen (1997), at a leaf temperature of 25 °C, V_{cr} and J_{mc} are equal to 20.2 $\mu\text{mol CO}_2$ [(g Rubisco)⁻¹ s⁻¹] and 156 mol electrons [(mol cyt *f*)⁻¹ s⁻¹], respectively. P_l [g N in PSI, PSII and LCHII (g total leaf N)⁻¹] is computed in a similar way, assuming scaling relationships between (i) J_{max} and cyt *f*, and (ii) the different thylakoid chlorophyll–protein complexes and cyt *f* (for details, see Eqn 6 and appendix B in Niinemets & Tenhunen 1997; see also Hikosaka & Terashima 1995). Nitrogen investment in carboxylation was also estimated directly from measured total N and Rubisco amounts and was compared with values calculated according to Niinemets & Tenhunen (1997).

Statistical analyses

Statistical analyses were performed with the Genstat 4.2 software developed by Genstat Committee; IACR-Rothamsted, Harpenden, UK. The residual maximum likelihood (REML) procedure was applied for the analysis of variance components on repeated measurements and unbalanced data sets. This procedure allowed overall tests of treatment (*T*), date (*D*) and treatment \times date interaction (*I*). Because the various parameters were observed through time on the same individuals, ante-dependence analysis (Gabriel 1962) was used to assess the times at which treatment effects occurred.

RESULTS

Comparison of leaf stage between the two years

The seasonal courses of global incident radiation and air temperature were very similar for the two years (Fig. 1). The average global irradiances from June to mid-September were 19.24 \pm 6.31 and 19.66 \pm 6.74 MJ m⁻² during 1999 and 2000, respectively. The average air temperatures for the same period were 19.1 \pm 2.7 and 18.9 \pm 3.0 °C in 1999 and 2000, respectively.

The time and duration of bud break (late April and early May) was also similar for the two years (Fig. 1). N_m decreased from 2.8 to 1.5% from the end of June to September. At a given date, values were similar in 1999 and 2000 (Fig. 2). Leaf N_m was greater when plants were transferred in late June 2000 (2.8%) than in late July 1999 (2.0%). Therefore, leaves did not have a comparable age or N status when the treatments were imposed during the two years.

LMA, starch and soluble sugars

In 1999, the LMA and the amount of starch and soluble sugars per unit leaf area were higher for leaves developed under sun conditions than for those developed in the shade (Fig. 3). LMA and non-structural carbohydrate values of

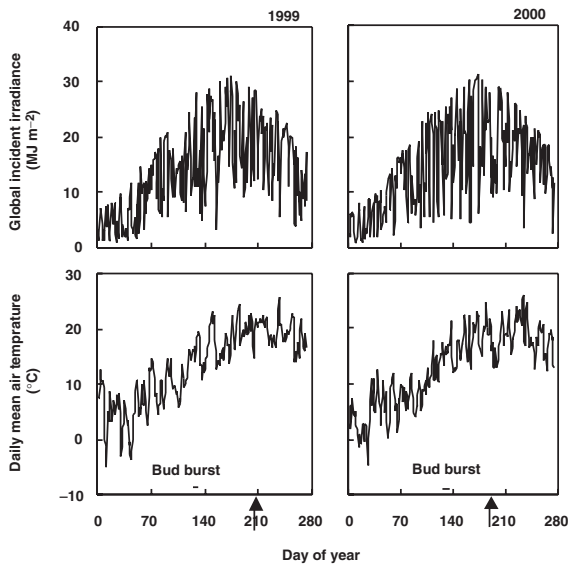


Figure 1. Seasonal courses of global incident radiation and daily mean air temperature from 1 January to 15 September in 1999 and 2000. Horizontal bars indicate the time and duration of bud burst. Arrows indicate the dates of transfers.

transferred leaves did not change after transfer. Starch exhibited a slight decrease with time in all treatments.

In 2000, LMA was constant or increased slightly in the HH, HL and LL treatments during the 2 month measurement period (Fig. 3). In contrast, LMA increased from 35 to 55 g m⁻² during the same period in the LH treatment. During the first month after transfer, the amount of starch remained roughly constant for the HH, HL and LL treatments, whereas it increased from around 4 to 6 g m⁻² in the LH treatment. The amount of starch per unit area decreased rapidly in all treatments after day 210. The

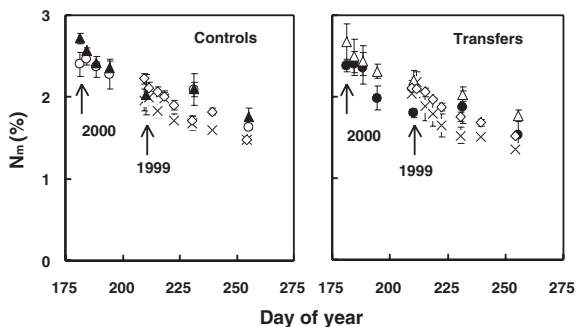


Figure 2. Temporal courses of nitrogen concentration (N_m) for leaves of the HH treatment in 1999 (\diamond) and 2000 (\circ); LL treatments in 1999 (\times) and 2000 (\blacktriangle); HL treatments in 1999 (\diamond) and 2000 (\bullet), and LH treatments in 1999 (\times) and 2000 (\triangle). Arrows indicate the dates of transfers. Bars indicate standard errors ($n = 4$ for all treatments in 1999; in 2000, $n = 3$ for HH and LL and $n = 4$ for HL and LH). Significant effect of treatments (T) was observed only in 1999 ($P = 0.014$); dates (D) always had a significant effect ($P < 0.001$), but the interaction (I) between treatments and dates was not significant (NS).

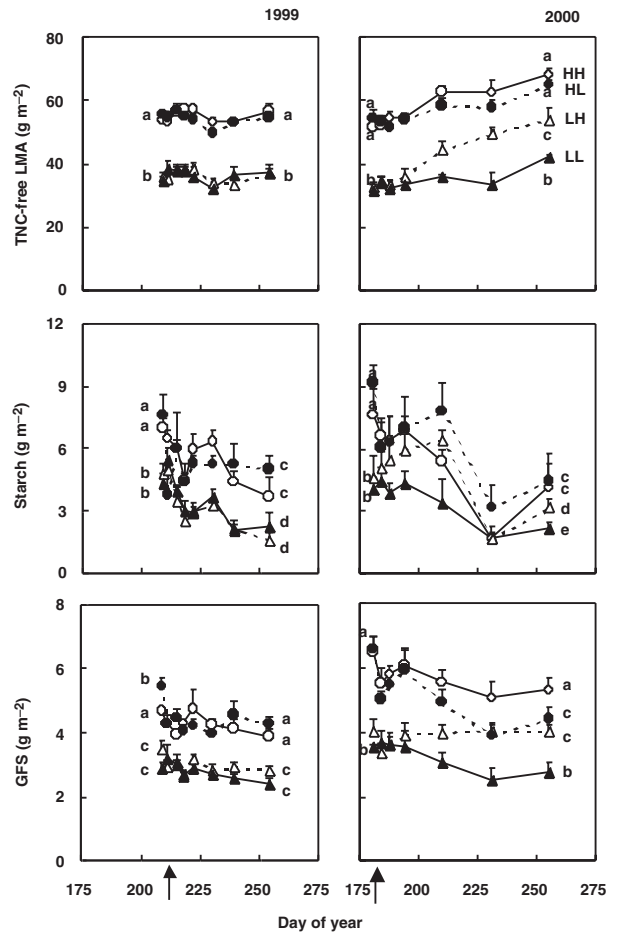


Figure 3. Temporal courses of the TNC-free leaf mass : area ratio (LMA), and the amount of starch and soluble sugars (GFS) per unit leaf area. Values are presented for high-to-high (\circ) and low-to-low (\blacktriangle) (both with solid lines), and high-to-low (\bullet) and low-to-high (\triangle) (both with dashed lines) treatments in 1999 and 2000. Arrows indicate the dates of transfers. Bars indicate standard errors ($n = 4$ for all treatments in 1999; in 2000, $n = 3$ for HH and LL and $n = 4$ for HL and LH). In 1999 and 2000, significant T ($P < 0.001$) and D ($P < 0.001$) effects were observed on LMA ($I = NS$ in both years). T , D ($P < 0.001$) and I ($P = 0.001$) had significant effects on starch amount in 1999. In 2000, T and D had a strong effect ($P < 0.001$) on starch ($I = NS$). In 1999, I was NS whereas T and D had significant effects ($P < 0.001$) on GFS. In 2000, T , D and I had strong effects on GFS ($P < 0.001$). Significant differences on a few dates are indicated by letters.

amount of soluble sugars per unit leaf area decreased slightly in the HH and LL treatments. In contrast, soluble sugar content increased with time for the LH treatment and decreased for the HL treatment as compared with the constant LL- and HH-grown plants.

Total leaf N, Rubisco and chlorophylls

In 1999, N_a was higher in sun than in shade leaves, and values decreased with time in all treatments (Fig. 4). N_a of transferred leaves did not change as compared with constant light leaves. However, in 2000, N_a increased by about

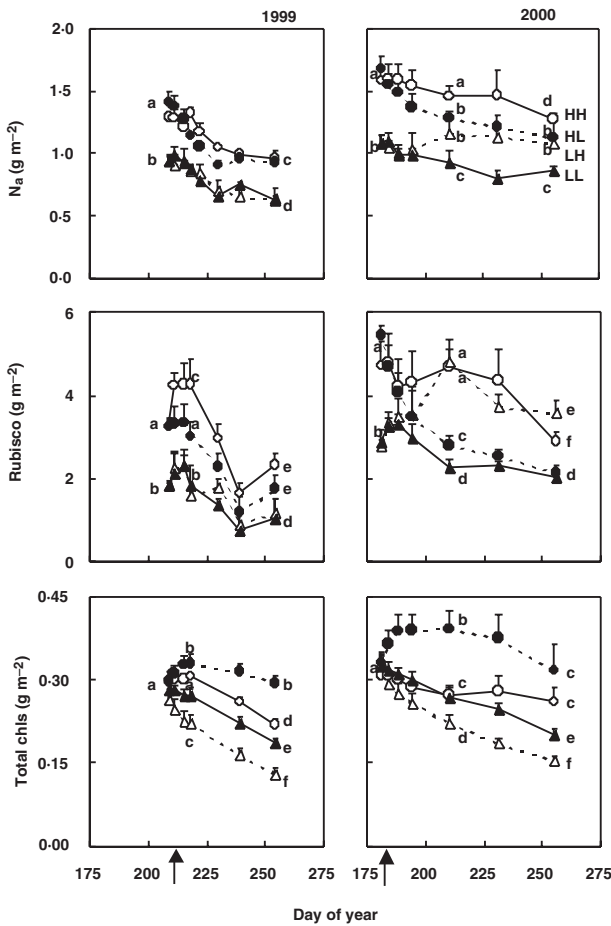


Figure 4. Temporal courses in the amount of total leaf nitrogen (N_a), Rubisco and total chlorophylls (Total chls) per unit leaf area in 1999 and 2000. Symbols as in Fig. 3; bars indicate standard errors. In 1999 and 2000, N_a was affected significantly by T and D ($P < 0.001$), but I was NS. T ($P < 0.001$) and D ($P = 0.001$) had significant effects on Rubisco in both years; I was only significant in 2000 ($P < 0.001$). In both years, T , D and I had strong effects on total chls ($P = 0.001$). Significant differences on a few dates are indicated by letters.

40% in the LH treatment and decreased by 30% in the HL treatment as compared with the corresponding controls (LL and HH, respectively).

The amount of Rubisco per unit leaf area decreased throughout the experiment for all treatments in 1999. Values in HL leaves became lower than those in HH leaves after transfer. In 2000, Rubisco increased for the LH treatment and decreased for the HL treatment. The amount of Rubisco was similar within 30 d of transfer in LH and HH treatments, and within 40 d in HL and LL treatments.

During both years, total chlorophyll levels changed within a few days of transfer. Before transfer, the amount of chlorophyll per unit area was similar between shade and sun leaves. However, after transfer, chlorophyll decreased significantly in LH treatments and increased in HL treatments.

Photosynthetic capacities

During both years, V_{cmax} and J_{max} were higher in sun than in shade leaves. V_{cmax} and J_{max} decreased with time in all treatments (Fig. 5). In 1999, the leaf photosynthetic capacities of transferred leaves remained close to the values of the corresponding controls after transfer. In contrast, the respiration rate of HL leaves decreased rapidly after transfer and was similar to that of LL leaves 1 week after transfer. The respiration rate of LH leaves did not differ from values for LL leaves during the study period.

In 2000, V_{cmax} decreased by around 40% in the HL treatment and increased by 25% in the LH treatment. Concurrently, J_{max} decreased by around 35% in HL and increased by 45% in LH treatment. Respiration rate acclimated fully within 2 weeks of transfer in both LH and HL treatments. Full acclimation was defined as occurring when plants transferred to the new light condition produced values for the various parameters that were the same as those for plants grown constantly under this condition.

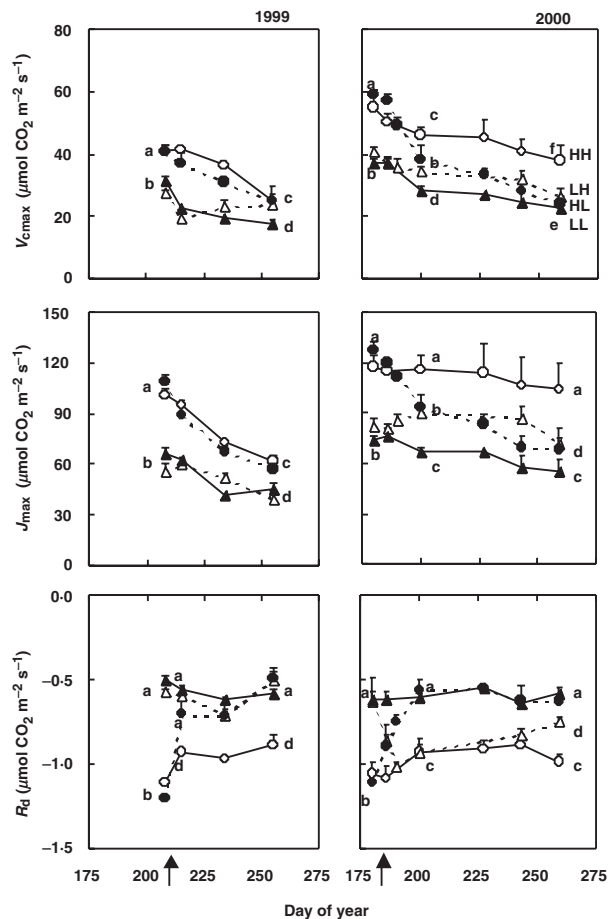


Figure 5. Temporal changes in the maximum carboxylation rate (V_{cmax}), the light-saturated electron transport rate (J_{max}) and the respiration rate (R_d) per unit leaf area in 1999 and 2000. Symbols as in Fig. 3; bars indicate standard errors. The effects of T , D and I were highly significant for all variables ($P < 0.001$) in 1999 and 2000. Significant differences on a few dates are indicated by letters.

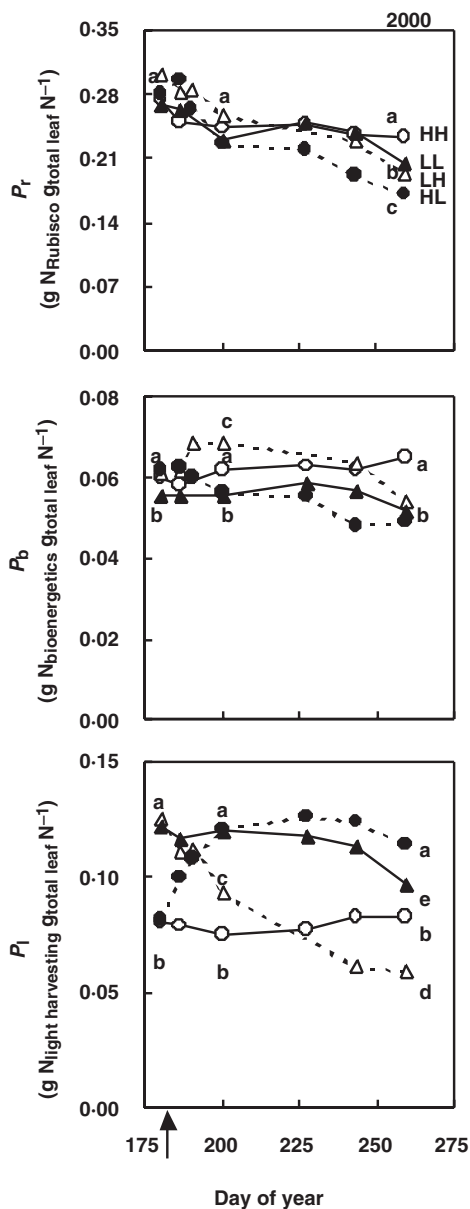


Figure 6. Temporal changes of the nitrogen investments into carboxylation (P_r), bioenergetics (P_b) and light harvesting (P_l) in 2000, computed according to Niinemets & Tenhunen (1997). Symbols as in Fig. 3. T had a smaller effect on P_r than D ($P = 0.042$ and $P < 0.001$), and P_b was affected more by T than by D ($P < 0.001$ and $P = 0.034$). I was NS for P_r and P_b . T , D and I had a highly significant effect on P_l ($P < 0.001$). Significant differences on a few dates are indicated by letters.

Leaf N partitioning within the photosynthetic apparatus

In 1999, leaf N partitioning between carboxylation and bioenergetics did not change significantly after transfer as compared with plants grown in constant LL and HH (not shown). However, in 2000, the fraction of leaf N invested into carboxylation decreased significantly for HL leaves as compared with HH leaves (Fig. 6). Concurrently, the frac-

tion of N invested into carboxylation did not change between LL and LH treatments. The fraction of leaf N invested in bioenergetics increased significantly in LH and decreased in HL trees after transfer as compared with constant LL and HH, respectively. These changes were significant 2 weeks after transfer for LH leaves (6.9% versus 5.6%) and 3 weeks after transfer for HL leaves (5.6% versus 6.2%). In 1999 (not shown) and 2000 (Fig. 6), the fraction of leaf N invested into light capture decreased significantly in LH and increased in HL treatments after transfer as compared with controls. For HL leaves, the fraction of leaf N invested into light capture became similar to values for LL leaves (12% instead of 8% initially) within 2 weeks of transfer. The delay for full acclimation of P_l in LH leaves was around 1.5 months.

After LH transfer, the relative changes in V_{cmax} resulted mainly from an increase in N_a , whereas P_r decreased slightly with time (Fig. 7). However, after HL transfer, the relative changes in V_{cmax} resulted from changes in N_a and P_r equally. Concurrently, changes in N_a and P_b drove the relative changes in J_{max} after HL transfer equally. After LH transfer, the relative changes in J_{max} resulted mainly from changes in P_b initially (until 20 d after transfer), and were then driven mainly by an increase in N_a .

For the LL, HH and HL treatments, the P_r values computed according to Niinemets & Tenhunen (1997) were well correlated to those computed directly from measured total N and Rubisco amounts (Fig. 8). However, P_r values estimated from Niinemets & Tenhunen (1997) were 45% lower than those computed directly. Furthermore, P_r values of

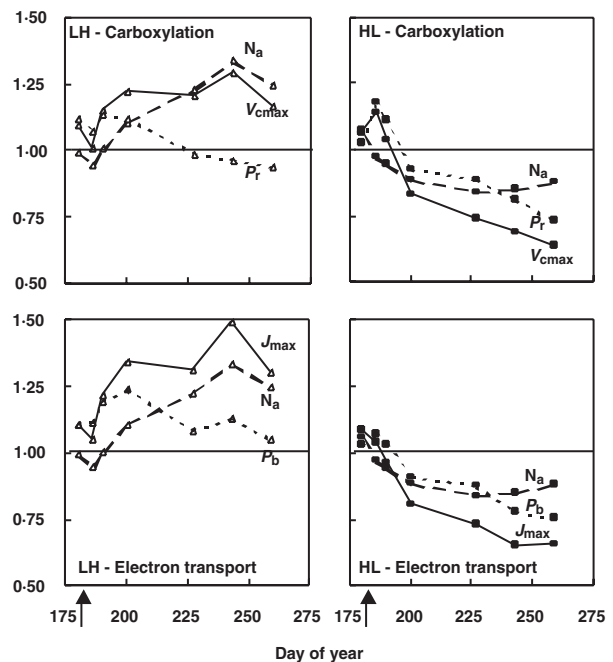


Figure 7. Variations in V_{cmax} and J_{max} and in their components N_a , P_r and P_b as a function of time after HL or LH transfer. Values for HL and LH leaves are normalized to those observed on control HH and LL leaves, respectively.

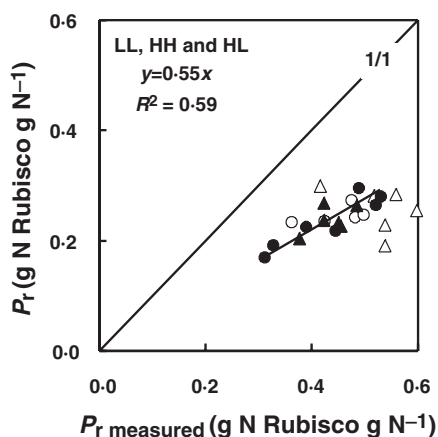


Figure 8. Comparison between the nitrogen investment into carboxylation computed according to Niinemets & Tenhunen (1997) (P_r) and that computed directly from measured total nitrogen and Rubisco amounts ($P_{r \text{ measured}}$). Symbols as in Fig. 3. The fitted curve and equation given in the graph correspond only to LL, HH and HL treatments

LH leaves computed according to Niinemets & Tenhunen (1997) decreased from 30 to 19% after transfer, whereas those computed directly from N and Rubisco amounts increased from 41 to 59%.

DISCUSSION

Acclimation ability in fully developed walnut leaves

In 2000, photosynthetic acclimation occurred in fully developed walnut (*Juglans nigra* × *regia*) leaves after a sudden change in light regime, and was demonstrated by the increase in photosynthetic capacity in the LH treatment and the decrease in the HL treatment. Acclimation of photosynthetic capacity in adult leaves was reported to occur in response to sudden changes in light microclimate in *Phaseolus vulgaris* (von Caemmerer & Farquhar 1984), *Lolium multiflorum* (Sebaa, Prioul & Brangeon 1987), *C. sativus* (Evans 1989), *Abies amabilis* (Brooks *et al.* 1994) and *G. max* (Pons & Pearcy 1994) after high-to-low light transfers, and in adult leaves of *Hedera helix* (Bauer & Thoni 1988), *Quercus rubra* and *Acer saccharum* (Naidu & DeLucia 1997) after low-to-high light transfers. In our study, photosynthetic capacity decreased by 35–40% in HL trees and increased by 25–45% in LH trees within 20 d of transfer, whereas full acclimation of V_{cmax} and J_{max} to the new light regime was not observed 50 d after transfer. The fact that the leaf structure is fixed after the completion of leaf development could explain the lack of full acclimation. This is consistent with the lack of full acclimation of photosynthetic capacity in *Q. rubra* and *A. saccharum* 1 month after a low-to-high light transfer (Naidu & DeLucia 1997). In *A. amabilis*, full acclimation was observed only 2–3 months after shading (Brooks *et al.* 1994). However, abscission of needles with a less favourable carbon balance,

together with the large gradient in needle properties within a single shoot, could lead to an illusion of perfect acclimation. In contrast, photosynthetic capacity acclimated almost fully to the new, low-light regime within 10 d in *P. vulgaris* (von Caemmerer & Farquhar 1984) and *L. multiflorum* (Sebaa *et al.* (1987), and within 12 d in *G. max* (Pons & Pearcy 1994). As anticipated by Pearcy & Sims (1994), these results suggest that the time required for the completion of photosynthetic acclimation is higher for fully developed leaves of woody (more than 1 month) than herbaceous (less than 2 weeks) species. However, we anticipate that studies on fast-growing trees (e.g. poplar) and slow-growing herbaceous species could lead to a distinction between species exhibiting low versus high photosynthetic activities rather than between woody versus herbaceous species.

In contrast to the dynamics of acclimation observed for V_{cmax} and J_{max} , the dark respiration rate of fully developed walnut leaves acclimated fully within 1–2 weeks of the HL or LH transfer. Such a short delay in the acclimation of R_d is consistent with results obtained in both woody (Naidu & DeLucia 1997) and herbaceous (Sims & Pearcy 1991; Pons & Pearcy 1994) species after low-to-high or high-to-low transfers. The increase and decrease in leaf dark respiration rate in LH and HL leaves, respectively, were probably due to the costs of carbohydrate processing and transport and/or protein turnover (Irving & Silsby 1988; De Visser, Spitters & Bouma 1992).

Given that climatic conditions were similar in 1999 and 2000, the comparison of the results obtained in both years shows that the capacity for photosynthetic acclimation depended on leaf age at the time of change in light regime. In walnut, fully developed leaves exhibited a poor capacity for photosynthetic acclimation when the transfer was made 91 d after bud burst (1999), whereas they were largely able to acclimate when the transfer was made 58 d after bud burst (2000). Leaf age and/or ontogenic status has been shown to strongly influence photosynthetic acclimation capacity in young versus adult leaves of *Fragaria virginiana* (Jurik, Chabot & Chabot 1979), *H. helix* (Bauer & Thoni 1988) and *Alocasia macrorrhiza* (Sims & Pearcy 1992). In contrast, both 1-year-old and 4-year-old leaves of *A. amabilis* acclimated after shading (Brooks *et al.* 1994). These results show that, along with leaf production and turnover, the acclimation capacity of leaves of different ages in response to changes in light regime should be taken into account when analysing the ecological roles of photosynthetic acclimation.

Changes in non-structural carbohydrates and leaf mass : area ratio

Foliar TNCs vary spatially along light gradients in tree canopies (Kull & Niinemets 1998; Niinemets & Kull 1998; Le Roux *et al.* 1999a). The amount of TNCs in leaves is generally assumed to reflect the carbon (C) balance between acquisition and export (Chapin, Schulze & Mooney 1990) in source–sink interactions. Changes in light regime during

2000 induced a modification of TNCs, which suggests that source–sink relationships in LH and HL leaves were modified. However, a decrease in photosynthetic capacity in HL leaves did not reduce starch pool of the leaves, as reported by Thorne & Koller (1974) for shaded plants of *G. max*. The high amount of starch found in HL leaves after transfer was presumably because of a decrease in C demand (respiration rate and protein synthesis), as well as higher chlorophyll content and electron transport capacity as compared with ‘true’ shade leaves. In contrast to *G. max* (Thorne & Koller 1974), the amount of GFS in HL leaves of walnut decreased through the experiment and was independent of starch content. In both the HL and the LH treatments, changes in GFS were concurrent with changes in N_a , suggesting a strong interaction between these two pools. Although C demand for respiration and protein synthesis increased during photosynthetic acclimation in LH leaves, limitation of C exportation could be responsible for starch accumulation, as reported by Silvius, Chatterton & Kremer (1979) for fully developed leaves of *G. max* transferred to bright light.

In many species, LMA varies according to the light environment and largely drives the differences observed for some leaf photosynthetic characteristics (e.g. N_a) within the tree canopy (Niinemets 1997; Niinemets & Kull 1998; Le Roux *et al.* 1999a; Rosati, Day & DeJong 2000). The TNC-free LMA quantifies the allocation of C to the leaf structures. Allocation of biomass to the leaf structures was higher in HH and HL than in LL and LH treatments, respectively. After transfer, TNC-free LMA in the HL treatment did not change as compared with values observed for the constant light-grown plants. This shows that physiological and biochemical, rather than structural, modifications controlled photosynthetic acclimation. However, in LH leaves, an increase in LMA occurred during photosynthetic acclimation. This increase was independent of the accumulation of TNC and protein, and could be due to a larger production of secondary metabolites like phenolics or flavonoids (Christian Jay-Allemand, personal communication).

Importance of concurrent changes in total leaf N and N partitioning between photosynthetic functions for photosynthetic acclimation

Generally, photosynthetic capacities per unit leaf area are tightly correlated to the amount of leaf N invested into the different photosynthetic functions (Evans & Seemann 1989). Thus, photosynthetic light acclimation in fully developed leaves following a change in light regime can be driven either by changes in N_a and/or by partitioning of total leaf N between the different pools of the photosynthetic machinery, as expressed by P_r and P_b . In our study, the relative changes in V_{cmax} or J_{max} resulted equally from changes in N_a and P_r or P_b after a HL transfer. The qualitative agreement observed for the HL treatment between the P_r values computed according to Niinemets & Tenhunen (1997) and those computed directly from the measured amounts of total N and Rubisco provided some

confidence in the observed behaviour of P_r . Only a few studies have actually surveyed the changes in photosynthetic capacity, N_a and leaf N partitioning after a change in light regime. In *G. max*, the decrease in V_{cmax} resulted from a decrease in both N_a and P_r after shading (Pons & Pearcy 1994). Concurrently, the decrease in J_{max} resulted mainly from changes in N_a , whereas P_b remained constant. In *A. amabilis*, Brooks *et al.* (1994) showed that acclimation of photosynthetic capacity resulted only marginally from changes in N_a after a high-to-low light transfer. Therefore, changes in both N_a and leaf N partitioning between the different photosynthetic functions can control photosynthetic acclimation to shading in fully developed leaves.

After the LH transfer, changes in V_{cmax} resulted mainly from an increase in N_a , whereas P_r computed according to Niinemets & Tenhunen (1997) decreased with time. An increase in N_a after a low-to-high light transfer was observed in *Q. rubra* and *A. saccharum* (Naidu & DeLucia 1997). Similarly, an increase in soluble proteins was observed in *H. helix* after an increase in light level (Bauer & Thoni 1988). However, to our knowledge, changes in the partitioning of leaf N have not been quantified accurately after a transfer of mature shade leaves to bright light. The discrepancy observed for the LH treatment between the P_r values computed according to Niinemets & Tenhunen (1997) (i.e. from the V_{cmax} values estimated on the basis of the CO_2 partial pressure in the substomatal spaces) and those computed directly from measured total N and Rubisco amounts suggests that (i) the investment of leaf N into carboxylation actually increased, and (ii) an enhanced limitation of photosynthesis by CO_2 diffusion into the leaves occurred when the photosynthetic capacity of LH leaves increased. Several studies demonstrated that a significant diffusive resistance to CO_2 exists between the intercellular spaces and carboxylation sites, which leads to underestimation of maximal carboxylation rate (Lloyd *et al.* 1992; Loreto *et al.* 1992; Epron *et al.* 1995). In our study, the internal conductance (g_i) to CO_2 transfer measured before transfer was much lower in shade (around $100 \text{ mmol m}^{-2} \text{ s}^{-1}$) than in sun (around $250 \text{ mmol m}^{-2} \text{ s}^{-1}$) leaves (C. Piel *et al.*, unpublished results). Using these g_i values to calculate V_{cmax} led to a 35–45% increase in both computed V_{cmax} and N investment into carboxylation, calculated from Eqn 1. This underlines the importance of internal conductance for the computation of V_{cmax} and N investment, as suggested by Niinemets & Tenhunen (1997).

The strong increase in the fraction of leaf N invested into light harvesting after a HL transfer is consistent with results reported by Evans (1989) in *C. sativus*, by Pons & Pearcy (1994) in *G. max* and by Brooks *et al.* (1994) in *A. amabilis*. In our study and that of Pons & Pearcy (1994), P_1 acclimated fully within <2 weeks of transfer. Such an increase in P_1 enhances the capture of the major limiting resource for shade leaves, i.e. light (Evans 1987; Evans & Seemann 1989). In contrast, the decrease of P_1 in LH leaves was less rapid, and P_1 values only acclimated fully 50 d after transfer. P_1 decreased only weakly in leaves of *Q. rubra* and *A. saccharum* transferred to bright light (Naidu & DeLucia 1998).

CONCLUSIONS

In this study, we demonstrated that concurrent changes in total leaf N and N investment in carboxylation, electron transport and light harvesting drove photosynthetic acclimation in mature leaves of hybrid walnut. The relative roles of the different changes differed between HL and LH transfers and depended on the time after transfer. Our results suggest that a strong limitation of photosynthesis by CO₂ diffusion occurred after a transfer to high light. The contrast between the data obtained in 1999 and in 2000 underlined the effect of leaf age on photosynthetic acclimation ability.

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