Photosynthetic light acclimation in peach leaves: importance of changes in mass:area ratio, nitrogen concentration, and leaf nitrogen partitioning

X. LE ROUX,1 A. S. WALCROFT,1 F. A. DAUDET,1 H. SINOQUET,1 M. M. CHAVES,2 A. RODRIGUES2 and L. OSORIO2

1 U.M.R. PIAF (INRA-Université Blaise Pascal), Domaine de Crouelle, 234 Avenue du Brezet, 63039 Clermont-Ferrand Cedex 02, France
2 Dept. Botânica e Engenharia Biológica, Instituto Superior de Agronomia, Tapada da Ajuda, 1399 Lisboa codex, Portugal

Received December 23, 1999

Summary Photosynthetic light acclimation of leaves can result from (i) changes in mass-based leaf nitrogen concentration, \( N_m \), (ii) changes in leaf mass:area ratio, \( M_a \), and (iii) partitioning of total leaf nitrogen among different pools of the photosynthetic machinery. We studied variations in \( N_m \) and \( M_a \) within the crowns of two peach (Prunus persica L. Batsch) trees grown in an orchard in Portugal, and one peach tree grown in an orchard in France. Each crown was digitized and a 3-D radiation transfer model was used to quantify the intracrown variations in time-integrated leaf irradiance, \(<\text{PAR}_a>\). Nitrogen concentration, leaf mass:area ratio, chlorophyll concentration, and photosynthetic capacity were also measured on leaves sampled on five additional peach trees in the orchard in Portugal. The data were used to compute the coefficients of leaf nitrogen partitioning among carboxylation, bioenergetics, and light harvesting pools. Leaf mass:area ratio and area-based leaf nitrogen concentration, \( N_a \), were nonlinearly related to \(<\text{PAR}_a>\), and photosynthetic capacity was linearly related to \( N_a \). Photosynthetic light acclimation resulted mainly from changes in \( M_a \) and leaf nitrogen partitioning, and to a lesser extent from changes in \( N_m \). This behavior contrasts with photosynthetic light acclimation observed in other tree species like walnut (Juglans regia L.) in which acclimation results primarily from changes in \( M_a \).

Keywords: chlorophyll, intra-crown variability, Juglans regia, leaf irradiance, photosynthesis, Prunus persica, radiation transfer model, specific leaf area, walnut.

Introduction Plant acclimation to the environment, i.e., the phenotypic response to different combinations of environmental conditions, is critical for plant productivity and survival, and thus strongly determines patterns of distribution and abundance of species (Woodward 1987). Because photosynthetic energy capture is crucial for green plants, acclimation of photosynthetic leaf traits to the environment is of particular importance for plant fitness. For a given nitrogen availability, leaf photosynthetic capacity generally acclimates to the prevailing light regime (Field and Mooney 1986, Evans 1989b). Single leaves can acclimate to the vertical gradient of mean leaf irradiance encountered within plant stands (e.g., Hirose and Werger 1987b, Ackerly 1992, Anten and Werger 1996), or to the heterogeneous light regime observed within individual tree crowns (e.g., DeJong and Doyle 1985, Le Roux et al. 1999a, 1999b). Generally, leaves developed at high leaf irradiance have a high nitrogen content and a high photosynthetic capacity per unit leaf area compared with shade leaves. This can significantly enhance carbon gain at the whole-plant level (Field 1983, Hirose and Werger 1987a, Leuning et al. 1995, Hollinger 1996).

Because more than 50% of total leaf nitrogen is allocated to the photosynthetic apparatus (Makino and Osmond 1991), photosynthetic capacity generally strongly correlates to the total amount of leaf nitrogen per unit leaf area (Field and Mooney 1986, Evans 1989b, Walcroft et al. 1997). Photosynthetic light acclimation of individual leaves can be driven by changes in leaf anatomical features (particularly changes in leaf mass:area ratio, \( M_a \)), or by changes in biochemical features (particularly total leaf nitrogen concentration, \( N_m \), and partitioning of total leaf nitrogen among the different pools of the photosynthetic machinery), or both (Boardman 1977, Björkman 1981, Anderson and Osmond 1987). Ecological studies have generally focused on anatomically based leaf light acclimation (e.g., Jarvis 1964, Nobel 1976, Chabot et al. 1979, Kellomäki and Oker-Blom 1981, Klinka et al. 1992). Some studies have also examined the relative importance of changes in \( M_a \) versus changes in photosynthetic capacity and respiration rate per unit leaf mass in controlling photosynthetic light acclimation, without distinguishing between changes in total leaf nitrogen concentration and changes in partitioning of total leaf nitrogen among the different photosynthetic pools (e.g., Marini and Marini 1983, Chazdon and Kaufmann 1993). Physiological studies have demonstrated the importance of biochemical changes such as the composi-
tion of chloroplast components and the partitioning of total leaf nitrogen among the different pools of the photosynthetic machinery for light acclimation, without considering fully the importance of changes in $M_a$ (e.g., Leong and Anderson 1984, Evans 1987, Anderson et al. 1988, Chow et al. 1988, Terashima and Evans 1988, Evans 1989a, Pons and Pearcy 1994). Consequently, comprehensive studies on the relative importance of concurrent changes in $M_a$, $N_a$, and leaf nitrogen partitioning in controlling the photosynthetic light acclimation of a given plant material in the field are scarce. This restricts our ability to understand photosynthetic light acclimation under natural conditions and to compare strategies of photosynthetic light acclimation of different species.

Following the seminal work by Pons and Pearcy (1994), Niinemets and Tenhunen (1997) studied the importance of changes in $M_a$, $N_a$, and leaf nitrogen partitioning for light acclimation in several other species (Niinemets et al. 1998), including *Juglans regia* L. (Le Roux et al. 1999a). In all of these species, light acclimation is dominated by adjustments in $M_a$ for leaf irradiances ranging from 5 to 30 mol m$^{-2}$ day$^{-1}$, suggesting that anatomical rather than biochemical changes generally govern leaf photosynthetic acclimation to high light. However, it remains to be determined if such behavior fully describes photosynthetic light acclimation in all tree species. For example, changes in leaf anatomy could be important for shade acclimation in leaves developed in the lower layers or inner parts of a dense canopy, whereas shade reacclimation occurring during canopy development when leaf area index is increasing could depend on nitrogen mobilization and reallocation as shown in soybean (*Glycine max* (L.) Merrill) (Pons and Pearcy 1994). Shade reacclimation is particularly important for continuously growing tree canopies such as peach, where many leaves within the canopy experience a changing light regime with time.

Photosynthetic light acclimation studies in peach indicate that (i) $M_a$, $N_a$ and nitrogen per unit leaf area, $N_a$, are linearly related to light exposure (expressed as hours at PAR > 100 µmol m$^{-2}$ s$^{-1}$), (ii) $N_a$ weakly varies with light conditions, and (iii) photosynthetic light acclimation in peach largely results from changes in $M_a$, and to a lesser extent from changes in leaf N partitioning (DeJong and Doyle 1985, Rosati et al. 1999, 2000). In these studies, light regime was not characterized as irradiance. Instead, data were collected based on six irradiance instead of the light exposure index on the linearity of the relationships between $M_a$, $N_a$, or $N_a$ and light regime, and (ii) the importance of changes in leaf nitrogen partitioning along a light gradient in peach tree canopies. Variations in $N_a$ and $M_a$ were studied within the crowns of two peach (*Prunus persica* L. Batsch) trees grown in an orchard in Portugal, and one peach tree grown in an orchard in France. Each crown was digitized and a 3-D radiation transfer model was used to quantify intra-crown variations in time-integrated leaf irradiance. Nitrogen concentration, mass:area ratio, chlorophyll concentration, and photosynthetic capacity were also measured on leaves sampled on five additional peach trees in the orchard in Portugal. The approach of Niinemets and Tenhunen (1997) was used to characterize the sources of photosynthetic light acclimation. In addition, the light acclimation process in peach was compared with that in walnut (*Juglans regia* L.) (Le Roux et al. 1999a, 1999b).

**Material and methods**

**Plant material**

Most measurements were made in July 1998 in a nectarine (*Prunus persica* var. Silver King) orchard near Montijo, Portugal (38° N, 9° W). The 3-year-old trees were grown on a podzolic sandy soil in a 1-ha plantation with a south–north row orientation. Additional measurements were made in May 1999 in a peach (*Prunus persica* var. Alexandra) orchard near Avignon, France (43.9° N, 4.8° E). The 6-year-old trees were grown on a silt–clay soil in a 0.25-ha orchard (5 x 5 m plantation). On both sites, fertilization and drip irrigation ensured adequate nutrient supply and non-limiting soil water conditions, respectively.

Leaf gas exchange and leaf nitrogen concentration were measured on leaves sampled from five trees in the orchard in Portugal. The within-crown spatial distributions of leaf mass:area ratio and nitrogen concentration in relation to light regime were studied on two additional trees in the same orchard, and on one tree in the orchard at Avignon.

**Digitization of tree crowns and simulation of the intra-crown radiation regime**

The architecture of the two trees investigated for spatial variations in leaf nitrogen in Portugal and of the tree at Avignon was measured by a 3-D digitizing technique (Sinouquet et al. 1997a). This allowed us to record simultaneously tree geometry, i.e., the spatial location of tree components, and tree topology, i.e., the physical connections between tree components. Spatial coordinates were recorded with an electromagnetic 3-D digitizer (Fastrak, Polhemus Inc., Colchester, VT), whereas tree topology was described as a multi-scale tree graph (Godin and Caraglio 1998). The tree graph allows the components of the tree to be defined at several scales, and describes connections between tree components in terms of succession and branching. For a complete description of the method, see Godin et al. (1999). The peach trees were described at three scales. At Scale 1, the trees were represented...
as a collection of axes (A), i.e., trunk and branches of different orders. At Scale 2, axes were considered to comprise segments (S), long shoots (L) and short shoots (D). Segments were used to describe the woody parts set during the previous years, whereas long and short shoots accounted for the leafy parts only. At Scale 3, long shoots were described in terms of internodes (I). At the internode level, stipules (F) were also recorded. Basal diameter was measured with a Vernier calliper as an attribute of axes, segments and long shoots. Leaf number was recorded as an attribute of short shoots and stipules that allowed the spatial co-ordinates of each leaf within the tree crowns to be determined. From intensive sampling (about 3000 leaves), the mean areas of leaves subtending long shoots, short shoots and stipules were estimated to be 22, 14 and 8 cm², respectively (C. Valancogne, INRA Bordeaux, unpublished data). At Montijo, leaf orientation was measured on all leaves of 10 spurs and 15 long shoots, randomly sampled in the tree canopies (550 leaves in total). The orientation of each leaf was measured with the 3-D digitizer by setting the pointer parallel to both the midrib and the lamina. Lamina inclination (i.e., the angle between leaf normal and the vertical direction) was derived from midrib inclination and rolling angles. For each leaf, length and width were measured with a ruler at the same time as the tree crowns were digitized. This allowed computation of the leaf inclination distribution by weighting individual leaf angles by leaf area.

The distribution of time-integrated leaf irradiance within the digitized tree crowns was computed with a 3-D version of the RIRI model (Radiation Interception in Row Intercropping, Sinoquet and Bonhomme 1992). The model is based on the turbid medium analogy and deals with direct and diffuse incident radiation and scattered radiation. The tree is abstracted by an array of 3-D cells that may be either empty or contain leaves. Radiation exchange factors between radiation sources (incident direct and diffuse radiation from the sky and scattered radiation from leaf area and soil surface) and receivers (leaf area, soil surface, and the sky for reflected radiation) are computed from a sample of beams coming from all sky directions. Beam interception within the cells is derived from the Beer-Lambert law. For each 3-D cell, the radiation balance including multiple scattering is solved by an approach similar to the radiosity method (Ozisik 1981). This allows the derivation of leaf irradiance in each 3-D cell. More details on the model are given in Sinoquet and Bonhomme (1992). The ability of the model to simulate the spatial and temporal trends of leaf irradiance within individual tree crowns has been tested in walnut (Sinoquet et al. 1997b).

The 3-D light model uses tree architecture, leaf optical properties, and leaf inclination distribution as input parameters. In this study, each tree crown was abstracted by a set of 0.25 × 0.25 × 0.25 m cells where leaves were allocated according to their spatial co-ordinates measured with the digitizer. The measured leaf angle distribution was used. Leaf reflectance and transmittance in the PAR waveband were assumed to equal 0.08. The model was run over the diurnal period with a 30-min time step, based on incident global and diffuse radiation data measured during a 1-week period before sampling.

**Measurements of leaf photosynthetic capacity**

Net CO₂ assimilation rate, A, stomatal conductance, and CO₂ partial pressure in the substomatal spaces, Cᵢ, were measured with a portable infrared gas analyzer (LI 6400, Li-Cor, Inc., Lincoln, NE) equipped with leaf chamber and a red/blue light source. Measurements were made on 14–17 fully expanded leaves chosen within the crowns of five trees in the orchard in Portugal. These leaves encompassed full sunlight and shade conditions. The key parameters of the Farquhar photosynthesis model (Farquhar et al. 1980, version proposed by Harley et al. 1992) were determined for each leaf. An A–Cᵢ response curve measured at high irradiance (PAR = 1200 µmol m⁻² s⁻¹) was used to infer the best fit value of maximum carboxylation rate V_max by non-linear least squares regression, using the SAS statistical software suite Ver. 1990 (SAS Institute Inc., Cary, NC). Only data collected at Cᵢ values below 25 Pa were used. On the same leaf, an A–PAR response curve performed at high CO₂ partial pressure (Cᵢ = 100 Pa) was used to infer the best fit value of electron transport capacity, J_max, by the same non-linear regression technique. For each response curve, eight measurements were made (Cᵢ = 100, 35, 30, 20, 15, 10, 7.5 and 5 Pa, and PAR = 1800, 1500, 1000, 500, 200, 100, 50, and 0 µmol m⁻² s⁻¹). A 15-min equilibration time was allowed before each measurement. Values of leaf dark respiration, R_d, were estimated by measuring CO₂ evolution rates before daybreak (around 0600 h). During A–Cᵢ responses, A–PAR responses, and R_d determinations, leaf temperature and air water vapor pressure deficit at the leaf surface were 30 ± 0.4 °C and 1.3 ± 0.2 kPa, respectively.

A detailed description of the version of the Farquhar photosynthesis model used is given by Harley et al. (1992), and model primary parameters including the kinetic constants for Rubisco are tabulated in Le Roux et al. (1999a).

**Measurements of Mₛ, Nₛ, and chlorophyll concentration**

To study the relationship between photosynthetic capacity and Nₛ, the 14 leaves used for determination of photosynthetic capacity were harvested immediately after the gas exchange measurements. To study the relationship between Nₛ and daily mean leaf irradiance, 16 leaves were sampled within the crown of the two digitized trees in Portugal on July 20, 1998 (32 leaves in total) and 21 leaves were sampled within the crown of the digitized tree at Avignon on May 20 1999. All the leaves were sampled between 1500 and 1600 h. These leaves were identified during the digitizing procedures, allowing them to be located within the reconstructed tree architectures to simulate their radiation regime.

The shape of each sampled leaf was drawn on paper, and leaves were then frozen in liquid nitrogen and lyophilized. In the laboratory, leaf shapes were used to measure fresh leaf areas with an area meter (Delta T Devices, Hoddeston, U.K.). Leaf dry mass was measured, and total leaf nitrogen concentration was determined with an elemental analyzer (Carlo Erba
Discs were taken from the leaves used for determination of photosynthetic capacity, and chlorophyll concentration was measured after extraction in 80% acetone as described by Arnon (1949).

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

The model proposed by Niinemets and Tenhunen (1997) was used to determine the coefficients for leaf nitrogen partitioning between carboxylation (mainly Rubisco) \( (P_c) \), 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, \( P_c \) defines the foliar nitrogen cost for overall light-saturated carboxylation capacity (i.e., influencing \( V_{cmax} \)), \( P_b \) determines the nitrogen cost for the capacity of electron transport and photophosphorylation (i.e., influencing \( J_{max} \)), whereas \( P_l \) determines the nitrogen cost for light harvesting. The computation of \( P_c \) (g N in Rubisco (g total leaf N)\(^{-1}\)) and \( P_b \) (g N in cytochrome f, ferredoxin NADP reductase, and coupling factor (g total leaf N)\(^{-1}\)) is given by:

\[
P_c = \frac{V_{cmax}}{(6.25V_{cmax}N_a)}, \tag{1}
\]

\[
P_b = \frac{J_{max}}{(8.06J_{max}N_a)}, \tag{2}
\]

where \( V_c \) is specific activity of Rubisco (i.e., the maximum rate of RuBP carboxylation per unit Rubisco protein in \( \mu \)mol CO\(_2\) (g Rubisco)\(^{-1}\) s\(^{-1}\)), \( J_{max} \) is the potential rate of photosynthetic electron transport per unit cytochrome f (mol electrons (mol cyt f)\(^{-1}\) s\(^{-1}\)), 6.25 (g Rubisco (g N in Rubisco)\(^{-1}\)) converts nitrogen content to protein content, and 8.06 (\( \mu \)mol cyt f (g N in bioenergetics)\(^{-1}\)) was used, assuming a constant 1:1:1.2 molar ratio for cyt f:ferredoxine NADP reductase:coupling factor (Niinemets and Tenhunen 1997). According to Niinemets and Tenhunen (1997, their Equations 4, 5 and 6 and Appendix B), at a leaf temperature of 30 °C, \( V_c \) and \( J_{max} \) are equal to 31.9 \( \mu \)mol CO\(_2\) (g Rubisco)\(^{-1}\) s\(^{-1}\) and 180.7 mol electrons (mol cyt f)\(^{-1}\) s\(^{-1}\), respectively. The value of \( P_l \) (g N in PSI, PSII and LCHII (g total leaf N)\(^{-1}\)) is computed in a similar way assuming scaling relationships (i) between \( J_{max} \) and cytochrome f, and (ii) between the different thylakoid chlorophyll-protein complexes and cytochrome f (for details, see Equation 6 and Appendix B in Niinemets and Tenhunen 1997).

Comparison with published data for peach and walnut trees

Rosati et al. (1999) reported values for (i) daily leaf irradiance, (ii) leaf mass:area ratio, (iii) nitrogen concentration, and (iv) maximal rate of carboxylation and light-saturated rate of electron transport, for leaves in full sunlight or deep shade within the canopy of the nectarine cultivar Fantasia grown at two nitrogen-supply rates. These published data were used to compute \( P_c \) and \( P_b \) to compare with our data.

Published data on photosynthetic light acclimation in walnut leaves (Le Roux et al. 1999a, 1999b) were also used for comparison. These data were obtained by the same methodology as in the present study. The leaves were sampled over the whole range of irradiance experienced within the canopy of a 20-year-old tree, and on greenhouse-grown seedlings subjected to two irradiance treatments.

Results

Simulated spatial variations in time-integrated leaf irradiance within the peach tree foliage

The two digitized peach trees at Montijo (Figure 1) had total leaf areas of 23.3 and 13.5 m\(^2\). Long-shoot leaves, spur leaves and stipule leaves represented 83, 12, and 5% of total leaf area, respectively. The measured leaf inclination distribution was close to the classical erectophile distribution (Figure 2). Total leaf area of the digitized tree at Avignon was 67.3 m\(^2\). Long shoot leaves and spur leaves represented 96.4 and 3.5% of to-
tal leaf area, respectively.

The simulated mean leaf irradiance over one week, \( \text{<PAR}_i \), varied within the tree canopy (Figure 3). At Montijo, the maximum and minimum values of \( \text{<PAR}_i \) were 28.2 and 3.8 mol m\(^{-2}\) day\(^{-1}\), respectively. Fifty percent of the total leaf surface experienced \( \text{<PAR}_i \) values ranging from 6.25 to 11.25 mol m\(^{-2}\) day\(^{-1}\) (Figure 3a). At Avignon, the maximum and minimum values of \( \text{<PAR}_i \) were 26 and 1.8 mol m\(^{-2}\) day\(^{-1}\), respectively. Forty-four percent of the total leaf surface experienced \( \text{<PAR}_i \) values ranging from 3.75 to 8.75 mol m\(^{-2}\) day\(^{-1}\) (Figure 3b). At Montijo, 99.7% of the sampled leaves experienced \( \text{<PAR}_i \) values between 3.8 and 26 mol m\(^{-2}\) day\(^{-1}\), whereas at Avignon, 77.3% of the sampled leaves experienced \( \text{<PAR}_i \) values between 2.2 and 14.1 mol m\(^{-2}\) day\(^{-1}\).

Variations in \( M_a \), \( N_a \) with leaf radiation regime

Relationships between \( N_m \), \( M_a \) or \( N_a \) and \( \text{<PAR}_i \) differed for Montijo and Avignon. At a given irradiance, higher \( M_a \) and lower \( N_m \) were found at Montijo than at Avignon, whereas \( N_a \) exhibited little variation at either location (Table 1). Normalization of the leaf characteristics versus \( \text{<PAR}_i \) relationships was achieved by using values obtained at 14 mol m\(^{-2}\) day\(^{-1}\) in both orchards as scaling factors (Table 1). The normalized \( N_m \) values varied with \( \text{<PAR}_i \) only weakly and linearly \((P = 0.0007)\) (Figure 4). In contrast, the normalized values of \( M_a \) and \( N_a \) exhibited nonlinear variations with \( \text{<PAR}_i \). The increases in \( M_a \) and \( N_a \) with increasing \( \text{<PAR}_i \) were adequately described by logarithmic functions \((P < 0.0001)\). The use of linear relationships led to lower \( r^2 \) values (0.39 versus 0.48 and 0.59 versus 0.69 for \( M_a \) and \( N_a \), respectively) and to biased residues (not shown). Both \( M_a \) and \( N_a \) varied strongly when \( \text{<PAR}_i \) increased from 2.5 to 6 mol m\(^{-2}\) day\(^{-1}\), whereas they

Table 1. Values of \( N_m \), \( M_a \) and \( N_a \) measured in leaves experiencing a mean irradiance of 14 mol m\(^{-2}\) day\(^{-1}\) on peach trees grown at Montijo and Avignon. These values were used to normalize data in Figure 4.

<table>
<thead>
<tr>
<th>Location</th>
<th>( N_m ) (g m(^{-2}))</th>
<th>( M_a ) (g m(^{-2}))</th>
<th>( N_a ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montijo (Portugal)</td>
<td>2.31</td>
<td>81.0</td>
<td>2.85</td>
</tr>
<tr>
<td>Avignon (France)</td>
<td>2.63</td>
<td>64.5</td>
<td>4.08</td>
</tr>
</tbody>
</table>

Figure 3. Frequency distribution of mean leaf irradiance (integrated over the week before sampling) within (a) the two peach tree crowns at Montijo, and (b) the peach tree crown at Avignon. Percentage refers to percent of total leaf area, and irradiance values represent the modes of classes (e.g., 5 for the 3.75–6.25 mol m\(^{-2}\) day\(^{-1}\) class).

Figure 4. Variations in (top) mass-based leaf nitrogen concentration, \( N_m \), (middle) leaf dry mass:area ratio, \( M_a \), and (bottom) area-based leaf nitrogen concentration, \( N_a \), with respect to mean daily leaf irradiance, \( \text{<PAR}_i \), as simulated by the RIRI model over a 1-week period before sampling. Data were obtained for 16 leaves in each digitized tree at Montijo (A) and for 21 leaves in the digitized tree at Avignon (O). All values are normalized for a leaf irradiance of 14 mol m\(^{-2}\) day\(^{-1}\) (see Table 1).
varied weakly with irradiance for \(<\text{PAR}_i\rangle\) higher than 6 mol m\(^{-2}\) day\(^{-1}\) (Figure 4). However, the correlations between \(N_m\), \(M_a\) or \(N_a\) and \(<\text{PAR}_i\rangle\) remained significant \((P = 0.045, 0.003\) and 0.0001\) when the analyses were restricted to \(<\text{PAR}_i\rangle\) higher than 6 mol m\(^{-2}\) day\(^{-1}\).

Variations in photosynthetic capacity, chlorophyll per area and leaf nitrogen partitioning

Parameters \(V_{\text{cmax}}\), \(J_{\text{max}}\), and \(R_d\) were linearly correlated to \(N_a\) \((P < 0.0001, 0.0001\) and 0.002, respectively\) (Figure 5). A threefold increase in these parameters was observed when \(N_a\) increased from 1.3 to 2.4 g N m\(^{-2}\). Total amount of chlorophyll \(a + b\) per unit leaf area was around 0.65 mmol m\(^{-2}\), and no significant \((P = 0.56)\) changes in the chlorophyll amount were observed within the tree canopy (Figure 6).

Leaf nitrogen partitioning between carboxylation, \(P_c\), bioenergetics, \(P_b\), and light capture pool, \(P_l\), differed significantly \((P\) from 0.04 to 0.0001\) with \(N_a\) (Figure 7). When \(N_a\) increased from 1.3 to 2.4 g N m\(^{-2}\), \(P_l\) decreased from 15 to 9\%, whereas \(P_c\) and \(P_b\) increased from 13 to 20\% and 3.9 to 4.7\%, respectively.

Discussion

Spatial variations in \(M_a\), \(N_m\) and \(N_a\) as a function of local radiation regime

The peach tree canopies exhibited moderate changes in \(M_a\) (i.e., from 65 to 88 g m\(^{-2}\), and from 40 to 70 g m\(^{-2}\) at Montijo and Avignon, respectively) and weak spatial variation in \(N_m\) (i.e., 2.5 to 3.1\% at Montijo, and 3.5 to 4.2\% at Avignon) despite strong intra-canopy light gradients (i.e., 4 to 28 mol m\(^{-2}\) day\(^{-1}\) and 1.8 to 26 mol m\(^{-2}\) day\(^{-1}\), respectively). Such weak spatial variation in \(N_m\) seems to be common in \(P. \) persica (DeJong and Doyle 1985, Rosati et al. 1999). The results obtained at Montijo are consistent with \(M_a\) data reported for \(P. \) persica, which typically range from around 50–60 g m\(^{-2}\) for leaves in inner parts of the canopy to 80–100 g m\(^{-2}\) for leaves in the outer parts of the canopy (Marini and Marini 1983, Rosati et al. 1999). In contrast, the \(M_a\) values of sun leaves at Avignon were low (70 g m\(^{-2}\)). In parallel, leaf nitro-
gen concentration was higher at Avignon than at Montijo. These differences in \( M_a \) and \( N_m \) between sites may be explained by (i) a cultivar effect, (ii) differences in climatic factors experienced during leaf ontogeny, or (iii) differences in nitrogen availability.

Our normalized values of \( N_m \) were linearly related to \(<\text{PAR}_i>\), whereas \( M_a \) and \( N_m \) were nonlinearly related to \(<\text{PAR}_i>\). In contrast, DeJong and Doyle (1985) and Rosati et al. (2000) observed a linear relationship between \( M_a, N_m \) and \( N_a \) and light exposure expressed as hours per day at PAR higher than 100 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). Nonlinear relationships between \( N_a \) and mean leaf irradiance have been reported for some canopies (e.g., Lemaire et al. 1991, Del Pozo and Dennet 1999), although linear relationships have also commonly been observed (e.g., Niinemets 1997, Le Roux et al. 1999b). Several authors have shown that, for a given species, linear relationships between \( N_a \) and \(<\text{PAR}_i>\) are observed in open stands, whereas nonlinear relationships are observed in dense stands, such as those of *Lysimachia vulgaris* L. (Hirose et al. 1988) or maize (Drouet and Bonhomme 1999). However, the data of DeJong and Doyle (1985) and Rosati et al. (2000) were obtained on 11-year-old trees, and the range of irradiances reported by the authors indicates that it is unlikely that differences in tree crown density can explain the discrepancy between the relationships. A reanalysis of our data showed that the relationships between \( N_m, M_a \) or \( N_a \) and the simulated daily number of hours at PAR higher than 100 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) were linear (Figure 8). Use of logarithmic relationships did not improve the regression coefficients (Table 2) because there was a nonlinear relationship between light exposure index and daily mean leaf irradiance \((y = 6.26\ln x - 6.43; r^2 = 0.87)\). Thus, the use of a light exposure index linearized the relationships between \( N_m, M_a \) or \( N_a \) and the radiation regime.

**Intra-crown variations in \( V_{\text{cmax}} \) versus changes in \( M_a \) versus changes in \( N_m \) versus changes in leaf nitrogen partitioning**

Despite the relatively weak spatial variations in \( N_m \) and \( M_a \), strong variations in photosynthetic capacity were observed within the canopy of the peach trees; namely, threefold increases in \( V_{\text{cmax}}, J_{\text{max}} \) and \( R_d \) versus a twofold increase in \( N_a \). The increase in dark respiration rate associated with the increased photosynthetic potential may reflect a photosynthetically-rate-dependent protein turnover or costs of assimilate transport, or both (Irving and Silsbury 1988, De Visser et al. 1992).

Our data allow assessment of the relative importance of changes in \( M_a, N_m \) and nitrogen partitioning for leaf photosynthetic light acclimation in peach. In this analysis, leaf photosynthetic light acclimation was assessed from the observed variation of \( V_{\text{cmax}} \) (and the related variables \( M_a, N_m \) and \( P_c \)) in response to variations in \(<\text{PAR}_i>\). The relative variations in \( V_{\text{cmax}} \) resulted mainly from changes in \( P_c \) and \( M_a \) and to a lesser extent from changes in \( N_m \) (Tables 3 and 4). The same conclusion was drawn for the irradiance ranges of 2.4 to 23.5 \( \text{mol m}^{-2} \text{day}^{-1} \) (Table 3) and 6 to 20 \( \text{mol m}^{-2} \text{day}^{-1} \) (Table 4), although variations in \( P_c \) were particularly important at low irradiance. Changes in \( V_{\text{cmax}} \) and \( N_m \) with increasing mean leaf irradiance were similar to changes reported for peach by Rosati et al. (1999). These authors found that changes in \( M_a \)

![Figure 8. Variations in (top) mass-based leaf nitrogen concentration, \( N_m \), (middle) leaf dry mass:area ratio, \( M_a \), and (bottom) area-based leaf nitrogen concentration, \( N_a \), with respect to the light exposure index (defined as the daily number of hours at PAR higher than 100 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)). Data were obtained for 16 leaves in each digitized tree at Montijo (A) and for 21 leaves in the digitized tree at Avignon (C). All values are normalized for a leaf irradiance of 14 \( \text{mol m}^{-2} \text{day}^{-1} \).](http://heronpublishing.com)

**Table 2. Regression coefficients \((r^2)\) of the relationships between \( N_m, M_a \) or \( N_a \) and light regime, with mean daily leaf irradiance or the light exposure index (i.e., daily number of hours at PAR higher than 100 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) as the independent variable. All the relationships are significant.**

<table>
<thead>
<tr>
<th></th>
<th>Linear regression</th>
<th>Logarithmic regression</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean daily irradiance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( N_m )</td>
<td>0.191</td>
<td>–</td>
</tr>
<tr>
<td>( M_a )</td>
<td>0.392</td>
<td>0.484</td>
</tr>
<tr>
<td>( N_a )</td>
<td>0.588</td>
<td>0.692</td>
</tr>
<tr>
<td><strong>Light exposure index</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( N_m )</td>
<td>0.214</td>
<td>–</td>
</tr>
<tr>
<td>( M_a )</td>
<td>0.394</td>
<td>0.410</td>
</tr>
<tr>
<td>( N_a )</td>
<td>0.594</td>
<td>0.61</td>
</tr>
</tbody>
</table>
were more important than changes in nitrogen partitioning for leaf light acclimation. Our results are qualitatively consistent with this conclusion, despite the higher relative importance of changes in \( P_c \) in our study (Table 3) (but note the variances associated with the relationships in Figures 4 and 5, and those associated with the results reported by Rosati, DeJong and colleagues). The major conclusion from these studies is that changes in both \( M_a \) and leaf nitrogen partitioning play a role in photosynthetic light acclimation in peach trees, and our study also shows that the relative role of variation in \( P_c \) is particularly important at low irradiance. This light acclimation process is consistent with results reported by Marini and Marini (1983) who found a 150 to 200% increase in light-saturated photosynthesis concurrently with a 33% increase in \( M_a \) for peach (their Figure 3b). Such behavior differs from that observed for other tree species like walnut (Table 4). In walnut, photosynthetic light acclimation essentially results from changes in \( M_a \), because changes in \( N_m \) and nitrogen partitioning are negligible. Control of photosynthetic light acclimation by \( M_a \) has been observed for many tree species (Gulmon and Chu 1981, Niinemets et al. 1998, Le Roux et al. 1999a, 1999b) and for several shade-tolerant herbaceous species (Nobel et al. 1975, Sims and Pearcy 1992). Comparison of the results obtained with peach and walnut, along with previously published data, shows that there is no universal rule concerning the relative importance of the factors controlling light acclimation of photosynthetic capacities.

The ability to adjust the allocation of total leaf nitrogen to the different pools of the photosynthetic machinery partly explains these different responses to shade. In peach trees, the relative amount of leaf nitrogen allocated to carboxylation (\( P_c \)) and electron carriers (\( P_b \)) increased with increasing irradiance. In contrast, the total amount of chlorophyll per unit leaf area was not significantly related to leaf radiation regime (which is consistent with earlier results published for \( P. persica \); see Marini and Marini 1983), so that the relative amount of leaf nitrogen allocated to chlorophyll–protein complexes (\( P_l \)) decreased with increasing irradiance. This is consistent with the expected optimal pattern of nitrogen partitioning that maximizes the daily CO\(_2\) carbon gain of individual leaves (Hikosaka and Terashima 1995). Such a qualitative trend was obtained for \emph{Phaseolus vulgaris} L. and \emph{Cucumis

### Table 3. Comparison of concurrent changes in \( V_{\text{cmax}} \) (µmol CO\(_2\) m\(^{-2}\) s\(^{-1}\)), \( M_a \) (g m\(^{-2}\)), \( N_m \) (%), and \( P_c \) along light gradients in peach trees obtained during this study (Montijo) and by Rosati et al. (1999). Changes were computed from equations given in Figures 4, 5 and 7 for our study, and from Figures 1 and 2 and Table 1 in Rosati et al. (1999). Values of integrated PAR were chosen according to data of Rosati et al. (1999).

<table>
<thead>
<tr>
<th>PAR(_i) = 2.4 mol m(^{-2}) day(^{-1})</th>
<th>PAR(_i) = 23.5 mol m(^{-2}) day(^{-1})</th>
<th>Ratio of value at 23.5 mol m(^{-2}) day(^{-1}) to value at 2.4 mol m(^{-2}) day(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peach (this study, Montijo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( V_{\text{cmax}} )</td>
<td>36.3</td>
<td>106.8</td>
</tr>
<tr>
<td>( M_a )</td>
<td>55.7</td>
<td>88.2</td>
</tr>
<tr>
<td>( N_m )</td>
<td>2.59</td>
<td>3.07</td>
</tr>
<tr>
<td>( P_c )</td>
<td>0.136</td>
<td>0.207</td>
</tr>
<tr>
<td>Peach (Rosati et al. 1999)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( V_{\text{cmax}} )</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>( M_a )</td>
<td>50</td>
<td>83</td>
</tr>
<tr>
<td>( N_m )</td>
<td>2.6</td>
<td>3.16</td>
</tr>
<tr>
<td>( P_c )</td>
<td>0.20</td>
<td>0.25</td>
</tr>
</tbody>
</table>

### Table 4. Comparison of concurrent changes in \( V_{\text{cmax}} \) (µmol CO\(_2\) m\(^{-2}\) s\(^{-1}\)), \( M_a \) (g m\(^{-2}\)), \( N_m \) (%), and \( P_c \) along light gradients in peach (Montijo) and walnut trees. Changes were computed from equations given in Figures 4, 5 and 7 for our study, and from equations given by Le Roux et al. (1999a, 1999b) for walnut. Values of integrated PAR were chosen according to data of Le Roux et al. (1999a).

<table>
<thead>
<tr>
<th>PAR(_i) = 6 mol m(^{-2}) day(^{-1})</th>
<th>PAR(_i) = 20 mol m(^{-2}) day(^{-1})</th>
<th>Ratio of value at 20 mol m(^{-2}) day(^{-1}) to value at 6 mol m(^{-2}) day(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peach (this study, Montijo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( V_{\text{cmax}} )</td>
<td>64.6</td>
<td>101.8</td>
</tr>
<tr>
<td>( M_a )</td>
<td>68.8</td>
<td>85.9</td>
</tr>
<tr>
<td>( N_m )</td>
<td>2.67</td>
<td>2.99</td>
</tr>
<tr>
<td>( P_c )</td>
<td>0.176</td>
<td>0.205</td>
</tr>
<tr>
<td>Walnut (Le Roux et al. 1999)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( V_{\text{cmax}} )</td>
<td>35.0</td>
<td>77.1</td>
</tr>
<tr>
<td>( M_a )</td>
<td>61.4</td>
<td>135.5</td>
</tr>
<tr>
<td>( N_m )</td>
<td>2.15</td>
<td>2.47</td>
</tr>
<tr>
<td>( P_c )</td>
<td>0.21</td>
<td>0.184</td>
</tr>
</tbody>
</table>
tius L. (Seemann et al. 1987, Evans 1989a) and for Alocasia macrorrhiza (L.) G. Don (Sims and Pearcy 1989). However, an inability to adjust leaf nitrogen partitioning has sometimes been observed. For instance, the fractional leaf nitrogen investments in Rubisco and bioenergetics are affected little, or not at all, by time-integrated leaf irradiance in Alocasia macrorrhiza (Seemann et al. 1987), in four temperate deciduous tree species (Niinemets et al. 1998) and in walnut (Le Roux et al. 1999a, 1999b). The carboxylation rate per unit nitrogen (V\textsubscript{cmax}/N\textsubscript{i}) and the electron transport capacity per unit nitrogen (J\textsubscript{max}/N\textsubscript{i}) decrease with increasing irradiance in some tree species (Thompson et al. 1988). The contradictory results obtained in Alocasia macrorrhiza by Seemann et al. (1987) and Sims and Pearcy (1989) illustrate that such results can depend on the experimental conditions.

Another explanation of such different leaf responses to shade is the effect of the radiation regime experienced during leaf ontogeny. As postulated by Pons and Pearcy (1994), changes in leaf anatomy may be important for shade acclimation in leaves that develop in the lower layers or inner parts of the canopy. Because most of the leaves sampled had developed when the tree canopy was more open. This could be the case in the peach trees studied in early summer, because most of the leaves sampled had developed when the tree canopy was more open.

Acknowledgments

The authors thank I. Ferreira (I.S.A., Lisbon, Portugal) for organizing the joint campaign in the peach orchard, N. Donès and S. Ploquin (INRA, Clermont Ferrand, France) for help during tree digitizing, E. Breia (ISA, Lisbon, Portugal) for help during leaf sample analysis, and C. Valancogne (INRA, Bordeaux, France) for providing climatic data and mean leaf area values for the trees studied. This work was supported by the FAIR1 project (CT95-0030-UE), the INRA–ICCTI (Instituto para a Cooperação Científica e Tecnológica Internacional) Cooperation, and fellowships provided by the French Embassy in Lisbon.

References


