Internal conductance under different light conditions along the plant profile of Ethiopian mustard (*Brassica carinata* A. Brown.)

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Abstract

This study focused on the internal conductance (*g*<sub>i</sub>) along the plant profile of Ethiopian mustard under two light conditions: (i) light from the top only (*I*<sub>1</sub>); (ii) light from the top integrated by supplementary lateral light along the whole plant profile (*I*<sub>2</sub>). Lateral light strongly increased the productivity (e.g. +104% of seed oil) and net photosynthesis (*A*). The latter appeared more driven by *g*<sub>i</sub> (*r* = 0.78**) than by stomatal conductance (*g*<sub>s</sub>) (*r* = 0.51*). Importantly, irradiance also considerably shortened the time from leaf appearance to senescence, which means that corresponding leaves in *I*<sub>1</sub> and *I*<sub>2</sub> had different ages. Therefore, since leaf age and irradiance have counteracting effects on *g*<sub>i</sub>, *I*<sub>1</sub> sometimes showed higher *g*<sub>i</sub> values than *I*<sub>2</sub>. With respect to irradiance, leaf age had clearly higher effects on *g*<sub>i</sub>, which radically declined from the top to the basal leaves, even under constant light conditions. The internal conductance caused a significant drawdown of CO<sub>2</sub> from the sub-stomatal cavity (*C*<sub>i</sub>) to the site of carboxylation (*C*<sub>c</sub>) that, in turn, led to a substantial underestimation of *V*<sub>cmax</sub> calculated using the A/C<sub>i</sub> model. Again, the trends of *g*<sub>i</sub> and *g*<sub>s</sub> were not consistent along the plant profile, and so the ratio between stomatal and internal limitations to *A* changed from top to bottom leaves, accordingly. This study suggests that *g*<sub>i</sub> may be a valuable trait for increasing photosynthetic capacity and productivity; nonetheless, it suggests caution in selecting leaves for high *g*<sub>i</sub>, as the latter can considerably change along the plant profile due to leaf age and irradiance effects.

Key words: Internal conductance, irradiance, leaf age, light, mesophyll conductance, photosynthesis, plant profile, stomatal conductance.

Introduction

Ethiopian (*E.*) mustard (*Brassica carinata*) is an interesting C<sub>3</sub> oil crop evolved through the inter-specific hybridization between *B. nigra* and *B. oleracea* (Hemingway, 1995). Because of the high erucic acid content of its seeds, it has recently attracted a growing interest in a number of non-food applications such as biodiesel, bio-polymers, lubricants, soaps, and surfactants (Becker, 1999). In addition, the residual defatted meal has some precursors of biologically active compounds (glucosinolate) which could be used as soil amendments for plant defence (FAIR-CT96-1946 Project report; Anon., 2000). Due to its higher tolerance of drought (Cardone *et al.*, 2003) and the lower tendency to pod-shattering, *E.* mustard could be very competitive against rapeseed under unfavourable environmental conditions. Furthermore, it produces a higher biomass than rapeseed that could eventually be processed into electricity and/or heat.

Studying internal conductance (*g*<sub>i</sub>) is of enormous importance in crops like *E.* mustard which are envisaged to be grown in environments where high water use efficiency is imperative. There is, in fact, evidence that increasing *g*<sub>i</sub> may improve photosynthesis and water use efficiency (Warren and Adams, 2006), while the increase in stomatal conductance (*g*<sub>s</sub>) generally involves significant transpiration costs that crops might be unable to support. This was emphasized in a number of studies (Evans and Vellen, 1996; Lauteri...
et al., 1997; Flexas et al., 2008) showing consistent and positive correspondence between water use efficiency and the \( g_i/g_e \) ratio.

There is increasing evidence that \( g_i \) can significantly decrease the CO\(_2\) concentration at the Rubisco site (for a review, see Ethier and Livingston, 2004) to an extent depending on water shortage, salinity, leaf age, mineral nutrition, etc. (Bernacchi et al., 2002; Long and Bernacchi, 2003). The irradiance can significantly modify the leaf anatomy and mesophyll structure (Nobel, 1991; Parkhurst, 1994) and thus it can be envisaged to affect also \( g_i \). Nonetheless, as far as is known (for a review, see Flexas et al., 2008), only eight studies out of over 130 articles on \( g_i \) documented the relationships between irradiance and \( g_i \) (Lloyd et al., 1992; Hanba et al., 2002; Piel et al., 2002; Gorton et al., 2003; Warren et al., 2003, 2007; Laisk et al., 2005), and, of these, only three involved herbaceous crops (Laisk et al., 2005; Flexas et al., 2007a; Evans et al., 2008). Moreover, most of these experiments used natural light gradients or examined instantaneous responses to irradiance (Evans et al., 2008). By contrast, the present study is a manipulative mid-term acclimation experiment, which should provide greater power to disentangle the various determinants of \( g_i \). Specifically, the experiment focused on the influence of irradiance upon \( g_i \) along the plant profile.

### Materials and methods

#### Site description

In 2007, 24 plants of E. mustard, variety CT 204, were grown in 0.21-m-diameter plastic pots (two plants per 6.6 l pot) in a 2×2.5 m (2 m height) controlled-environment room where relative humidity, temperature, and photoperiod could be regulated and monitored. Light was provided by six groups of 12 independently controllable neon tubes (Philips Master TL-D 58W/840). Pots were weighed twice a week and watered immediately after, thus maintaining the soil moisture constantly close to the field water capacity, the latter being determined gravimetrically. The soil substrate was a mixture (2:1, v/v) of sand and neutral peat (46% C; 0.8% N; pH 6). The Ruakura solution, a nutrient solution (data not shown). Briefly, the allometric models for leaves and siliqua were obtained from the linear relationship between their actual areas as measured by an area meter (Li-3000; LI-COR, Nebraska, USA), and the estimated areas, which were calculated from the rectangles subtending

![Fig. 1. Light extinction along the plant profile under higher (I2) and lower (I1) light conditions. Light intensity was maintained fairly constant in I2 plants by a supplementary lateral light. The arrows indicate the exact position of leaves taken for measurements. Numbers between brackets indicate the cumulative intercepted PAR (photosynthetically active radiation) within each layer (L1–L3 delimited by horizontal broken lines) of I1 and I2 plants. A trapezoidal rule was used to integrate PAR values over the plant profile.](https://academic.oup.com/jxb/article-abstract/60/8/2341/2110161)
them. The seed area was calculated assuming the seed shape is a sphere.

In all plants, three arbitrary and equally dimensioned (0.3 m height) plant layers (L1–L3) were characterized on the base of their productivity parameters (biomass, siliqua, seed, and oil production) and physiological traits. The seed oil was analysed according to the Soxhlet’s method (Soxhlet, 1879). To extract oil completely, 0.5 g seed flour was mixed with Na-sulphate anhydrous and quartz sand then treated in the Soxhlet device for 6 h in a solution of 200 ml n-hexane. Because of the low seed production in each single layer, the amount of flour was slightly lower than indicated in the official Soxhlet method. For this reason, the Soxhlet device was first subjected to a test run in order to correct systematic errors due to the small samples.

**Physiological parameters**

Leaf gas-exchange measurements were performed by a portable open-path system (CIRAS-2; PP-Systems). During measurements the air flow entering the chamber was 250 cm$^3$ min$^{-1}$, RH ~60%, leaf temperature 24±0.5 °C (mean ±SD), the latter calculated using the energy balance method. Leaf-to-air vapour pressure deficit was between 0.8 kPa and 1.0 kPa.

In three layers (L1–L3) of three labelled plants per treatment ($n$=3), the two main parameters representing photosynthetic capacity, i.e. the maximum carboxylation rate ($V_{c_{\text{max}}}$) and the electron transport rate ($J_{\text{max}}$), were determined at 7 and 21 d after the start of treatment (DAT) (36 $A/C_i$ curves in total) at a constant PPFD of 1500 μmol photons m$^{-2}$ s$^{-1}$ provided by an LED light unit above the cuvette. $V_{c_{\text{max}}}$ and $J_{\text{max}}$ were calculated using the biochemical model proposed by Farquhar et al. (1980), which represents the mechanistic relationship between net photosynthesis ($A$) and the intercellular CO$_2$ concentration ($C_i$). Briefly, the model relies on the concept that $A$ is co-adjusted by three processes: (i) the activity and kinetics of Rubisco ($W_i$); (ii) the regeneration of ribulose-1,5-bisphosphate (RuBP) that is supported by the electron transport chain ($W_j$); (iii) the availability of triose phosphates for the Calvin cycle ($W_p$):

$$A = [1-(\Gamma^* / C_i)] \min(W_c, W_j, W_p) - R_d$$

where $\Gamma^*$ is the CO$_2$ compensation point in the absence of dark respiration; $R_d$ is the day respiration, i.e. the mitochondrial respiration during illumination. In this experiment, the apparent $\Gamma^*$ (Γ$^*_{\text{n}}$) and $R_d$ were determined in the three plant layers (L1–L3) of the I1 plants using the method described by Laisk (1977), i.e. the determination of the intercept of four $A/C_i$ curves generated at different low PPFDs (from 100 to 500 μmol photons m$^{-2}$ s$^{-1}$) for $C_i$ values below 15 Pa. To calculate the true $\Gamma^*$, $\Gamma^*_{\text{n}}$ was corrected according to von Caemmerer and Evans (1991) as: $\Gamma^* = \Gamma^*_{\text{n}} + (R_d/g_i)$; where $g_i$ is the internal conductance. Since $\Gamma^*$ and $R_d$ were not significantly different along the plant profile, their average values were taken for the subsequent calculations: $\Gamma^*$ and $R_d$ were 35.8±3.2 μmol CO$_2$ mol$^{-1}$ and 0.80±0.11 μmol CO$_2$ m$^{-2}$ s$^{-1}$ (mean ±SD), respectively. These values were also used for I2 as previous studies showed that both $\Gamma^*$ and $\Gamma^*_{\text{n}}$ were not significantly affected by irradiance level (Piel et al., 2002; Warren et al., 2007).

Each point of the $A/C_i$ curve took about 4 min. Measurements started at $C_{\text{a}}$, the [CO$_2$] in the incoming air, of about 400 μmol mol$^{-1}$. First, $C_{\text{a}}$ was progressively reduced to 200 and 100 μmol mol$^{-1}$ and then gradually increased up to about 2000 μmol mol$^{-1}$ across the series of 400, 500, 600, 800, 1000, and 1600 μmol mol$^{-1}$ using a CO$_2$ mass flow controller (generally 10 data points for each $A/C_i$ curve). Each data point was recorded after equilibration to a steady state (CV <2% was considered tolerable). The software Photosyn Assistant (Dundee Sci., Scotland, UK) was used to fit the model and calculate the photosynthetic parameters. Before running the model, it was assumed that the threshold between $W_c$ and $W_j$ was 20–25 Pa (Wullschleger, 1993; Manter and Kerrigan, 2004).

Finally, chlorophyll index (SPAD-502 chlorophyll meter; Minolta), which is widely known to be related to leaf nitrogen content (Debaeye et al., 2006), was measured in the leaves used for gas-exchange analysis.

**Soluble sugars extraction, photosynthetic pigments, and carbon isotope composition**

Two leaf discs (15 mm diameter) were collected about 4 h after each $A/C_i$ curve: one disc was used to determine the relative water content, i.e. the ratio of tissue fresh weight minus dry weight to tissue turgid weight minus dry weight, the specific leaf area (SLA, leaf area/dry mass) and physiological traits. The seed area was calculated assuming the seed shape is a sphere. The seed was extracted sugars through the absorbance at 530 nm. The solution was sequentially filtered with 0.22 μm filter. The rest of the solution was maintained at rest (Summer, 1921). The rest of the solution was maintained at rest (Summer, 1921). The rest of the solution was maintained at rest (Summer, 1921). The rest of the solution was maintained at rest (Summer, 1921). The rest of the solution was maintained at rest (Summer, 1921). The rest of the solution was maintained at rest (Summer, 1921). The rest of the solution was maintained at rest (Summer, 1921). The rest of the solution was maintained at rest (Summer, 1921). The rest of the solution was maintained at rest (Summer, 1921). The rest of the solution was maintained at rest (Summer, 1921). The rest of the solution was maintained at rest (Summer, 1921). The rest of the solution was maintained at rest (Summer, 1921). The rest of the solution was maintained at rest (Summer, 1921). The rest of the solution was maintained at rest (Summer, 1921).
where $\delta_a$ and $\delta_p$ are the carbon isotope composition of source air and plant material, respectively, relative to Vienna-Pee Dee Belemnite.

Chla and Chlb, and Car were extracted by 10 ml ethanol mix (95%, v/v) into a cold shaded mortar. The extract was centrifuged at 3000 g for 10 min, then 1 ml of supernatant was diluted in 4 ml of ethanol 95% (v/v), and finally, the absorbance was determined at 750, 665, 649, and 470 nm through a spectrophotometer (Perkin-Elmer Lambda 1 UV/VIS) (Aranjuelo et al., 2007). Lichtenthaler’s equation (Lichtenthaler, 1987) was used to calculate Chl$\alpha$ and Chlb, and Car.

**Internal conductance and $C_c$**

As given by Brugnoli et al. (1988) and further developed by Lauteri et al. (1997), $g_c$ was calculated through the discrepancy between $\Delta$ determined in soluble sugars ($\Delta_s$) and the expected $\Delta$ ($\Delta_c$) calculated using the Farquhar model (Farquhar et al., 1982):

$$g_c = \frac{[b-e_s-a_1](A/C_s)/[(\Delta_s-\Delta_c)-(f\Gamma^c/C_s)]}{\delta_c}$$

where, $b$ is the discrimination associated with carboxylation reactions; $e_s$, the fractionation occurring during the dissolution of CO$_2$; $a_1$, the discrimination caused by the diffusion of CO$_2$ in the liquid phase; $f$, the discrimination during photorespiration. Details on values and references were reported elsewhere (Monti et al., 2006). The concentration of CO$_2$ at the site of carboxylation ($C_c$) was then obtained as $C_c = C_i - A/g_c$. Once $C_c$ was calculated, $V_{\text{cmax}}$ and $J_{\text{cmax}}$ were recalculated using the $A/C_c$ model ($V_{\text{cmax, c}}$; $J_{\text{cmax, c}}$).

Stomatal and internal limitations to photosynthesis were calculated as proposed by Jones (1985). In short, the drawdown of CO$_2$ from the leaf surface to the site of carboxylation is a function of stomatal and internal conductance, i.e.

$$C_c = C_i - (A/g_c) - (A/g_s)$$

Assuming that mitochondrial respiration is not significantly changing during the experimental period, the relationship between $A$ and $V_{\text{cmax}}$ can be expressed (Farquhar et al., 1980) as:

$$\Delta A / \delta A_{\text{max}} = A / V_{\text{cmax}}$$

where $A_{\text{max}}$ is the light-saturated net photosynthesis. As $\delta C_c$ can also be expressed as:

$$\delta C_c = [(A/g_c)(\delta g_s/g_s)] + [(A/g_s)(\delta g_i/g_i)] - [(1/g_s) + (1/g_i) \times \delta A]$$

Combining the last three equations gives the following:

$$(\delta A/A) = [(l_s(g_s/g_s) + h_s(A/g_s) + h_i(V_{\text{cmax}}/V_{\text{cmax}})]$$

Therefore, the stomatal ($l_s$) and internal ($l_i$) limitations can be calculated as:

$$l_s = [(g_{\text{tot}}/g_s)(\delta A/C_c)]/[g_{\text{tot}}(\delta A/C_c)];$$

$$l_i = [(g_{\text{tot}}/g_i)(\delta A/C_c)]/[g_{\text{tot}}(\delta A/C_c)]$$

where $g_{\text{tot}}$ is the total conductance to CO$_2$ ($1/g_{\text{tot}} = 1/g_s + 1/g_i$).

**Statistical analysis**

All data were subjected to statistical analysis according to the general linear model for repeated measurements performed by SYSTAT 10.2 (Systat Software, Inc., Chicago, IL, USA). The Bartlett’s test ($P \leq 0.05$) was used to test the homogeneity of the variance. The LSD Fisher’s test ($P \leq 0.05$) was applied to separate the significantly different means. The statistical significance of the correlation coefficients was tested according to Pearson’s correlation test ($P \leq 0.05$). To solve the area under a curve (see Fig. 1), the trapezoidal rule was used.

**Results**

**Light effect on growth and production**

Increasing the light distribution along the plant profile visibly modified the cycle length and productivity: I2 took 14 d less than I1 to complete the cycle (Fig. 2) and it produced 47%, 140%, and 61% more biomass, siliqua, and seeds, respectively (Fig. 3). In addition, I2 had a higher oil content (%) than I1, with a consequent double oil yield (Fig. 3).

![Fig. 2.](https://example.com/2341.png) Length of the different phenological stages (CETIOM scale) under the two light treatments. The higher irradiance (I2) clearly reduced the total cycle length.
on average). The relative water content averaged 0.85 ± 0.09 and was not influenced by irradiance or by leaf position along the plant profile. The oil content (%) was also modified by light intensity: 25% higher oil content was found in I2 compared with I1 (Fig. 3).

**Light effects on** $g_i$

Since the light regime substantially modified the cycle length, the corresponding leaves in I1 and I2 had different ages. Therefore, the differences in $g_i$ between I1 and I2, within each layer, probably reflected cumulative effects of leaf age and irradiance. Nonetheless, a reliable estimate of the leaf age effects on $g_i$ could be obtained in I2, since these plants maintained a constant light level along the profile (Fig. 1). Similarly, the effects of the leaf age on $g_i$ were quantified through the comparison of the same leaf at 7 and 21 DAT.

The internal conductance drastically declined from top to basal leaves irrespective of irradiance level (Fig. 4). Therefore, leaf age effects are likely to have prevailed over irradiance effects in changing $g_i$. Nevertheless, the influence of irradiance upon $g_i$ was not negligible, as testified by the significant interaction between irradiance and plant layer. The reason behind this interaction was that $g_i$ was significantly higher in L3 of I2 with respect to I1, while the opposite occurred in L1. By contrast, there were no significant differences in $g_i$ between I1 and I2, in L2 (Fig. 4). The interaction between irradiance and plant layer was also significant 14 d later when, however, it was no longer possible to measure L1 due to the advanced senescence of the basal leaves. Besides, in L2, $g_i$ was higher in I1, while in L3 it not longer differed between I1 and I2 (Fig. 4).

**Photosynthesis and $g_i$**

Internal conductance had a significant impact on $A$, which appeared more driven by $g_i$ than by $g_s$ (Fig. 5). Overall, $g_i$ was fairly consistent with $A$, being higher in L3 and then gradually decreasing downward (Table 1). Moreover, since $A$ declined downward more rapidly in I2 than in I1, the leaf-age effect on $A$ was likely to be more prevalent than that of irradiance. A result which is consistent with the effects of leaf age on $g_i$; however, unlike for $g_i$, I2 showed higher $A$ values than I1 also in L2 (Table 1).

The leaf Chl and Car contents were also rather consistent with $A$. Specifically, Chla, Chlb, and Car did not change between L3 and L2, while it declined drastically in L1 (Table 2). Conversely, no significant effects of the irradiance were observed on Chla, Chlb, and Car. Similarly, SPAD values were only slightly influenced by the irradiance; however, unlike photosynthetic pigments, SPAD values
radically decreased downward, thus revealing a likely decline in leaf N-content parallel to leaf ageing (Table 2).

Stomatal conductance \((g_s)\) was weakly influenced by the irradiance, and it appeared significantly related to \(g_l\) (Fig. 5). Nonetheless, unlike \(g_c\), \(g_s\) remained rather constant between L3 and L2 in I2. More than that, \(g_l\) decreased more markedly than \(g_c\) from the apex to the basal leaves, with the result that the ratio between \(g_l\) and \(g_c\) significantly changed with the position of the leaf and, to a lesser extent, with the irradiance (Fig. 6). Specifically, in L3, \(g_l\) was about three times larger than \(g_c\) both in I1 and I2, while in L2 \(g_l\) was much larger than \(g_c\) only in I2. In the basal leaves, \(g_l\) and \(g_c\) were nearly equivalent (Fig. 6). The diffusive limitations to \(A\) through stomata \((l_i)\) averaged \(21\%\) and they were significantly higher than internal limitation \((l_{im})\) (Fig. 6). Overall, \(l_i\) values were higher in the upper layer and then decreased downward, contrary to that observed for \(l_{im}\).

The latter, however, was not negligible, ranging from \(9\%\) to \(15\%\) in L3 and L1, respectively. The irradiance effects on diffusive limitations to \(CO_2\) were generally negligible; the only significant effect was in L2 where \(l_{im}\) was significantly higher in I2 than I1, both on DAT 7 and DAT 21 (Fig. 6).

The drawdown of \(CO_2\) from the atmosphere to the carboxylation site was significantly influenced by \(g_c\). \(C_c\) resulted in being \(7\% - 9\%\) lower than \(C_l\) in L2 and L3, and \(12\% - 14\%\) lower in L1 (Table 1). This led to a significant underestimation of \(V_{cmax}\) as calculated by the conventional \(A/C_c\) model, and, to a lesser extent, of \(J_{max}\) as well (Table 1). Importantly, the correlation between \(V_{cmax}\) and \(J_{max}\) significantly improved \((r=0.90**) once the two parameters were recalculated using the \(A/C_c\) model.

### Discussion

This study revealed that light distribution along the plant profile can enormously affect biomass, seed and oil yields, and, to a lesser extent, the fatty acid composition. Therefore, plant density and its architecture should be taken into account by agriculturists and breeders in establishing the optimal growing conditions towards maximizing the productivity of this crop.

A bottom-up comparison of leaves under two light conditions was the original goal of this study. Nonetheless, irradiance had an unexpected strong influence upon the plant growth that caused an early maturation of I2 plants. Consequently, the difference between corresponding leaves of I1 and I2 were probably the consequence of the cumulative effects of the irradiance and leaf age. Anyway, since I2 plants were subjected to constant light intensities along the whole profile, the ageing effects on \(g_l\) could be reasonably estimated along the profile of I2 plants or, possibly, by measuring the same leaf at 7 d and 21 d after the treatment started.

Generally, \(g_l\) increases until the leaf is fully expanded and then decreases parallel to the leaf senescing (Hanba et al., 2002; Ethier et al., 2006). Similarly, the present results show

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**Table 1.** Net assimilation rate \((A)\), \(CO_2\) concentration at the substomatal cavity \((C_c)\) and at the chloroplasts \((C_l)\) and maximum carboxylation \((V_{cmax,Cc})\), and electron transport \((J_{max,Cc})\) rates

The two latter parameters were calculated on \(C_c\) basis \((A/C_c\) curve). The underestimation \((\%)\) of \(V_{cmax}\) and \(J_{max}\) if calculated using the \(A/C_c\) curve is given in parenthesis. Different letters indicate vertical significant differences \((P<=0.05, LSD Fishers’s test)\) within each DAT (days after the start of treatment; \(n=3\)).

<table>
<thead>
<tr>
<th>DAT</th>
<th>Light</th>
<th>Layer</th>
<th>(A)</th>
<th>(C_l)</th>
<th>(C_c)</th>
<th>(V_{cmax,Cc})</th>
<th>(J_{max,Cc})</th>
<th>(V_{cmax}/J_{max})</th>
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<tbody>
<tr>
<td>7</td>
<td>I1</td>
<td>L3</td>
<td>8.6</td>
<td>b</td>
<td>319</td>
<td>a</td>
<td>285 ab</td>
<td>108 (22%) a</td>
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<td></td>
<td></td>
<td>L2</td>
<td>6.2</td>
<td>c</td>
<td>328</td>
<td>a</td>
<td>289 ab</td>
<td>93 (19%) b</td>
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<tr>
<td></td>
<td></td>
<td>L1</td>
<td>5.6</td>
<td>c</td>
<td>316</td>
<td>a</td>
<td>302 a</td>
<td>70 (41%) c</td>
</tr>
<tr>
<td></td>
<td>I2</td>
<td>L3</td>
<td>10.5</td>
<td>a</td>
<td>320</td>
<td>b</td>
<td>274 b</td>
<td>113 (21%) a</td>
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<td></td>
<td></td>
<td>L2</td>
<td>8.7</td>
<td>b</td>
<td>327</td>
<td>a</td>
<td>287 a</td>
<td>97 (22%) b</td>
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<td></td>
<td></td>
<td>L1</td>
<td>3.2</td>
<td>d</td>
<td>327</td>
<td>a</td>
<td>293 a</td>
<td>65 (35%) c</td>
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<tr>
<td>21</td>
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<td>L3</td>
<td>8.3</td>
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<td>319 a</td>
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<td>316 a</td>
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<td>302 a</td>
<td>93 (26%) a</td>
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<td></td>
<td></td>
<td>L2</td>
<td>7.1</td>
<td>c</td>
<td>334</td>
<td>a</td>
<td>306 a</td>
<td>69 (31%) b</td>
</tr>
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</table>
Table 2. Chlorophyll (Chla and b), carotenoid (Car) contents, and SPAD values determined at 7 d and 21 d after the start of treatment (DAT). L1, L2, and L3 indicate the plant layers from bottom to top, respectively; I1 and I2, less and more illuminated plants, respectively. Different letters indicate vertical significant differences (P = 0.05, LSD Fisher’s test; n=3) within each DAT.

<table>
<thead>
<tr>
<th>DAT</th>
<th>Light</th>
<th>Layer</th>
<th>Chla (mg g⁻¹)</th>
<th>Chlb (mg g⁻¹)</th>
<th>Chla/b (mg g⁻¹)</th>
<th>Car (mg g⁻¹)</th>
<th>SPAD (adim.)</th>
</tr>
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<tbody>
<tr>
<td>7</td>
<td>I1</td>
<td>L3</td>
<td>9.9 a</td>
<td>3.1 a</td>
<td>3.2 a</td>
<td>2.6 a</td>
<td>58 a</td>
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<td></td>
<td>L2</td>
<td></td>
<td>9.2 a</td>
<td>3.0 a</td>
<td>3.2 a</td>
<td>3.0 a</td>
<td>43 b</td>
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<tr>
<td></td>
<td>L1</td>
<td></td>
<td>6.8 b</td>
<td>2.0 b</td>
<td>3.4 a</td>
<td>1.9 b</td>
<td>32 c</td>
</tr>
<tr>
<td>I2</td>
<td>L3</td>
<td></td>
<td>10.3 a</td>
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<td>2.7 a</td>
<td>62 a</td>
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<tr>
<td></td>
<td>L2</td>
<td></td>
<td>10.0 a</td>
<td>3.1 a</td>
<td>3.2 a</td>
<td>2.6 a</td>
<td>46 b</td>
</tr>
<tr>
<td></td>
<td>L1</td>
<td></td>
<td>4.0 c</td>
<td>1.4 c</td>
<td>3.4 a</td>
<td>2.1 b</td>
<td>39 c</td>
</tr>
<tr>
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<td>I1</td>
<td>L3</td>
<td>10.5 a</td>
<td>3.8 a</td>
<td>2.7 b</td>
<td>2.7 a</td>
<td>56 a</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td></td>
<td>10.1 a</td>
<td>3.4 a</td>
<td>3.0 b</td>
<td>2.7 a</td>
<td>45 b</td>
</tr>
<tr>
<td></td>
<td>L1</td>
<td></td>
<td>11.2 a</td>
<td>3.7 a</td>
<td>3.0 b</td>
<td>2.6 a</td>
<td>59 a</td>
</tr>
<tr>
<td>I2</td>
<td>L3</td>
<td></td>
<td>8.7 b</td>
<td>2.4 b</td>
<td>3.6 a</td>
<td>1.9 b</td>
<td>40 b</td>
</tr>
</tbody>
</table>

In spite of a considerable change in \( g_i \) and \( A \), \( C_i \) and \( C_c \) remained fairly constant along the plant profile (\( C_i/C_c \) ratio of 1.08±0.14 SD), irrespective of the light treatment. It derives that \( C_c \) was never significantly related to \( A \); when \( A \) changed, \( g_i \) and \( g_s \) scaled accordingly and so \( C_c \) remained constant. A fact that would indicate, presumably, a feedforward mechanism on the diffusive stomatal limitation to \( \text{CO}_2 \) through the internal \( \text{CO}_2 \) concentration. The drawdown from \( C_i \) to \( C_c \) (on average 30 \( \mu \text{mol mol}^{-1} \)) was pretty low if compared with the average value of 226 species calculated by Warren (2008a). Nonetheless, it was not negligible as it determined a substantial underestimation of \( V_{\text{cmax}} \) and, to a lesser extent, of \( J_{\text{max}} \) as calculated by the conventional \( A/C_i \) model. Specifically, \( V_{\text{cmax}} \) and \( J_{\text{max}} \) calculated on a \( C_i \) basis were on average 30% lower \((r=0.91**) and 1.08 \((r=0.67**) \) higher than \( V_{\text{cmax}} \) and \( J_{\text{max}} \) calculated on the \( C_i \) basis, respectively. These two coefficients were slightly lower \((V_{\text{cmax}}) \) than or very similar \((J_{\text{max}}) \) to those calculated \((1.62 \text{ and } 1.08, \text{ respectively}) \) in a recent review (Warren, 2008a). Moreover, the correlation between \( V_{\text{cmax}} \) and \( J_{\text{max}} \) increased when the \( A/C_i \) model was used instead of the \( A/C_c \) model. As also ascertained in other herbaceous crops (Monti et al., 2006), not \( g_i \) taking into account may lead to the decrease in \( A \) to be attributed to biochemical constraints, while diffusive limitations could be the major cause.

The sum of stomatal and non-stomatal limitations was about 30% with little variation along the plant profile. Nevertheless, the diffusive limitations to \( \text{CO}_2 \) were modified by the significant interaction between leaf age and irradiance. As a result, the relative importance of stomatal and non-stomatal limitations changed accordingly. Specifically, in the two upper layers, the diffusive limitations to \( \text{CO}_2 \) in mesophyll were about half those in stomata, while in the bottom leaves (L1) they were nearly equal or even higher in the mesophyll (Fig. 6).

Contrasting effects occurred on \( g_i \) along the plant profile because \( g_i \) is known to decrease with leaf ageing (Bernacchi et al., 2005; Grassi and Magnani, 2005; Flexas et al., 2007b), while it increases with irradiance (Terashima et al.,...
The age effects were further evident both in I1 and I2. (i) At the top (L3), the leaves were still young both in I1 and I2. In this layer, gs was mostly influenced by light and, to a much lesser extent, by leaf age. Thus it follows that irradiance effects on gs could be reliably estimated in this layer, since the counteracting effects of leaf age on gs can be assumed to be negligible. In this layer, gs and irradiance were found to be positively related in agreement with other studies (Boardman, 1977; Warren et al., 2003; Evans et al., 2008; Flexas et al., 2008). (ii) In the intermediate layer (L2), I2 had significantly older leaves than I1. The negative age effects offset or prevailed over the positive effects of irradiance on gs, with the result that 21 DAT the internal conductance was higher in I1. (iii) In the basal leaves (L1), red light than mesophyll receptors. and mesophyll, as guard cells could be more sensitive to far-

There is evidence that gs and gr are positively related (Loreto et al., 1992; Centritto et al., 2003; Warren, 2008b). Nonetheless, Warren (2008b) demonstrated that this relationship is not ubiquitous as no relationship was found between gs and gr when gs was modulated by the atmospheric water deficit. The present study, though it showed gs and gr being generally related (r = 0.51*), seems somehow to support the results of Warren (2008b) since gs and gr were sometimes independent in responding to irradiance. Under I2 conditions, gr remained fairly stable from L3 to L2 to then decreased appreciably in L1; conversely, gs declined by 25% from L3 to L2, and then it decreased drastically from L2 to L1 (from 0.335 to 0.074 mol m⁻² s⁻¹). Consequently, the responsiveness of stomata and mesophyll to light changed along the plant profile and so the gs/gr ratio changed accordingly. The reason behind the different responses of gs and gr to light was not clear. Whether or not the mesophyll can drive gs is still an open matter. Recent studies suggest that stomatal response to light is mostly driven by a signal generated by the mesophyll (Mott et al., 2008). Nonetheless, stomata also responded to red-light, probably through a mechanism regulated by the chloroplasts of the guard cells (Tominaga et al., 2001). As a result, mesophyll should have little or no effect on gs. Furthermore, Messinger et al. (2006) showed that stomatal response to red-light was evident even when Cj was maintained constant through regulating the ambient CO₂. Of course, the fact remains that other mechanisms might influence the relationship between stomata and mesophyll. Therefore, since the light source used in the present experiment (neon tubes) is characterized by a prevailing far-red-to-orange light spectrum, an hypothesis could be that this specific light spectrum, or the still uncertain responsiveness to blue-light of the mesophyll and stomata (Marten et al., 2007; Loreto et al., 2008), was the cause of the differing behaviour of stomata and mesophyll, as guard cells could be more sensitive to far-red light than mesophyll receptors.

In summary, four main findings could be underlined: (i) productivity was strongly increased by the lateral light through the rise in photosynthetic rates of basal and intermediate leaves as well as other green organs (Ruuksa et al., 2004); (ii) irrespective of irradiance level, gs declined drastically from the top to the bottom leaves thus suggesting a likely prevalent effect of leaf age compared with irradiance—for this reason, the positive effects of irradiance on gs are visible only in the top leaves; (iii) once again, gs led to significant underestimation of Vcmax which was higher related to Jmax once it was recalculated using the A/Cc curve; (iv) gs and gr were significantly related and they scaled with the photosynthetic capacity, but the ratio between stomatal and non-stomatal limitations was not constant along the profile.

References


