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Limitation of coffee leaf photosynthesis by stomatal conductance and light availability under different shade levels

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Abstract In agroforestry systems, the effect of shade trees on coffee net photosynthesis (A_n) has been the object of debates among coffee scientists. In this study, we undertook over 600 coffee A_n "spot" measurements under four different artificial shade levels (100, 72, 45 and 19%) of full solar irradiance) and analyzed limitations to A_n by low light availability (photon flux density, PFD) and stomatal conductance (g_s) . These gas exchange measurements were carried out during two consecutive coffee growing seasons in a commercial plantation in the Orosi valley of Costa Rica. Levels of A_n were related to PFD and g_s in order to calculate envelope functions which were used to establish PFD or g_s limitations to A_n . Under the growing conditions of the present trial, mean leaf A_n remained stable for growth irradiance (GI) as low as 45% of full sun and decreased by $\sim 20\%$ at 19% GI. Limitation to $A_{\rm n}$ due to g_s was strong in full sun and decreasing with increasing shade levels. On the other hand, limitation due to PFD remained at a similar level for all shade treatments. These different evolutions of limitations of A_n by PFD and g_s in response to shade explain the absence of a decrease in coffee leaf A_n with a shade level up to 55%. Consequently,

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these results confirm that Arabica coffee is a shade-adapted plant with leaves that can maintain a high photosynthetic performance under low light availability.

Keywords Coffea Arabica · Costa Rica · Gas exchange · Microclimate

Introduction

Light and CO₂ availability in the mesophyll of plant leaves are the main determinants of their net carbon assimilation rate (A_n) . Leaf photosynthesis can therefore be estimated as the minimum rate permitted by the limitations imposed by either (1) photosynthetic photon flux density (PFD) or (2) the CO_2 concentration in the mesophyll (C_i) to the biochemical response of A_n to each of these two variables (Farquhar et al. 1980). Leaf interception of PFD depends on its position within the plant canopy and on full solar irradiance. Light availability can also be reduced by the surrounding vegetation in agroforestry systems. On the other hand, the CO_2 concentration in the air surrounding a leaf is relatively constant and variations in C_i are mainly determined by limitations to CO₂ diffusion from the air to the leaf mesophyll. These diffusional limitations are mainly modulated by the stomatal conductance (g_s) which responds to microclimatic conditions and plant water status (Larcher 2003).

Arabica coffee (*Coffea arabica* L.) evolved in the Ethiopian forest understorey (Kumar 1979) and is thus considered to be a shade plant (Beer et al. 1998; DaMatta 2004; Fahl et al. 1994; Ramalho et al. 1999; Rhizopoulou and Nunes 1981). Nevertheless, shading reduces flower bud formation (Cannell 1976) and can also reduce the whole-tree carbon assimilation (DaMatta 2004; DaMatta et al. 2007). This may result in reduced yield whereas

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under optimal growing conditions yields are higher in full sun (Beer et al. 1998). Coffee has low rates of net carbon assimilation (A_n), typically bellow 11 μ mol m⁻² s⁻¹ (Franck et al. 2006; Silva et al. 2004) which has been related to diffusive rather than biochemical limitations (Chaves et al. 2008; DaMatta et al. 2007). Actually, the stomatal limitations are associated with a strong stomatal sensitivity to increasing leaf-to-air vapor pressure deficit (VPD₁) along the day (Ronquim et al. 2006; Vaast et al. 2007) and result in large reductions of A_n , particularly in the afternoon (DaMatta and Ramalho 2006). Moreover, this species is prone to photoinhibition of photosynthesis when exposed to full solar irradiance as coffee A_n saturates at low irradiance (Ramalho et al. 2000). Nevertheless, photoinhibitory limitations of A_n in full sun have been shown to be of secondary importance as compared to g_s limitations in commercial Arabica coffee varieties according to Chaves et al. (2008) who attributed this feature to an acclimation to high irradiance in order to prevent photoinhibition. On the other hand, shade can result in An limitations due to insufficient light interception (DaMatta 2004, DaMatta et al. 2007). Although coffee leaves exhibit typical shade acclimation features, theoretically allowing them to maintain A_n in low light (Hollies 1967), Araújo et al. (2008) recently found a low physiological plasticity to low light in coffee leaves located inside the canopy, resulting in reduced A_n as compared to exposed leaves. Limitation of photosynthesis by low light availability has been proposed as one of the main reasons for lower yields of coffee grown in agroforestry systems in optimal coffee production areas such as southeastern Brazil (DaMatta et al. 2007) and the Central American highlands (Beer et al. 1998). Under suboptimal (hotter and drier) growing conditions, coffee production in full sun is lower than in the shade (De Freitas et al. 2003; van Kanten and Vaast 2006) which has been related to the high sensitivity of coffee g_s to VPD₁ (DaMatta and Ramalho 2006; Ronquim et al. 2006; Vaast et al. 2007). As shade trees reduce wind speed and leaf temperature while increasing air humidity, and hence reducing VPD₁ and the stomatal limitations of coffee A_n , agroforestry systems have been recommended for suboptimal growing conditions (DaMatta 2004; DaMatta et al. 2007; Vaast et al. 2007). Nevertheless, DaMatta et al. (2007) have emphasized that, under optimal or near-optimal edapho-climatic conditions, shade provides little, if any, benefit to the crop. However, agroforestry systems have other benefits such as (1) positive financial impacts by the reduction of biennial bearing and by the generation of extra income from associated trees (fruit, fuel wood and timber) and (2) the enhanced conservation of natural resources and biodiversity (Beer et al. 1998; Vaast et al. 2007). Moreover, shade has been shown to enhance coffee bean size (Franck 2005) as well as cup quality (Vaast et al.

2006). These shade advantages have led to a renewed interest in coffee cultivation in agroforestry systems in the Americas, even under optimal coffee growing conditions (Beer et al. 1998; DaMatta et al. 2007).

Certainly, g_s and PFD availability can be considered the main limiting factors of coffee A_n in full sun and shade, respectively. Therefore, the present study focuses on analysing the limitations of coffee leaf A_n by these two factors over a range of light availability. To that effect, envelope curves were adjusted for the response of A_n to either g_s or PFD following the approach developed by Jarvis (1976) in order to analyze and model the effect of climatic variables on leaf g_s . Envelope curves are equations that are fitted to the maximum values of a dependent variable $(A_n$ in this case) in response to a targeted range of values of an independent variable (g_s and PFD in this case) and allow fixing an "upper limit" for the dependent variable. Data points located in the very proximity of these curves are considered not to be limited by any other factor but the analyzed independent variable.

Our working hypothesis was that, even under the optimal coffee growing conditions of the Orosi valley of Costa Rica, the negative effect of low light availability in agroforestry systems for coffee A_n is compensated by (1) an attenuation of stomatal limitations, and (2) an efficient acclimation of the photosynthetic apparatus to low light. This hypothesis was tested by measuring the gas exchange of leaves of coffee plants grown under four growth irradiance levels, ranging from 19% to full solar irradiance, during two consecutive rainy seasons (i.e. the optimal growth conditions for coffee) and by determining the proportion of measurements that were limited by either g_s or PFD. The contribution of low light availability and low g_s to the limitation of the potential A_n under these growth irradiance levels and their daily evolution are discussed.

Materials and methods

Experimental site and plant material

Measurements were performed on coffee plants (*Coffea* arabica L.) of the highly productive, dwarf cv. "Caturra" in a homogenous commercial orchard in the Orosi valley of Costa Rica (9.79°N, 83.82°W; 1,108 m above sea level) planted in 1999 on an Inceptisol. The coffee plants were in their second (2003) and third (2004) production cycles at a 1 m \times 2 m spacing on east–west oriented rows.

Growth irradiance treatments

Four growth irradiance (GI) treatments were established in December 2002. Each treatment included eight coffee rows of ten plants each. Treatments consisted in full solar irradiance (GI₁₀₀) and three shade treatments with 72% (GI₇₂), 45% (GI₄₅), and 19% (GI₁₉) of the full solar irradiance. GI treatments were achieved by constructing shade houses covered with black shade screens, with the required light transmittances, positioned at a minimum height of 3 m.

Measurements

In order to assess the effect of shade on fruit set, the total amount of fruits present on eight randomly selected plants within each GI treatment were counted after flowering in May of 2003.

Measurements of instantaneous net CO2 assimilation rate (A_n) and stomatal conductance to H₂O (g_s) were performed with a CO₂/H₂O infrared gas analyzer (LCPro, ADC Bio-Scientific Ltd., Hoddesdon, UK) connected to a broadleaf chamber under ambient microclimatic conditions. These measurements were performed during the rainy seasons of 2003 (14 days between August and October) and 2004 (9 days between August and September) on fully developed exposed leaves (third to sixth pairs of leaves from the branch tip) sampled on plagiotropic branches located in the middle part of the canopy. Gas exchange measurements were performed in the morning (AM: 6:00-8:30), midday (MM: 10:00-12:30) and afternoon (PM: 13:30-16:00). Leaf temperature (T_1) , incident photon flux density (PFD), air temperature and humidity (RHa) were recorded during each gas exchange measurement. RH_a and T_1 were also used to calculate leaf-to-air vapor pressure deficit (VPD_{1-a}). A total of 604 gas exchange measurements were performed during the two years: 187 for GI_{100} , 139 for GI_{72} , 140 for GI_{45} and 138 for GI₁₉.

Data analysis

For each GI treatment, A_n data were plotted as a function of PFD and g_s in order to construct envelope functions. Based on the studies of Causton and Dale (1990), a monomolecular function was selected to relate A_n to PFD:

$$A_{\rm n} = A^{\rm max} \left(1 - e^{-k\rm PFD} \right) - R_{\rm d} \tag{1}$$

where A^{max} is the maximum light saturated gross CO₂ assimilation rate, R_d is the day respiration and k is a coefficient logarithmically related to the initial slope of the response of A_n to PFD (i.e. maximal quantum use efficiency). R_d was estimated for each GI treatment in the same experimental plot in a previous work by Franck (2005) and light saturated net photosynthesis rate (A_n^{max}) was calculated by subtracting R_d from A^{max} ($A_n^{\text{max}} = A^{\text{max}} - R_d$). To relate A_n to g_s , we assumed the often observed linear relationship between these traits

(Leuning 1995) with a slope (*m*) representing the maximal intrinsic water use efficiency (WUE):

$$A_{\rm n} = m \times g_{\rm s} - R_{\rm d}.\tag{2}$$

In order to fit these envelope functions to the data sets derived from each GI treatment, the independent variables (PFD or g_s) were divided into 12 classes encompassing an equal number of data. First, the envelope functions were fitted to the maximum A_n data of these classes. Based on this first fitting, the data closest to the values predicted by the function were included, one at a time, re-fitting the envelope function each time new data was included. This procedure was repeated until the addition of new data produced a residual exceeding 5% of the predicted value and the parameter values adjusted in the previous step were retained. The A_n values calculated with Eq. 1 were termed A^{PFD} and those calculated with Eq. 2 were termed A^{gs} . Sigmaplot 2000 software (SPSS Inc., Chicago, IL, USA) was used for fitting the envelope functions. Four categories of A_n data were then selected: non-limited (Lim^{None}), limited by light availability (Lim^{PFD}), limited by stomatal conductance (Limgs) and limited by another factor (Lim^{Other}). We established a saturated PFD value (PFD^{sat}) from Eq. 1 as the PFD level for which the predicted $A_{\rm n} = 0.95 A_{\rm n}^{\rm max}$. Then, the $A_{\rm n}$ data of each GI treatment were assigned to one of the previously mentioned categories by means of the following assumptions:

• $\operatorname{Lim}^{\operatorname{None}}: A_n \ge 0.95 A_n^{\max}$

Excluding Lim^{None} data:

- $\operatorname{Lim}^{\operatorname{PFD}}: A_{n} \geq 0.95A^{\operatorname{PFD}} \wedge \operatorname{PFD} < \operatorname{PFD}^{\operatorname{sat}}$
- $\operatorname{Lim}^{gs}: A_n \ge 0.95A^{gs}$
- $\operatorname{Lim}^{\operatorname{Other}}: A_n < 0.95A^{\operatorname{PFD}} \wedge A_n < 0.95A^{\operatorname{gs}}$

In all GI treatments, some data recorded at low PFD fulfilled the conditions of both LIM^{PFD} and LIM^{gs}. In these cases, data were assigned to the category for which the A_n value estimated by each envelope function (A^{PFD} or A^{gs}) was closest to the measured value. Two approaches were used to assess to what extent the limitations of A_n could be related to the analyzed limiting factors (PFD, g_s or "Other"). First, the proportion of measurements within each of the limitation categories was calculated and used to assess the frequency with which each factor limited A_n during the day [Lim_{Freq} (%)]. Then, the absolute contribution of each factor to the limitation of A_n (Lim_{Abs}) was estimated with the following equation:

$$\operatorname{Lim}_{\operatorname{Abs}}^{i} = \frac{\operatorname{Lim}_{\operatorname{Freq}}^{i}}{100} \left(A^{\max} - \overline{A_{n}^{i}} \right)$$
(3)

where $\text{Lim}_{\text{Abs}}^{i}$ (µmol CO₂ m⁻² s⁻¹) is the contribution of the limiting factor "i" (PFD, g_{s} or Other), $\text{Lim}_{\text{Freq}}^{i}$ is the frequency (%) with which the limiting factor "i" limits A_{n} Fig. 1 Effects of growth irradiance [100%: GI₁₀₀ (plus); 72%: GI72 (multiplication symbol); 45%: GI₄₅ (open squares); and 19%: GI19 (filled squares)] and period of day [morning (AM), midday (MM) and afternoon (PM)] on photon flux density (PFD; a), leaf temperature $(T_1; \mathbf{b})$, leaf-to-air vapor pressure deficit (VPD₁; c), stomatal conductance $(g_s; \mathbf{d})$ and net photosynthesis $(A_n; \mathbf{e})$ of Coffea arabica leaves. Mean values are presented \pm standard error; uppercase letters indicate significant differences $(\alpha < 0.05)$ between treatments for each factor (GI and period of day); for cases with significant interaction between factors (A_n) and PFD); lowercase letters indicate significant differences between treatment combinations; each period of day \times GI treatment represents an average of 50 data



and $\overline{A_n^i}$ is the mean A_n value of the data limited by the factor "i".

Analysis of variance (ANOVA) with two factors (GI and period of day) was performed and means were compared with the Fisher LSD method by means of the InfoStat statistical software (InfoStat 2008).

Results

Effect of growth irradiance on fruit set, microclimate and gas exchange

Shade had a strong and significant effect on flowering intensity and consequently fruit set of 4,620 \pm 821 (fruits per plant) for GI₁₀₀, 3,052 \pm 525 for GI₇₅, 1,500 \pm 183 for GI₅₀ and 605 \pm 139 for GI₂₅.

GI treatments affected PFD in the expected proportions throughout the day (Fig. 1a) achieving the highest levels at MM and similar levels at AM and PM ($\sim 57\%$ and $\sim 50\%$

of MM, respectively). For all GI treatments, VPD₁ and T_1 also exhibited their highest levels at MM and their PM values were higher than AM values (Fig. 1b, c). VPD₁ and T_1 linearly decreased with shade regimes; VPD₁ decreased to half its value from GI_{100} down to GI_{19} whereas T_1 decreased by $\sim 3^{\circ}$ C between those extreme GI treatments (Fig. 1b, c). Regarding gas exchange, the daily mean A_n was similar in the range $GI_{100}\text{-}GI_{45}$ and decreased by $\sim\!1.2~\mu\text{mol}~CO_2~m^{-2}~s^{-1}$ to a mean value of 5.0 ± 2.0 μ mol CO₂ m⁻² s⁻¹ at GI₁₉ (Fig. 1e). GI and the period of day had a significant interaction for this variable inducing different patterns of daily An evolution; at the higher GI treatments (GI₁₀₀ and GI₇₂), A_n was highest at AM and decreased to similar levels at MM and PM, whereas for GI₄₅ A_n was similarly higher at AM and MM than PM. For GI_{19} , the highest A_n levels were observed at MM and the lowest at PM (Fig. 1e). Regarding g_s , this trait was lowest at MM and PM and highest at AM irrespective of GI treatment; g_s was similar for all shade treatments and significantly lower in full sun (Fig. 1d).



Fig. 2 *Coffea arabica* leaf net photosynthesis (A_n) as a function of photon flux density (PFD) under 100% (**a**), 72% (**b**), 45% (**c**) and 19% (**d**) of full solar irradiance. Different *symbols* represent the data for which A_n was estimated to be limited by PFD (*open circles*), stomatal conductance (*open triangles*), another factor (*multiplication symbol*) and not limited (*plus*); *full line* was fitted to A_n data limited by PFD and non limited A_n data

Limitations to net photosynthesis

Envelope functions relating A_n versus PFD and A_n versus g_s are shown on Figs. 2 and 3, respectively. Parameters of the A_n versus PFD envelope function showed that reducing GI, through enhanced shading, linearly reduced A_n^{max} but increased the quantum use efficiency at low light, which resulted in lower PFD^{sat} (Figs. 2, 4a). On the other hand, the linear A_n versus g_s envelope function showed that when GI was reduced through increasing shading, the slope (m) of this function decreased linearly (Figs. 3, 4b).

The frequency of limitations (Lim_{Freq}) of A_n by PFD was negatively correlated to GI, increasing from 31 to 40% between GI_{100} and GI_{19} (Fig. 5d). The opposite was observed for the frequency of A_n limitations by g_s which was positively correlated to GI, increasing from 19% of the measurements in GI_{19} to 44% of the measurements in GI_{100} (Fig. 5d). "Other" limiting factors were estimated to increase from 17% at GI_{100} to 30% at GI_{19} (Fig. 5d). PFD limitation of A_n was more frequent at AM, g_s limitation at MM and PM while "other" limitations showed a stable frequency ($\sim 25\%$) except for higher levels at PM for low GI treatments (Fig. 5a-c). Cumulated absolute limitations to A_n (Lim_{Abs}) strongly increased from 2.7 µmol $CO_2 \text{ m}^{-2} \text{ s}^{-1}$ at GI_{19} to 5.1 µmol $CO_2 \text{ m}^{-2} \text{ s}^{-1}$ at GI_{100} (Fig. 5h). This difference was mainly caused by g_s limitations to A_n , which linearly increased from 0.5 to 3.0 μ mol CO₂ m⁻² s⁻¹ between GI₁₉ and GI₁₀₀. On the



Fig. 3 *Coffea arabica* leaf net photosynthesis (A_n) as a function of stomatal conductance (g_s) under 100% (**a**), 72% (**b**), 45% (**c**) and 19% (**d**) of full solar irradiance. Different *symbols* represent the data for which A_n was estimated to be limited by PFD (*open circles*), g_s (*open triangles*), another factor (*multiplication symbol*) and not limited (*plus*); *full line* was fitted to A_n data limited by g_s

other hand, PFD and "Other" limitations remained stable among different GI treatments (~1.0 µmol CO₂ m⁻² s⁻¹ reduction for each; Fig. 5h). Regarding the evolution of Lim_{Abs} during the day, the most important difference was found for MM with a fivefold increase from GI₁₉ to GI₁₀₀ (Fig. 5f). A clear prevalence of PFD limitations were observed at AM, especially at low GI (Fig. 5e, g) whereas at MM and PM periods, g_s was the main limiting factor at high GI levels. "Other" limiting factors remained low for most of the period-of-day × GI combinations but strongly increased for low GI levels at PM, exhibiting a maximum reduction of 2.7 µmol CO₂ m⁻² s⁻¹ for GI₁₉.

Discussion

Acclimation of leaf gas exchange to sun and shade

The presently observed acclimation of Arabica coffee leaves to shade (Figs. 1, 4) has been reported by several authors and related to coffee origin in the understorey of the Ethiopian rainforest (Rhizopoulou and Nunes 1981). The stable A_n for shade levels up to 55% may be partly explained by the acclimation of leaves to shade through (1) an increased quantum use efficiency and (2) a reduction in PFD^{sat} (Figs. 2, 4a). These photosynthetic shade-acclimation features have enabled coffee leaves to maintain photosynthesis under low GI, hereby compensating for the concomitant reduction of A_n^{max} (Figs. 2, 4a). Although



Fig. 4 Parameters of the envelope functions fitted to relate *Coffea arabica* leaf net photosynthesis (A_n) to **a** photon flux density: maximum light saturated $A_n [A_n^{max} (open squares)]$ and saturating light dose (PFD^{sat}) and to **b** slope [k (multiplication symbol)] of the

monomolecular equation relating A_n to PFD; and stomatal conductance: slope [*m* (*open circles*)] of the linear equation; as a function of growth irradiance



Fig. 5 Cumulated frequency of the limitation of *Coffea arabica* leaf net photosynthesis (**a**–**d**) and cumulated absolute limitation of net photosynthesis (**e**–**h**) by PFD (*dark gray*), g_s (*light gray*) and other factors (*dotted*) as a function of growth irradiance and during the morning (*AM* **a**, **e**), Midday (*MM* **b**, **f**), afternoon (*PM* **c**, **g**) and the whole day (*Day* **d**, **h**)

these results are in general agreement with plant acclimation of A_n to shade (Boardman 1977) and have been previously reported for coffee (Fahl et al. 1994; Franck 2005; Hollies 1967), the present observation of a maintained A_n with shade levels up to 55% is in contradiction with the inefficiency of shade-acclimation of coffee leaves from shaded canopy positions in maintaining A_n observed on another variety of arabica coffee in Viçosa, Brazil at similar shade levels (Araújo et al. 2008). These authors (Araújo et al. 2008) found no difference in quantum use efficiency and light saturated A_n between shade and sun leaves, which also contradicts the present results, and may reflect differences in light acclimation between shaded leaves inside the coffee canopy from results obtained from exposed coffee leaves submitted to artificial shading (Fahl et al. 1994 and the present results). Nevertheless, in another artificial shading study, Chaves et al. (2008) reported a low coffee leaf plasticity to contrasting irradiances. In any case, the present shade-acclimation of A_n to PFD does not fully explain the effect of shade on coffee leaf A_n , otherwise all the data recorded for all GI treatments would fit to the monomolecular functions depicted in Fig. 2.

The observed reduction in g_s with increasing GI (Fig. 1) is consistent with the lower g_s values previously measured in sun coffee leaves as compared to shade leaves (Chaves et al. 2008; Fanjul et al. 1985; Vaast et al. 2007; van Kanten and Vaast 2006). In the case of A_n , sustained levels up to 55% shade are in agreement with similar and recent results in Brazil. Nonetheless, the present results have significantly higher daily average values of $\sim 6 \,\mu mol$ $CO_2 \text{ m}^{-2} \text{ s}^{-1}$ (Fig. 1) than the ones (~2 and ~3 µmol $CO_2 \text{ m}^{-2} \text{ s}^{-1}$) measured in Viçosa, Brazil by Chaves et al. (2008) and Araújo et al. (2008), respectively. Similar relative differences were found between the g_s values measured in the present study and in the Brazilian studies (Araújo et al. 2008; Chaves et al. 2008). These higher values of g_s may be related to the more favorable climatic conditions of the rainy season of Orosi, resulting in VPD₁ values of about half those measured by Chaves et al. (2008). This may also explain the higher A_n due to lower diffusional limitations. PFD and T_1 were also significantly lower in the present study as compared to optimal coffee growing conditions of Viçosa, Brazil (Chaves et al. 2008) which may have reduced photoinhibitory and temperature

stress to A_n (Araújo et al. 2008; DaMatta et al. 2007). On the other hand, the A_n values measured for GI_{100} at AM (Fig. 1e) coincide with those measured by Steiman et al. (2007) for "Tipica" arabica coffee leaves. The MM and PM A_n levels for GI₄₅ through GI₁₀₀ (Fig. 1e) are in line with results of De Freitas et al. (2003). The positive linear relationship observed between the slope (m) of the linear $A_{\rm n}$ versus $g_{\rm s}$ equation in response to GI (Fig. 4b) indicates that acclimation of coffee leaves to full sun results in an increased maximal intrinsic WUE in line with results by Niinemets and Tenhunen (1997) for leaves of Acer saccharum, a temperate shade-tolerant species. Aranda et al. (2005) also found higher $A_n:g_s$ levels for seedlings of the sun plant, Quercus suber, growing in high light rather than in low light environments. Nevertheless, unlike the present results in coffee and the study by Niinemets and Tenhunen (1997), this difference was due to a higher intercept and not to a higher slope of the linear A_n versus g_s function.

Limitations to photosynthesis by light availability and stomatal conductance

The use of envelope functions allowed the screening of the large dataset of gas exchange measurements for PFD and g_s and quantifying limitations to A_n (Figs. 2, 3). To our knowledge, this is the first example of the use of this simple approach for assessing environmental and physiological limitations of photosynthesis.

As hypothesized by DaMatta (2004), g_s limitations of A_n prevailed at high GI whereas PFD limitations of A_n were more frequent at lower GI (Fig. 5d). Nevertheless, the absolute limitation of A_n progressively increased between the lowest and the highest GI levels (Fig. 5e-h) which explains that, although A_n^{max} was 23% higher for GI₁₀₀ than GI_{45} (Fig. 4a), their mean A_n was practically the same (Fig. 1). The cumulated frequency of different limitations to A_n measurements under all GI treatments was shown to be equally high ($\sim 90\%$; Fig. 5d). This indicates that the higher absolute limitation of A_n at higher GI levels (Fig. 5h) was due to the degree of limitation by environmental and physiological factors and not to their frequency. This positive correlation between the intensity of limitation to A_n and GI level was mainly related to g_s whereas absolute limitations by PFD remained at a stable value of ~1.0 μ mol CO₂ m⁻² s⁻¹ (Fig. 5h). This g_s limitation was principally observed for higher GI levels at MM and PM (Fig. 5), confirming the high sensitivity of g_s to VPD₁ (Fig. 1) of coffee leaves (DaMatta and Ramalho 2006; Ronquim et al. 2006; Vaast et al. 2007). In the case of extremely high shade such as GI₁₉, the absolute reductions in A_n were as expected mainly attributable to low PFD levels at AM (Figs. 1, 5e). Actually, as shade levels increased, the typical daily pattern of A_n showing higher

levels in the morning and low levels from noon onwards (Ronquim et al. 2006; Vaast et al. 2007) presently observed in GI100, progressively evolved into a "bell shaped" pattern with highest values at noon in GI_{19} (Fig. 1e). Although this feature can be associated with a reduction of g_s limitations to A_n during MM, due to a shade-induced reduction in VPD₁ and PFD limitations at AM and PM (Fig. 1a, c), A_n of GI₁₉ was estimated to be mainly restricted by "other" factors during PM (Fig. 5g). This "other" factor may be related to sink limitation to A_n due to the lower fruit set, and hence sink demand, induced by shade. Several authors have related low fruit load to sink feedback down-regulation of A_n of coffee leaves (Cannell 1971; DaMatta et al. 2008; Vaast et al. 2005; Franck et al. 2006; Ronchi et al. 2006). Furthermore, Franck et al. (2006) observed that this down-regulation was more severe in the afternoon and related to a build up of soluble sugars in the leaves due to a rapid fulfillment of fruit carbon demand during morning hours. Recently, DaMatta et al. (2008) showed that sinklimitation to coffee leaf A_n is mediated by a reduction of g_s and independent of carbon metabolism. If this were the case in the present study, limitations by low sink demand should have been captured within the data considered to be limited by g_s and the data classified as limited by "other" factors could not be attributed to sink-limitations. Factors other than PFD, g_s and sink demand would hence limit coffee leaf afternoon A_n in the shade. Given the fact that low values of T_1 , which could have been this "other" factor for the GI₁₉ and PM conditions, were not observed, and that T_1 was rather in the range considered to be optimal for coffee photosynthesis (Nunes 1988; Figure 1b), the present results still point toward a sink limitation to A_n with decreasing GI and toward PM. DaMatta et al. (2008) studied the effect of varied source-sink relationships on A_n , carbon metabolism and g_s under clear sky, full sun conditions. Our results suggest that sink limitation under shade may involve different mechanisms which would be independent of g_s . In any case and based on the results of DaMatta et al. (2008), part of the g_s limitations presently reported may be related to low sink demand rather than unfavorable climatic conditions (i.e. high VPD₁). Some agroforestry practices such as pruning or using deciduous shade trees for enhancing light interception by coffee trees during the dry season and thereby increasing floral induction are applied by farmers in Central America (Beer et al. 1998). These techniques may partly alleviate the sink limitations to A_n imposed by low fruit load observed under constant shade along the year as applied in the present study. Based on the negative relationship between GI level and fruit load, it can be hypothesized that sink limitation to An would prevail at low GI and may have been partly classified as g_s limitations in shaded treatments. In the case of higher GI levels, "other" limitations to An could mainly

be attributed to photoinhibition (Ramalho et al. 1999) and high T_1 stress (Nunes 1988).

Conclusions

The use of envelope curves allowed to quantify the effects of limitations by stomatal conductance and light availability to coffee leaf photosynthesis under different shade levels showing that growth irradiance levels as low as 45% did not limit the photosynthesis of exposed coffee leaves even under the optimal coffee growing conditions of Orosi Valley, Costa Rica. This feature is mainly related to (1) an attenuation of stomatal limitation due to a reduction of VPD₁ by shade which compensates for the reduction in light saturated net photosynthetic rate due to shade acclimation and (2) an acclimation of the photosynthetic apparatus which enables coffee leaves to maintain similarly low PFD limitations in shade as in full sun, by increasing maximal quantum use efficiency. Low sink demand, resulting from reduced fruit set in response to increasing shade, may explain part of the stomatal and "other" limitations to A_n in the shade. This aspect merits a more detailed study of (1) the interaction of shade and sourcesink relationships on coffee leaf photosynthesis and (2) agroforestry practices to increase fruit load. Nonetheless, the present results confirm our hypothesis that, even under the optimal coffee growing conditions of the Orosi Valley, and with even lower climatic stress conditions than the ones observed in the optimal coffee growing region of Viçosa in Brazil, shade of up to 55% is beneficial for coffee leaf photosynthesis as it maintains A_n with respect to full sun via a significant attenuation of stomatal stress. The present results at the leaf scale need to be up-scaled and validated at the canopy level before they can be translated into recommendations in terms of agricultural management practices.

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