What limits photosynthesis of coffee leaves under sun and shade?

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Abstract

The effect of the shade of trees on coffee net photosynthesis (A_n) in agroforestry systems has been the object of debate 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 (PFD) and stomatal conductance (g_s). These gas exchange measurements were carried out during two consecutive active coffee berry growth seasons in a commercial plantation in the Orosi valley of Costa Rica. Levels of A_n under the different shade treatments were related to PFD and g_s in order to calculate envelope functions which were used to establish PFD or g_s limitation to A_n. Under the conditions of the present trial, mean leaf A_n remained at a stable level up to 45 % growth irradiance (GI) and decreased by ~ 20 % at 19 % GI. g_s strongly limited A_n in full sun and this effect decreased linearly with increasing shade level. 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 may explain the absence of a decrease in coffee leaf A_n with shade level up to 55%.

Resumen

El efecto sobre la fotosíntesis del cafeto (A_n) de la sombra proyectada por los árboles asociados en cafetales cultivados en sistemas agroforestales ha sido objeto de controversia en el ámbito científico ligado a la cafeicultura. El presente estudio se basa en más de 600 mediciones instantáneas de A_n realizadas bajo cuatro niveles de sombra artificial (100, 72, 45 y 19% de pleno sol) con el fin de analizar limitaciones de la A_n por parte de la radiación fotosintéticamente activa (PFD)y la conductancia estomática (g_s). Dichas mediciones se llevaron a cabo en una plantación comercial del valle de Orosi, Costa Rica, durante la fase de crecimiento activo de los frutos en dos temporadas consecutivas. Los niveles de A_n bajo los distintos niveles de sombra fueron puestos en relación con PFD y g_s permitiendo ajustar curvas envoltorio utilizadas para determinar los valores de A_n limitados por PFD o g_s, respectivamente. Bajo las condiciones del estudio, el valor medio de A_n se mantuvo a un nivel similar hasta niveles de un 55% de sombra y disminuyó en ~ 20% a niveles de sombra de 81%. En pleno sol, g_s redujo fuertemente el nivel de A_n, efecto que disminuyó en forma lineal al aumentar el nivel de sombra. Las limitaciones de A_n por PFD, por otra parte, se mantuvieron en un nivel similar para todos los niveles de sombra estudiados. Estas diferencias en la respuesta de las limitaciones de A_n por PDF y g_s podrían explicar la ausencia de reducción de A_n foliar del cafeto en respuesta niveles de sombra de hasta 55%.

Introduction

Coffee (*Coffea arabica* L.) originated in Ethiopia where it grows in shaded environments characteristic of tropical rainforest understorey (Kumar 1979). This is why coffee plants show typical features of shade adaptation and has been classified as a shade (Ramalho et al. 1999; Rhizopoulou and Nunes 1981) or shade facultative species (Fahl et al. 1994). This shade

adaptation favors coffee cultivation in agroforestry systems which actually is regaining popularity among growers in Central America (Beer et al. 1997). Da Matta (2004) raised the question of the ecophysiological constraints on the production of shaded and un-shaded coffee in a recent review article in which they postulated that photosynthesis (A_n) would benefit mostly from the shade of associated trees under sub-optimal conditions (i.e. hotter and/or dryer climates) where it would buffer the negative effects of temperature and high vapor pressure deficit (VPD). On the other hand, these authors suggest that, under optimal coffee growing conditions, the shade of trees would result in a limitation of coffee photosynthesis as compared to full sun due to low light availability (Da Matta 2004). Actually, it has been documented that one of the main factors restricting coffee photosynthesis in full sun is low stomatal conductance (Fanjul et al. 1985) induced by high VPD (Vaast et al. 2007). Nevertheless, photosynthesis limitation due to low light availability under shade conditions is less "straightforward" in view of the high acclimation capacity to shade of the coffee leaf photosynthetic apparatus (Fahl et al. 1994; Franck 2005; Hollies 1967).

In this study, gas exchange was measured under four growth irradiance levels ranging from 19% to full solar irradiance over two consecutive rainy seasons under the optimal coffee growing conditions of the Orosi valley of Costa Rica. The contribution of low light availability and low stomatal conductance 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; 1108 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 x 2 m spacing with east-west oriented rows.

Growth irradiance treatments

Four growth irradiance (GI) treatments were established in December 2002. Each treatment included eight coffee rows of 10 plants each. Treatments consisted in full solar irradiance (GI_{100}) and three shade treatments with 72% (GI_{72}), 45% (GI_{45}), and 19% (GI_{19}) of the full solar irradiance. GI treatments were achieved by constructing shade houses covered with black shade screens with the required light transmittances which were positioned at a height of at least 3 m.

Measurements

Measurements of instantaneous net CO_2 assimilation rate (A_n) and stomatal conductance to H_2O (g_s) were performed with a CO_2/H_2O infrared gas analyser (LCPro, ADC BioScientific Ltd., Hoddesdon, U.K.) connected to a broadleaf chamber and 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 (3rd to 6th pair of leaves from the branch tip) sampled on plagiotropic, mid-height branches. 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_l), incident photon flux density (PFD), air humidity and temperature (used to calculate air vapor pressure deficit [VPD]) were recorded during each gas exchange measurement. 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 GI_{19}.

Data analysis

For each GI treatment, the 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), we chose the monomolecular function for relating A_n to PFD:

$$A_n = A^{max}(1 - e^{-k\,PFD}) - R_d \qquad \text{Eqn 1}$$

where A^{max} is the maximum light saturated gross CO_2 assimilation rate, R_d is day respiration and k is a coefficient logarithmically related to the initial slope of the response of A_n to PFD (i.e. 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} ($A_n^{max} = A^{max} - R_d$). For relating A_n to g_s, we assumed the often observed linear relationship between these traits (Leuning 1995) with a slope (m), representing the intrinsic water-use-efficiency (WUE):

$$A_n = m \cdot g_s - R_d \qquad \text{Eqn 2}$$

In order to fit these envelope functions to the data sets for 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 the time, re-fitting the envelope function each time a new data was included. This procedure was repeated until the addition of a new data caused one data to exceed a 5 % difference from the predicted value. The A_n values calculated with Eqn 1 were termed A^{PFD} and those calculated with Eqn 2 were termed A^{gs}. Sigmaplot 2000 software (SPSS Inc., Chicago, IL, USA) was used for fitting the envelope curves. Three categories of A_n data were then selected: non limited (Lim^{None}), limited by light availability (Lim^{PFD}), limited by stomatal conductance (Lim^{gs}) and limited by another factor (Lim^{Other}). We established a saturated PFD value (PFD^{sat}) from Eqn 1 as the PFD level for which the predicted $A_n = 0.95\ A_n^{max}$. Then, the A_n data of each GI treatment were assigned to one of the previously mentioned categories by means of the following assumptions:

- Lim^{None}: $A_n \geq 0.95\ A_n^{max}$

Excluding Lim^{None} data:

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

In all GI treatments, some data recorded at low PFD fulfilled the conditions of both LIM^{PFD} and LIM^{gs}. In these cases, the data were assigned to the category for which the estimated A_n value (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 each of the analyzed limiting factors (PFD, g_s or "Other"). First the proportion of measurements of each of the limitation categories was calculated and used to assess the frequency with which each factor limited A_n along 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:

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

where Lim^{i}_{Abs} (μmol CO_2 m^{-2} s^{-1}) is the contribution of the limiting factor 'i' (PFD, g_s or Other), Lim^{i}_{Freq} is the frequency (%) with which the limiting factor 'i' limits A_n and $\overline{A^{i}_{n}}$ is the mean A_n value of the data limited by the factor 'i'.

Results

Microclimatic and gas exchange traits

GI treatments affected PFD in the expected proportions throughout the day (Table 1) achieving the highest levels at MM and similar levels at AM and PM (~57% and ~50% of MM, respectively). For all GI treatments, vapor pressure deficit (VPD) and T_l also exhibited their highest levels at MM and their PM values were higher than AM values (Table 1). VPD and T_l, linearly decreased in response to decreasing GI, but this effect was more pronounced for VPD which decreased to half its value from GI_{100} to GI_{19} whereas T_l was only decreased by 10% (~3 ºC) between those treatments (Table 1). Concerning gas exchange, the daily mean A_n was similar for GI_{100} - GI_{45} and decreased by ~ 1.2 μmol CO_2 m^{-2} s^{-1} to a mean value of 5.0±2.0 μmol CO_2 m^{-2} s^{-1} at GI_{19} (Table 1). GI treatments induced different patterns of daily A_n evolution; for the higher GI_{100} and GI_{72} treatments, A_n was highest at AM and decreased to similar levels at MM and PM, whereas for GI_{45} A_n was similarly higher at AM and MM than PM. GI_{19} exhibited the highest A_n levels at MM and the lowest at PM (Table 1). Concerning g_s, this trait was lowest at MM and highest at AM for all treatments and decreased with increasing GI (Table 1).

Table 1. Effects of growth irradiance (100%: GI_{100}; 72%: GI_{72}; 45%: GI_{45}; and 19%: GI_{19}) and period of day on photon flux density (PFD), vapor pressure deficit (VPD), leaf temperature (T_l), net photosynthesis (A_n) and stomatal conductance (g_s) of *Coffea arabica* leaves. Mean values are presented ± standard deviation; each period of day x GI treatment represents an average of 50 data.

Trait	Treatment	Morning	Midday	Afternoon	Day
PFD (μmol photons m^{-2} s^{-1})	GI_{100}	738±537	1288±632	661±414	893±557
	GI_{72}	539±391	891±449	470±348	636±424
	GI_{45}	327±142	568±276	301±215	407±233
	GI_{19}	147±61	257±82	131±77	178±125
VPD (kPa)	GI_{100}	1.15±0.93	2.37±1.4	1.67±1.07	1.73±1.24
	GI_{72}	0.71±0.28	2.00±1.33	1.41±0.79	1.50±1.08
	GI_{45}	0.57±0.18	1.54±0.76	1.25±0.53	1.24±0.66
	GI_{19}	0.43±0.12	1.06±0.44	0.87±0.21	0.88±0.38
T_l (ºC)	GI_{100}	27.1±5.2	33.4±6.1	30.3±5.2	30.3±6.0
	GI_{72}	24.1±3.6	32.4±6.9	29.1±4.0	29.5±6.0
	GI_{45}	22.3±2.1	31.9±5.4	28.8±3.2	28.9±5.3
	GI_{19}	20.8±1.9	30.6±4.8	26.4±2.4	27.3±4.9
A_n (μmol CO_2 m^{-2} s^{-1})	GI_{100}	7.6±2.7	5.7±3.1	5.7±2.5	6.3±2.9
	GI_{72}	7.1±2.6	6.2±2.8	5.8±2.8	6.2±2.8
	GI_{45}	6.8±1.6	6.5±1.7	5.8±1.8	6.2±2.0
	GI_{19}	4.7±1.8	6.6±1.3	3.5±1.2	5.0±2.0
g_s (mol H_2O m^{-2} s^{-1})	GI_{100}	0.35±0.20	0.15±0.12	0.19±0.22	0.23±0.20
	GI_{72}	0.45±0.20	0.20±0.12	0.21±0.16	0.25±0.18
	GI_{45}	0.44±0.15	0.19±0.10	0.24±0.10	0.27±0.16
	GI_{19}	0.40±0.23	0.28±0.10	0.25±0.11	0.29±0.14

Limitations to net photosynthesis

The results of the fitting of the envelope functions for the relationship of A_n vs. PFD and A_n vs. g_s are shown on figures 1 & 2, respectively. The parameters of the A_n vs. PFD envelope function showed that reducing GI through shading linearly reduced A_n^{max} but increased the quantum use efficiency at low light, which resulted in lower PFD^{sat} (Figures 1 & 3). On the other hand, the linear A_n vs. g_s envelope function showed that when GI was reduced through shading, the slope

(*m*) of this function linearly decreased (Figures 2 & 3b), indicating that intrinsic WUE decreased with decreasing GI.

Regarding Lim_{Freq}, limitation by PFD was estimated to linearly increase with decreasing GI from 31% to 40% between GI_{100} and GI_{19} (Figure 4d). The opposite was observed for the limitation by g_s which linearly increased with increasing GI from 19% in GI_{19} to 44% in GI_{100} (Figure 4d). "Other" limiting factors were estimated to increase from 17% at GI_{100} to 30% at GI_{19} (Figure 4d).

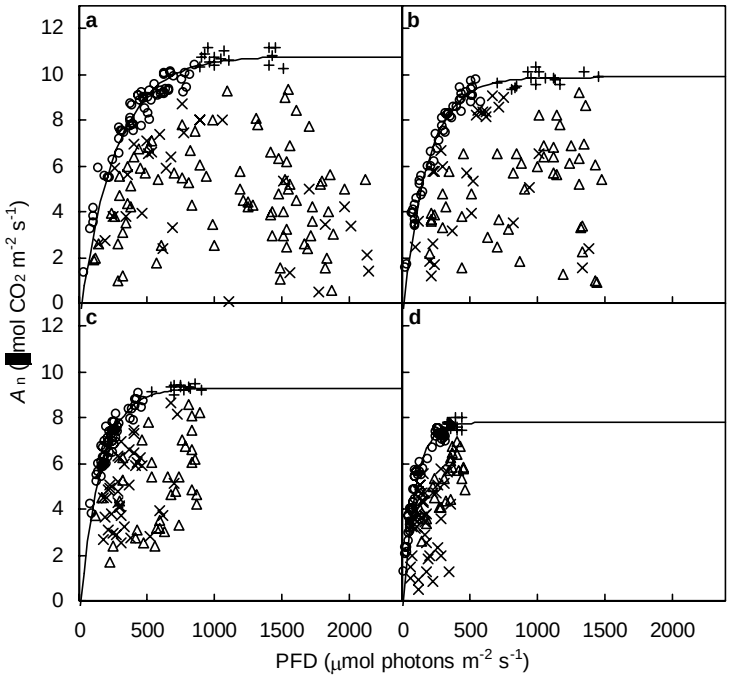

Figure 1. *Coffea arabica* leaf net photosynthesis (A_n) as a function of photon flux density (PFD) under 100% (**a**), 72% (**b**), 45% (**c**) and 19% (**d**) of full solar irradiance. Different symbols represent the data for which A_n was estimated to be limited by PFD (○), stomatal conductance (Δ), another factor (x) and not limited (+); full line was fitted to A_n data limited by PFD and non limited A_n data.

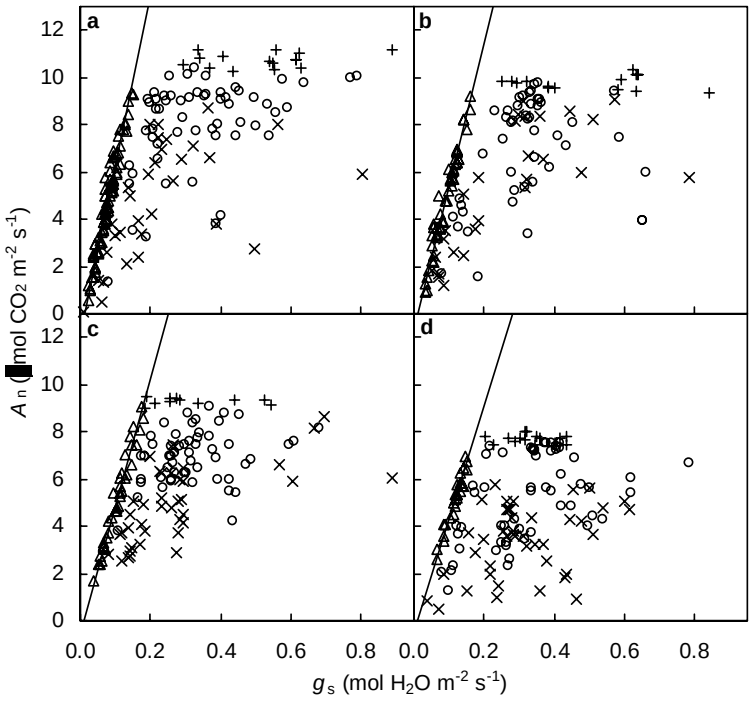

Figure 2. *Coffea arabica* leaf net photosynthesis (A_n) as a function of stomatal conductance (g_s) under 100% (**a**), 72% (**b**), 45% (**c**) and 19% (**d**) of full solar irradiance. Different symbols represent the data for which A_n was estimated to be limited by PFD (○), g_s (Δ), another factor (x) and not limited (+); full line was fitted to A_n data limited by g_s.

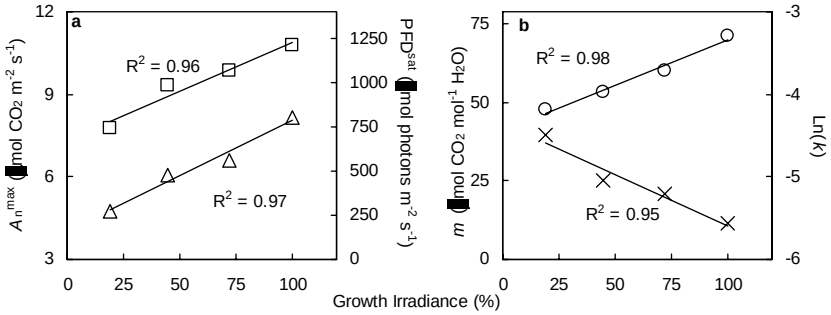

Figure 3. Parameters of the envelope functions fitted to relate *Coffea arabica* leaf net photosynthesis (A_n) to (**a**) photon flux density: maximum light saturated A_n (A_n^{max}: □) and saturating light dose (PFDsat) and to (**b**) slope (*k*: ∆) of the monomolecular equation; and stomatal conductance: slope (*m*: ○) of the linear equation, as a function of growth irradiance.

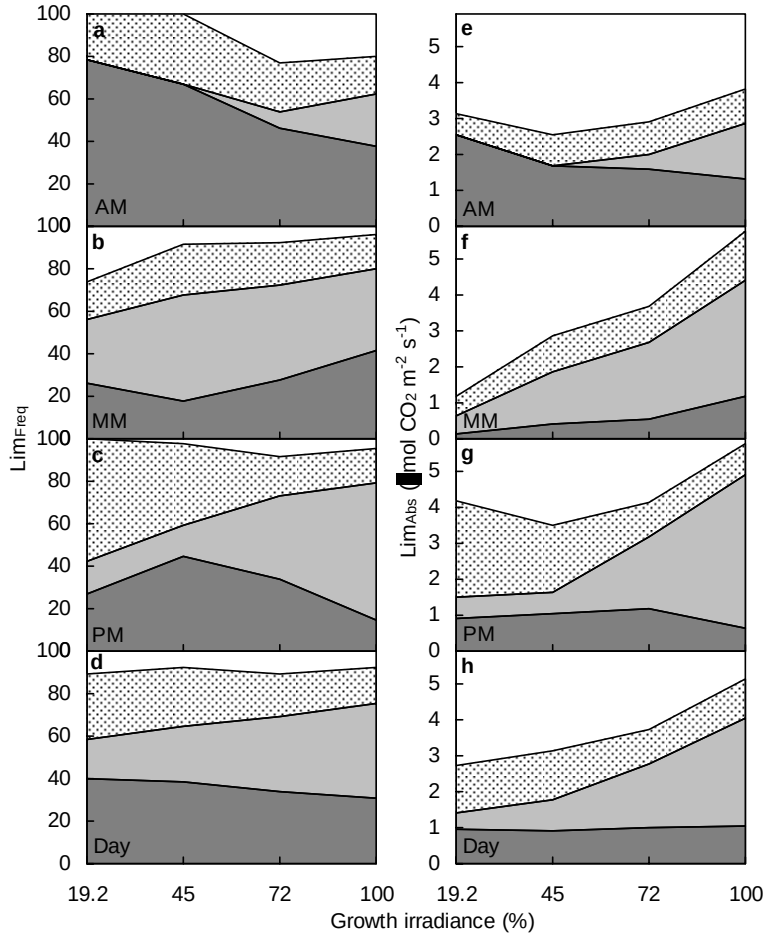

Figure 4. Cumulated frequency of the limitation of *Coffea arabica* leaf net photosynthesis (**a**-**d**) and cumulated absolute limitation of net photosynthesis (**e**-**h**) by PFD (dark grey), g_s (light grey) and other factors (dotted) as a function of growth irradiance and during the morning (AM: **a** & **e**), Midday (MM: **b** & **f**), afternoon (PM: **c** & **g**) and the whole day (Day: **d** & **h**).

PFD limitation of A_n was more frequent at AM, g_s limitation at MM and PM while "other" limitations showed a stable frequency (~25%), but increased for low GI treatments at PM (Figure 4a-c). Cumulated Lim_{Abs} strongly increased with increasing GI level from 2.7 μmol CO_2 m^{-2} s^{-1} at GI_{19} to 5.1 μmol CO_2 m^{-2} s^{-1} at GI_{100} (Figure 4h). This difference was mainly caused by g_s limitations to A_n, which linearly increased from 0.5 to 3.0 μmol CO_2 m^{-2} s^{-1} between GI_{19} and GI_{100}, whereas PFD and other limitations remained stable among different GI treatments, both at a level of ~ 1 μmol CO_2 m^{-2} s^{-1} (Figure 4h). Concerning limitations along the day, the most important difference was found for MM where limitations to A_n linearly increased from GI_{19} to

GI_{100} by a factor of five (Figure 4f). A clear prevalence of PFD limitations were observed at AM, especially at low GI (Figure 4e & g). At PM, g_s was the main limiting factor for high GI levels and, unlike for the rest of the period of day x GI combinations, "other" limiting factors strongly increase for low GI levels, exhibiting a peak of 2.7 μmol CO_2 m^{-2} s^{-1} for GI_{19}.

Discussion

The similar daily levels of A_n measured for GI_{100}, GI_{72} and GI_{45}, despite the significant reduction in PFD (Table 1) indicate a high acclimation of coffee leaves to shade. This acclimation of Arabica coffee to shade has been reported by several authors and related to coffee origin in the understorey of the Ethopian rainforest (Rhizopoulou and Nunes 1981). The stable A_n for shade levels up to 55% presently observed may be partly explained by the acclimation of leaves to shading through (i) an increased quantum use efficiency and (ii) a reduction in PFD^{sat} (Figure 1 & 3a). Opposite to these positive photosynthetic shade-acclimation features, the reduction in A_n^{max} (Figure 1 & 3a) would favor GI_{100}, provided saturating PFD levels prevail along the day and production cycle. Although these features of coffee acclimation to shade 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), they do not fully explain the effect of shade on coffee leaf A_n otherwise all the data recorded for all GI treatments should fit to the monomolecular functions depicted in Figure 1. On the other hand, the linear increment in the slope of the A_n vs. g_s linear equation with increasing GI (Figure 3b) is a clear indicator of a stomatal acclimation of coffee leaves to full sun by increasing the intrinsic WUE (Table 1) and is consistent with the lower g_s values previously measured in coffee sun leaves as compared to shade leaves by other authors (Fanjul et al. 1985; Gutierrez and Meinzer 1994; Vaast et al. 2007). The presently observed higher frequency of g_s limitation for high GI treatments and higher frequency of PFD limitation for lower GI treatments (Figure 4d) is in agreement with the conclusion of the review on coffee ecophysiological constraints by Da Matta (2004). On the other hand, the absolute limiting effect on A_n (Figure 4e-h) increases with increasing GI which explains that, although A_n^{max} was 23% higher for GI_{100} than GI_{45} (Figure 3), their mean A_n was practically the same (Table 1). The frequency of non-limited A_n measurements under all GI treatments showed to be equally scarce (Figure 4d) implying that the increment of the absolute limitation to A_n with increasing GI (Figure 4h) is due to a higher degree of reduction of A_n when it was limited by some factor. Actually, this increasing extent of limitation with increasing exposure to sun could be mainly related to a higher amount of restriction owed to g_s as observed when comparing Figure 4d with Figure 4h. This higher g_s limitation was principally observed for higher GI levels, and especially for GI_{100}, at MM and PM (Figure 4), suggesting a relationship to higher VPD levels (Table 1). Actually, a high sensitivity of g_s to VPD has been previously reported (Nunes 1988; Vaast et al. 2007). In the case of extreme shade, the GI_{19} treatment showed that absolute reductions of A_n were expectedly mainly attributable to low PFD levels at AM (Table 1, Figure 4e) but, despite even lower PFD levels, in the afternoon A_n was mainly restricted by "other" factors (Figure 4g). 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. Actually, Franck et al. (2006) related low fruit load to sink feedback down-regulation of A_n of coffee leaves and observed that this down-regulation increased along the day. Based on micrometeorological data (Table 1) and the fact that fruit-set was linearly reduced by decreasing GI (data not shown) it may be hypothesized that, besides PFD and g_s, "other" limitations to A_n would mainly consist of photoinhibition (Ramalho et al. 1999) and high T_l (Nunes 1988) for high GI and MM, whereas sink limitation would be the main component of "other" limitations for low GI and PM.

Conclusions

Under the present conditions, growth irradiance levels as low as 45% did not limit photosynthesis of exposed coffee leaves. This feature may be mainly related to an attenuation of stomatal limitation due to a reduction of VPD by shade. The acclimation of the photosynthetic apparatus of exposed coffee leaves to shade induces a reduction of light saturated photosynthesis but allows them to efficiently photosynthesize in shade keeping similar levels of light limitation at growth irradiance levels of 19%. Nevertheless, at this lower growth irradiance level, other factors, probably related to low sink demand due to a low fruit set, induce coffee leaf photosynthesis to be reduced as compared to growth irradiance levels ≥ 45%. Although measurements at the leaf scale need to be up-scaled to canopy level, they show that, even under the present and optimal coffee growing conditions of the Orosi Valley, leaf photosynthesis is still limited by stomatal conductance in full sun as compared to growth irradiance levels of 45%, hence resulting in similar photosynthetic level.

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