Stomatal conductance in Amazonian tree saplings in response to variations in the physical environment

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Abstract

In juvenile trees growing at the rainforest understory, light is the most limiting factor for growth. It has been assumed that stomata quickly respond to light irrespective of the physical conditions prevailing before leaf illumination. Nevertheless, so far this issue has not been addressed for saplings of Amazonian tree species. The aim of this study was to determine how stomatal conductance (g_s) and photosynthetic parameters of Amazonian saplings respond to diurnal variation in the physical environment and to rainfall seasonality. Light-saturated net photosynthetic rate (P_{Nmax}) and g_s at light saturation (g_{smax}) were measured in the dry (August) and rainy (January) season of 2008 in saplings of 10 Amazonian tree species (*Minquartia guianensis*, *Myrcia paivae*, *Protium apiculatum*, *Guatteria olivacea*, *Unonopsis duckei*, *Rinorea guianensis*, *Dicypellium manausense*, *Eschweilera bracteosa*, *Gustavia elliptica*, and *Tapura amazonica*). At the forest understory, variables of the physical environment were measured. Rainfall seasonality did not affect P_{Nmax} and g_{smax}, nor was the effect of species on P_{Nmax} and g_{smax} significant ($p>0.05$). The g_s and P_{Nmax} increased as the forest understory became brighter and warmer; as a result, P_{Nmax} and g_{smax} were higher at midday than early in the morning or in the afternoon. However, contrary to expectations, neither changes in air vapor pressure deficit nor air CO_2 concentration at the forest understory affected stomatal opening. More investigation is needed to elucidate the role of environmental factors in modulating stomatal movements in juvenile trees growing beneath the dense canopy of tropical rainforests.

Additional key words: atmospheric variables; photosynthesis; red to far-red ratio; sunflecks; understory CO_2.

Introduction

Most factors related to the physical environment affect stomatal functioning (Mansfield *et al.* 1990, Buckley 2005) and thereby carbon uptake. In many species, stomata open in the morning and close in the afternoon to reduce water loss (Mansfield *et al.* 1990, Camargo and Marenco 2012). Besides light conditions, stomatal opening is affected by $[CO_2]$, temperature, relative humidity, and leaf-air vapor pressure difference (Stålfelt 1962, Okamoto *et al.* 2009). Thus, when air CO_2 concentration ($[CO_2]_{air}$), soil moisture, humidity, and temperature are not limiting, it should be expected that light leads to stomatal opening (Shimazaki *et al.* 2007, Lawson 2009).

Beneath the forest canopy, seedlings and saplings cope with dimly lit conditions. Hence stomatal functioning and the photosynthetic machinery of juvenile trees need to be fine-tuned with the environment to maximize carbon gain. Because the low light conditions at the forest understory, a large portion of carbon gain depends on sunflecks, which may contribute up to 90% of daily PAR (Pearcy 1990). As photosynthesis depends on stomatal functioning, it is imperative to assume that stomata respond rapidly to the light stimulus for maximum sunfleck efficiency. Indeed, several authors have reported a fast stomatal response to light (*e.g.*, Pearcy *et al.* 1997, Vialet-Chabrand *et al.* 2013).

For understory plants, Singsaas *et al.* (2000) found that g_s and photosynthesis decline in the afternoon, which was associated with an increase in vapor pressure deficit. Mendes and Marenco (2010) also found that when assessed at the same light intensity, saplings growing beneath the forest canopy have lower both g_s and photosynthetic rates in the afternoon. For saplings of Amazonian tree species that grow at the forest understory, it is still unknown how variations in light intensity, temperature, and air humidity affect stomatal functioning.

Materials and methods

Study area and plant material: The study was conducted 60 km north of Manaus, at the Tropical Forest Experiment Station (Reserve ZF-2, 02°36'21" S, 60°08'11" W) of the National Institute for Research in the Amazon (INPA). Data were collected in January (hereinafter referred to as the wet season) and August (herein referred to as the dry season) of 2008. The study area is a pristine terra-firme (105 m a.s.l.) rainforest in central Amazonia. The area has a humid tropical climate and an annual rainfall of about 2,300 mm, with a rainy season from November to May and a dry period (≤100 mm per month) between June and September. October is a dry-rainy transition month. Mean temperature is about 25°C. Above the canopy, maximum mean PAR is about 1,000 µmol(photon) m^{-2} s^{-1}, relative humidity (RH) varies from 70% at noon to 100% at night (Magalhães *et al.* 2014). Beneath the forest canopy, air humidity is high and the red to far-red ratio (R:FR) is very low because of the dense canopy foliage. The soil type is an Oxisol (Yellow Latosol according to the Brazilian classification) with a clay texture, low fertility, and pH 4.5. At the first 10 cm from the soil surface, soil density is 0.9–1.0 g cm^{-3}, water content (v/v) is about 65% at soil saturation, and 35% at the permanent wilting point (Ferreira *et al.* 2002).

In this study, we used ten species of canopy trees in the juvenile phase (saplings) with a height between 1 and 3 m. As the experiment was carried out under natural conditions, the number of species was severely limited by natural constraints suffered by selected saplings (*e.g.*, snapped stems caused by branches or trees fall, pathogen infection, herbivore attack or simple defoliation by wild animals). Only species with at least three plants and enough foliage for collecting data in both rainfall seasons were included in the study; they were: *Minquartia guianensis* Aubl. (Olacaceae), *Myrcia paivae* O. Berg (Myrtaceae), *Protium apiculatum* Swart (Burseraceae), *Guatteria olivacea* R.E. Fries (Annonaceae), *Unonopsis duckei* R.E.Fries (Annonaceae), *Rinorea guianensis* Aubl. (Violaceae), *Dicypellium manausense* W.A. Rodrigues (Lauraceae), *Eschweilera bracteosa* (Poepp. ex O.Berg) Miers (Lecythidaceae), *Gustavia elliptica* S.A. Mori (Lecythidaceae), and *Tapura amazonica* Poepp. & Endl. (Dichapetalaceae).

In this study, we hypothesized that variation in light intensity, ambient [CO_2], and temperature at the forest understory affect stomatal opening of saplings in their natural environment. We also tested the hypothesis that rainfall seasonality affects photosynthetic rates of Amazonian saplings. Thus, the aim of this study was to determine how g_s and photosynthetic rates respond to rainfall seasonality and to diurnal variation in the physical environment.

Seasonal and diurnal variation in g_{smax} and P_{Nmax}: Light-saturated net photosynthetic rate (P_{Nmax}) and stomatal conductance at light saturation (g_{smax}) were measured with a portable photosynthesis system (*Li-6400*, *Li-Cor*, NE, USA) in two fully expanded leaves per plant and three plants per species. Each plant was measured at two occasions, the dry (August) and wet (January) season of 2008. In each season, measurements were taken from 06:00 to 17:00, at about 20–30 min intervals, because of the time needed to travel from one plant to the next to be measured. During gas-exchange measurements, the leaf was exposed to irradiance of 250 µmol(photon) m^{-2} s^{-1} to slowly induce stomatal opening for the first 8 min. Once g_s reached a steady state condition, PAR was increased to 500 µmol m^{-2} s^{-1} for 1 min and then to 1,000 µmol m^{-2} s^{-1} (light saturation). It took about 10 min to collect the data from each leaf. In the leaf chamber, P_{Nmax} and g_{smax} were measured at saturating light, 70% RH, 28°C, and [CO_2] of 380 ppm. As the leaves were kept at light saturation just for a short period of time (2 min), no photosynthetic rate decline at the higher PAR values (photoinhibition) was observed.

To determine when P_{Nmax} and g_{smax} reached maximum values, equations fitted for P_{Nmax} and g_{smax} were solved (*i.e.*, $\partial P_{Nmax}/\partial t$ and $g_s/\partial t = 0$). Leaf thickness and specific leaf area (SLA) were also assessed in leaves similar in appearance to those used in gas-exchange measurements. The SLA was calculated as the leaf area/mass ratio in five leaves per plant and five 240-mm^2 discs per leaf (avoiding the midrib). In order to determine if leaf thickness (succulence) affected gas exchange, fresh leaf thickness (L_T) was measured using digital calipers (accuracy of 0.01 mm). After pigment extraction in aqueous 80% acetone, absorbance was measured at 646 and 663 nm with a spectrophotometer (*SP-2000 UV*, *Shanghai Spectrum,* Shanghai, China). Leaf chlorophyll [Chl ($a+b$)] content was calculated using the equations described elsewhere (Wellburn 1989). Leaf dry mass was obtained after drying the leaves at 72°C until reaching constant mass. Leaf nitrogen was determined using the classic Kjeldahl method and leaf phosphorus using ammonium molybdate and the absorbance measured at 660 nm (*Shimadzu UVmini-1240, Shimadzu Corp.*, Kyoto, Japan), and finally, as a

supplementary information, we also recorded SPAD values of leaves (*SPAD-502*, *Minolta Camera Co.*, Osaka, Japan).

The physical environment: The fraction of sky visible (FSV, the relationship between the openings in the forest canopy and the open sky above the canopy) was determined in the wet and dry season of 2008 with a canopy analyzer (*LAI-2000 Plant Canopy Analyzer, Li-Cor*, NE, USA) using two synchronized sensors. One sensor was used to collect data at the forest understory (six FSV readings, forming a circle around each sapling) and the second, operating in the remote mode and installed on the top of the observation tower, to log FSV values above the forest canopy. Air temperature (T_{air}), PAR, RH, and rainfall data were recorded in the dry and wet season of 2008 above the forest canopy, at the observation tower; PAR above the canopy (PAR_{can}) was measured using a quantum sensor (*Li-190 SA*, *Li-Cor*, NE, USA), whereas PAR at the forest understory (PAR_{und}) was estimated as the product of FSV and PAR_{can} (*i.e.*, $PAR_{und} = FSV \times PAR_{can}$). The accuracy of this calculation was validated in previous experiments (Mendes *et al.* 2013). T_{air} and RH data were collected at 30-min intervals with a sensor (*Humitter 50Y, Vaisala Oy*, Finland) connected to a datalogger (*Li-1400, Li-Cor*, NE, USA). At the forest understory and during daytime, T_{air} and RH data were also collected (as described before) to calculate daytime understory air vapor pressure deficit (VPD_{und}). Saturation vapor pressure (VP_{sat}) was calculated as follows (Buck 1981): VP_{sat} (kPa) = 0.61365 exp[$17.502 T_{air}/(240.97 + T_{air})$], where T_{air} is in ºC.

Results

Physical environment: Rainfall was 355 mm in January (wet season) and 107 mm in August (dry season), which was within the historical mean recorded in the study region. Average PAR_{und} was 45% higher during the dry season than in the rainy period (*i.e.*, 0.53 *vs.* 0.29 mol m^{-2} day^{-1}), and in comparison with the dry period, PAR_{can} was 34% lower during the wet season (20.8 *vs.* 31.5 mol m^{-2} day^{-1}). Above the forest canopy, monthly mean T_{air} was 24.1°C in January and 26.3°C in August. Monthly mean RH was 74 and 90% in August and January, respectively. At the forest understory, ambient [CO_2] was higher in the morning and the afternoon than at midday (Fig. 1*A*). VPD_{und} was 20–25% higher in the dry season, and it increased during the day from zero at night to about 300–350 Pa at midday, following an opposite trend than ambient [CO_2] (Fig. 1). In the forest understory, T_{air} ranged from 22°C (night time) to 28°C at noon, whereas RH remained above 80% throughout the study period. At the understory, mean daytime T_{air} and RH were similar in both seasons (25.5°C and 94.5%). Most PAR_{inst} values were below 20 μmol m^{-2} s^{-1}; occasional sunflecks occurred during gas-exchange measurements. Rainfall seasonality

VPD_{und} was obtained as $VP_{sat} - (VP_{sat} \times RH)$. Instantaneous understory light conditions during gas exchange measurements (PAR_{inst}) were recorded using an external quantum sensor (*Li-190SA*) mounted on the *Li-6400* IRGA head. In both rainfall seasons, we determined soil water content (after drying the soil samples at 105°C until constant mass) in 30 soil samples (on each season) collected at 100-mm depth and close to the plants used in the study; mean soil water tension for each season was calculated as described elsewhere (Ferreira *et al.* 2002). To correlate with g_s data in the dry season we measured (1 m above the ground and before gas-exchange measurements) diurnal variation in ambient [CO_2] at the forest understory with a portable photosynthesis system (*Li-6400, Li-Cor*, NE, USA).

Statistical analyses: The data were subjected to analysis of variance (*ANOVA*). As the same set of plants was measured in both seasons, a complete randomized design with three replications (plants) was used and data were analyzed using two-way repeated-measures *ANOVA*. The effect of understory light, [CO_2], T_{air}, and VPD on photosynthetic parameters were examined by linear regression analysis, whereas the effect of time of the day was modeled using polynomial regression. As there was a diurnal effect on gas-exchange data, only data collected between 10:00 to 15:00 (when g_s was higher) were used to assess rainfall seasonality. The *SAEG 9.0* package of the Federal University of Viçosa-Brazil was used for statistical analyses.

led to variations in soil water content, from 47.6% (v/v, soil water tension of 10 kPa) in the dry season to 55.5% (v/v) in the rainy season with soil water tension of 6.3 kPa ($p<0.01$, $n = 30$); soil moisture content at field capacity was 60% (v/v) and the mean mass of oven dried soil per volume of soil core was 0.95 g cm^{-3}. There was little difference in FSV values between seasons or sampling sites indicating all plants shared rather similar understory light conditions.

Seasonal and diurnal variation in g_{smax} and P_{Nmax}: P_{Nmax}, g_{smax}, and Chl content and SPAD values did not differ among species ($p>0.05$, data not shown), neither between seasons (Table 1). In addition, SLA, L_T, and the leaf nitrogen and phosphorus contents (determined only in the dry season) showed only small variation among species ($p>0.05$). The mean values (± SD) were: SLA of 17.4 ± 4.6 m^{-2} kg^{-1}, L_T of 0.17 ± 0.04 mm, leaf phosphorus of 0.40 ± 0.07 mg g^{-1}, and leaf nitrogen concentration of 18.2 ± 0.49 mg g^{-1}. Thus, data across species were pooled to examine the effect of atmospheric variables on stomatal functioning. The highest values of P_{Nmax} and g_{smax} occurred
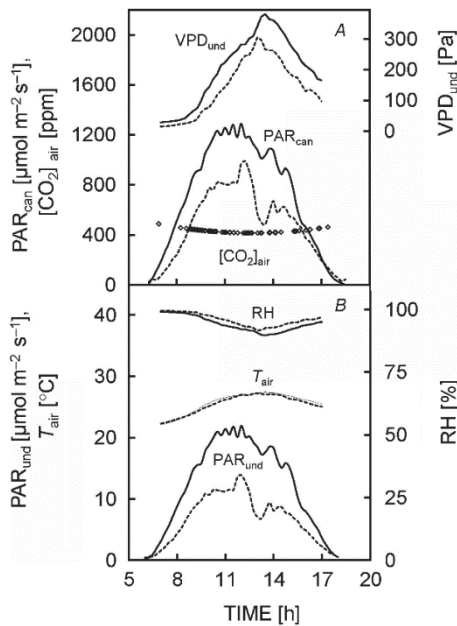
Fig. 1. Diurnal course of PAR above the forest canopy (PAR$_{can}$), understory air vapor pressure deficit (VPD$_{und}$), and air CO_2 concentration ([CO_2]$_{air}$) in the dry season (*A*); air temperature (T_{air}), estimated daily PAR at the forest understory (PAR$_{und}$), and air relative humidity (RH) at the forest understory (*B*). *Solid line* (dry season) and *dashed line* (rainy season).

at midday (Fig. 2) with a positive correlation between P_{Nmax} and g_{smax} (r^2 = 0.57, $p \leq 0.01$). Furthermore, irrespective

Table 1. Light-saturated net photosynthetic rate (P_{Nmax}), stomatal conductance at light saturation (g_{smax}), fraction of sky visible (FSV), chlorophyll ($a+b$) concentration, and SPAD values in saplings of ten Amazonian tree species. Values are means ± SE (n = 30). Means followed by *same letter* within a row do not differ significantly ($p>0.05$) by *t*-test.

Parameter	January	August
P_{Nmax} [μmol m^{-2} s^{-1}]	3.86 ± 0.21[a]	3.22 ± 0.24[a]
g_{smax} [mol m^{-2} s^{-1}]	0.096 ± 0.01[a]	0.105 ± 0.01[a]
FSV [unitless]	0.014 ± 0.001[a]	0.017 ± 0.001[a]
Chlorophyll $a+b$ [μmol m^{-2}]	431.1 ± 20.1[a]	431.7 ±19.94[a]
SPAD values [unitless]	50.7 ± 5.2[a]	48.14 ± 7.97[a]

of [CO_2] in the leaf chamber (50–380 ppm), early in the morning and at dusk, stomata were insensitive to light, even after illuminating the leaf at 250–500 μmol(photon) m^{-2} s^{-1} for 60 min (data not shown).

As PAR$_{inst}$ and air temperature at the forest understory increased, P_{Nmax} and g_{smax} also increased (Fig. 3). However, contrary to expectations, diurnal variation in [CO_2]$_{air}$ or VPD$_{und}$ did not affect photosynthesis or stomatal conductance (Fig. 4). Although stomata were responsive to variation in PAR$_{inst}$, they did not respond to variations in PAR$_{und}$ values across plant microsites in the experiment area (data not shown). On the other hand, once stomata were prone to open (*e.g.*, midday) the typical stomatal response to light and [CO_2] was observed (Nascimento and Marenco 2013).

Discussion

Seasonal and diurnal variation in g_{smax} and P_{Nmax}: The lack of effect of rainfall seasonality on P_{Nmax} and g_{smax} can be attributed to the rather high soil water content recorded in the dry season. The occasional rainfall events that occurred in this season saved the saplings from experiencing water stress. In the dry season, the soil water content (v/v) corresponded to about 50% of field capacity (*i.e.*, 60% at field capacity *vs.* 48% in the dry season, assuming soil water content of 35% at the permanent wilting point; Ferreira *et al.* 2002) which suggested that soil water was readily available to plants during the dry period. However, it does not necessarily mean than taller trees respond in the same way. Canopy trees are exposed to higher temperatures and lower RH, which may ultimately lead to stomatal closure, particularly after midday when VPD is higher (Johnson *et al.* 2012, Manzoni *et al.* 2013). Contrary to observations made in other part of the Amazon region with a pronounced dry season (Miranda *et al.* 2005), in central Amazonia, the predawn leaf water potential tends to remain high (−0.26 MPa) even during the dry season (Magalhães *et al.* 2014).

It is assumed that stomata quickly respond to variation in light intensity (Shimazaki *et al.* 2007), even to sunflecks

of short duration (Pearcy 1990). Thus, the absence of stomatal response to light either early in the morning or at dusk (Fig. 2) was rather unexpected. Often high epidermal backpressure restrains stomatal opening (Grantz 1990, Buckley 2005). As stomata failed to open under the rather warm and dry conditions prevailing in the leaf chamber early in the morning and at late-afternoon, we concluded that hydropassive mechanisms were not the main factors inducing stomatal closure at dawn or dusk. Doughty *et al*. (2006) and Mendes and Marenco (2010) also observed higher photosynthetic rates at midday.

As the leaves showed similar Chl contents and similar SLA, nitrogen and phosphorus values, diurnal variation in g_{smax} and P_{Nmax} did not seem to be related to the intrinsic leaf characteristics, but somehow to environmental factors. Besides the effect of light, variation in P_N and g_s can be also associated with changes in humidity (Aliniaeifard and van Meeteren 2013) and temperature (Neilson and Jarvis 1975, Peak and Mott 2010). Stomatal opening depends on the activity of a light-modulated proton pump and occurs in response to an osmotic increase in turgor pressure of guard cells (Shimazaki *et al.* 2007). The activity of the proton pump and the conductance of K$^+$ channels is
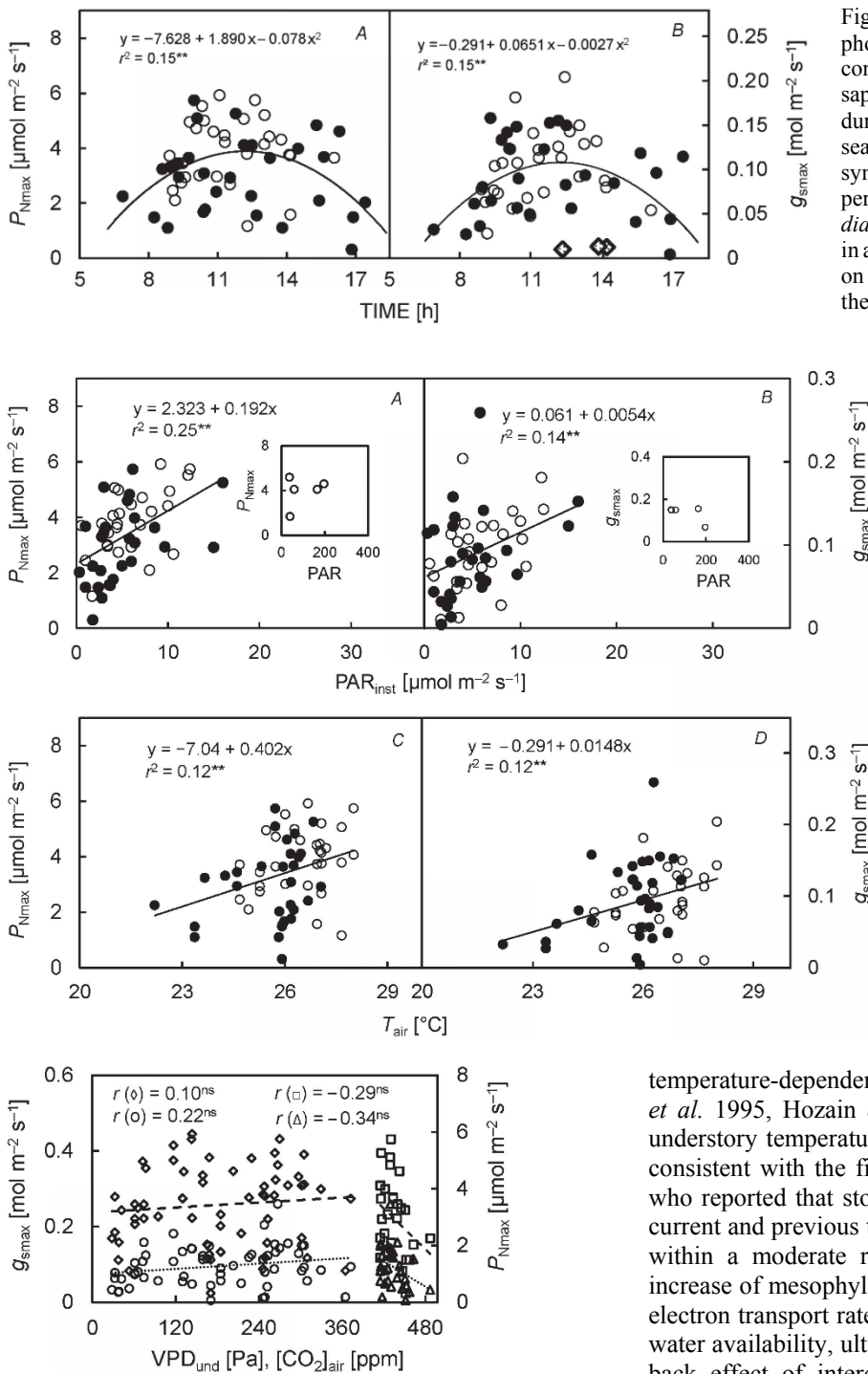
Fig. 2. Diurnal course of light-saturated net photosynthetic rate (P_{Nmax}) (*A*) and stomatal conductance at light saturation (g_{smax}) (*B*) in saplings of ten Amazonian tree species, during the rainy (*open circle*, ○) and dry season (*closed circle*, ●) of 2008. Each symbol represents the mean of two leaves per plant. [**] – significant at $p \leq 0.01$. The *diamonds* (panel *B*) indicate leaves located in a dimly lit environment (<4 μmol m^{-2} s^{-1}), on which stomata did not respond to light in the illuminated leaf chamber.

Fig. 3. Light-saturated net photosynthetic rate (P_{Nmax}) (*A*) and stomatal conductance at light saturation (g_{smax}) (*B*) in response to variation in instantaneous PAR recorded during gas-exchange measurements (PAR_{inst}); and P_{Nmax} and g_{smax} in response to variation in air temperature (T_{air},) at the forest understory (*C,D*) in saplings of ten Amazonian tree species, during the rainy (*open circle*, ○) and dry season (*closed circle*, ●) of 2008. The insets (*A,B*) represent sunflecks that occurred during gas-exchange measurements. Each symbol represents the mean of two leaves per plant. [**] – significant at $p \leq 0.01$.

Fig. 4. Stomatal conductance at light saturation (g_{smax}, *open circle*, ○) and light-saturated net photosynthetic rate (P_{Nmax} *open diamond*, ◊) in response to variation in understory air vapor pressure deficit (VPD_{und}), and g_{smax} (*open triangle*, Δ) and P_{Nmax} (*open square*, □) in response to variation air CO_2 concentration ($[CO_2]_{air}$) at the forest understory in saplings of ten Amazonian tree species. VPD_{und} data were collected during dry and rainy of 2008, whereas $[CO_2]_{air}$ data were collected only in the dry season of 2008. For gas-exchange data, each symbol represents the mean of two leaves per plant; ns – not significant (p>0.05).

temperature-dependent (Racker and Hinkle 1974, Ilan *et al.* 1995, Hozain *et al.* 2010). The positive effect of understory temperature on stomatal aperture we found is consistent with the finding of Neilson and Jarvis (1975) who reported that stomatal opening depends on both the current and previous temperature. Increase in temperature within a moderate range (23–28°C) often leads to an increase of mesophyll conductance, Rubisco activity, and electron transport rate (Warren 2008), which, under good water availability, ultimately increases g_{smax}, *via* the feedback effect of intercellular CO_2 concentration (C_i) on stomatal opening.

Stomata failed to open at dusk and early in the morning in well-illuminated leaves. However, in several experiments conducted in central Amazonia, saplings rapidly respond to light and open as the forest understory becomes warmer and brighter (*e.g.*, Nascimento and Marenco 2013, Magalhães *et al.* 2014). This indicates that atmospheric conditions play a key role in regulating stomatal conductance. Indeed, light and temperature (and even R:FR, Casal 2013) might regulate stomatal functioning by entraining a

circadian rhythm (Heintzen *et al.* 1994, Webb 2003, Mas and Yanovsky 2009). If stomatal movement is under circadian regulation, it does not necessarily mean that stomatal aperture accurately tracks solar time (*i.e.*, open in the morning and close in the afternoon) because the phases of the circadian oscillator are daily reset by external signals (*e.g.*, light, temperature, R:FR) to keep synchrony with the environment (Kojima *et al.* 2011, Casal 2013). Therefore, changes in environmental conditions (*e.g.*, low light, cooler temperature, low R:FR) may delay the end of the subjective night, and hence stomata may remain closed long after sunrise. This might explain why some stomata (indicated by *diamonds* in Fig. 2*B*) failed to open at midday (it might also explain the low r^2 values in Fig. 2), when understory irradiance was very low. It demonstrates that the light environment before gas-exchange measurements plays a key role in guard cells response to illumination. This suggests that the carbon gain during sunflecks depends not only on intensity and duration of sunflecks (Pearcy 1990), but also on the atmospheric conditions preceding the sunfleck event. It is plausible to conclude that in comparison with stomata of sun leaves, under deep shade conditions, stomata delay their response to direct light, even at midday.

Why do stomata close at the forest understory? It is widely accepted that stomata close to avoid transpiration, and thereby to reduce water stress (Jones 1998). If water economy is the main reason for the stomata to close, they should remain permanently open under the high humidity (low VPD, Aliniaeifard and van Meeteren 2013) and well-watered conditions of the Amazonian forest understory (Magalhães *et al.* 2014), but they do not. Enhanced nutrient availability *via* a continuous xylem flux (Caird *et al.* 2007) and improved transport of oxygen for cell respiration of sapwood (Daley and Phillips 2006) have been associated with night time stomatal opening. Although some species show partial stomatal aperture at night (Caird *et al.* 2007), Amazonian trees do not seem to share this feature (Doughty *et al.* 2006). Indeed, there is no obvious reason (*i.e.*, neither low air humidity nor low soil moisture) for the stomata to close at night. Based on these observations we could hypothesize that stomata close under low light and high humidity to block the entry of pathogens. Although the majority of fungi use appressoria to penetrate directly the leaf epidermis, some of them (*e.g.*, *Puccinia triticina*, *Puccinia graminis* var. *tritici*, *Uromyces viciae-fabae)* predominantly or exclusively penetrate the leaf through the stomatal pore (*e.g.*, Mendgen *et al.* 1996, Song *et al.* 2011). On the other hand, foliar bacteria depend on natural openings, such as stomata, hydathodes or lenticels, to enter into the leaf. For example, numerous plant pathogenic bacteria penetrate the leaf *via* stomata, including *Pseudomonas syringae* pv. *tomato*, *Pseudomonas syringae* pv. *lachrymans*, *P. syringae* pv. *mors-prunorum*, *P. cichorii*, *P. syringae* pv. *syringae*,

Xanthomonas campestris pv. *pruni*, and *P. syringae* pv. *avenae* (Huang 1986, Kumar *et al.* 2012).

It has been found that stomata may limit bacterial invasion by remaining closed when environmental conditions favor bacterial dissemination and penetration (Melotto *et al.* 2008). In *Arabidopsis*, defense genes that anticipate pathogen infection at dawn are under the circadian control (Wang *et al.* 2011). Whether a circadian clock plays a role in avoiding pathogen attacks under the humid and dimly lit conditions of the rainforest understory, it is an issue that remains to be elucidated. It has been found that far-red radiation induces stomatal closing (Roth-Bejerano and Itai 1981, Talbott *et al.* 2002), perhaps because abscisic acid content increases under low R:FR (Casal 2013). Hence the effect of a low R:FR value on g_s may help to explain why some leaves located in deep shade environments failed to open at midday (*diamonds* in Fig. 2*B*; PAR <4 μmol m^{-2} s^{-1}; R:FR value <0.25; R:FR = (lnPAR − 0.278)/4.39, Capers and Chazdon 2004).

Stomatal response to air vapor pressure and air CO$_2$ concentration: Even when stomata were responsive to variation in PAR$_{inst}$, neither g_{smax} nor P_{Nmax} responded to variations in PAR$_{und}$, [CO$_2$]$_{air}$ or VPD$_{und}$. Contrary to expectations, early in the morning and late in the afternoon, the low VPD$_{und}$ failed to stimulate stomatal opening (Aliniaeifard and van Meeteren 2013). This indicated that stomata were more sensitive to subtle changes in the before-measurement intensity light that impinged on the leaf surface than to overall variations in background brightness, air humidity or CO$_2$ concentration in forest understory. The nil effect of L_T on g_{smax} or P_{Nmax}, indicated that the path length for CO$_2$ diffusion was little affected by leaf succulence. No effect of rainfall seasonality on stomatal functioning negated our initial hypothesis and allowed us to conclude that variations in g_{smax} or P_{Nmax} observed during the study period depended on atmospheric variables (*e.g.*, light and temperature) rather than on changes in leaf water potential, which often varied little over seasons, from −0.26 MPa (dry period) to −0.13 MPa in the rainy season (Magalhães *et al.* 2014). Absence of correlation between P_{Nmax} or g_{smax} and the ambient [CO$_2$] confirms that the leaf epidermis is almost impermeable to ambient CO$_2$ (Boyer *et al.* 1997).

We concluded that diurnal variations in the physical environment, particularly, in light and temperature, led to diurnal changes in photosynthesis and stomatal conductance. As soil moisture and humidity remained high during the whole day at the forest understory, there was no obvious reason for the stomata to close at the forest understory (*e.g.*, at dusk and dawn). More investigation is needed to elucidate the role of environmental factors, particularly PAR, light quality (R:FR), RH, and temperature in modulating stomatal movements in juvenile trees growing beneath the dense canopy of tropical rainforests.

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BOOK REVIEW

Allakhverdiev S. I., Rubin A. B., Shuvalov V. A. (ed.): **Contemporary Problems of Photosynthesis** (Современные проблемы фотосинтеза). Vol. I, 554 pp. and Vol. 2, 535 pp. – Institute of Computer Science, Moscow-Izhevsk 2014, ISBN: 978-5-4344-0181-4. RUB 960 (Vol. I), RUB 940 (Vol. II).

This two-volume book, which belongs to the Series "Interdisciplinary Questions of Biology, Mathematics, Physics, Chemistry and Medicine" (Междисципли-нарные вопросы биологии, математики, физики, химии и медицины), is a compendium of 31 chapters, contributed by various authors noticeable in the area of photosynthesis research. Its editors are internationally acknowledged experts in the area of photosynthesis. The unusual feature of the book is that it is published in two languages. Twenty-two of its thirty-one chapters are in Russian and the remaining nine in English. However, each Russian chapter has an English abstract and each English chapter has a Russian abstract. The book targets primarily, but not exclusively, a Russian readership. In a way, we may view it as a tribute to the great tradition of Russian science in the areas of photobiophysics and photobiology, and their natural extension to the science of photosynthesis, as personified by a constellation of outstanding 20th century scientists in these fields. One may risk to single out the top Russian scientists S.I. Vavilov, A.N. Terenin, A.A. Krassnovsky, and V. Evstigneev, and the top Russian-American scientist E.I. Rabinowitch, whose scientific progenies are among the contributors to these two volumes.

The structure of the photosynthetic apparatus is known today to nearly atomic detail. In conjunction with the emergence of powerful new methodologies, this allows for the design of more incisive experiments and the extraction of far more detailed information from the experimental results. These capabilities are clearly evident in the chapters of this book, which address a number of front-line research topics in photosynthesis. It begins with a general overview chapter on chlorophyll *a* fluorescence *in vivo*, dedicated to D.E. Walker, a pioneer in photosynthesis research. This is followed by reviews on the potential of optical spectroscopy (femtosecond-resolved, as well as resonance Raman) for probing structure relaxation dynamics in pigment-protein complexes, as a result of photosynthetic electron transfer; the potential of non-optical spectroscopy, such as *neutron scattering* for probing biomolecular and membrane dynamics, and time-resolved *electron paramagnetic resonance* (EPR) for tracking free radicals and triplet states of excited pigments. Several chapters address physiological and ecological aspects, including regulated responses of photosynthetic organisms to environmental stresses, such as high light, high salt, and elevated CO_2 concentrations, as well as to evolutionary topics pertaining to the emergence of chlorophylls, of reaction centers, and of the oxygen-evolving complex. Finally, there are chapters that offer mathematical modeling of photoinduced electron transport, of coupled proton transport, and of ATP synthesis; and a chapter on synthesized biomimetic oxo-manganese molecules for *in vitro* photo-oxidation of water.

Photosynthesis is a fast advancing scientific field, as it may be judged from the number of scientific papers that appear each month in the scientific literature. This well-organized book succeeds in providing a timely and authoritative snapshot of several "burning" questions in this area. Its educational value is unquestionable. To both emerging, as well as established, Russian investigators, it provides a reference framework for the rapidly advancing front of photosynthesis research. Important contributing factors to that are the extensive reference lists at the end of each chapter. The international reader, however, would have benefitted more if instead of the short abstracts, one to two page long summaries, in English, would have accompanied each Russian chapter.

I do highly recommend the Editors to produce another book, fully in English, so that it can reach the international community as well.

In spite of these reservations, I do recommend this book to all photosynthesis-inclined physical scientists and thinkers.

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