DOI: 10.1007/s11099-011-0077-0 PHOTOSYNTHETICA 49 (4): 619-626, 2011

# Chloroplastidic pigments, gas exchange, and carbohydrates changes during *Carapa guianensis* leaflet expansion

F.K.C. MORAES, G.L.S. CASTRO, D.D. SILVA JÚNIOR, H.A. PINHEIRO+, and R.A. FESTUCCI-BUSELLI

*Centro de Tecnologia Agropecuária, Instituto Sócioambiental e dos Recursos Hídricos, Universidade Federal Rural da Amazônia. Tancredo Neves Av. 2501, Belém, PA 66077-530, Brazil*

## Abstract

Changes in chloroplastidic pigments, gas exchange and carbohydrate concentrations were assessed during the rapid initial expansion of *C. guianensis* leaflet. Leaves at metaphyll stage were tagged and assessments were carried out 14, 17, 20, 23, 27, and 31 days later. Pigments synthesis, distribution and accumulation were uniform among leaflet sections (basal, median and apical). Chlorophyll (Chl) *a*, Chl *b*, Chl (*a*+*b*), and total carotenoids (Car) concentrations were significantly increased after 27 days from metaphyll, and the most expressive increases were parallel to lower specific leaflet area. Chl *a/b* was lower on day 14 and it was increased on subsequent days. Negative net photosynthesis rate ($P_N$), and the lowest stomatal conductance ($g_s$) and transpiration ($E$) were registered on day 14, following significant increases on subsequent days. The Chl (*a*+*b*) and Chl *a* effects on $P_N$ were more expressive until day 20. Intercellular to ambient $CO_2$ concentration ratio ($C_i/C_a$) was higher on day 14 and lower on subsequent days, and no stomatal limitation to $CO_2$ influx inside leaflets was observed. Leaflet temperature was almost constant (*ca.* 35°C) during leaflet development. Sucrose and starch concentrations were increased in parallel to increases in $P_N$. Altogether, these results highlight the main physiological changes during *C. guianensis* leaflet expansion and they should be considered in future experiments focusing on factors affecting $P_N$ in this species.

*Additional key words:* carbohydrates; chlorophyll; leaf age; leaf expansion; leaf gas exchange.

## Introduction

During leaf expansion, chloroplastidic pigments are synthesized and their concentrations are increased from immature (young) to mature leaves, as previously showed in *Hevea brasiliensis* (Miguel *et al.* 2007), *Rhamnus alaternus* (Varone and Gratani 2009), and *Quercus ilex* (Gratani and Bonito 2009). The synthesis, distribution and accumulation patterns of chlorophylls and carotenoids vary according to both plant species (genetic factor) and environmental conditions. In the later, the incidence of photosynthetically active radiation during leaf expansion is closely associated to chloroplast and chloroplastidic pigment synthesis (Tardieu *et al.* 1999).

Among chloroplastidic pigments, Chl *a* is the most important pigment involved in capture, storing and energy transfer during the photochemical pathway of net photosynthesis (Nelson and Yocum 2006, Fiedor *et al.* 2008). Chl *b* shows an accessory function in photosynthesis and it is synthesized from Chl *a*. Several reactions are necessary to convert Chl *a* (methyl group) into Chl *b* (aldehyde group) (Tanaka and Tanaka 2006). Besides Chls *a* and *b*, Car are structurally related to photosystems I and II and they play an important role in thylakoid membrane stabilization and fluidity as well as in exceeded energy dissipation as heat (Havaux 1998, Nelson and Yocum 2006). Regarding the important structural and functional roles of chloroplastidic pigments on photosynthetic pathway, one could expect that increases in chloroplastidic pigment concentrations (Chl *a*, Chl *b*, and Car) may contribute, in some extent, to increasing $P_N$ in expanding leaves.

*Received* 15 February 2011, *accepted* 9 August 2011.
+Corresponding author; tel.: + 55 91 3210 5271, e-mail: hugo.pinheiro@ufra.edu.br
*Abbreviations:* Car – carotenoids; Chl – chlorophyll; $C_i/C_a$ – intercellular to ambient $CO_2$ concentration ratio; DM – dry mass; $E$ – transpiration rate; FM – fresh mass; $g_s$ – stomatal conductance to water vapor; PAR – photosynthetically active radiation; $P_N$ – net photosynthetic rate; SLA – specific leaflet area; Suc – sucrose concentration; $T_{leaf}$ – leaflet temperature.
*Acknowledgement:* Scholarships were granted by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior/CAPES (F.K.C. Moraes) and Conselho Nacional de Desenvolvimento Científico e Tecnológico/CNPq (D.D. Silva Júnior, G.L.S. Castro).

In *Q. ilex*, the $P_N$ to Chl relationship during leaf expansion was linear and maximum $P_N$ was coincident to maximum total Chl content at 56 days after bud break (Gratani and Bonito 2009). Similar $P_N$ increases in response to changes in Chl content were observed in *R. alaternus* expanding leaves (Varone and Gratani 2009). Besides Chl, changes in $P_N$ during leaf expansion are also dependent on minimum stomatal aperture that allows an adequate $CO_2$ influx inside leaves. Simultaneous increases in both $P_N$ and $g_s$ were registered in expanding leaves of *R. alaternus* (Varone and Gratani 2009) and *Q. ilex* (Gratani and Bonito 2009), and in both species the maximum $P_N$ was coincident to maximum $g_s$. The intercellular $CO_2$ concentration effects in $P_N$ may be associated either to increases in mesophyll $CO_2$ conductivity or in ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylative activity in expanding leaves of *Prunus persica* and *Olea europaea* (Marchi *et al.* 2008). On the other hand, $g_s$ increases during leaf expansion may be a strategy for controlling leaf temperature through transpiration, allowing metabolic processes to proceed at adequate rates. In *Q. ilex*, the maximum $E$ was coincident to maximum $g_s$ and the relationship between $g_s$ and $E$ was linear during leaf development (Gratani and Bonito 2009).

In *Carapa guianensis* Aubl., a Meliaceae species found in tropical regions (*e.g.* Amazon forest), the leaflets in expansion show leaf color changes which may vary from brown (immature) to light green (in maturation) to dark green (mature). Empiric observations indicate that color transitions are fast, happening within 30 days after the metaphyll phase. These color transitions, in special from light to dark green, may be associated to increased chloroplastidic pigments (Chl *a* and Chl *b*) synthesis, distribution and accumulation. Regarding the necessity of structured and functional photosynthetic apparatus to efficiently photosynthesize, it is possible that Chl concentrations, $g_s$, and $C_i/C_a$ changes during leaflet expansion influence $P_N$ in some extent. Consequently, the carbohydrates (sucrose and starch) biosynthesis, distribution, and accumulation may also vary significantly during leaflet expansion. Therefore, this work aimed to evaluate the magnitude of changes in chloroplastidic pigment synthesis, distribution, and accumulation, leaflet gas exchange, and carbohydrate (sucrose and starch) concentration during the fast initial (31 days) expansion of *C. guianensis* leaflets. These results should be considered in future experiments focusing intrinsic and extrinsic factors influencing net photosynthesis in this species.

## Materials and methods

**Plant material and growth conditions**: Andiroba (*Carapa guianensis* Aubl.) seeds were obtained from adult trees at the campus of Federal Rural University of Amazon, Belém-PA, north Brazil (01º28'03"S, 48º29'18"W). After imbibition in distilled water for 24 h at room temperature, seeds were planted in polyethylene bags (15 × 27 cm, diameter × height) for germination. Considering both leaf number and stem height, uniform seedlings were transferred to 20-L polyethylene pots (31 × 35 cm, diameter × height) filled with 16 kg of sifted yellow loam latossol. One seedling per pot was used for experimental setup. Acidity and nitrogen of the substrate were respectively adjusted by adding 2.7 g dolomite calcareous and 1.65 g urea per pot. Throughout experimental period, plants were grown under greenhouse conditions under averages of diurnal photosynthetically active radiation (PAR) of 880 μmol(photon) $m^{-2}$ $s^{-1}$, air temperature of 33°C and relative air humidity of 73%, characterizing a typical tropical day. Irrigation was performed daily to maintain the soil near to field capacity and weeds were manually controlled.

When plants were eight month old, leaves at metaphyll stage (Fig. 1) containing at least six leaflets were tagged for analysis. The changes in chloroplastidic pigments, gas exchange and carbohydrates were started two weeks later, when leaflets showed minimum leaflet area for analyses. Therefore, physiological and biochemical assessments were exactly performed at 14, 17, 20, 23, 27, and 31 days after metaphyll identification. Two leaflets (the second leaf from the stem apices) per plant were selected to perform gas-exchange measurements and sampling for biochemical analyses. After analyses, an average of the results was considered an individual replicate and a total of six plants (replicates) were assayed.

**Specific leaflet area (SLA)**: This variable was used as an indicative of leaflet expansion (Miyazawa *et al.* 1998). Leaflet fragments were collected using a blade and their

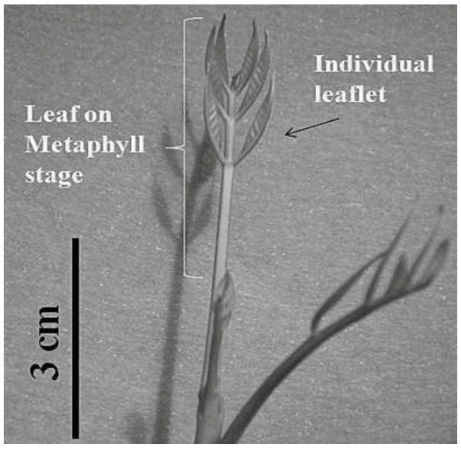


Fig. 1. Morphological aspect of metaphyll leaf stage containing three leaflet-pairs. Image is representative of six replicates and it was taken from stem apices.

total areas (TA) were determined using a portable leaf area meter *AM300* (*ADC BioScientific Ltd.,* Hoddesdon, UK). Each leaflet fragment was individually packaged in a paper bag and oven-dried at 72°C until constant mass, following the determination of its dry mass (DM). SLA was calculated as SLA = TA/DM.

**Leaflet pigments**: To verify if chloroplastidic pigment synthesis, distribution and accumulation happens uniformly through leaflet, sampled leaflets were sectioned in basal (BS), median (MS), and apical (AS) sections. From each section, corresponding to 1/3 leaflet length, the main vein was discarded and tissue fragments (0.1 g) were immediately frozen at –20°C until assays. Chl *a*, Chl *b*, Chl (*a*+*b*), and Car were determined according to Lichthenthaler (1987) and as exactly modified by Costa *et al.* (2010). Pigment concentrations were expressed in g(pigment) $kg^{-1}$(fresh mass, FM). Chl *a* and Chl *b* data were used to calculate Chl *a/b* ratio as the quotient between Chl *a* and Chl *b* concentrations. The obtained data were also used to verify changes in their concentrations during the fast leaflet expansion.

**Leaflet gas exchange**: Net photosynthetic rate ($P_N$), stomatal conductance to water vapour ($g_s$), transpiration rate ($E$), and intercellular to ambient $CO_2$ concentration ratio ($C_i/C_a$) were measured between 08:00 and 10:00 h with a portable open-path infrared gas analyzer *LCpro* (*ADC BioScientific Ltd.,* Hoddesdon, UK). Measurements were carried out at ambient $CO_2$ concentration [*ca.* 362 μmol $mol^{-1}$ ($CO_2$)] and PAR was adjusted to 900 μmol (photon) $m^{-2}$ $s^{-1}$ simulating natural PAR occurring between 08:00 and 10:00 h in tropical regions. Leaflet temperature ($T_{leaf}$) during measurements was *ca.* 35°C. The effect of Chl *a* concentration on $P_N$ was also examined by the recalculation of the net $CO_2$ assimilation rate on Chl *a* basis [μmol($CO_2$) $mg^{-1}$(Chl *a*) $h^{-1}$] as reported by Lichtenthaler *et al.* (2007).

**Carbohydrates**: Leaflet samples were oven-dried until constant mass (at 72°C) and 0.1 g from the resultant grounded tissue was used for assays. Starch was extracted using 80% ethanol (v/v) and its content was determined according to McCready *et al.* (1950). Sucrose was extracted using MCW (methanol:chloroform:water, 12:5:3, v/v/v) and color reaction was performed according to van Handel (1968). The results were expressed in mg(carbohydrate) $g^{-1}$(dry mass, DM).

**Statistics**: Changes in pigments, leaflet gas exchange and carbohydrates during leaflet expansion were tested by *ANOVA* and averages were compared using *Newman-Keuls*' test ($p$<0.05). Statistical analysis was performed using *Systat 11* package (*Systat Software Inc.*, Chicago, USA).

## Results

**Specific leaflet area (SLA)** decreased over experimental time. The highest SLA [425.7 $cm^2$ $g^{-1}$(DM)] was registered 14 days after metaphyll leaf stage and the lowest SLA was registered on days 27 [169.1 $cm^2$ $g^{-1}$(DM)] and 31 [150.2 $cm^2$ $g^{-1}$(DM)] (Fig. 2). A tendency of SLA stabilization was observed on days 27 and 31.

**Chloroplastidic pigments**: Chl (*a*+*b*) and Chl *a* concentrations did not differ between leaflet sections (AS, MS, and BS) on the same experimental day (Fig. 3). Car was significantly lower in MS than in both AS and BS on day 14, while differences between leaflet sections were not observed on subsequent days (Fig. 3). These results may evidence uniform synthesis and distribution of chlorophylls and carotenoids through leaflet lamina. Thus, variations of pigment concentrations during leaflet expansion may be adequately quantified by the mean of pigment concentrations of all assessed sections (Fig. 4), or alternatively by pigment concentration on an individual leaflet section.

Until day 23 after metaphyll stage, leaflet concentrations of Chl (*a*+*b*), Chl *a* and Car did not differ significantly ($p$<0.05), and their averages were respectively 0.67, 0.45, and 0.15 [g(pigment) $kg^{-1}$(FM)] (Fig. 4). Chl (*a*+*b*) increased to 1.14 [g(pigment) $kg^{-1}$(FM)] and 1.59 [g(pigment) $kg^{-1}$(FM)] respectively on days 27 and 31. Chl *a* increased to 0.85 [g(Chl *a*) $kg^{-1}$(FM)] on day 27 and 1.15 [g(Chl *a*) $kg^{-1}$(FM)] on day 31. Chl *a/b* ratio was lower on day 14 and higher on days 27 and 31 (Fig. 4). During leaflet expansion, increasing Car concentrations were also observed, with the highest mean value [0.44 g(Car) $kg^{-1}$(FM)] registered on day 31 (Fig. 4).

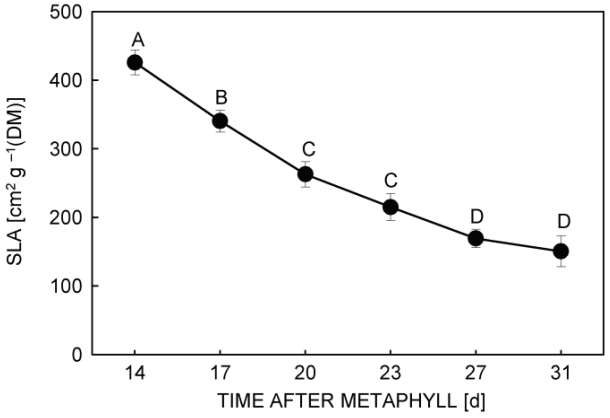


Fig. 2. Changes in specific leaflet area (SLA) during rapid *C. guianensis* leaflet expansion after metaphyll leaf stage. Data are the mean of six replicates (± SD). Mean values with the same capital letters are not significantly different (*Newman Keuls*' test, $p$≥0.05).

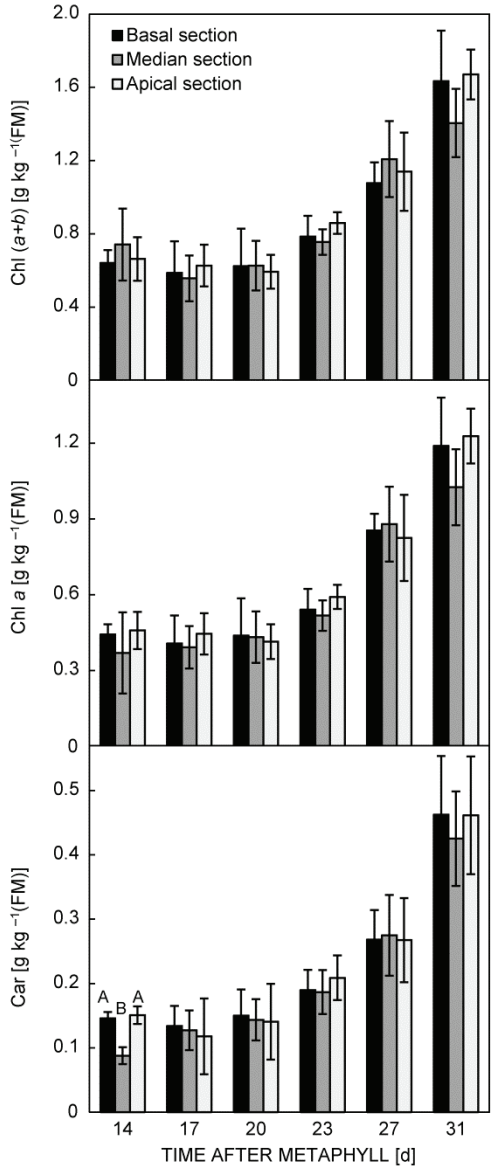


Fig. 3. Distribution of chlorophyll (Chl) (*a*+*b*), Chl *a* and total carotenoids (Car) through leaflet sections during rapid *C. guianensis* leaflet expansion after metaphyll leaf stage. Data are the mean of six replicates (± SD). Mean comparisons were performed into the same experimental day. Mean values without letters or with the same capital letters are not significantly different (*Newman Keuls*' test, $p \geq 0.05$).

Although pigment synthesis stabilization was unreached over the experimental period, the great changes in leaflet coloring, in special from light to dark green (Fig. 5), happen in higher Chl concentrations.

**Leaflet gas exchange**: Significant differences in leaflet gas exchange were observed during leaflet expansion. Negative $P_N$ –2.23 [μmol($CO_2$) $m^{-2}$ $s^{-1}$] was just registered 14 days after metaphyll stage. $P_N$ varied from 0.54 [μmol($CO_2$) $m^{-2}$ $s^{-1}$] on day 17 to 6.78 [μmol($CO_2$) $m^{-2}$ $s^{-1}$] on day 31 (Fig. 6). Similar $P_N$ changes were

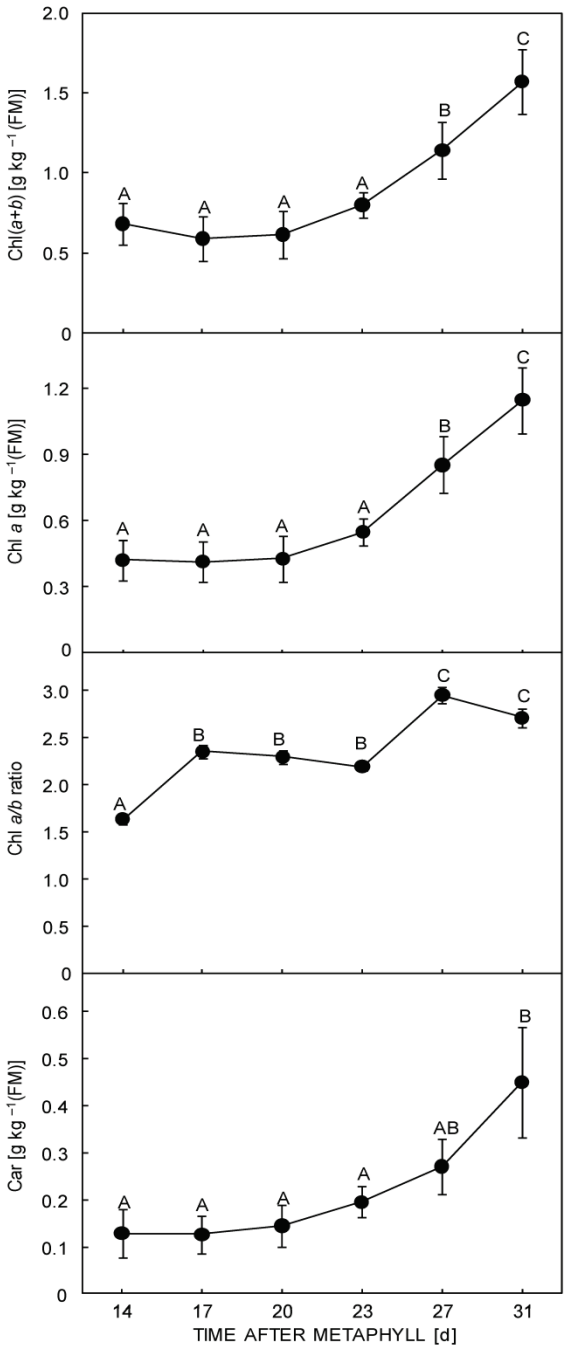


Fig. 4. Changes in chlorophyll (Chl) (*a*+*b*), Chl *a*, Chl *a*/*b*, and total carotenoids (Car) during rapid *C. guianensis* leaflet expansion after metaphyll leaf stage. Data are the mean of six replicates (± SD). Mean values with the same capital letters are not significantly different (*Newman Keuls*' test, $p \geq 0.05$).

observed when its values were expressed in [μmol($CO_2$) $g^{-1}$(DM) $s^{-1}$]; however, $P_N$ ranged from –0.047 [μmol ($CO_2$) $g^{-1}$(DM) $s^{-1}$] on day 14 to 0.14 [μmol($CO_2$) $g^{-1}$(DM) $s^{-1}$] on day 31 (data not shown). On the other hand, when $P_N$ values were expressed in [μmol($CO_2$) $mg^{-1}$(Chl *a*) $h^{-1}$], it was shown that Chl *a* significantly influenced $P_N$ until day 20, while no further increases in

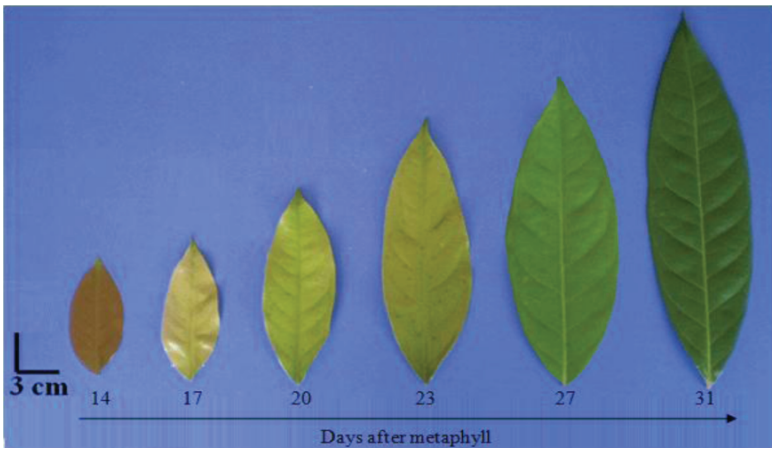


Fig. 5. Changes in leaflet lamina color during *C. guianensis* leaflet expansion after metaphyll leaf stage. Image is representative of six replicates.

$P_N$ were observed on subsequent days (Fig. 6). The lowest averages of $g_s$ [*ca.* 38.4 mmol($H_2O$) $m^{-2}$ $s^{-1}$] and $E$ [*ca.* 0.86 mmol($H_2O$) $m^{-2}$ $s^{-1}$] were registered on days 14 and 17 (Fig. 6). Higher averages of $g_s$ were registered on days 23, 27, and 31 [*ca.* 135 mmol($H_2O$) $m^{-2}$ $s^{-1}$] and higher $E$ [*ca.* 2.97 mmol($H_2O$) $m^{-2}$ $s^{-1}$] was registered on day 31 (Fig 6). The $C_i/C_a$ was 1.19 [mol($CO_2$) $mol^{-1}$($CO_2$)] on day 14, while it remained nearly constant [*ca.* 0.80 mol($CO_2$) $mol^{-1}$($CO_2$)] on subsequent experimental days (Fig 6). $T_{leaf}$ did not differ significantly during leaflet expansion, and its average was *ca.* 35°C (Fig 6).

**Sucrose and starch**: The lowest sucrose concentrations [*ca.* 14.5 mg(sucrose) $g^{-1}$(DM)] were found on days 14 and 17 and the highest ones on days 27 and 31 [*ca.* 60.2 mg(sucrose) $g^{-1}$(DM)]. Intermediate sucrose concentrations [*ca.* 39.5 mg(sucrose) $g^{-1}$(DM)] were found on days 20 and 23 after metaphyll (Fig. 7). Starch accumulation was clearly evident regardless of experimental day (Fig. 7). Its averages were significantly lower until 20 days after metaphyll [*ca.* 35.4 mg(starch) $g^{-1}$(DM)], reaching its maximum concentration [58 mg(starch) $g^{-1}$(DM)] on day 31 (Fig. 7).

## Discussion

During the *C. guianensis* leaflet evaluation time of 31 days, SLA decreased with leaflet expansion and values tended to stabilization in the last experimental days (27 and 31) (Fig. 2). This indicates a greater accumulation of DM per area unit which might suggest a thicker leaflet and consequently a greater mesophyll cell number (Miyazawa *et al.* 1998). Even though SLA tended to stabilization after experimental day 27, it would be necessary to analyze leaflet expansion for a long period of time (> 31 days) to conclude about its full expansion. During the fast leaflet expansion (31 days), it was always observed remarked changes in chloroplastidic pigment concentrations, leaflet gas exchange and carbohydrates concentration.

Here, we examined if chloroplastidic pigment synthesis, distribution and accumulation changes through leaflet lamina sections. The unobserved changes in Chl *a*, Chl *b*, Chl (*a*+*b*) and Car concentrations between different sections (basal, median, and apical) of leaflet lamina over 31 days (Fig. 3) indicated that synthesis, distribution and accumulation of chloroplastidic pigments may happen uniformly through leaflet lamina, independently of evaluation time. These results were similar when pigments were expressed on area unit basis, because SLA did not vary between leaflet sections (data not shown). Regarding the physiological point of view, the chloroplastidic pigment uniform distribution in leaflet is an important feature for an efficient light energy capture to the photochemical reactions of net photosynthesis, increasing ATP and NADPH production which may be used in atmospheric $CO_2$ fixation (Nelson and Yocum 2006). Therefore, tissue samples from any leaflet section may be used in future experiments to estimate both leaflet chlorophylls and total carotenoids concentration in this species.

Since pigment concentrations did not differ significantly between leaflet sections, the effect of leaflet expansion on chloroplastidic pigments concentrations was examined by assessing the average of pigments between the sections for every experimental day. Although Chls *a* and *b* and Car have been detected in the leaflet during all evaluation data, it was observed that their concentrations were kept almost constant until day 23 (23 days after metaphyll identification), following significant average increases ($p$<0.05) on days 27 and 31 (Fig. 4). Therefore, Chl synthesis, distribution, and accumulation patterns in *C. guianensis* were not linear during the fast leaflet expansion, and pigment concentrations were higher in older expanded leaflets (days 27 and 31), matching with leaflet color transition from light to dark green (Fig. 5). This trend was similar to those previously observed in *Q. ilex* (Gratani and Bonito 2009) and *R. alaternus*

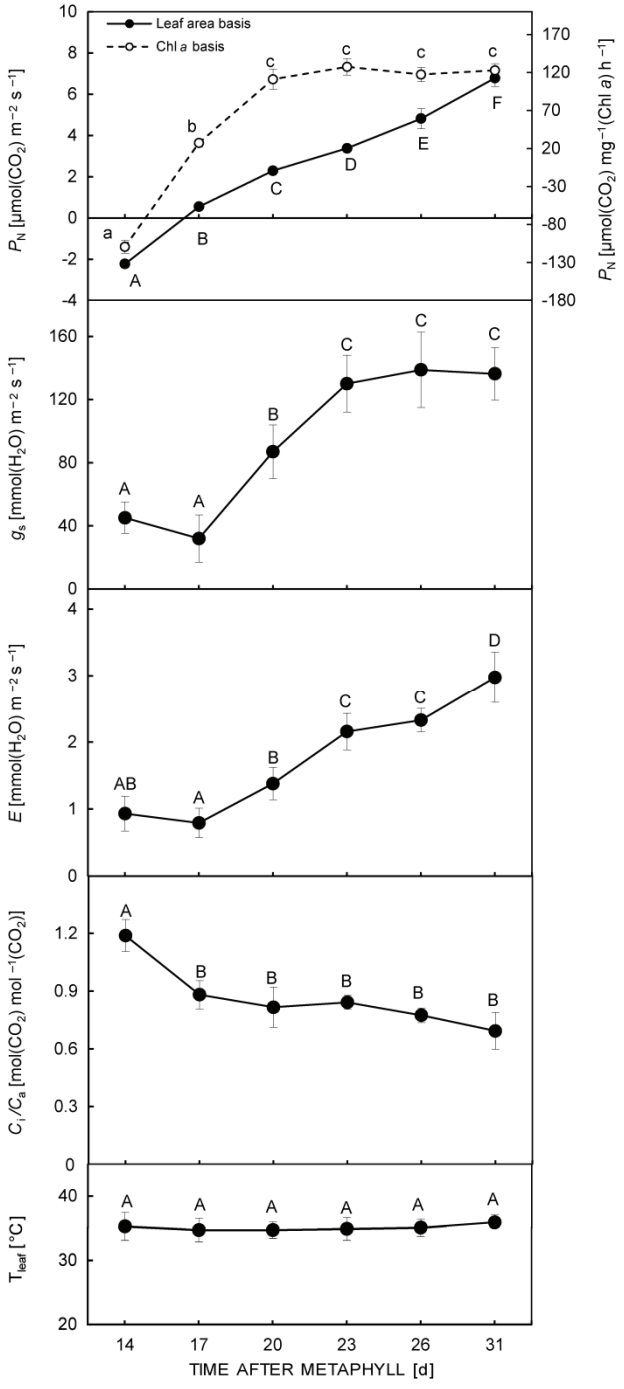


Fig. 6. Changes in net photosynthetic rate ($P_N$), stomatal conductance to water vapor ($g_s$), transpiration ($E$), and intercellular to ambient $CO_2$ ratio ($C_i/C_a$) during rapid *C. guianensis* leaflet expansion after metaphyll leaf stage. Data are the mean of six replicates (± SD). Mean values with the same capital or small letters are not significantly different (*Newman Keuls*' test, $p \geq 0.05$).

(Varone and Gratani 2009), even though these species show different branch morphology and habitat.

Considering that Chls synthesis, distribution and accumulation changed significantly during rapid leaflet development, changes in $P_N$ (and carbohydrates concentration) in response to increased Chls concentration may also occur in some extent. Indeed, $P_N$ varied from negative (day 14) to positive values (after day 16) (Fig. 6). The negative $P_N$ registered on day 14 and the low sucrose and starch contents (Fig. 7) indicated that young leaflet (until day 14 after metaphyll) behavior as strong sink, as previously reported for *H. brasiliensis* (Miguel *et al.* 2007). Although positive $P_N$ values have been recorded after day 16, the exactly moment of sink-source transition was undetermined. According to Turgeon (1989), the sink-source transition in dicotyledonous species may be reached when leaves are 30–60% expanded. If we consider that *C. guianensis* leaflet expansion was reached on day 31 with the SLA stabilization, we can estimate that *C. guianensis* leaflets may start acting as a source 23 days after metaphyll leaf stage, when $P_N$ was up to 3.0 [μmol(photon) m$^{-2}$ s$^{-1}$] and both sucrose and starch concentrations were significantly higher than those observed on days 14 and 20. However, for a precise inference about sink-source transition it would be necessary to quantify total foliar area, biomass (dry and wet), growing rate (absolute and relative) (Gratani and Bonito 2009), and determine the exact moment in which leaflets achieve positive net carbon balance (Marchi *et al.* 2005a,b).

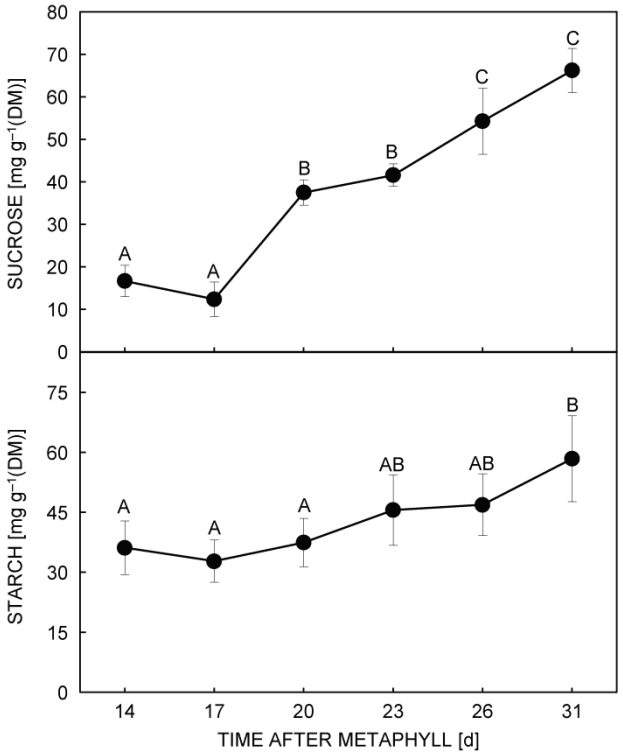


Fig. 7. Changes in sucrose and starch concentrations during rapid *C. guianensis* leaflet expansion after metaphyll leaf stage. Data are the mean of six replicates (± SD). Mean values with the same letters are not significantly different (*Newman Keuls*' test, $p \geq 0.05$).

As observed in several species, Chl *a* increases may directly affect $P_N$ during leaflet expansion (Miguel *et al.* 2007, Varone and Gratani 2009, Gratani and Bonito 2009). Here, the negative $P_N$ found on day 14 was unrelated to Chl (*a*+*b*) or Chl *a* concentrations because positive $P_N$ values between days 17 and 23 were registered under unchanged Chl concentrations (compared to day 14). Furthermore, the $P_N$ increases from day 17 to 23 were not followed by significant ($p$<0.05) increases in Chl (*a*+*b*) and Chl *a* concentrations. At least in part, this response may be explained by the differential Chl *a* and *b* synthesis during *C. guianensis* leaflet expansion, as evidenced by changes in Chl *a/b* ratio (Fig. 4). Therefore, the lower Chl *a/b* ratio on day 14 [1.64 unit(Chl *a*) unit$^{-1}$ (Chl *b*)] than in other experimental days is an indicative that photosystems may not be fully structured to maximize light capture and energy transfer during the earlier stages of leaflet expansion (until 14 days after metaphyll). On the other hand, when $P_N$ values were expressed on Chl *a* basis [μmol ($CO_2$) mg (Chl *a*)$^{-1}$ h$^{-1}$], the results clearly showed that Chl *a* concentration significantly influenced $P_N$ until day 20 (Fig. 6). Altogether, these results highlight the important role of Chl *a* as the main molecule involved in energy capture, storing and transfer between photosystems (Nelson and Yocum 2006, Fiedor *et al.* 2008) and indicates that photosystems structure and functionality is variable during *C. guianensis* leaflet expansion as previously reported for developing leaves of *H. brasiliensis* (Miguel *et al.* 2007).

Linear relationship between $P_N$ and $g_s$ was observed in *Q. ilex* (Gratani and Bonito 2009) and similar increase patterns in both $P_N$ and $g_s$ during leaf development were observed in *P. persica*, *O. europaea* (Marchi *et al.* 2008) and *R. alaternus* (Varone and Gratani 2009). In this work, the lowest $P_N$ and $g_s$ values were recorded on days 14 and 17 and the highest ones from day 23 (Fig. 6). The stomatal conductance is generally associated with variations in $P_N$ because it can limit (low $g_s$) or facilitate (high $g_s$) $CO_2$ influx inside leaves. Thus, $CO_2$ influx inside leaflets could be negatively affected by decreased $g_s$ on days 14 and 17. Nevertheless, the lower $g_s$ (day 14) was registered under higher $C_i/C_a$ (1.2 mol mol$^{-1}$), while subsequent increases in $g_s$ (after day 19) were coincident to slight decreases in $C_i/C_a$ (compared to day 14). Once $C_a$ was almost constant during the experimental period [*ca.* 362 μmol mol$^{-1}$($CO_2$)], the higher $C_i/C_a$ on day 14 was better associated to higher $C_i$ [*ca.* 428 μmol mol$^{-1}$($CO_2$)], while lower $C_i/C_a$ on day 31 was better associated to lower $C_i$ [*ca.* 240 μmol mol$^{-1}$($CO_2$)]. The $g_s$ changes during leaflet expansion did not limit $CO_2$ availability inside leaflets (Flexas and Morano 2002, Zhang *et al.* 2008), thus the lower $P_N$ on day 14 under the higher $C_i/C_a$ may be related to limited Rubisco amount and activity (Marchi *et al.* 2008).

Similar trend of increments in both $g_s$ and $E$ have been reported in expanding leaves of *H. brasiliensis* (Miguel *et al.* 2007) and *R. alaternus* (Varone and Gratani 2009). In *Q. ilex*, $E$ and $g_s$ relationship was linear (Gratani and Bonito 2009). Here, both $g_s$ and $E$ increased during leaflet expansion and their variation patterns were relatively similar. Once plants were grown under non-limiting soil water, the continued increases in $E$ during leaflet expansion seems to be an efficient strategy to regulate leaflet temperature, as clearly evidenced by the unchanged values of $T_{leaf}$ (*ca.* 34°C) (Fig. 6). Thus, changes in $E$ during *C. guianensis* leaflet expansion should be considered in future experiments that focus on estimation of whole-plant transpiration and water economy under either full-irrigation or water-limited conditions.

In conclusion, the results showed here underline that chloroplastidic pigment synthesis, distribution and accumulation through leaflet lamina was uniform. It also indicated that these pigments were not linearly synthesized during rapid (31 days) leaflet expansion and the differential Chl *a* and *b* synthesis assessed as Chl *a/b* ratio was an indicative that photosystems structure and functionality may be variable during *C. guianensis* leaflet expansion. As a consequence, the effects of Chl *a* on $P_N$ were more evident in the earlier stages of leaflet expansion (until day 20). Finally, the leaflet temperature was efficiently regulated by transpiration regardless of leaflet age. Altogether, the results are of crucial importance for future experiments aiming a better understanding about intrinsic and extrinsic factors affecting $P_N$ of *C. guianensis*.

## References


Costa, M.A., Pinheiro, H.A., Shimizu, E.S.C., Fonseca, F.T., Santos Filho, B.G., Moraes, F.K.C., Figueiredo, D.M.: Lipid peroxidation, chloroplastidic pigments and antioxidant strategies in *Carapa guianensis* (Aubl.) subjected to water-deficit and short term rewetting. – Trees **24**: 275-283, 2010.

Fiedor, L., Kania, A., Mysliwa-Kurdzel, B., Orzel, L., Stochel, G.: Understanding chlorophylls: Central magnesium ion and phytyl as structural determinants. – Biochim. Biophys. Acta **1777**: 1491-1500, 2008.

Flexas, J., Medrano, H.: Drought-inhibition of photosynthesis in $C_3$ plants: stomatal and non-stomatal limitations revisited. – Ann. Bot. **89**: 183-189, 2002.

Gratani, L., Bonito, A.: Leaf traits variation during leaf expansion in *Quercus ilex* L. – Photosynthetica **47**: 323-330, 2009.

Havaux, M.: Carotenoids as membrane stabilizers in chloeoplasts. – Trends Plant Sci. **3**: 147-151, 1998.

Lichthenthaler, H.K.: Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. – Method. Enzymol. **148**: 350-382, 1987.

Lichtenthaler, H.K., Ač, A., Marek, M.V., Kalina, J., Urban, O.: Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. – Plant Physiol. Biochem. **45**: 577-588, 2007.

Marchi, S., Sebastiani, L., Gucci, R., Tognetti, R.: Changes in sink-source relationships during shoot development in olive. – J. Amer. Soc. Hortic. Sci. **130**: 631-637, 2005a.

Marchi, S., Sebastiani, L., Gucci, R., Tognetti, R.: Sink-source transition in peach leaves during shoot development. – J. Amer. Soc. Hortic. Sci. **130**: 928-935, 2005b.

Marchi, S., Tognetti, R., Minnocci, A., Borghi, M., Sebastiani, L.: Variation in mesophyll anatomy and photosynthetic capacity during leaf development in a deciduous mesophyte fruit tree (*Prunus persica*) and an evergreen sclerophyllous Mediterranean shrub (*Olea europaea*). – Trees **22**: 559-571, 2008.

McCready, R.M., Guggloz, J., Silveira, V., Owens, H.S.: Determination of starch and amylose in vegetables: applications to peas. – Anal. Chem. **22**: 1156-1158, 1950.

Miguel, A.A., Oliveira, L.E.M., Cairo, P.A.R., Oliveira, D.M.: [Photosynthetic behavior during the leaf ontogeny of rubber tree clones [*Hevea brasiliensis* (Wild. *ex*. Adr. de Juss.) uell.Arg.] in Lavras MG.] – Cienc. Agrotec. **31**: 91-97, 2007. [In Portuguese.]

Miyazawa, S.-I., Satomi, S., Terashima, I.: Slow leaf development of evergreen broad-leaved tree species in Japanese warm temperate forests. – Ann. Bot. **82**: 859-869, 1998.

Nelson, N., Yocum, C.F.: Structure and Function of Photosystems I and II. – Annu. Rev. Plant Biol. **57**: 521-565, 2006.

Tanaka, A., Tanaka, R.: Chlorophyll metabolism. – Curr. Opin. Plant Biol. **9**: 248-255, 2006.

Tardieu, F., Granier, C., Muller, B.: Modelling leaf expansion in a fluctuating environment: are changes in specific leaf area a consequence of changes in expansion rate? – New Phytol. **143**: 33-43, 1999.

Turgeon, R.: The sink-source transition in leaves. – Annu. Rev. Plant Physiol. Plant Mol. Biol. **40**: 119-138, 1989.

van Handel, E.: Direct microdetermination of sucrose. – Anal. Biochem. **22**: 280-283, 1968.

Varone, L., Gratani, L.: Leaf expansion in *Rhamnus alaternus* L. by leaf morphological, anatomical and physiological analysis. – Trees **23**: 1255-1262, 2009.

Zhang, S.B., Hu, H., Li, Z.R.: Variation of photosynthetic capacity with leaf age in an alpine orchid, *Cypripedium flavum*. – Acta Physiol. Plant. **30**: 381-388, 2008.