RESEARCH ARTICLE

Moderate warm temperature improves shoot growth, affects carbohydrate status and stimulates photosynthesis of sweet orange plants

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ABSTRACT

Citrus plants were grown under two thermal conditions for evaluating carbon metabolism acclimation to moderate warm temperature (30/20ºC, day/night), and its likely impact on plant growth. As reference, plants were grown at 25/20ºC, in which they were subjected to optimum temperature for photosynthesis during the diurnal period (25ºC). Higher photosynthetic rates were found at 30/20ºC as compared to 25/20ºC in both mature and young leaves, being this response associated with higher stomatal conductance. After 30 days of thermal treatment, plants grown at 30/20ºC presented higher shoot growth as compared to those at 25/20ºC. The carbohydrate concentration decreased in stem and root tissues, while it increased in leaf tissues under moderate warm conditions. Both mature and young leaves showed higher photoassimilate consumption/exportation at 30/20ºC than at 25/20ºC. In this paper, we have proven that citrus plants present a positive balance in carbon metabolism as an acclimation mechanism to temperature changes, with plants presenting increased photosynthesis. Such photosynthetic acclimation was associated with improved vegetative growth, being both mature and young tissues sensitive to changes in thermal regimen.

Keywords: citrus, biomass, carbohydrate, leaf gas exchange, acclimation.

INTRODUCTION

Perennial plants are subjected to large changes in environmental conditions during their life cycle, which may occur either among seasons in a single year or among several years. Thus, photosynthetic characteristics have shown high phenotypic plasticity to temperature changes. Plants grown at high temperatures usually present higher optimal temperature and less photosynthetic inhibition than those at low ones (Hikosaka et al., 2006; Yamori et al., 2010). This behavior is related to changes in the activation energy (Hikosaka et al., 2006), in CO_2 concentration in the chloroplast (Sage and Kubien, 2007), and in the balance between the maximum rate of RuBP carboxylation ($V_{c,max}$) and the maximum electron transport rate for RuBP regeneration (J_{max}) (Onoda et al., 2005). As tree species might adjust their metabolism to seasonal changes in the surrounding environment, the physiological acclimation is essential for improving solar energy capture and then the carbon uptake throughout the plant cycle, especially in evergreen species, such as citrus trees.

Several reports have pointed out the important role of temperature as a meteorological element causing significant changes in citrus photosynthesis. Currently,

we know that low nocturnal temperatures inhibit photosynthesis by decreasing $V_{c,max}$ and J_{max} (Ribeiro et al., 2009a). The specific regulation imposed by low soil/substrate and low air temperatures during nighttime was further evaluated by Magalhães Filho et al. (2009) and Santos et al. (2011). These authors revealed that the low substrate temperature negatively affected $V_{c,max}$ and J_{max}, and that it is more limiting to citrus photosynthesis than the low air temperature. Considering high temperatures, Guo et al. (2006) found a significant reduction in citrus photosynthesis, with impairment of primary photochemistry under 38ºC. Citrus respiration is also affected by temperature, being stimulated in leaf temperatures higher than 35ºC (Ribeiro et al., 2006a). As exposed leaves of field-grown trees commonly face temperatures higher than 35ºC under subtropical conditions (Ribeiro et al., 2005), citrus plants have to deal with both low and high temperatures during their life span.

Although high and low temperature effects on citrus physiology have been reported, it is difficult to establish the importance of such physiological responses for plant growth, as most studies have not addressed the morphological or biometric changes caused by sub or supraoptimal temperatures (Guo et al., 2006; Machado et al., 2005; 2010; Ribeiro et al., 2006a; 2009a; Santos et al., 2011). In addition, most of the available literature reports the physiological responses of tree species to extreme temperature conditions, for instance near to heat damage or freezing (Ahrens and Ingram, 1988; Hoch and Körner, 2009). In fact, citrus trees are subjected to moderate seasonal changes in environmental temperature under subtropical conditions (Ribeiro et al., 2006b).

Air temperature frequently reaches 30ºC during the Spring and Summer in many of the main citrus-growing regions (Habermann and Rodrigues, 2009; Rabe et al., 2003; Ribeiro et al., 2005; 2006b). If the optimal temperature for sweet orange photosynthesis is around 25ºC (Machado et al., 2005; Ribeiro et al., 2004), and orange trees are productive (Habermann and Rodrigues, 2009), with vigorous vegetative growth (Espinoza-Núñez et al., 2011) under warmer conditions, we may argue that citrus plants have the potential for acclimation to changing environmental conditions between seasons, maintaining the carbon uptake even under nonoptimal conditions, e.g. moderate to high air temperatures. This issue is especially important as plants would face increments in global temperature in near future and would have their crop production impacted (Magrin et al., 2007)

In this study, we hypothesized that citrus plants present a positive acclimation of carbon metabolism to moderate warm condition, with this response being associated with improved vegetative growth. This hypothesis was tested under controlled conditions by evaluating the photosynthesis of both mature and young leaves, plant growth, and carbohydrate status.

MATERIAL AND METHODS

Plant material and growth conditions: Sweet orange plants (*Citrus sinensis* [L.] Osbeck) var. Valencia grafted on Rangpur lime (*Citrus limonia* Osbeck) were grown in plastic bags (1.5 L), which were filled with organic substrate composed of *Pinus* bark (Multicitrus, Terra do Paraíso Ltda., Brazil) under greenhouse conditions, with air temperature varying between 17.6 (minimum) and 34.8ºC (maximum). Plants were fertilized with 200 mL of nutrient solution every two days and did not show any visual symptom of mineral stress. Details of the nutrient solution were shown by Magalhães Filho et al. (2009).

Seven-month old plants presenting similar vegetative vigor (with three young shoots, having around 2 cm in length) and photosynthesis were selected and transferred to a growth chamber PGR15 (Conviron, Canada), where the temperature regimens (day/night) were not imposed simultaneously. Plants were subjected to 25/20ºC and 30/20ºC for 30 days. Not including the temperature, other environmental conditions inside the growth chamber were maintained similar between thermal treatments: photosynthetic photon flux density (PPFD) at the plant height of 800 μmol m^{-2} s^{-1}; 1.2 kPa air vapor pressure deficit (VPD), and a 12-hour photoperiod (from 0700h to 1900h). Those values of PPFD and VPD were not limiting for citrus plants (Machado et al., 2005). Substrate temperature was also monitored throughout the experiment, with the equilibrium between air and substrate temperatures being achieved rapidly inside the growth chamber.

Photosynthesis and pigments: Leaf gas exchange and chlorophyll fluorescence were evaluated with an infrared gas analyzer, model LI-6400 (LI-COR, Lincoln NE, USA), equipped with a modulated fluorometer (6400-40 LCF) after 30 days of thermal treatment, in both mature (developed before thermal treatment) and young (developed during the thermal treatment) leaves. Leaf gas exchange was also evaluated before the beginning of the experimental period for selecting homogeneous plants.

Measurements of leaf CO_2 assimilation (P_N), stomatal conductance (g_S), and transpiration (E) were taken under PPFD of 800 μmol m^{-2} s^{-1} at 0900h, 1100h, 1300h, 1700h

and 1900h. Dark respiration was also evaluated at 0700h and 2000h. All measurements were taken with a coefficient of variation (CV) lower than 5% and when temporal stability was achieved. In order to normalize the growth temperature effect, photosynthesis was evaluated at the optimal temperature of 25ºC (Machado et al., 2005; Ribeiro et al., 2004) in both regimens, as carried out by Bueno et al. (2011). Diurnal-integrated CO_2 assimilation (P_D) was calculated by integrating P_N between 0700h and 2000h (Ribeiro et al., 2009b).

The potential quantum efficiency of photosystem II (F_V/F_M) was measured by applying a saturation pulse (λ=630 nm, Q~6000 μmol m^{-2} s^{-1}, 0.8 second) in dark-adapted (30 minutes) leaves (Van Kooten and Snel, 1990). Measurements were taken at 1500h in leaves similar to those used in leaf gas exchange measurements.

The photosynthesis biochemistry was evaluated through the response of P_N to changes in intercellular CO_2 concentration (C_i) in both mature and young leaves, under PPFD of 1,000 μmol $m^{-2}s^{-1}$ and 25º C leaf temperature. The intercellular CO_2 concentration was changed by decreasing the air CO_2 concentration (C_a) from 400 to 50 μmol mol^{-1} (four steps) and then increasing from 400 to 2,000 μmol mol^{-1} (eight steps), in accordance to the procedure recommended by Long and Bernacchi (2003). The maximum rate of RuBP carboxylation ($V_{c,max}$) and that of electron transport driving RuBP regeneration (J_{max}) were estimated as previously reported (Ribeiro et al., 2009a). No limitation by triose-P use was detected in response curves, and the stomatal limitation (L) of photosynthesis was calculated according to Long and Bernacchi (2003).

Concentration of chlorophyll (Chl.) *a*+*b* was calculated after pigment extraction from leaf discs (10 cm^2) with acetone (80%, v/v). After centrifugation (2,000 g for five minutes), the supernatant absorbance was measured with a spectrophotometer at 646 and 663 nm. The equations proposed by Lichtenthaler and Wellburn (1983) were used for calculating the pigment concentrations.

Leaf water potential: The leaf water potential was monitored throughout the experimental period and only data of the 30th day of thermal treatment are herein presented. Measurements were taken with a pressure chamber model 3005 (Soilmoisture Equipment Corp., Santa Barbara CA, USA) at 1500h in leaves similar to those evaluated for photosynthesis.

Leaf, stem, and root carbohydrates andphotoassimilate exportation/consumption: The concentrations of carbohydrates in leaves, stems, and roots were evaluated at the end of each thermal treatment, when the growth of citrus plants was measured. For leaves, samplings were taken at an interval of 24 hours (at 0700h of two consecutive days) for calculating the photoassimilate exportation/consumption. Sampled leaves were similar to those evaluated for photosynthesis.

Leaf, stem, and root samples were collected and dried in an oven with forced-air circulation (60ºC) until they were in a constant weight. Afterwards, samples were ground and used in carbohydrate analyses. The total soluble carbohydrates (SC) were assayed from 75 mg samples after their extraction in a methanol:chloroform:water solution (12:5:3, v/v), according to Bieleski and Tunner (1966). After extraction and concentration of samples at 55ºC, the SC concentration was quantified according to Dubois et al. (1956). The starch concentration was quantified in samples of 10 mg in the insoluble fraction of SC extraction. Starch (Sta) was determined by the enzymatic method proposed by Amaral et al. (2007). The concentration of nonstructural carbohydrates (NSC) was calculated as the sum of SC and Sta fractions.

The leaf photoassimilate exportation/consumption (PEC) was calculated according to Ribeiro et al. (2012): $PEC=[(NSC_D+P_D)-NSC_{D+1}]$, where NSC_D and NSC_{D+1} are the concentration of NSC at the early morning (0700h) of two consecutive days, and P_D is the diurnal-integrated CO_2 assimilation at the day D. The P_D values in g CO_2 m^{-2} were converted to g CH_2O m^{-2} using a factor of 0.68 as multiplier, and the NSC in mass basis was converted in leaf area basis using the specific leaf mass.

Plant growth: The dry matter of leaves (LDM), stem (SDM), and roots (RDM) was evaluated before and after each thermal treatment, being the growth rate (g day^{-1}) of each fraction calculated. The dry matter of young leaves was also evaluated (LDM_Y), and LDM considered the sum of mature and young leaves. Plant fractions were collected, dried in an oven with forced-air circulation at 60º C, and finally they were weighed.

The length of young shoots (L_Y) was measured with a measuring tape and the young (LA_Y) and total leaf areas (LA, sum of mature and young leaf areas) with a digital planimeter model LI-3100 (LI-COR, USA). As measurements were done before and after the thermal treatments, growth rates were also estimated in cm day^{-1} and cm^2 day^{-1}.

Data analysis: The experiment was arranged in a randomized block design and we considered two sources of variation: thermal regimen (25/20 *versus* 30/20ºC) and leaf type (mature *versus* young). Data were subjected to the ANOVA procedure, and the mean values (n=3 to n=4) were compared by Tukey's test when appropriated ($p \leq 0.06$).

RESULTS

Photosynthesis: from stomatal to biochemical aspects: The diurnal dynamics of leaf gas exchange was similar between thermal regimens (Figure 1); however, plants presented high CO_2 assimilation (P_N), stomatal conductance (g_S) and transpiration (E) when grown at 30/20ºC. Besides the lower leaf water potential at 30/20ºC, g_S in both mature and young leaves remained higher as compared to plants at 25/20ºC (Figures 1C and D). Nonsignificant changes were noticed in dark respiration between thermal regimens or between leaf ages (Figures 1A and B).

The differences between thermal treatments were more pronounced in young leaves (Figure 1), i.e., leaves developed during the experimental period. The diurnal-integrated P_N values were higher under moderate warm conditions, with differences of 22% in mature leaves and 60% in the young ones (Figure 2).

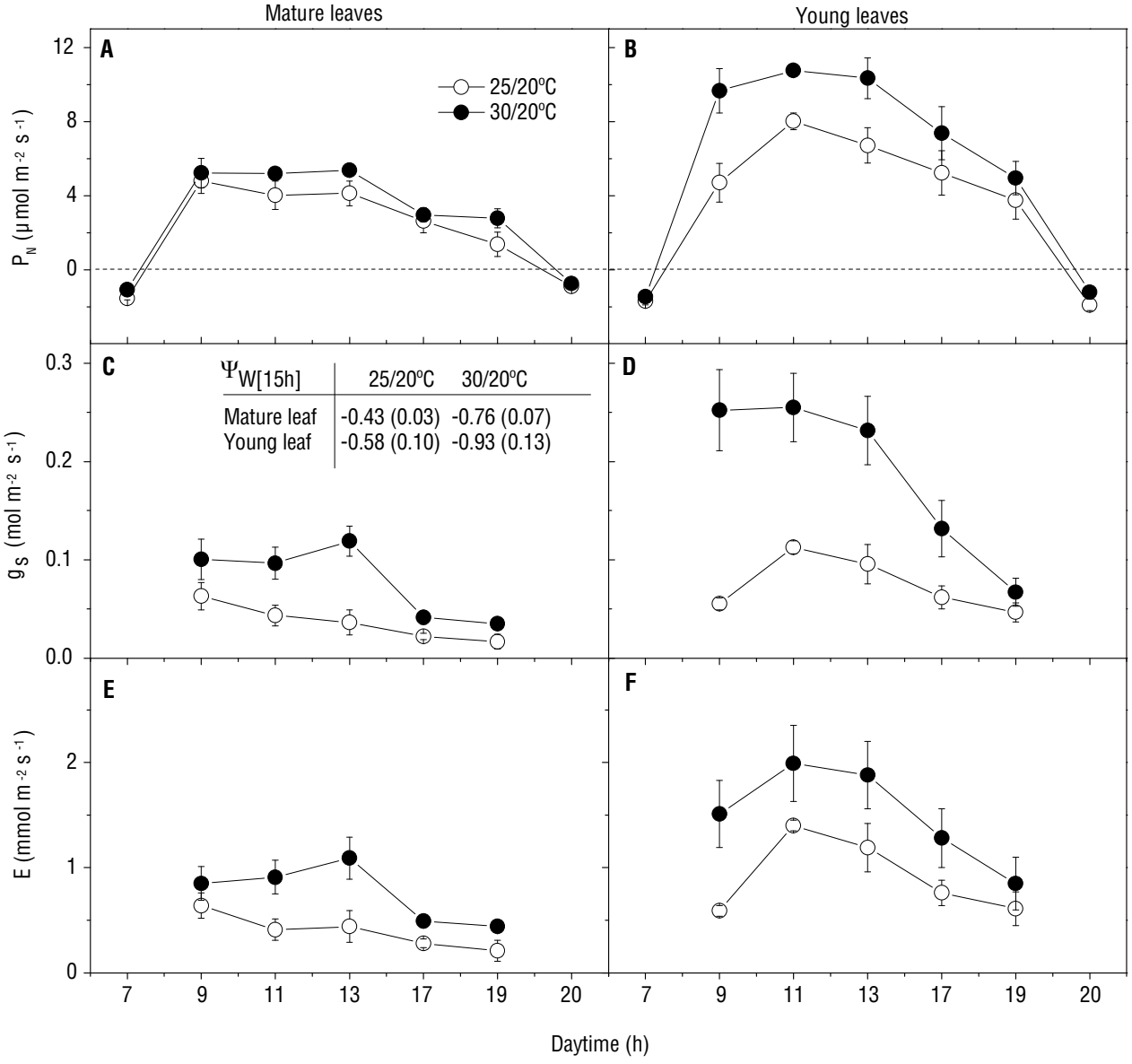

Figure 1. Diurnal changes in leaf CO_2 assimilation (P_N, in A, B), stomatal conductance (g_S, in C, D), transpiration (E, in E, F) in mature (A, C, E) and young (B, D, F) leaves of Valencia orange plants grown at the temperature regimes (day/night) of 25/20ºC (open circles) and 30/20ºC (closed circles). Leaf water potential measured at 1,500 hours ($\Psi_{w\ [15h]}$) is shown in C. Each symbol represents the mean value of four replications (±SD). Measurements were taken at the 30th day of treatment.

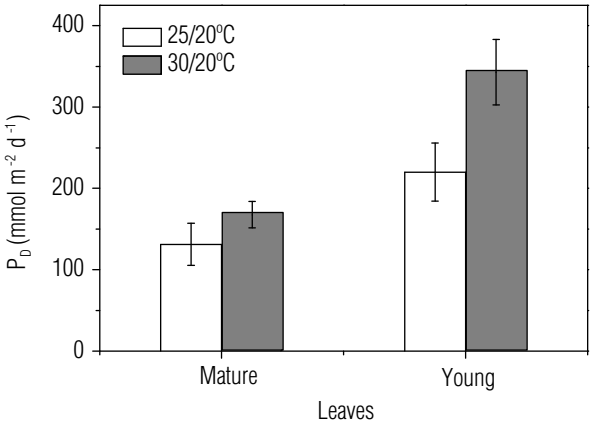

Figure 2. Diurnal-integrated CO_2 assimilation (P_D) in mature and young leaves of Valencia orange plants grown at the temperature regimes (day/night) of 25/20ºC and 30/20ºC. Each histogram represents the mean value of four replications (±SD). Measurements were taken at the 30th day of treatment.

Although the growth temperature of 30/20ºC had reduced the photochemical efficiency of plants in both mature and young leaves as compared to 25/20ºC, F_V/F_M values were always higher than 0.77, regardless thermal condition and leaf age (Table 1). On the other hand, the concentration of Chl. *a+b* in mature leaves was increased at 30/20ºC (Table 1).

Table 1. The potential quantum efficiency of photosystem II (F_V/F_M), chlorophyll concentration, the maximum rate of RuBP carboxylation ($V_{c,max}$), maximum rate of electron transport driving RuBP regeneration (J_{max}), and stomatal limitation of photosynthesis (L) in mature and young leaves of Valencia orange plants grown at two temperature regimens.

Variables*	Mature leaves		Young leaves	
	25/20ºC	30/20ºC	25/20ºC	30/20ºC
F_V/F_M (15 hours)	0.83±0.01^{aA}	0.81±0.01^{bA}	0.81±0.01^{aB}	0.78±0.01^{bB}
Chlorophyll *a+b* (mg g^{-1})	3.9±0.6^{bA}	6.4±0.3^{aA}	3.4±0.4^{aA}	4.1±0.4^{aB}
$V_{c,max}$ (μmol m^{-2} s^{-1})	42.8±9.5^{aB}	31.8±4.3^{bB}	78.2±0.9^{aA}	43.8±2.3^{bA}
J_{max} (μmol m^{-2} s^{-1})	125.7±1.1^{aB}	90.3±8.9^{bB}	179.8±8.4^{aA}	108.8±7.0^{bA}
L (%)	46.5±8.9^{aA}	26.7±6.9^{bA}	27.5±5.9^{aB}	15.5±3.8^{bB}

*Different lower case letters indicate significant difference between growth temperatures within the same leaf type, while different capital ones indicate significant difference between leaf types within the same growth temperature. Mean values of four replications (±standard deviation). Measurements were taken at the 30th day of treatment.

Considering the photosynthesis biochemistry, the maximum rate of RuBP carboxylation ($V_{c,max}$) and the maximum electron transport rate driving RuBP regeneration (J_{max}) of both mature and young leaves were higher in plants grown at 25/20ºC when compared to 30/20ºC (Table 1). However, the stomatal limitation of photosynthesis (L) was lower at 30/20ºC (Table 1). When comparing leaf ages, $V_{c,max}$, J_{max}, and g_S conductance were always higher in young leaves (Figure 2; Table 1).

Carbohydrates in plant tissues: Mature leaves had higher concentration of carbohydrates when plants were grown under warmer conditions, being the starch concentration at 30/20ºC around 4.5 times higher than the one found at 25/20ºC (Figure 3A). Young leaves followed the same pattern of response to thermal treatment; however, the difference in starch concentration was less pronounced (+2.7 times) between temperature regimes (Figure 3B). In contrast to leaf tissues, stem and roots presented lower carbohydrate concentrations at 30/20ºC. In stem and roots, the starch was the most responsive carbohydrate to changing thermal regimen (Figures 3C and D).

The PEC, a measure of leaf carbohydrate dynamics, was increased under warmer conditions in both mature (+29%) and young (+55%) leaves (Figure 4). An interesting finding is the high PEC capacity of young leaves with around 30 days-old, which was twice higher when compared to mature leaves at 30/20ºC.

Changes in plant growth due to temperature regimens: Moderate warm conditions imposed by the 30/20ºC thermal regimen increased the plant growth as compared to the 25/20ºC one (Figure 5). Considering the plant tissues developed during the thermal treatment and the reference as 25/20ºC, we noticed significant increases in length (+41%), leaf area (+39%), and leaf dry matter (+22%) in young shoots under 30/20ºC (Figure 5). As compared to 25/20ºC, the total leaf area was higher in 30/20ºC as well as the leaf dry matter (+23%) and the stem dry one (+37%). Nonsignificant changes were found in root dry matter due to thermal treatments (Figure 5C).

DISCUSSION

Plant growth and photosynthetic responses to changes in thermal regimen: Citrus growth was increased when plants were grown under moderate warm conditions, and this was more evident in young tissues, i.e., young shoots (Figures 5A and B).

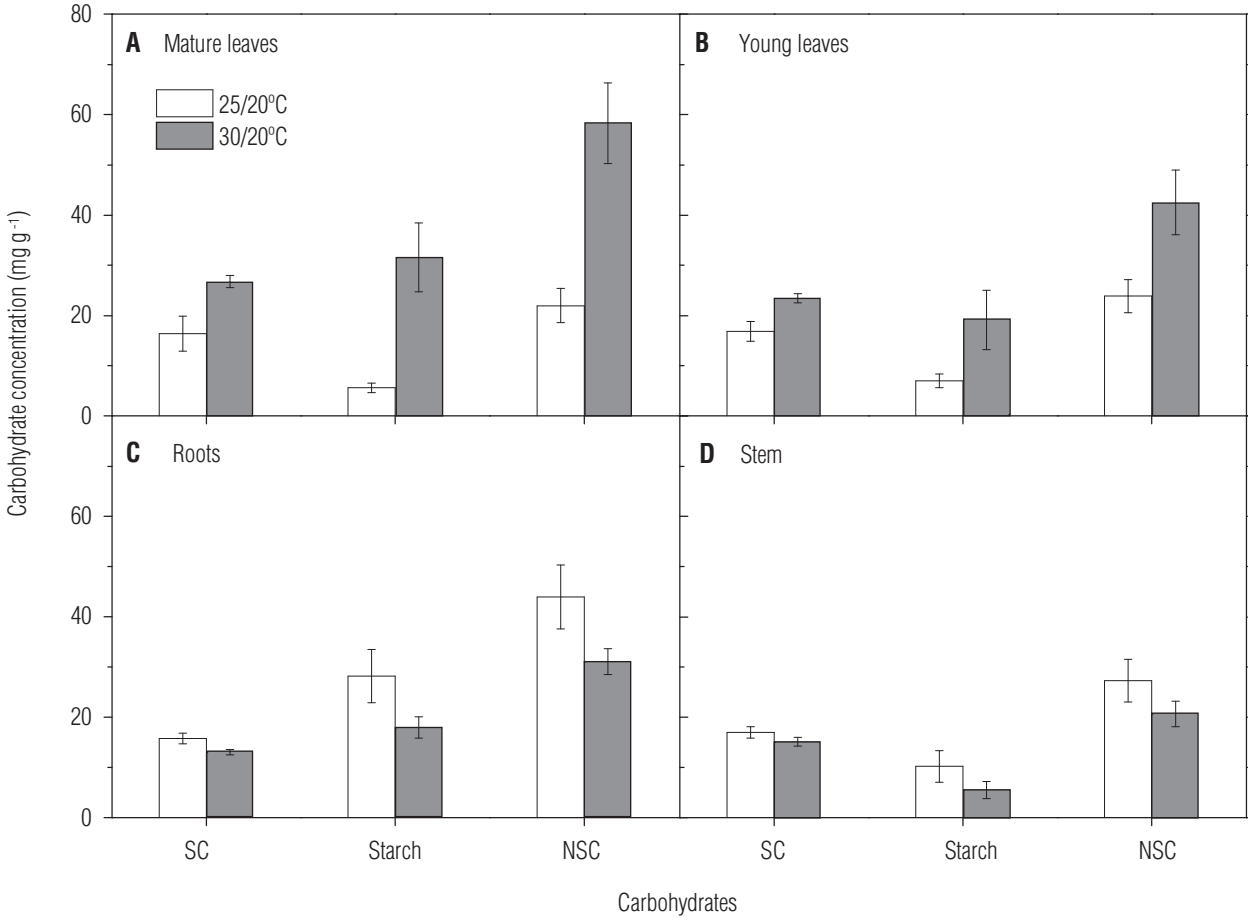

Figure 3. Concentrations of total soluble carbohydrates (SC), starch and nonstructural carbohydrates (NSC) in mature (A) and young (B) leaves, roots (C), and stems (D) of Valencia orange plants grown at the temperature regimes (day/night) of 25/20ºC and 30/20ºC. Each histogram represents the mean value of four replications (±standard deviation). Measurements were taken at the 30th day of treatment.

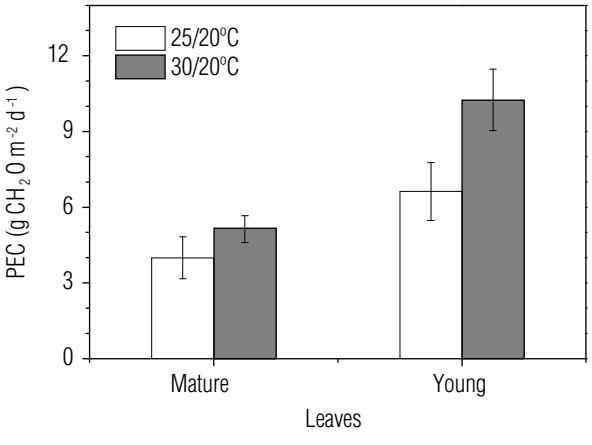

Figure 4. Leaf photoassimilate exportation/consumption (PEC) in mature and young leaves of Valencia orange plants grown at the temperature regimes (day/night) of 25/20ºC and 30/20ºC. Each histogram represents the mean value of four replications (±standard deviation). Measurements were taken at the 30th day of treatment.

Mature tissues such as stems have also presented higher growth rate under 30/20ºC as compared to 25/20ºC (Figure 5C). In fact, we found significant growth response to changes of only 2.5ºC in average temperature (25.0 *versus* 22.5ºC). This result indicates that little changes in growth temperature have large effects in citrus growth, even with changes occurring near the optimal temperature range for citrus species (Davies and Albrigo, 1994). Also, biomass accumulation in young shoots determined the plant growth as the roots were not affected by changes in growth temperature and the stem presented marginal variation (Figure 5C).

The leaf area large increase in young shoot caused an increase in the total leaf area of citrus plants, which obviously improve carbon uptake of the entire plant canopy. Although the optimum temperature for 25ºC photosynthesis (Machado et al., 2005; Ribeiro et al., 2004) was given at 25/20ºC, orange plants exhibited higher photosynthetic rates when grown under warmer conditions, i.e., 30/20ºC (Figures 1A, B and 2).

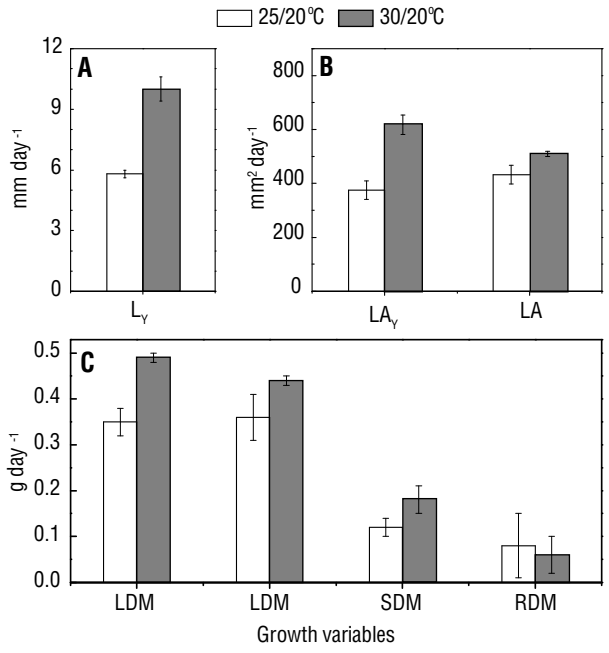

Figure 5. Plant growth of Valencia orange plants grown at the temperature regimens (day/night) of 25/20ºC and 30/20ºC: length of young shoots (L_Y, in A), leaf area of young shoots (LA_Y, in B) and total leaf area (LA, sum of mature and young leaves, in B); leaf dry matter of young shoots (LDM_Y, in C); total leaf dry matter (LDM, sum of mature and young leaves, in C); stem dry matter (SDM, in C); and root dry matter (RDM, in C). Growth rates were calculated considering the measurements taken at the beginning (before thermal treatment) and end (after 30 days of thermal treatment) of the experimental period. Each histogram represents the mean value of four replications (±standard deviation).

When temperature regimens were compared, we noticed larger differences in photosynthesis of leaves that developed during the experimental period, i.e. young leaves that were fully expanded and were 30-days old. Those leaves had an important role in carbon gain as they exhibited higher photosynthetic rates (more than two-fold) compared to the mature ones (Figures 1A and B).

Regardless the temperature regimen, the highest photosynthetic rates of young leaves were associated with higher stomatal conductance, higher maximum rate of RuBP carboxylation, and also higher maximum rate of electron transport driving RuBP regeneration when compared to mature leaves (Figures 1C and D; Table 1). The potential quantum efficiency of photosystem II (F_V/F_M) of young leaves was lower than in mature leaves (Table 1). In spite of this reduction, young leaves had relative high photochemical activity, with F_V/F_M values varying between 0.78 and 0.81 when both temperature regimens were considered. Accordingly, F_V/F_M values of healthy and sun leaves were around 0.8 (Critchley, 1998), and we have commonly found F_V/F_M of 0.76 in nonstressed citrus plants (Ribeiro et al., 2009a).

Despite leaf age, the highest photosynthetic rates found at 30/20ºC were well-associated with high stomatal conductance, even with orange plants presenting lower leaf water potential under moderate warm conditions (Figures 1 and 2). As the biochemical indexes $V_{c,max}$ and J_{max} and also the photochemical index F_V/F_M do not justify the highest photosynthesis under warmer conditions, we may argue that the most important factor regulating photosynthetic performance in this study was the stomata. In fact, the stomatal limitation of photosynthesis was lower under moderate warm conditions (Table 1), where orange plants exhibited higher photosynthetic rates in both mature and young leaves (Figure 1). Furthermore, the young leaves presented lower stomatal limitation of photosynthesis as compared to the mature ones (Table 1).

One would expect that warm temperatures cause reduction in stomatal conductance due to increased evaporative demand and consequent imbalance of leaf water relations (Jones, 1998). However, we controlled the air relative humidity in order to maintain the air water VPD around 1.0±0.2 kPa and then prevent stomatal closure. As a consequence of higher stomatal conductance, citrus plants presented higher intercellular CO_2 concentration (C_i) at 30/20ºC than at 25/20ºC (data not shown). For instance, the difference of C_i between the temperature regimens reached 110 μmol mol^{-1} in mature leaves when measurements were taken at 01 PM. Besides the stomatal limitation, our data do not exclude the importance of mesophyll conductance in regulating citrus photosynthesis (Flexas et al., 2008). In fact, Junqueira (2012) has reported higher sensitivity of mesophyll conductance of orange leaves to changes in growth temperature. The important role of the diffusive limitation of photosynthesis (considering stomata and mesophyll) was recently reported in coffee plants, another tree species largely cultivated under subtropical conditions (Batista et al., 2012).

Besides improving photosynthesis, the higher stomatal conductance under warm conditions also increased the leaf transpiration of both mature and young leaves (Figures 1E and F). As plants were well-hydrated, the transpiration-induced decrease in leaf water potential did not represent any imbalance in plant water relations, being Ψ_W relatively high when considering values found in well-hydrated citrus trees under field conditions, around -1.2 MPa (Ribeiro and Machado, 2007).

One may not disregard the influence of daily temperature variation on citrus photosynthesis (Bueno et al., 2011), which is likely mediated through changes in hormonal balance. As daily temperature variation was higher at 30/20ºC as compared to 25/20ºC (10 *versus* 5ºC), we may suggest the effect of thermoperiodism as an additional factor leading to enhanced plant growth and then high photosynthesis by increasing sink demand. Thermoperiodism affects gibberellins metabolism by decreasing catabolism and increasing biosynthesis through changes in gene expression (Penfield, 2008).

Carbohydrate changes due to growth temperature: stems and roots as source organs: The improved plant growth was associated with increased photosynthesis and then we may argue that the carbon invested in plant structure was originated from the atmosphere. In fact, the high photosynthesis of mature and young leaves under warm conditions increased the carbohydrate concentration in both leaf types (Figures 3A and B). The leaf concentrations of SC and starch were both increased and lead to a large enhancement of NSC in mature leaves, which was almost three-times higher at 30/20ºC (Figure 3A). A similar pattern was noticed in young leaves, in which the main carbohydrate fraction affected by temperature changes was the starch, but the increase of NSC was less intense when compared to mature leaves (Figure 3B).

Starch concentration commonly increases in storage organs to serve the purpose of reserving when plants are experiencing some kind of growth limitation (Dickson, 1991). The question is: why did starch concentration in young leaves increase in orange plants experiencing active growth as noticed at 30/20ºC? Mature and young leaves presented higher carbohydrate concentration and improved PEC under warmer conditions (Figures 3A, B and 4). These responses were in accordance to the higher plant growth at 30/20ºC as compared to 25/20ºC (Figure 5). As leaves are the primary sources of carbon and have to supply the other plant organs with photoassimilates, we may suggest that the high carbohydrate concentration under moderate warm conditions enabled the maintenance of PEC.

According to Smith and Stitt (2007), the photoassimilate partitioning is driven by plant carbon demand and requires a complex control to supply the immediate demand during the diurnal period with sucrose and also to anticipate the nocturnal demand through starch accumulation in leaf tissues. These assumptions were confirmed in this study, in which the highest concentrations of both sucrose and starch were found in plants growing at 30/20ºC (Figures 3A and B).

Our data revealed the importance of evaluating the carbohydrate availability from the static (carbohydrate concentration itself) and dynamic (daily carbohydrate changes) points of view. Recently, Nebauer et al. (2011) and Ribeiro et al. (2012) have reported that citrus photosynthesis is regulated by leaf carbohydrate changes rather than by absolute carbohydrate concentration, with the photoassimilate consumption/exportation presenting a positive association with photosynthesis and plant growth. We also reported the important role of carbohydrate stored in roots and stems for shoot growth when the carbon and energy demands cannot be supported by photosynthetic rates (Dickson, 1991). This is a very common situation in tree species, which present in general low photosynthesis as compared to other C_3 and C_4 species with rapid growth habit. Regarding the carbon sources, Bueno et al. (2011) also reported a significant reduction in starch concentration of citrus roots when plants exhibited improved shoot growth.

The shoot development in parallel to the nonapparent root growth suggests that the photoassimilates were driven to young shoots, the main sink organ in both temperature regimes. In fact, Bevington and Castle (1985) have reported that the canopy growth induces inhibition of root growth and this is in accordance to the growth pattern reported herein.

Besides the increased stomatal conductance, we may argue that photosynthesis was stimulated by the sink demand under warm conditions (Iglesias et al., 2002; Ribeiro and Machado, 2007; Ribeiro et al., 2012). The physiological mechanisms determining high photosynthesis may be related to increased expression of genes related to photosynthesis and increased phosphorus recycling between stromal and cytosol compartments (Foyer and Galtier, 1996; Koch, 1996; Paul and Pellny, 2003). With regards to the gene expression, the candidate signaling molecules are sucrose, glucose, and fructose (Rolland et al., 2006; Smith and Stitt, 2007). Our data revealed that soluble sugars were increased under warm conditions; however, their role on gene expression related to photosynthesis must be proven in further studies.

In conclusion, we have shown that citrus plants present a positive acclimation of carbon metabolism to moderate warm condition, with plants presenting high photosynthesis supported by increased stomatal conductance. Such photosynthetic acclimation was associated with improved vegetative growth, being both mature and young tissues sensitive to changes in thermal regime. Under such condition of active growth, roots and stems are important sources of carbohydrates for plant growth.

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