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Daily Temperature Amplitude Affects the Vegetative Growth and Carbon Metabolism of Orange Trees in a Rootstock-Dependent Manner

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Abstract Both instantaneous and average growth temperatures affect plant metabolism, and the physiological importance of daily variations in temperature is frequently underestimated. To improve our understanding of the environmental regulation of citrus trees, we hypothesized that vegetative growth would be stimulated in orange plants subjected to large daily temperature variations, even without changes in the average daily air temperature or the amount of energy given by degree-days. This hypothesis was tested with orange plants grafted onto Rangpur lime or Swingle citrumelo rootstocks and grown for 20 days under thermal regimes (day/night) of 25/25°C or 32.5/17.5°C. Such regimes imposed growth conditions with daily temperature variations of 0 and 15°C. Plant growth, photosynthesis, respiration, and carbohydrate availability in leaves, stems, and roots were measured under both thermal conditions. The daily temperature variation affected the carbon metabolism of young citrus trees; plants grown under daily variation of 15°C used more of the carbon stored in mature leaves and roots and the energy generated by respiration for the biosynthesis of vegetative structures, such as leaves and branches. Thus, there was a significant increase in the leaf area of plants subjected to high daily temperature variation. Current photosynthesis was similar in the two thermal regimes; however, the photosynthetic rates increased under the 15°C variation when measurements were normalized to 25°C. In addition to the stimulatory effect of the source–sink relationship on photosynthesis, we suggest a probable involvement of hormonal regulation of plant growth through gibberellin metabolism. The rootstock affected the response of the canopy to daily temperature amplitude, with the Rangpur lime improving plant growth through higher carbohydrate availability in roots. This is the first report that highlights the importance of daily temperature variations for citrus growth and physiology under nonlimiting conditions.

Keywords Carbohydrate · *Citrus sinensis* · Environment · Growth · Photosynthesis

Introduction

The reduction of citrus photosynthesis due to increasing instantaneous temperature is well known, regardless of the average growth temperature; however, plants grown under warmer conditions exhibit better photosynthetic performance (Ribeiro and others 2004). Such an increase of photosynthesis under warmer conditions has been associated with increases in vegetative growth (Ribeiro and others 2004; Ramos 2009), and it is probably driven by changes in the source–sink relationship of citrus plants that occur through modifications in leaf carbohydrate content (Iglesias and others 2002; Li and others 2003; Ribeiro and Machado 2007).

Plant growth is regulated by the synthesis (photosynthesis) and consumption (respiration) of organic substances, as well as by the partitioning of photoassimilates. According to Loomis and Amthor (1999), vegetative growth requires high rates of respiration for the synthesis of new biomass and consumption of carbohydrates derived

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from photosynthesis and/or hydrolysis of more complex carbohydrates stored in plant organs such as leaves, stems, and roots. Similar to photosynthesis, respiration is a physiological process that is dependent on temperature (Amthor 2000); however, although increases in temperature stimulate plant respiration, photosynthesis is negatively affected in tree species such as *Citrus* spp. in temperatures higher than 30°C (Ribeiro and others 2004; Machado and others 2005; Pimentel and others 2007). As an overall consequence of varying temperature, the carbon balance and plant growth are changed.

Plant responses to extreme temperatures have been studied, with papers reporting disturbances in physiology, growth, and development of tree species (Vu and others 2002; Penfield 2008; Allen and Vu 2009; Hoch and Körner 2009; Xu and others 2011). However, little information is available on physiological and morphological responses to small changes in temperature within the physiological range, that is, nonlimiting conditions (Penfield 2008). A recent study revealed that photosynthesis in citrus trees was twofold higher when the plants were grown under an average air temperature of 25°C as compared with 22.5°C (Ramos 2009). The 2.5°C increase also improved plant growth and occurred in parallel with an increase in the daily temperature variation from 5 to 10°C.

Most available reports dealing with temperature effects on plant growth and physiology have not separately addressed the importance of average temperature and daily temperature variation (Ribeiro and others 2004; Xu and Zhou 2005). Indirect evidence has suggested a stimulation of citrus vegetative growth when only the daily temperature amplitude varied (Susanto and others 1992). Because the plants had similar nutritional status and leaf contents of chlorophyll and carbohydrates, the reason for the increased vegetative growth under higher daily temperature variations was unclear.

In general, plant growth patterns depend on the carbon balance in the tissues, and both source and sink organs play an important role in maintaining this balance. To improve our understanding of the environmental regulation of citrus trees, we hypothesized that vegetative growth is stimulated in plants grown under a higher daily temperature variation. This response is a consequence of changes in the carbon balance, as Valencia orange trees show increases in photosynthesis and photoassimilate consumption even when the average daily air temperature and amount of available energy are unchanged. As *Citrus* species are commercially grafted onto rootstocks that cause differential responses to varying environmental conditions (Magalhães Filho and others 2008; Machado and others 2010; Castle 2010), the above hypothesis was tested with Valencia orange scions grafted onto Rangpur lime or Swingle citrumelo rootstocks.

Materials and Methods

Plant Material

Six-month-old Valencia orange plants [*Citrus sinensis* (L.) Osb.] were grafted onto Rangpur lime (*Citrus limonia* Osbeck) or Swingle citrumelo [*Citrus paradisi* Macf. × *Poncirus trifoliata* (L.) Raf.] rootstocks. The plants were grown in plastic pots (7 l) containing an organic substrate composed of pine bark and vermiculite (Multplant citrus, Terra do Paraíso Ltda., Holambra, SP, Brazil) under greenhouse conditions. Plants were irrigated daily and fertilized every 3 days with 100 ml of a nutrient solution prepared according to Ramos (2009).

Thermal Treatments

Plants with vegetative vigor and of similar size and physiological condition (determined by measurements of photosynthesis) were transferred to a growth chamber (model PGR14, Conviron, Winnipeg, Manitoba, Canada), where they were subjected to a 5-day period of preacclimation at 25/20°C (day/night). After this time, morphological and physiological traits of six plants from each rootstock were measured to characterize the initial condition. At the beginning of the thermal regimes, the plants photosynthesized 5.3 ± 0.3 μmol m^{-2} s^{-1}, had 11 ± 2 leaves, were 42.5 ± 2.8 cm tall, had stems that were 10.3 ± 1.3 mm in diameter, and had three young shoots with a length of approximately 1 cm. Regarding dry matter partitioning, the plants had 4.4 ± 0.9 g of leaves, 21.8 ± 2.5 g of stem, and 32.9 ± 4.7 g of roots. The above values refer to the average of both thermal regimes and both rootstocks.

The environmental conditions other than temperature were held constant and nonlimiting throughout the experimental period. The photosynthetically active radiation (Q) was 800 μmol m^{-2} s^{-1}, the air vapor pressure deficit (VPD) was lower than 1.5 kPa, and the photoperiod was 12 h (from 7:00 to 19:00 h). According to Machado and others (2005) and Ribeiro and Machado (2007), these Q and VPD values were not limiting for citrus photosynthesis. After the preacclimation period, the plants were grown for 20 days under two thermal regimes (day/night) with the same average air temperature of 25°C: 25/25°C and 32.5/17.5°C, which had daily temperature variations of 0 and 15°C, respectively. In addition to the same average air temperature, the plants received the same amount of available energy under both thermal conditions. Considering the basal temperature of 13°C for sweet orange plants (Davies and Albrigo 1994), the thermal regimes caused an accumulation of approximately 245 degree-days over 20 days.

Biometry

Evaluations were performed before (as previously described in the Thermal Treatments subsection) and after each thermal regime. The young and mature leaves, stem, and roots of each plant were collected and dried in an oven with forced-air circulation (model MA032, Marconi, Piracicaba, SP, Brazil) at 60°C to determine the dry matter of the roots (RDM), stems (SDM), young branches (SDM_Y), mature leaves (LDM_M), and young (LDM_Y) leaves. The dry matter partitioning was calculated considering the underground (UnDM = RDM) and aboveground biomass (AbDM = $LDM_M + LDM_Y + SDM + SDM_Y$). The total leaf area (LA) of each plant was determined indirectly through the LDM and the specific leaf mass (SLM) as LA = LDM/SLM. The numbers of leaves and shoots were determined by simple counting, and the shoot length and plant height were determined with a measuring tape. The stem diameter was measured with digital calipers.

Leaf Water Potential and Photosynthetic Pigments

The leaf water potential was measured with a pressure chamber (model 3005, SoilMoisture Equipment Corp., Santa Barbara CA, USA) on leaves similar to those evaluated for gas exchange. Leaf water potential measurements were obtained at 6:30 h. Using the method described by Lichtenthaler (1987), chlorophyll *a* (chl *a*) and *b* (chl *b*) and xanthophyll + carotenoid ($x + c$) contents were evaluated in 0.78-cm^2 discs of young and mature leaves.

Leaf Gas Exchange

The leaf gas exchange was evaluated with an infrared gas analyzer (model Li-6400F, LI-COR, Lincoln, NE, USA), and the following physiological variables were studied: leaf CO_2 assimilation (Pn), stomatal conductance (g_s), and respiration (Rd). The diurnal course of Pn and g_s was measured, with evaluations every 2 h from 7:00 to 19:00 h. Measurements of Rd were obtained at 6:30 and 13:30 h. Leaf gas exchange was measured in both young and mature leaves after 15 days of thermal treatment. All measurements were obtained under the following conditions: 380 μmol mol^{-1} air CO_2, 800 μmol m^{-2} s^{-1} Q, and 25°C leaf temperature under the 25/25°C regime or 32.5°C leaf temperature under the 32.5/17.5°C regime. All evaluations were performed when the total coefficient of variation was lower than 5%, with temporal stability of CO_2 and H_2O_V fluxes.

The diurnal-integrated CO_2 assimilation (Pn_i) was calculated for both young and mature leaves. The plant diurnal-integrated CO_2 assimilation (Pn_P) was estimated by considering Pn_i and the leaf area of young and mature leaves. The diurnal (Rd_D) and nocturnal (Rd_N) respiration were estimated in both young and mature leaves by considering Rd measurements obtained at 13:30 and 6:30 h as representative of the diurnal and nocturnal conditions, respectively. The photoperiod of 12 h and the leaf area of both young and mature leaves were also considered. The daily leaf respiration (Rd_L) was estimated as the sum of Rd_D and Rd_N. The variables Pn_P, Rd_D, Rd_N, and Rd_L were estimated for the entire plant and also on a dry matter basis.

Photosynthetic Activity under Normalized Conditions

In an attempt to normalize the effect of thermal regimes on photosynthesis, measurements of leaf gas exchange and photochemistry were obtained at 25°C in plants growing under the 25/25°C and 32.5/17.5°C thermal regimes. The photosynthetic apparatus was evaluated in young leaves after 15 days of thermal treatment. Measurements were obtained at a leaf temperature of 25.0 ± 0.4°C, which was controlled by varying the temperature of the Li-6400F sampling chamber. The other environmental conditions were Q of 800 μmol m^{-2} s^{-1} and $[CO_2]_{air}$ of 380 μmol - mol^{-1}, and the leaf-to-air vapor pressure difference was lower than 1.5 kPa. As previously stated, measurements were obtained with a total coefficient of variation lower than 5% and under temporal stability. In addition to Pn and g_s, the intercellular CO_2 concentration (Ci) and the instantaneous carboxylation efficiency (Pn/Ci) were evaluated.

Photochemistry was also studied through measurements of chlorophyll fluorescence with a modulated fluorometer (LCF 6400-40) attached to the Li-6400F system. Leaf tissues were dark-adapted for 30 min, and then we used the saturation pulse method ($\lambda = 630$ nm, Q $\sim$ 6,000 μmol m^{-2} s^{-1}, 0.8 s) to evaluate the primary photochemistry. Photosystem I (PSI) was excited with far-red light ($\lambda = 740$ nm, Q $\sim$ 5 μmol m^{-2} s^{-1}, 2.0 s), and the following photochemical variables were evaluated: the potential (F_V/F_M) and effective ($\Delta F/F'_M$) quantum yield of photosystem II (PSII), the photochemical (q_P) and nonphotochemical (NPQ) fluorescence quenching, and the apparent electron transport rate (ETR). Details about the calculation of the above variables are given in Rohacek (2002). The ETR was separated into two components for the evaluation of electron flow to carboxylation (ETR_C) and oxygenation (ETR_O) activities of Rubisco (Krall and Edwards 1992).

Leaf nitrogen concentration was evaluated under each thermal regime with the micro-Kjeldahl method (Bataglia and others 1983). Leaf samples were collected and dried as previously described in the Biometry subsection. The photosynthetic nitrogen-use efficiency (PNUE) was calculated on the basis of the SLM, leaf N concentration, and Pn under normalized conditions.

Carbohydrate Concentration and Dynamics

Carbohydrates were measured in young and mature leaves, stems, and roots. Samples were collected before and after each thermal treatment at 6:30 h. The leaves were similar in age and canopy position to those assessed for gas exchange. Plant tissues were dried at 60°C, macerated in a mortar, and stored. Soluble carbohydrates (SC), sucrose (Suc), starch (Sta), and total nonstructural carbohydrates (TC = SC + Sta) were evaluated. The SC and Suc fractions were extracted according to Bieleski and Turner (1966). SC was determined by the phenol-sulfuric method (Dubois and others 1956), and Suc was determined according to Van Handel (1968). The Sta fraction was evaluated by the enzymatic method proposed by Amaral and others (2007). We also evaluated the carbohydrate changes in mature leaves, stems, and roots by calculating the differences in carbohydrate contents before and after each thermal treatment (a 20-day interval). Values were expressed as mg g^{-1} day^{-1}, and negative values represent the consumption of carbohydrates during a day.

Data Analysis

A randomized block experimental design was used, and data were subjected to an analysis of variance (ANOVA) that considered thermal regimes and rootstocks as sources of variation. When significant differences between thermal regimes or rootstocks were detected, the mean values were compared by the Tukey test at the 0.05 probability level.

Results

Plant Growth

Significant vegetative growth occurred during the 20 days of study. Plants subjected to 15°C of daily temperature variation had greater increases in shoot length (+29%), number of leaves (+21%), and leaf area (+46%) than the plants subjected to 0°C of variation, regardless of which rootstocks were used (Fig. 1a, b, e). Larger increases in leaf and shoot dry matter were found in plants grown under 15°C of variation than in those grown under 0°C of variation (Fig. 1c, d). When comparing rootstocks, Rangpur lime induced more vegetative growth than Swingle, regardless of the daily temperature amplitude (Fig. 1). Dry matter partitioning was changed in both thermal regimes, with plants showing increases in aboveground dry matter from 44 to 52%. Rootstocks and changes in daily temperature amplitude did not affect plant carbon partitioning between shoots and roots.

Plant Water Status, Photosynthetic Pigments, and Leaf Nitrogen Content

The leaf water potential measured at predawn was not affected by rootstocks or daily temperature variations. In general, the leaf water potential varied by around -0.20 ± 0.06 MPa in mature leaves and around -0.30 ± 0.09 MPa in young leaves. Leaf photosynthetic pigments were not affected by thermal regimes or rootstocks. Mature leaves had greater pigmentation than young leaves, with mean values varying from 26.8 ± 1.9 to 15.5 ± 1.7 mg cm^{-2} for chl *a*, 17.3 ± 2.4 to 9.9 ± 1.1 mg cm^{-2} for chl *b*, and 7.0 ± 1.8 to 4.2 ± 0.8 mg cm^{-2} for $x + c$. Thermal regimes and rootstocks did not affect the leaf nitrogen concentration, which was around 31.7 ± 1.9 mg g^{-1}.

Leaf Gas Exchange

On a dry matter basis, the Pn_P was reduced under diurnal variation of 15°C only on plants grafted onto Rangpur lime (Table 1). The Pn_P by the entire plant was affected only by the rootstock; it was higher on plants grafted onto Rangpur lime (Table 1). On average, plants grafted onto Rangpur lime assimilated about 38% more CO_2 per day than those grafted onto Swingle citrumelo. The average stomatal conductance throughout the diurnal period was not significantly changed by thermal regimes or rootstocks (data not shown).

The Rd_D, Rd_N, and Rd_L on a dry matter basis were not affected by daily temperature amplitude on plants grafted onto Rangpur lime. The only significant response was noticed in the Rd_D of plants grafted onto Swingle citrumelo, which increased under 15°C of diurnal variation (Table 1). In general, shoot respiration was stimulated by increasing the daily temperature variation, when considering the entire plant canopy. Whereas such stimulation in plants grafted onto Rangpur lime occurred during the diurnal (Rd_D) and nocturnal (Rd_N) periods, plants grafted onto Swingle citrumelo showed increases only in Rd_D (Table 1). Thus, the Rd_L was higher in plants grown under 15°C of temperature variation than those grown under 0°C of variation (Table 1). In general, plants grafted onto Rangpur lime exhibited higher respiration rates than those grafted onto Swingle, regardless of the thermal condition or time of day.

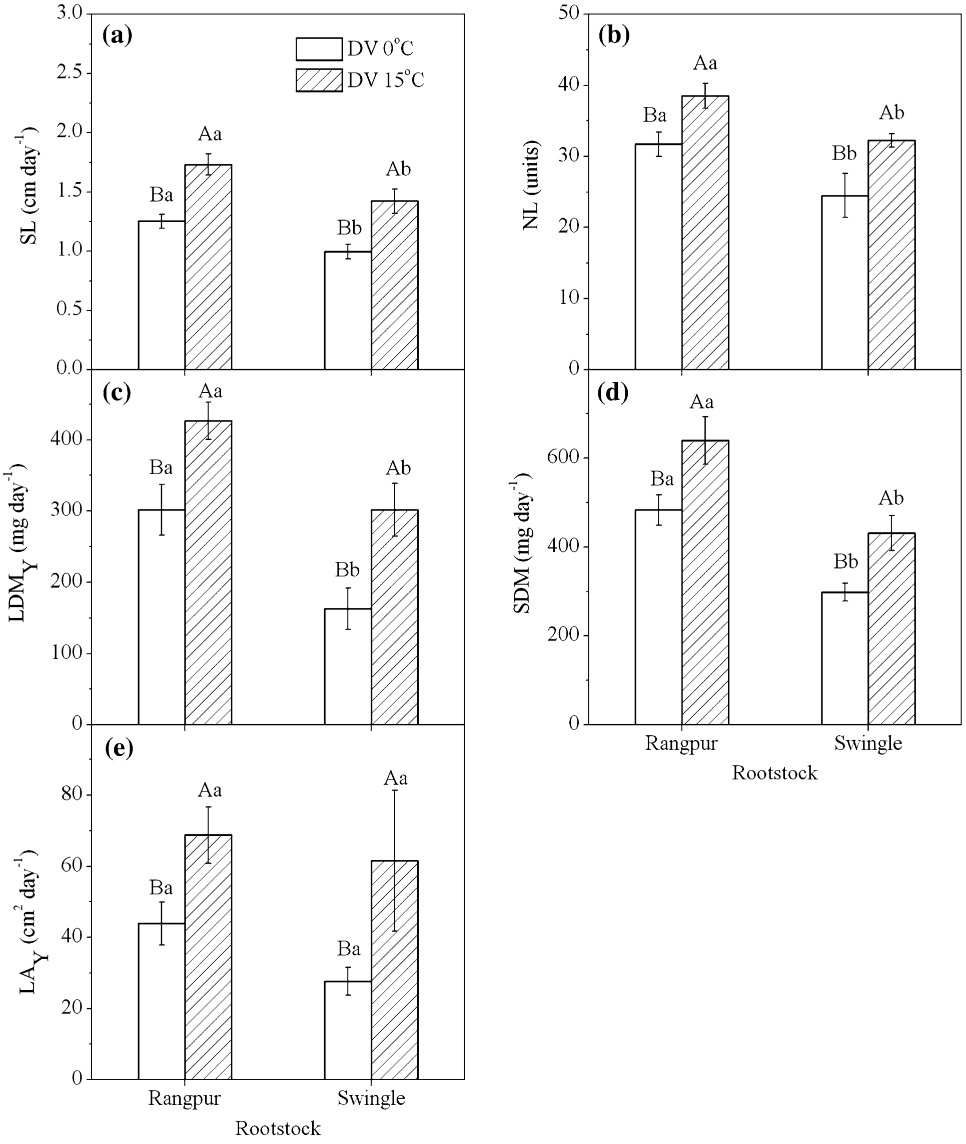

Fig. 1 Growth of Valencia orange trees grafted onto Rangpur lime or Swingle citrumelo rootstocks as affected by daily temperature variation (DV) of 0 and 15°C. **a** Shoot length (SL). **b** Number of leaves (NL) developed during thermal treatments. **c** Leaf dry matter (LDM$_Y$). **d** Shoot dry matter (SDM$_Y$). **e** Leaf area (LA$_Y$). Plants were grown for 20 days under thermal regimes of 25/25°C (DV = 0°C) or 32.5/17.5°C (DV = 15°C). Different capital letters indicate statistical differences ($p < 0.05$) between temperature amplitudes for the same rootstock, whereas lowercase letters represent differences between rootstocks over the same temperature amplitude. Values shown are the means of four replications ± SD

Table 1 Plant diurnal-integrated CO_2 assimilation (Pn_P), diurnal (Rd_D) and nocturnal (Rd_N) leaf respiration, and daily leaf respiration (Rd_L) of Valencia orange trees grafted onto Rangpur lime or Swingle citrumelo rootstocks grown under thermal regimes with 0 or 15°C of daily temperature variation (DV)

Variables[1]	Rangpur		Swingle	
	DV 0°C	DV 15°C	DV 0°C	DV 15°C
Pn_P (mmol g^{-1} day^{-1})	3.30 ± 0.41^{Aa}	2.27 ± 0.22^{Bb}	2.86 ± 0.35^{Aa}	2.78 ± 0.13^{Aa}
Rd_D (mmol g^{-1} day^{-1})	2.62 ± 0.42^{Aa}	2.41 ± 0.14^{Aa}	1.87 ± 0.23^{Bb}	2.50 ± 0.37^{Aa}
Rd_N (mmol g^{-1} day^{-1})	1.56 ± 0.19^{Aa}	1.60 ± 0.10^{Aa}	1.73 ± 0.29^{Aa}	1.66 ± 0.09^{Aa}
Rd_L (mmol g^{-1} day^{-1})	4.18 ± 0.56^{Aa}	4.01 ± 0.24^{Aa}	3.60 ± 0.49^{Aa}	4.16 ± 0.28^{Aa}
Pn_P (mmol day^{-1})	30.2 ± 3.8^{Aa}	27.9 ± 2.7^{Aa}	18.6 ± 2.3^{Ab}	22.2 ± 1.0^{Ab}
Rd_D (mmol day^{-1})	24.0 ± 3.8^{Ba}	29.6 ± 1.8^{Aa}	12.2 ± 1.5^{Bb}	20.0 ± 3.0^{Ab}
Rd_N (mmol day^{-1})	14.3 ± 1.8^{Ba}	19.7 ± 1.2^{Aa}	11.3 ± 1.9^{Aa}	13.2 ± 0.8^{Ab}
Rd_L (mmol day^{-1})	38.2 ± 5.1^{Ba}	49.3 ± 3.0^{Aa}	23.4 ± 3.2^{Bb}	33.2 ± 2.2^{Ab}

Mean values ± SD ($n = 3$). Different capital letters indicate statistical differences ($p < 0.05$) for the daily temperature variation on the same rootstock, whereas different lowercase letters represent statistical differences between rootstocks over the same temperature range

[1] Variables are given on a dry matter basis (mmol g^{-1} day^{-1}) and also considering the total leaf dry matter of each plant (mmol day^{-1})

Photosynthetic Activity Under Normalized Conditions

When evaluating the normalized photosynthesis at 25°C, plants subjected to the thermal regime with 15°C temperature variation exhibited higher photosynthetic performance than those subjected to the thermal regime with 0°C of variation (Fig. 2; Table 2). Improvements derived from high daily temperature variation were found in the biochemistry of photosynthesis, with plants showing higher photosynthesis (Pn), PNUE, ETR_C, and Pn/Ci in both rootstocks (Fig. 2).

Nonsignificant changes were observed in g_s and other photochemical variables (Table 2) such as F_V/F_M, apparent ETR, q_P, NPQ, and ETR_O. Although we have found increases in the effective quantum yield of PSII ($\Delta F/F'_M$) under 15°C temperature variation, this positive response did not affect ETR (Table 2).

Carbohydrates in Leaves, Stems, and Roots

Leaf tissues were more sensitive to the thermal regimes in plants grafted onto Swingle citrumelo than in those grafted onto Rangpur lime, with both young and mature leaves showing reductions in TC due to increasing temperature amplitude (Fig. 3b, d). In addition to this reduction in TC, leaves of plants grafted onto Swingle presented decreases in SC concentration under a daily temperature variation of 15°C (Fig. 3d). Accordingly, mature leaves of plants grafted onto the Rangpur lime also showed increases in Sta content when grown under 15°C temperature variation.

The effects of thermal regimes were more evident on stem tissues; the carbohydrate concentration increased in plants subjected to 15°C temperature variation (Fig. 3e, f). When comparing thermal regimes, the largest changes in stem carbohydrates were noticed in the SC and TC fractions. There was an increase in Suc concentration when plants were grown under 15°C temperature variation (Fig. 3e, f). Plants grafted onto Rangpur lime presented reductions in root carbohydrate concentration under 15°C temperature variation (Fig. 3g), but this response was not found in plants grafted onto Swingle citrumelo (Fig. 3h). In general, we also noticed higher root carbohydrate availability in Rangpur lime compared with Swingle citrumelo (Fig. 3g, h). Whereas the TC in the roots of Swingle was lower than 40 mg g^{-1}, this fraction in the roots of Rangpur lime reached values higher than 80 mg g^{-1}. Another interesting finding is that both rootstocks showed significant increases (+57%) in their Suc fractions when plants were grown under 15°C temperature variation (Fig. 3g, h).

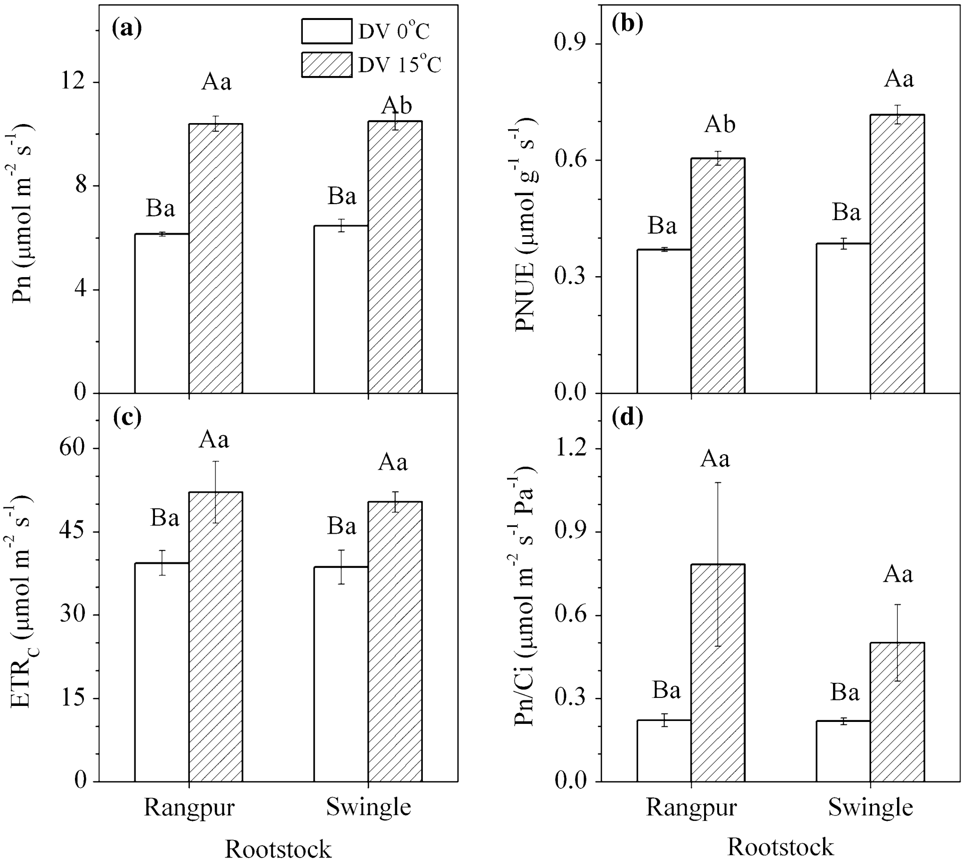

Fig. 2 Normalized (at 25°C) photosynthetic activity of Valencia orange trees grafted onto Rangpur lime or Swingle citrumelo rootstocks as affected by daily temperature variation (DV) of 0 and 15°C. **a** Leaf CO_2 assimilation (Pn). **b** Photosynthetic nitrogen use efficiency (PNUE). **c** Electron flow for carboxylation of Rubisco (ETR_C). **d** Instantaneous carboxylation efficiency (Pn/Ci). Plants were grown for 20 days under thermal regimes of 25/25°C (DV = 0°C) or 32.5/17.5°C (DV = 15°C). Different capital letters indicate statistical difference ($p < 0.05$) between temperature amplitudes for the same rootstock, whereas lowercase letters represent differences between rootstocks over the same temperature amplitude. Values shown are the means of three replications ± SD

Table 2 Stomatal conductance (g_s), potential (F_V/F_M) and effective ($\Delta F/F'_M$) quantum yield of photosystem II, apparent electron transport rate (ETR), photochemical (q_p) and nonphotochemical (NPQ) quenching, and electron flow for oxygenation of Rubisco (ETR_O) in Valencia orange trees grafted onto Rangpur lime or Swingle citrumelo grown under thermal regimes with 0 or 15°C of daily temperature variation (DV)

Variables[1]	Rangpur		Swingle	
	DV 0°C	DV 15°C	DV 0°C	DV 15°C
g_s (mol m^{-2} s^{-1})	0.113 ± 0.065^{Aa}	0.062 ± 0.023^{Aa}	0.169 ± 0.063^{Aa}	0.091 ± 0.038^{Aa}
F_V/F_M	0.73 ± 0.01^{Aa}	0.72 ± 0.01^{Aa}	0.70 ± 0.02^{Aa}	0.75 ± 0.04^{Aa}
$\Delta F/F'_M$	0.18 ± 0.02^{Ba}	0.26 ± 0.01^{Aa}	0.18 ± 0.03^{Ba}	0.22 ± 0.02^{Ab}
ETR (μmol m^{-2} s^{-1})	54.6 ± 5.2^{Aa}	67.5 ± 12.9^{Aa}	52.1 ± 7.5^{Aa}	65.0 ± 6.0^{Aa}
q_p	0.45 ± 0.04^{Aa}	0.48 ± 0.04^{Aa}	0.51 ± 0.06^{Aa}	0.44 ± 0.07^{Aa}
NPQ	2.10 ± 0.38^{Aa}	2.52 ± 0.13^{Aa}	3.11 ± 0.99^{Aa}	2.46 ± 0.56^{Aa}
ETR_O (μmol m^{-2} s^{-1})	15.3 ± 3.0^{Aa}	15.4 ± 7.4^{Aa}	12.9 ± 4.1^{Aa}	14.7 ± 4.7^{Aa}

[1] Mean values ± SD ($n = 3$). Different capital letters indicate statistical differences ($p < 0.05$) for the daily temperature variation on the same rootstock, whereas different lowercase letters represent statistical differences between rootstocks over the same temperature range

As a measure of plant carbohydrate dynamics, we calculated the variation in Sta concentration during the experimental period. This index revealed the effects of thermal regimes on the source–sink relationship. The Sta consumption increased significantly in mature leaves when plants were grown under 15°C temperature variation, regardless of the rootstock (Fig. 4). No unique pattern of response to temperature variation was found between rootstocks when considering the variation of Sta in the stems and roots. The high daily temperature variation reduced by half the Sta consumption in stem tissues and increased (by almost twofold) the Sta consumption in the roots of plants grafted onto Rangpur lime (Fig. 4).

Discussion

Our data reveal that orange plants are sensitive to changes in daily temperature amplitude, as they show a significant enhancement of vegetative growth with increased temperature variation (Fig. 1). To our knowledge, this is the first report of the isolated effects of temperature variation on plant growth under nonlimiting conditions. In fact, previous papers have reported physiological and/or morphological changes in plants subjected to variations in both mean air temperature and daily temperature (Ribeiro and others 2004; Xu and Zhou 2005) and underestimated the isolated effects of daily temperature variation. Indirect evidence of the effects of temperature variation had been reported by Susanto and others (1992), with *Citrus grandis* plants showing a large total leaf area under high temperature variation. However, the physiological bases of improved vegetative growth were not clarified in that study.

Higher dry matter accumulation was measured in plant tissues that developed during the thermal treatment with 15°C temperature variation as compared with the treatment with 0°C temperature variation, regardless of rootstock (Fig. 1). Interestingly, the positive effects of the increased temperature variation were found in thermal regimes in which the same amount of heat energy was supplied, that is, around 245 degree-days. Such results suggest an increase in energy use efficiency for biomass production. Plant growth is a consequence of the overall carbon balance and involves photosynthesis, respiration, and carbon partitioning (Loomis and Amthor 1999; Amthor 2000). Because photosynthesis on either a dry matter basis or entire plant basis did not explain the growth response to thermal treatments (Table 1), our data indicate that the effects of temperature variation on plant growth were not mediated by improvements in photosynthetic rates.

Regarding respiration rates on a dry matter basis, nonsignificant changes were found due to temperature variation in both rootstocks. The only exception was for the diurnal respiration (Rd_D) of plants grafted on Swingle citrumelo, which increased under 15°C of daily variation (Table 1). However, this is not a true physiological response to temperature variation as evaluations were taken in different instantaneous temperatures (25°C in 25/25°C regime and 32.5°C in 32.5/17.5°C regime). This question is discussed again with physiological data taken under normalized conditions.

On the entire shoot basis, the temperature variation affected citrus respiration, and this effect varied according to the rootstock (Table 1). Whereas plants grafted onto Rangpur lime showed increases in both diurnal and nocturnal respiration rates, plants grafted onto Swingle citrumelo presented increases only in diurnal respiration. Because the plants showed increased dry matter production during the thermal treatment, we may argue that there is a stimulatory effect of temperature variation on growth respiration (Amthor 2000). Respiration is a critical

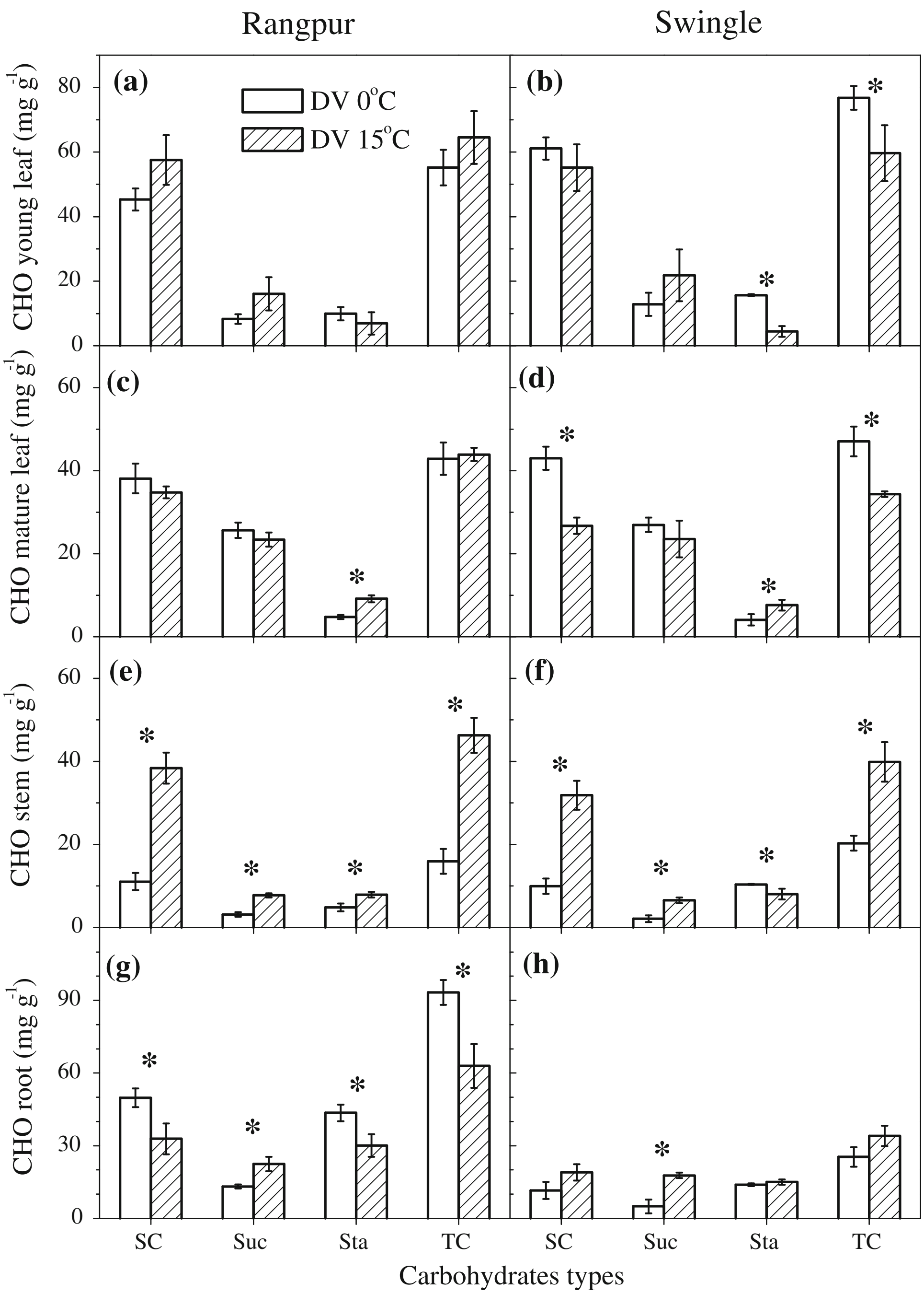

Fig. 3 Changes in the soluble carbohydrate (SC), sucrose (Suc), starch (Sta), and total nonstructural carbohydrate (TC) concentrations of Valencia orange trees grafted onto Rangpur lime (**a**, **c**, **e**, **g**) or Swingle citrumelo (**b**, **d**, **f**, **h**) rootstocks due to daily temperature variation (DV) of 0 and 15°C. **a**, **b** Young leaves developed during the thermal treatment. **c**, **d** Mature leaves. **e**, **f** Stems. **g**, **h** Roots. Plants were grown for 20 days under thermal regimes of 25/25°C (DV = 0°C) or 32.5/17.5°C (DV = 15°C). Asterisks indicate statistical difference ($p < 0.05$) between temperature amplitudes. Values shown are the means of three replications ± SD

physiological process related to metabolic energy production and to the availability of carbon skeletons for the synthesis of structural carbohydrates involved in plant growth.

Because respiration was affected, significant variation should occur in plant carbohydrates, mainly in storage organs. In fact, carbohydrate availability was also affected by the daily temperature variation, with stems and roots showing higher concentrations of Suc under 15°C temperature variation (Fig. 3e–h). Other responses indirectly related to sink demand were the reductions in Sta, SC, and TC in the roots of plants grafted onto Rangpur lime and grown under 15°C temperature variation (Fig. 2g). Rangpur roots have higher carbohydrate content than Swingle roots, and plants grafted onto the former rootstock showed larger increases in stem SC and TC under 15°C temperature variation. As carbohydrates available in storage organs are metabolized for the supply of sink demand, our data indicate that the growth of plants grafted onto Rangpur lime is more responsive to daily temperature amplitude because of its higher constitutive concentration of carbohydrates in stems and roots (Figs. 1a–d, 3e–h).

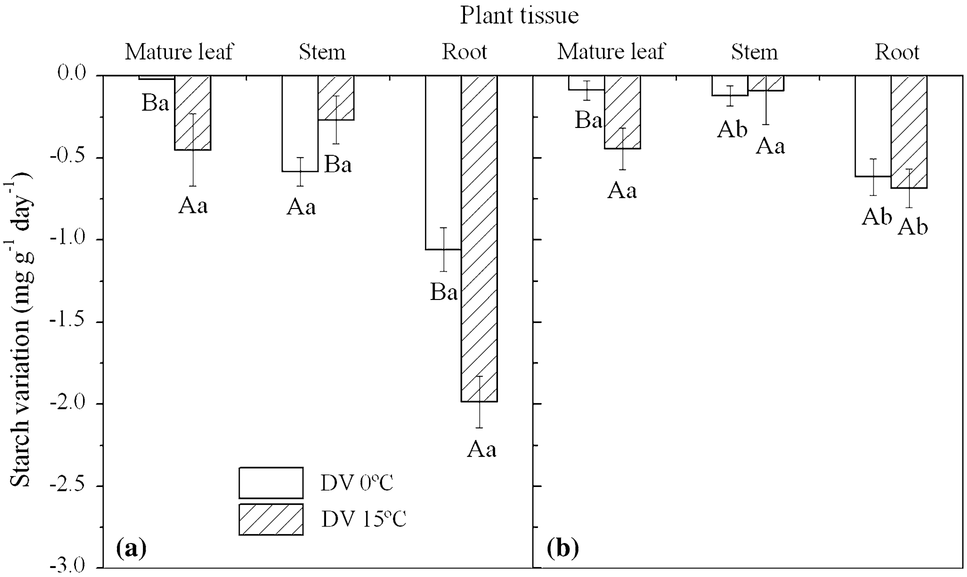

Fig. 4 Starch variation in mature leaves, stem, and roots of Valencia orange trees grafted onto Rangpur lime (**a**) or Swingle citrumelo (**b**) rootstocks arising from daily temperature variation (DV) of 0 and 15°C. Plants were grown for 20 days under thermal regimes of 25/25°C (DV = 0°C) or 32.5/17.5°C (DV = 15°C). Different capital letters indicate statistical differences ($p < 0.05$) between temperature amplitudes for the same rootstock, whereas lowercase letters represent differences between rootstocks for the same temperature amplitude. Values shown are the means of three replications ± SD. Negative values indicate starch consumption

According to Smith and Stitt (2007), the supply of carbon for plant growth requires an elaborate balance between Suc and Sta availabilities. Suc should meet the immediate sink demand during the diurnal period, whereas during the nocturnal period, Sta should anticipate the sink demand for the following day. As plant growth improved under 15°C temperature variation, the high availability of Suc in the stems and roots may be an important mechanism for supporting sink demand (Fig. 3e–h). The increases in the Sta concentration of mature leaves in plants grown under 15°C temperature variation may be essential to address the sink demand, which imposed a significant increase in Sta consumption in both rootstocks (Figs. 3c, d, 4). The twofold increase in the consumption of Sta stored in Rangpur roots under 15°C temperature variation also emphasizes the importance of the root system as a source of carbon and energy for plant growth (Fig. 4).

An interesting finding is the ability of Rangpur roots to accumulate and metabolize significant amounts of Sta when there is a sink demand. This relationship between stored carbohydrates and sink demand has previously been reported in citrus plants by Goldschmidt (1999), who found that carbon reserves were remobilized for use in growing organs. This process was more pronounced in plants grafted onto Rangpur lime. Accordingly, Quaggio and others (2004) found that the Rangpur lime rootstock induced more canopy growth than Swingle citrumelo. The low plant growth rate of trees grafted onto Swingle citrumelo is attributed to its genetic background, as the low growth rate is a characteristic inherited from the *Poncirus trifoliata* rootstock (Castle 2010).

The high demand for carbohydrates due to shoot growth under 15°C temperature variation can stimulate photosynthetic activity through the source–sink relationship (Iglesias and others 2002; Ribeiro and Machado 2007). Although current photosynthesis (Table 1) was not affected, normalized measurements at 25°C revealed interesting responses, indicating a true physiological change due to daily temperature variation (Fig. 2). Photosynthesis increased by 1.6 times when plants were grown under 15°C temperature variation, regardless of the rootstock. This improvement in photosynthetic capacity was supported by increases in Pn/Ci and ETR_C. Leaf nitrogen concentration was not limiting for citrus plants (Bondada and Syvertsen 2005; Mattos Jr. and others 2007) and varied around 32 mg g^{-1}. Because the proportion of nitrogen incorporated into Rubisco in C3 plants varies between 20 and 30% (Warren and Adams 2004; Feller and others 2008) and because the leaf nitrogen content was not modified by thermal treatments, we suggest that increases in PNUE and in photosynthesis were caused by more investment of nitrogen in Rubisco in plants growing under 15°C temperature variation.

The thermal regime with 15°C temperature variation improved plant growth and induced a systemic improvement in plant metabolism, with similar levels of plant photosynthesis maintained at 25 and 32.5°C (Table 1) and increased photosynthetic rates under normalized conditions (Fig. 2). According to Penfield (2008), the perception of temperature changes by plants is complex, particularly when the effects of small temperature changes on plant growth and physiology are considered. Given the proposed stimulation of photosynthesis by the source–sink relationship, we suggest that the growth of plants increased under 15°C temperature variation and that this effect is probably mediated by hormonal changes.

Recently, Penfield (2008) suggested gibberellin metabolism as an essential pathway for plant signaling under temperature changes. Gibberellins have an important role in thermoperiodism, as they improve plant growth when the daily temperature increases (Moe 1990; Grindal and others 1998; Penfield 2008). This response is based on reductions in gibberellin catabolism and increases in gibberellin biosynthesis through the enhancement of gene expression (Vidal and others 2003; Stavang and others 2005). Plants grown under 15°C temperature variation had higher SC contents in their stems and increased photosynthesis under normalized conditions (Figs. 2, 3). The positive associations among carbohydrate availability, photosynthesis, and gibberellins found by Moreno and others (2011) provide additional evidence for the effects of temperature amplitude on the hormonal balance of citrus plants.

The effects of thermoperiodism on the biological clock should also be considered in future research because of the sensitivity of the biological clock to temperature (Penfield 2008). Some studies have indicated improvements in plant performance under changing environments, with circadian genes regulating Sta metabolism, biomass production (Ni and others 2009), and hypocotyl elongation (Downson-Day and Millar 1999). Dodd and others (2005) have suggested that the synchrony between plant biological rhythms and environmental changes is an important element in metabolism regulation, with synchronized plants fixing more CO_2 and growing faster than plants not synchronized with the surrounding environment.

In conclusion, an increase in the daily temperature variation affects the carbon metabolism of young citrus trees, with plants using the carbon stored in the mature leaves and roots and the energy released by respiration for the biosynthesis of vegetative structures such as leaves and branches. Consequently, there is a significant increase in leaf area with temperature variation of 15°C, and the actual plant photosynthesis is maintained similar under thermal regimes (day/night) of 25/25°C and 32.5/17.5°C. In addition to the stimulatory effect of the source–sink relationship on photosynthesis, gibberellin metabolism likely regulates plant growth. The rootstock affected the canopy sensitivity to daily temperature variation, with Rangpur lime improving plant growth through carbohydrate availability in the roots.

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