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Low substrate temperature imposes higher limitation to photosynthesis of orange plants as compared to atmospheric chilling

C.M.A. SANTOS, R.V. RIBEIRO, J. R. MAGALHÃES FILHO, D.F.S.P. MACHADO,
and E.C. MACHADO[+]

*Laboratory of Plant Physiology "Coaracy M. Franco", Agronomic Institute (IAC), P.O. Box 28, 13012-970,
Campinas/SP, Brazil*

Abstract

The aim of this study was to evaluate the effects of low air temperature during nocturnal (T_N) and diurnal (T_D) periods as well as the substrate temperature (T_S) on photosynthesis of 'Valencia' orange tree grafted on Rangpur lime rootstock. The experiment was carried out in a growth chamber with seven-month-old plants. The plants were exposed to the following temperature regimes: low substrate temperature (LT_S, with: T_D = 28°C, T_N = 20°C, T_S = 10°C); low air temperature during night (LT_N, with: T_D = 28°C, T_N = 10°C, T_S = 26°C); low temperature during nighttime and also low substrate temperature ($LT_{SN,}$ with: T_D = 28°C, T_N = 10°C, T_S = 10°C); low air temperature during both diurnal and nocturnal periods (LT_{ND}, with: T_D = 17°C, T_N = 10°C, T_S = 26°C); and finally to low air temperature (night and day) and low substrate temperature (LT_{SND}, with: T_D = 17°C, T_N = 10°C, T_S = 10°C). As reference (control), plants were subjected to T_D = 28°C, T_N = 20°C, and T_S = 26°C. Measurements of leaf gas exchange, photochemical activity and carbohydrate concentrations were performed after six days of exposure to each thermal treatment. Compared to the control, all thermal regimes caused reductions in photosynthesis due to diffusive and metabolic limitations. The photoinhibition was transient in plants exposed to night and substrate low temperatures, whereas it was severe and chronic in plants subjected to chilling during the diurnal period. However, the lowest photosynthesis was observed in plants with low substrate temperature of 10°C (in LT_S, LT_{SND} and LT_{SN} treatments), regardless of air temperature. The occurrence of cold night and/or its combination with low substrate temperature caused accumulation of starch in leaves. When considering carbohydrate concentrations in stems and roots, it was not possible to establish a clear response pattern to chilling. In conclusion, the low substrate temperature causes a greater reduction of CO_2 assimilation in citrus plants as compared to the occurrence of low air temperature, being such response a consequence of diffusive and biochemical limitations.

Additional key words: carbohydrates; chlorophyll fluorescence; *Citrus sinensis*; gas exchange.

Introduction

One of the major citrus-producing areas in Brazil is located at the Central region of the State of Sao Paulo, where the air temperature of the coldest month varies between –3°C and 18°C. Plants do not face a regular dry season and the temperature of the warmest month is above 22°C (Ribeiro *et al.* 2006). In such areas, the soil temperature (10-cm depth) ranges from 9°C in the winter to 27°C in the summer season (Ribeiro 2006). During the winter, the growth of citrus plants is nonapparent ('winter rest'), being this response ascribed to the air low air

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[+]Corresponding author; fax: +55 19 21370706; e-mail: caruso@iac.sp.gov.br

Abbreviations: C – control; C_C – CO_2 concentration in chloroplast; C_i – intercellular CO_2 concentration; g_m – mesophyll conductance; g_s – stomatal conductance; ETR – apparent electron transport rate; F_V/F_M – maximum quantum efficiency of PSII; $\Delta F/F_M$'– effective quantum efficiency of PSII; LT_N – low nocturnal air temperature; LT_{ND} – low air temperature during both nocturnal and diurnal periods; LT_S – low substrate temperature; LT_{SN} – low substrate temperature and low nocturnal air temperature; LT_{SND} – low substrate temperature and cold conditions during both diurnal and nocturnal periods; NPQ – nonphotochemical quenching; P_N – leaf net CO_2 assimilation rate; P_{NI} – diurnal CO_2 assimilation; PPFD – photosynthetic photon flux density; PSII – photosystem II; q_P – photochemical quenching; T_D – diurnal air temperature; T_N – nocturnal air temperature; T_S - substrate temperature.

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temperature (Reuther 1977, Ramos *et al.* 2010). However, as evergreen plants, orange trees are photosynthetically active throughout the year. In a subtropical climate citrus plants show higher photosynthetic performance in the spring and summer seasons, when carbon uptake is two to three-fold higher than in the winter (Machado *et al.* 2002, Ribeiro and Machado 2007, Ribeiro *et al.* 2009a,b). Among the environmental factors that regulate the physiology and growth of orange trees, the temperature has a decisive role on photosynthesis (Machado *et al.* 2002, 2010; Magalhães Filho *et al.* 2009), also affecting the vegetative growth (Ramos *et al.* 2010), flowering and fruit production (Habermann and Rodrigues 2009).

Low CO_2 assimilation occurs in the winter even on days with diurnal air temperature varying between 20°C and 26°C (Ribeiro *et al.* 2009a), *i.e.* close to the optimum temperature range for citrus between 25°C and 30°C (Machado *et al.* 2005). Ribeiro *et al.* (2009a) suggested that the reduction of CO_2 assimilation in winter would be due to the low night and soil temperatures. In addition, low photosynthesis may be related with reduced plant growth rate as a consequence of low temperature and the decreased day length in winter (Goldschmidt and Golomb 1982, Bevington and Castle 1985, Machado *et al.* 2002, Ribeiro and Machado 2007). In fact, low sink activity decreases the demand for photoassimilates, which can lead to reductions in photosynthesis of evergreen species such as citrus (Paul and Foyer 2001, Iglesias *et al.* 2002). During the winter, plants face cold nights and also low temperatures during diurnal period, when there is high solar radiation. In such situation, the occurrence of photoinhibition is possible during daytime (Long *et al.* 1994), being an additional limitation to photosynthesis.

We have found some studies evaluating the seasonal changes in citrus photosynthesis (Machado *et al.* 2002, Ribeiro *et al.* 2009a,b), the effects of low substrate temperature (Magalhães Filho *et al.* 2009) and low night temperature (Allen *et al.* 2000, Machado *et al.* 2010) on photosynthesis of tree species. However, the relative importance of low air temperature in relation to low substrate temperature for photosynthesis is not yet understood in citrus species, an important crop grown worldwide.

The objective of this research was to compare the effects of low air temperature (day/night) and low substrate temperature in some physiological variables related to photosynthesis in orange plants. Carbohydrate concentration in leaves, stems, and roots and diffusive, biochemical and photochemical aspects of photosynthesis were evaluated in plants subjected to various thermal regimes, in which low air temperature occurred during diurnal and/or nocturnal periods in combination or not with low substrate temperature.

Materials and methods

Plants and thermal treatments: The experiment was carried out with seven-month-old 'Valencia' sweet orange [*Citrus sinensis* (L.) Osb.] scions grafted on Rangpur lime (*Citrus limonia* Osb.) rootstocks. The plants were grown in plastic pots (7 L) containing soilless substrate (95% pine bark and 5% vermiculite). The plants were watered daily and 200 mL of a nutrient solution was supplied twice a week (Magalhães Filho *et al.* 2009). Before the beginning of the experimental period, plants had a total dry mass of 24.3 ± 2.7 g, being 62 ± 2%, 19 ± 2%, and 18 ± 2% allocated into roots, branches and leaves, respectively.

The plants were grown under greenhouse conditions during 30 days, where the air temperature varied between 18°C and 35°C and the maximum photosynthetic photon flux density (PPFD) was about 900 μmol m^{-2} s^{-1}. Then, the plants were transferred to a growth chamber (*PGR14*, *Conviron*, Winnipeg, Manitoba, Canada) under the following conditions: photoperiod of 12 h, air temperature of 25 ± 1/20 ± 1°C (day/night), air vapor pressure deficit (VPD) of 1 kPa and PPFD of 800 μmol m^{-2} s^{-1}. After the first day under the conditions described above, the plants were subjected to six thermal treatments resulting from the combination of two substrate temperatures (T_S, 10°C and 26°C), two nocturnal air temperatures (T_N, 10°C and 20°C) and two diurnal air temperatures (T_D, 17°C and 28°C), as described in Table 1. One hour prior to the end of night period, the air temperature was increased to 28 ± 1°C in the C, LT_N, LT_S, and LT_{SN} treatments or to 17 ± 1°C in the LT_{ND} and LT_{SND} treatments. With exception of the temperature, the other environmental conditions in the growth chamber were kept constant throughout the experimental period.

During six consecutive days, the substrate temperature was maintained at 10ºC or 26ºC, whereas the diurnal and nocturnal temperatures remained constant (10ºC or 20ºC for T_N, and 17ºC or 28ºC for T_D) during a period of 12 h. The substrate temperature was controlled by immersing the pots in a container filled with water. The pots were involved in plastic bags to avoid a direct contact of cold water with roots and also to avoid hypoxia. The substrate temperature was monitored with a digital thermometer in all treatments, with a maximum variation of ± 0.5°C throughout the experimental period.

Gas exchange and chlorophyll fluorescence: On the sixth day of treatment, measurements of leaf gas exchange were taken from 8:00 to 18:00 h, in 2-h intervals. Data were grouped in the measurements during the morning (8:00–12:00 h) and afternoon (14:00–18:00 h). Leaf CO_2 assimilation (P_N), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were evaluated with a portable photosynthesis analyzer (*LI-6400, LI-COR,* Lincoln, NE, USA) in fully expanded two-month-

Table 1. Description of temperature conditions in which citrus plants were subjected for six days. C – control; LT_S – low substrate temperature; LT_N – low nocturnal air temperature; LT_{SN} – low substrate and low nocturnal air temperature; LT_{ND} – low air temperature during both nocturnal and diurnal periods; LT_{SND} – low substrate temperature and low diurnal and nocturnal temperature, T_D – diurnal temperature, T_N – nocturnal temperature, T_S – substrate temperature.

Thermal treatments	T_D [°C]	T_N [°C]	T_S [°C]
C	28	20	26
LT_S	28	20	10
LT_N	28	10	26
LT_{SN}	28	10	10
LT_{ND}	17	10	26
LT_{SND}	17	10	10

old leaves. The conditions inside the *LI-6400* chamber were the same as the inside the growth chamber, so that the measurements were taken under identical environmental conditions. The diurnal CO_2 assimilation (P_{NI}) was calculated from the integration of the diurnal curve of P_N, being expressed in mmol m^{-2} d^{-1}. Mesophyll conductance (g_m) and CO_2 concentration in the chloroplast ($C_C = C_i - g_m/P_N$) were estimated according to Harley *et al.* (1992), using the variable J method. We calculated the instantaneous carboxylation efficiency by the ratio P_N/C_C.

As measurements of gas exchange, photochemical activity was evaluated during the morning (8:00–12:00 h) and afternoon (14:00–18:00 h). Measurements were also taken in dark-adapted tissues before the beginning of the light period (6:00 h) and at 16:00 h, after 30 min of dark adaptation. Chlorophyll fluorescence emission was evaluated with a modulated fluorometer (*PAM-2000*, *Walz*, Effeltrich, Germany) by the method of saturation pulse ($\lambda < 710$ nm, PAR ~ 10,000 μmol(photon) m^{-2} s^{-1}, 0.8 s). The minimum (F_0) and maximum (F_M) fluorescence signals were measured after 30-min dark adaptation. After 1 h under light conditions (PPFD of 900 μmol m^{-2} s^{-1}), the instantaneous- (F) and maximum (F_M') fluorescence signals were measured. From the fluorescence signals described above, we calculated the maximum- (F_V/F_M, where $F_V = F_M - F_0$) and effective ($\Delta F/F_M$', where $\Delta F = F_M' - F$) quantum efficiency of photosystem II (PSII), the apparent electron transport rate (ETR = $\Delta F/F_M' \times$ PPFD $\times 0.5 \times 0.84$) and the nonphotochemical- [NPQ = $(F_M - F_M')/F_M'$)] and photochemical [$q_P = (F_M' - F)/(F_M' - F_0')$] quenching (Roháček 2002). For the calculation of ETR, we considered equal distribution of electrons between photosystem (PS) I and PSII (0.5) and a light absorption of 0.84 (Roháček 2002). F_0' was measured using far-red light ($\lambda = 735$ nm, PPFD ~ 50 μmol(photon) m^{-2} s^{-1}, 3.0 s). We estimated the number of electrons transported through PSII per molecule of CO_2 fixed as the ratio ETR/P_G (Erismann *et al.* 2008), where $P_G = P_N + R_D$, P_G is the gross assimilation of CO_2 and R_D is mitochondrial respiration measured after 30 min under dark condition.

Carbohydrate determinations: The carbohydrate concentration in leaves, stem and roots were quantified after six days of thermal treatment. Tissue samples were harvested at 6:00 h, frozen (–70°C) and then stored at –20°C. The leaves in which carbohydrate was quantified were similar in age and in canopy position to those assessed for the gas exchange and chlorophyll fluorescence measurements. Sucrose and soluble sugars were extracted through the MCW method (Bieleski and Turner 1966) and quantified according to Handel (1968) for sucrose and Dubois *et al.* (1956) for soluble sugars. Starch was quantified using the enzymatic method proposed by Amaral *et al.* (2007).

Statistical analyses: Data were subjected to the analysis of variance, considering the six thermal treatments and evaluation time (morning and afternoon) as sources of variation. Mean values of 12 (gas exchange and chlorophyll fluorescence) and 4 (carbohydrates) repetitions were compared by the *Tukey* test ($p<0.05$). Statistical analyses were performed using the *SisVar 5.0* software (*Ufla*, Lavras, Brazil). The overall plant response to thermal treatments was also evaluated through a radar graph.

Results

The thermal regimes affected leaf gas exchange significantly (Fig. 1), with control plants showing the highest rates of CO_2 assimilation (P_N) compared to the other treatments (Fig. 1*A*). Although environmental conditions have remained constant, the P_N of control, LT_S and LT_N plants were higher in the morning than in the afternoon. When plants were subjected to cold diurnal and nocturnal periods (LT_{ND}), P_N decreased only in relation to the control and LT_N plants. The effects of combining cold night and low substrate temperature were cumulative and P_N was significantly lower in LT_{SN} as compared to LT_S and LT_N (Fig. 1*A*). Differences among thermal treatments were more evident when evaluating the diurnal-integrated CO_2 assimilation (P_{NI}), with plants showing reductions of 28%, 41%, 60%, 69%, and 77% when subjected to LT_N, LT_{ND}, LT_S, LT_{SN}, and LT_{SND} treatments, respectively (data not shown).

Regardless daytime and place (air/substrate), the low temperature decreased the diffusion of gases through the stomata, as indicated by low g_s (Fig. 1*B*). However, those reductions were more pronounced in plants subjected to low substrate temperature, *i.e.*, LT_S, LT_{SN}, and LT_{SND}

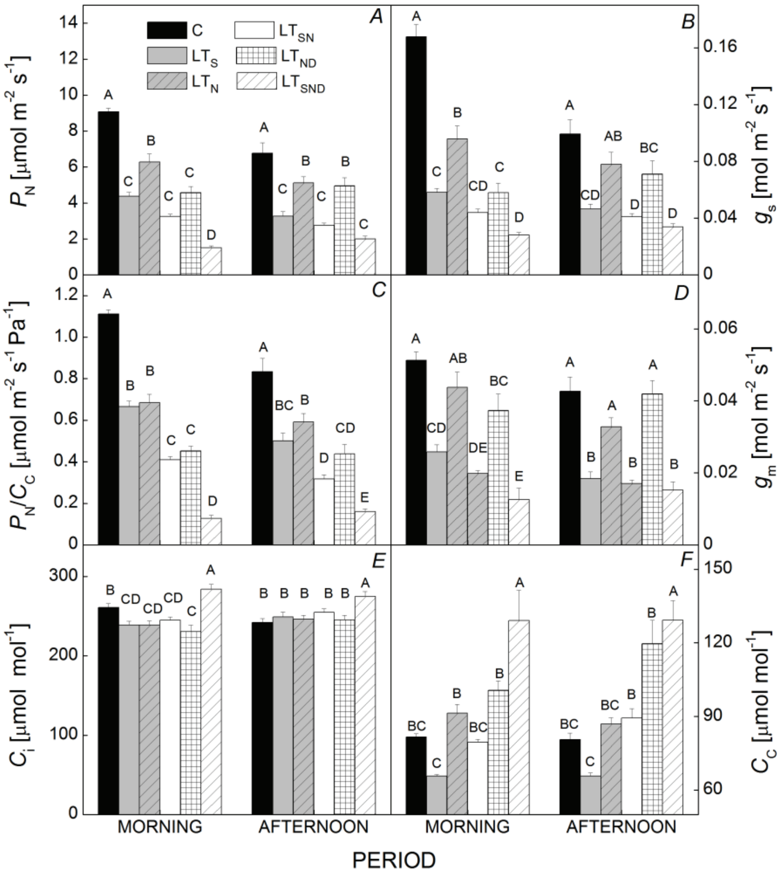

Fig. 1. Leaf CO_2 assimilation (P_N, *A*), stomatal- (g_s, *B*) and mesophyll (g_m, *C*) conductance, apparent carboxylation efficiency (P_N/C_C, *D*), concentration of CO_2 in intercellular spaces (C_i, *E*) and in chloroplasts (C_C, *F*) of 'Valencia' orange trees as affected by thermal regimes (diurnal/nocturnal/substrate): control (28/20/26°C); LT$_S$ (28/20/10°C); LT$_N$ (28/10/26°C); LT$_{SN}$ (28/10/10°C); LT$_{ND}$ (17/10/26°C); and LT$_{SND}$ (17/10/10°C). *Bars* indicate the mean value of 12 repetitions ± SD. *Different letters* indicate significant differences between treatments by the *Tukey* test ($p<0.05$).

treatments. There was a linear relationship between g_s and P_N ($r = 0.9$, $p<0.001$, $P_N = 1.01 + 52.88\ g_s$), suggesting that the low P_N was related to the decrease of g_s. The response of g_m to thermal treatments was similar to g_s, indicating a strong dependence in relation to substrate temperature as compared to air temperature. Thus, the decrease of P_N was related to increases in the resistance to CO_2 diffusion from the atmosphere to the chloroplasts (Fig. 1*A*,*B*,*C*).

The apparent carboxylation efficiency (P_N/C_C) and P_N had similar responses to thermal treatments, with the highest P_N/C_C values being observed in control plants (Fig. 1*A*,*D*). However, no significant differences were found between LT$_S$ and LT$_N$ treatments and also between LT$_{SN}$ and LT$_{ND}$. C_i was increased due to low temperature only in plants subjected to LT$_{SND}$ treatment (Fig. 1*E*). In general, thermal treatments imposing low temperature during day and night caused large increases in C_C (Fig. 1*F*).

The maximum quantum efficiency of PSII (F_V/F_M) was affected by thermal regimes, with F_V/F_M of plants subjected to LT$_S$, LT$_N$ and LT$_{SN}$ varying around 0.7 (Fig. 2*A*). A significant reduction was observed in F_V/F_M of plants subjected to LT$_{ND}$ and LT$_{SND}$, reaching 0.63 and 0.51 respectively and suggesting the occurrence of photoinhibition (Fig. 2*A*). The decline in F_V/F_M in both treatments was associated with an intense reduction of F_M (data not shown). The highest effective quantum efficiency of PSII ($\Delta F/F_m'$) was observed in the control plants and in those ones under LT$_S$ treatment (data not shown), causing higher rates of apparent electron transport (ETR) as compared to the other plants (Fig. 2*B*). As plants had a significant reduction in P_N (Fig. 1*A*) and maintained the photochemical activity (Fig. 2*A*,*B*), the highest ETR/P_G was found in LT$_S$ treatment (Fig. 2*C*). Reductions in ETR/P_G were found only in plants grown under LT$_{ND}$ and LT$_{SND}$ conditions (Fig. 2*C*). The photochemical quenching of fluorescence (q_P) was decreased significantly in LT$_N$, LT$_{SN}$, LT$_{ND}$ and LT$_{SND}$ (Fig. 2*D*). In the morning, the nonphotochemical quenching of fluorescence (NPQ) increased only in plants of LT$_{SND}$ treatment (Fig. 2*E*), remaining similar in the other treatments. In general, there was an increase in NPQ during the afternoon and the highest NPQ values were found in LT$_{SN}$, LT$_{ND}$, and LT$_{SND}$ treatments (Fig. 2*E*).

Aiming to evaluate the overall photosynthetic response to the thermal treatments, we presented the relative changes among physiological variables of citrus plants in a radar graph (Fig. 3). Data analysis revealed that the most deleterious treatment was the LT$_{SND}$, which caused severe impairment of citrus photosynthesis (Fig. 3). The thermal treatments LT$_S$ and LT$_{SN}$ also induced significant restriction of photosynthetic activity. The plants subjected to LT$_S$ presented the highest

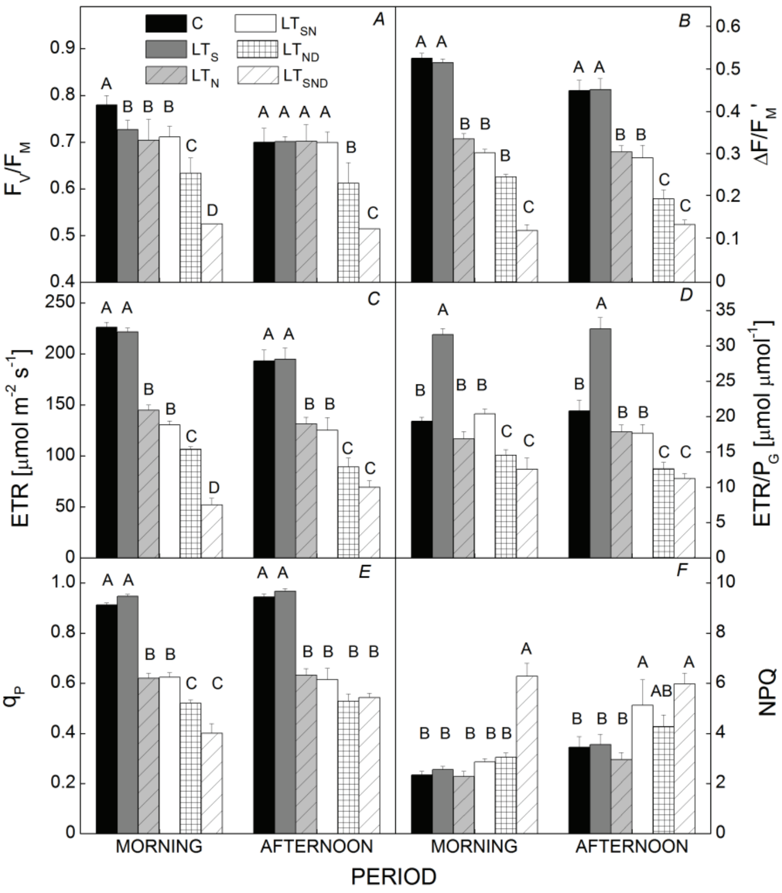

Fig. 2. Potential- (F_V/F_M, *A*) and effective ($\Delta F/F_M$', *B*) quantum efficiency of PSII, apparent electron transport rate (ETR, *C*) and ratio between ETR and the gross CO_2 assimilation (ETR/P_G, *D*) photochemical- (q_P, *E*) and nonphotochemical (NPQ, *F*) quenchings of 'Valencia' orange trees as affected by thermal regimes (diurnal/nocturnal/substrate): control (28/20/26°C); LT_S (28/20/10°C); LT_N (28/10/26°C); LT_{SN} (28/10/10°C); LT_{ND} (17/10/26°C); and LT_{SND} (17/10/10°C). *Bars* indicate the mean value of four (in *A*) or 12 (*B*, *C*, *D*, *E*, and *F*) repetitions ± SD. *Different letters* indicate significant differences between treatments by the *Tukey* test ($p<0.05$).

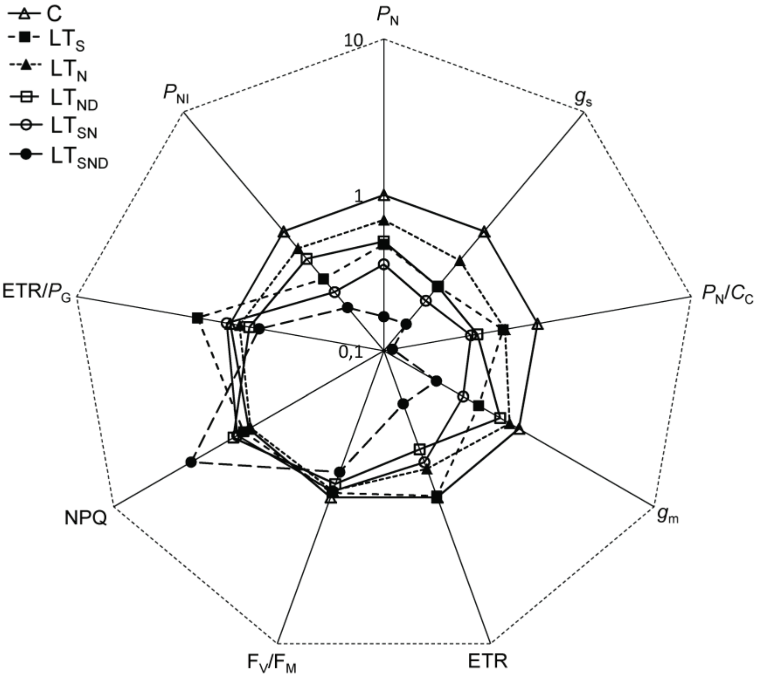

Fig. 3. Overall representation of physiological responses to thermal treatments. Data refer to the relative changes of the following variables: leaf CO_2 assimilation rate (P_N), diurnal-integrated CO_2 assimilation (P_{NI}), stomatal conductance (g_s), mesophyll conductance (g_m), instantaneous carboxylation efficiency (P_N/C_C), maximum quantum efficiency of PSII (F_V/F_M), apparent electron transport rate (ETR), nonphotochemical quenching (NPQ), ratio between apparent electron transport rate and gross CO_2 assimilation (ETR/P_G). C (control, 28/20/26°C); LT_S (28/20/10°C); LT_N (28/10/26°C); LT_{SN} (28/10/10°C); LT_{ND} (17/10/26°C); and LT_{SND} (17/10/10°C).

ETR/P_G (Fig. 3), whereas the plants under LT_{SND} conditions exhibited the highest NPQ, an important mechanism of photoprotection under stressful conditions.

The amount of soluble sugars in leaves, stems, and roots was not significantly affected by thermal treatments (Fig. 4*A*,*D*,*G*). As for soluble sugars, nonsignificant changes were found in sucrose content of stem tissues (Fig. 4*E*). However, sucrose contents in leaves and roots were increased in LT_N and LT_{SN} treatments (Fig. 4*B*,*H*). The leaf starch content was increased in LT_N and LT_{SN} treatments (Fig. 4*C*) and three situations were noticed when considering starch in stem tissues: (*1*) the highest contents occurred in LT_S; (*2*) intermediate contents occurred in LT_N, LT_{SN}, and LT_{ND}; and (*3*) the lowest contents were found in control and LT_{SND} plants (Fig. 4*F*). In roots, increases in starch content were observed in plants subjected to LT_S, LT_N, and LT_{ND} treatments (Fig. 4*I*).

Discussion

Limitations to photosynthesis caused by thermal regimes: In general, decreases in P_N were more pronounced in thermal treatments with low substrate temperature (LT_S, LT_{SN}, and LT_{SND}), being a consequence of low g_s, g_m and P_N/C_C (Figs. 1, 3). Increases in stomatal limitation due to low substrate temperature can be related to hydraulic and/or chemical signals (Allen and Ort 2001). Cold substrates can cause increases of abscisic acid synthesis in roots, which may be transported to the leaves and cause stomatal closure. Also, low substrate temperature can decrease cytokinin transport and/or affect cytokinin metabolism in leaves (Veselov *et al*. 2005, Zhou *et al*. 2007, Zhang *et al*. 2008). Regarding hydraulic signaling, low substrate temperature causes an increase in water viscosity due to increased hydrogen bond strength, reducing the plant hydraulic conductivity (Kramer 1983). In addition, it is known that low temperature decreases the activity of aquaporins in the plasma membrane of roots (Maruel *et al*. 2008), also decreasing plant hydraulic conductivity, g_m and the water absorption from soil or substrate (Tournaire-Roux *et al*. 2003, Magalhães Filho *et al*. 2009). Another consequence of low temperature is the suberization of endoderm cells, an additional factor leading to reduction in root hydraulic conductivity (Lee *et al*. 2005).

The root and/or air cooling caused metabolic limitation to photosynthesis (lower P_N/C_C, Figs. 1*D*, 3), which can be related to the lower carboxylation efficiency and/or lower RuBP regeneration. It is known that those biochemical reactions in citrus are sensitive to low night temperature (Machado *et al*. 2010) and/or low substrate temperature (Magalhães Filho *et al*. 2009), with plants being subjected to both limiting factors under subtropical conditions (Ribeiro *et al*. 2009a,b). Increases of C_i

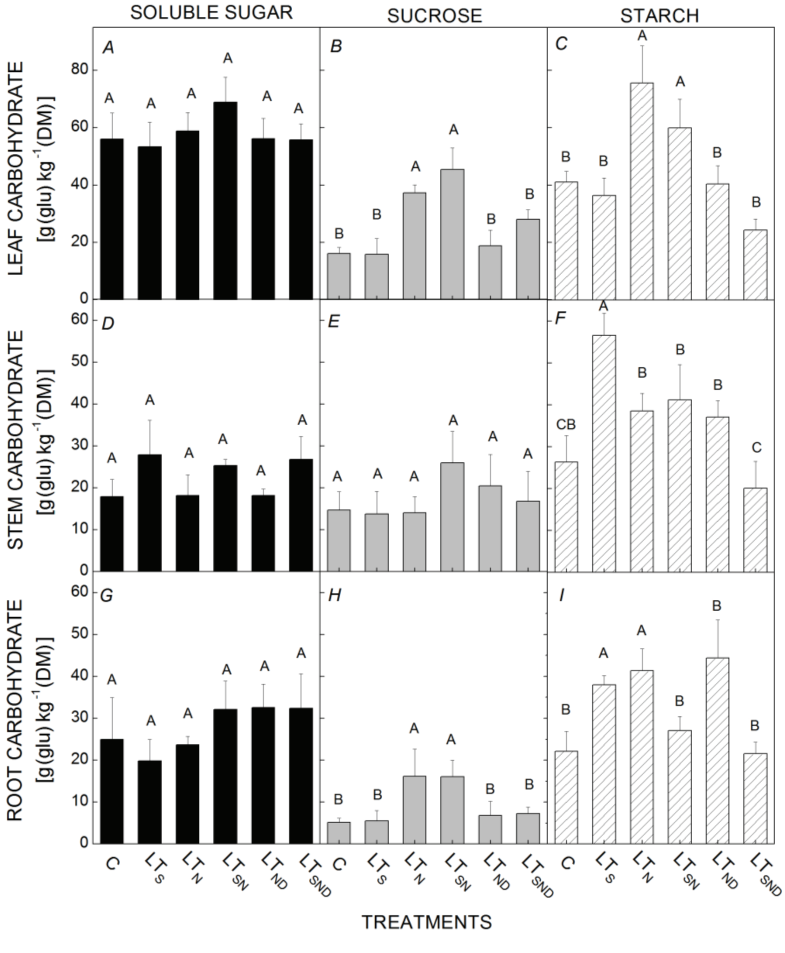

Fig. 4. Concentrations of soluble sugars (*A*,*D*,*G*), sucrose (*B*,*E*,*H*) and starch (*C*,*F*,*I*) in leaves (*A*,*B*,*C*), stems (*D*,*E*,*F*) and roots (*G*,*H*,*I*) of 'Valencia' sweet orange trees as affected by thermal regimes (diurnal/nocturnal/substrate): control (28/20/26°C); LT_S (28/20/10°C); LT_N (28/10/26°C); LT_{SN} (28/10/10°C); LT_{ND} (17/10/26°C); and LT_{SND} (17/10/10°C). *Bars* indicate the mean value of four repetitions ± SD. *Different letters* indicate significant differences between treatments by the *Tukey* test ($p<0.05$).

occurred only in plants subjected to LT_{SND} regime; a response associated to low g_m (Fig. 1). Despite low g_m, the amount of C_C was higher in LT_{SND} treatment than in control plants, which in turns is a consequence of sharp decline in P_N/C_C (Fig. 1).

Decreases in P_N of LT_{SN} and LT_{SND} plants were more pronounced than in LT_S, suggesting a cumulative effect when plants are additionally exposed to chilling during nocturnal and diurnal periods (Fig. 1*D*).

Plants subjected to LT_S, LT_N, and LT_{SN} treatments exhibited a slight photoinhibition (Critchley 1998); however, such reduction of potential photochemistry and also decreases in effective quantum efficiency of PSII did not limit P_N. In fact, ETR was sufficient for CO_2 fixation, with ETR/P_G values higher than 10 in all thermal treatments (Fig. 2*B,C*). The decrease of F_V/F_M ensures continuity of the PSII functionality, maintaining a high amount of reduced Q_A^- (Osmond and Grace 1995, Allen and Ort 2001). Excessive radiation induces the synthesis of anteraxanthin and zeaxanthin from violaxanthin, which are correlated with an increase of NPQ and decline of F_V/F_M (Long *et al.* 1994). The energy dissipation through xanthophylls cycle and the reduction of energetic pressure through F_V/F_M decline are interpreted as strategies for photoprotection. However, we may consider that such mechanism was efficient only in plants of the LT_S treatment, which showed photochemical activity similar to the control plants (Figs. 2, 3).

High q_P value in plants under LT_S and control conditions indicates a high efficiency of consumption of ATP and NADPH by coupled biochemical reactions. However, the ratio ETR/P_G significantly higher in LT_S treatment (Fig. 2*C*) indicates an increased proportion of electrons driven to alternative sinks, such as photorespiration and Mehler reaction (Allen and Ort 2001, Medina *et al.* 2002, Ribeiro *et al.* 2009b). The activity of alternative electron sinks allows the reoxidation of Q_A, maintaining the photochemical activity even with reductions in photosynthesis (Asada 2000). Magalhães Filho *et al.* (2009) also observed a significant increase in the activity of alternative electron sinks with small variation of NPQ in plants subjected to root chilling, as noticed herein (Fig. 2*C,E*).

The nonsignificant changes in ETR/P_G and concomitant decreases in q_P and ETR when comparing the control plants to those ones subjected to LT_N and LT_{SN} treatments were surprising responses (Fig. 2*B,C,D*), suggesting an internal regulation for maintaining a balance between biochemical and photochemical activities (Fig. 2).

Effects of thermal regimes on carbohydrate metabolism: Leaf concentration of soluble sugars was similar in all treatments; however there were differences when considering the concentration of sucrose and starch in leaves (Fig. 4). The increases of leaf sucrose and starch concentrations in plants of LT_N and LT_{SN} treatments (Fig. 4*B,C*) were probably related to the inhibition of sucrose transport to growing tissues and/or changes in carbon partitioning for starch synthesis (Foyer 1988, Iglesias *et al.* 2002, Ribeiro and Machado 2007).

The accumulation of starch in roots of plants in LT_{ND} (Fig. 4*I*) may be explained by the low demand for carbohydrates by growing shoots and leaves under low air temperature, being the carbon driven to the roots (Goldschmidt and Koch 1996). In fact, it is known that the citrus growth is reduced when daytime temperature varies around 17ºC and nighttime one around 10ºC (Goldschmidt and Golomb 1982, Bevington and Castle 1985).

Leaf carbohydrates in plants subjected to LT_S treatment were similar to control plants (Fig. 4*A,B,C*), even with a significant decrease in P_N (Fig. 1*A*). As starch contents in stems and roots were higher in the plants of LT_S treatment when compared to the control ones, we may argue that the production of photoassimilates was higher than the consumption (Fig. 1*A*).

Root growth occurs continuously, varying only the growth rate depending on water availability, aeration and temperature. According to Castle (1980), temperature should be above 13°C and below 36°C for citrus plants. As root system represents around 60% of plant dry matter, the main sink of photoassimilates was probably inhibited by temperature of 10°C.

In general, the carbohydrate concentration in plant tissues depends on the organ's size as well as on the balance between supply and utilization of photosynthetic products. The thermal regimes studied herein affected differently both components of such balance and a clear response pattern was not identified when considering our treatments and plant carbohydrates. In fact, the chilling effects in orange trees cannot be evaluated by punctual analysis of carbohydrate concentration in citrus organs, being necessary the study of carbohydrate variation for clarifying the source-sink relationship under low temperature.

From controlled to field conditions: Thermal treatments herein correspond to some situations that plants may face under field conditions. Cold nights occur during the winter with the arrival of cold air masses in growing regions. Under such conditions (similar to LT_N treatment) decreases in photosynthetic production were reported even if the subsequent days have nonlimiting temperatures (Ribeiro and Machado 2007, Ribeiro *et al.* 2009a,b). Low temperatures during diurnal and nocturnal periods and high soil temperature occur during the transition from summer to autumn. This situation was simulated by LT_{ND} treatment, in which photosynthesis was reduced in 40% as compared to the control condition. Low soil temperatures and sunny days with high temperature can occur in late winter, when air temperature is rising but soil is cold yet. Such condition was simulated with the LT_S treatment, in which the substrate temperature was 10ºC. Finally, the most deleterious situation was represented by LT_{SND} treatment (Fig. 3), in which plants faced low air and substrate temperatures as frequently occurs during a typical winter day under subtropical climates. Due to LT_{SND} treatment, plants showed a reduction of 77% in photosynthesis as compared to the control condition. Similar results were observed by Ribeiro *et al.* (2009a,b), evaluating the seasonal variation of P_{NI} in young citrus trees. On average, the low substrate temperature reduced by a half the photosynthesis of citrus trees if compared LT_S to control, LT_{SN} to LT_N, and LT_{SND} to LT_{ND}.

Conclusions: The low substrate temperature causes a greater reduction to citrus CO_2 assimilation as compared to the low air temperature, being this response induced by diffusive and biochemical limitations. Chilling affects negatively the photochemical activity; however, the apparent electron transport rates do not impose extra limitation to CO_2 assimilation under low temperature. The content of carbohydrates in citrus organs is not a good indicator of chilling effects in orange trees.

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