Photosynthesis and water relations of well-watered orange plants as affected by winter and summer conditions

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Abstract

The aim of this study was to evaluate how the summer and winter conditions affect the photosynthesis and water relations of well-watered orange trees, considering the diurnal changes in leaf gas exchange, chlorophyll (Chl) fluorescence, and leaf water potential (Ψ) of potted-plants growing in a subtropical climate. The diurnal pattern of photosynthesis in young citrus trees was not significantly affected by the environmental changes when compared the summer and winter seasons. However, citrus plants showed higher photosynthetic performance in summer, when plants fixed 2.9 times more CO_2 during the diurnal period than in the winter season. Curiously, the winter conditions were more favorable to photosynthesis of citrus plants, when considering the air temperature (< 29 ºC), leaf-to-air vapor pressure difference (< 2.4 kPa) and photon flux density (maximum values near light saturation) during the diurnal period. Therefore, low night temperature was the main environmental element changing the photosynthetic performance and water relations of well-watered plants during winter. Lower whole-plant hydraulic conductance, lower shoot hydration and lower stomatal conductance were noticed during winter when compared to the summer season. In winter, higher ratio between the apparent electron transport rate and leaf CO_2 assimilation was verified in afternoon, indicating reduction in electron use efficiency by photosynthesis. The high radiation loading in the summer season did not impair the citrus photochemistry, being photoprotective mechanisms active. Such mechanisms were related to increases in the heat dissipation of excessive light energy at the PSII level and to other metabolic processes consuming electrons, which impede the citrus photoinhibition under high light conditions.

Additional key words: *Citrus sinensis*, chlorophyll fluorescence, ecophysiology, gas exchange, seasonality.

Introduction

Large variations in environmental conditions are found in subtropical climates, where significant changes in solar radiation and temperature occur during diurnal and seasonal cycles. In fact, soil water availability also has

seasonal variation, occurring significant reduction of rainfall during winter (Ribeiro et al. 2006). This negative effect can be overcome by irrigation, a common practice under nursery of semi-controlled conditions. Therefore, we may argue that solar radiation and temperature are the main environmental elements affecting plant metabolism in well-hydrated plants in both winter and summer. In general, plants are subjected to cooler temperatures during winter, when there is a reduction of solar energy availability in relation to a summer season under subtropical climates.

In citrus plants, some authors have reported decrease in root permeability and in plant hydraulic conductance due to low temperatures (Syvertsen et al. 1983; Moreshet and Green 1984). As a consequence of low temperature in plant water relations, the reduction of stomatal conductance of citrus plants was noticed during winter (Ribeiro and Machado 2007). Reduced stomatal aperture may impair leaf photosynthesis by decreasing CO_2 availability to Rubisco (Jones 1985; Vu 1999; Machado et al. 2002; Medina et al. 2002). Cool temperatures also modify the biochemical reactions underlying CO_2 fixation (Allen and Ort 2001), with citrus plants showing the reduction in RuBP carboxylation and regeneration during winter (Ribeiro and Machado 2007).

As the citrus photosynthesis has a low light saturation point, the higher radiation loading during the summer season will probably be in excess under subtropical conditions, where the daily-integrated global solar radiation (395 to 1100 nm) may reach 35 MJ m^{-2} d^{-1} (Ribeiro et al. 2005). This harmful condition has the potential to cause photoinhibition of photosynthesis, as verified in citrus plants in other environmental conditions (Veste et al. 2000; Medina et al. 2002; Jifon and Syvertsen 2003). Since the optimum temperature range for citrus photosynthesis is between 25 and 30 ºC (Ribeiro et al. 2004; Machado et al. 2005; Guo et al. 2006), high air temperature is another important environmental element during the summer season. As heat stress consequences, citrus plants showed decrease in carboxylation efficiency (Ribeiro et al. 2004; Hu et al. 2007). In exposed canopy positions, leaf temperature reached around 42 ºC and the maximum difference between leaf and air temperatures was around 7.5 ºC during the summer season (Ribeiro et al. 2005).

The above seasonal changes in radiation loading and temperature will probably change the photosynthetic performance of well-watered citrus plants, considering both the maximum values and the diurnal dynamics of leaf gas exchange. Based on this supposition, the aim of this study was to evaluate how the summer and winter conditions affect the photosynthesis and water relations of well-watered orange plants, considering the diurnal changes in leaf gas exchange, chlorophyll fluorescence, and leaf water potential of potted-plants growing in a subtropical climate.

Materials and methods

One-year old 'Valencia' sweet orange (Citrus sinensis [L.] Osb.) scions grafted onto 'Cleopatra' mandarin (Citrus reticulata Blanco) rootstocks were grown in plastic pots (36 L) containing a mixture of soil:sand:manure (2:1:1) and fertilized according to van Raij et al. (1992). Plants were irrigated daily and exposed to natural environmental conditions in Piracicaba, SP, Brazil (22º42'S, 47º30'W, 576 m a.s.l.). This region has Cwa-type climate according to Köppen classification, with rainy summers and dry winters, and mean air temperature in the warmest month higher than 22 ºC. Plants were grown under these conditions from January 2003 to June 2004. Air temperature (T_{AIR}, ºC), photosynthetic photon flux density [PPFD, μmol(photon) m^{-2} s^{-1}], leaf temperature (T_{LEAF}), and leaf-to-air vapor pressure difference (VPD, kPa) were evaluated during the measurements of leaf gas exchange in both winter and summer. These environmental and plant-related data were monitored with an infrared gas analyzer (LI-6400, Li-Cor, Lincoln, USA). Plants were irrigated every two days throughout the experimental period.

Measurements of leaf gas exchange and chlorophyll (Chl) fluorescence were taken in sun-exposed and fully expanded leaves (around 6 months old) throughout the diurnal period (in intervals of 1 to 2 h) during the winter and summer seasons. The evaluations of leaf gas exchange and Chl fluorescence were performed simultaneously on a clear day (without clouds) in each season.

Leaf gas exchange was measured with an infrared gas analyzer (LI-6400), previously calibrated against standards of CO_2 and water vapor and zeroed using CO_2- and H_2O-free air. The diurnal courses of leaf CO_2 assimilation (P_N), stomatal conductance (g_S), transpiration (E), and intercellular CO_2 concentration (C_i) were evaluated. Water use efficiency (WUE) was calculated as WUE=P_N/E. Measurements were recorded when the total coefficient of variation (CV) was less than 0.5 %. The air pumped into the LI-6400 was passed through a buffering gallon (5 L) to reduce the time for measurement stabilization. The T_{LEAF} was measured on the abaxial leaf surface with a thermocouple built into the LI-6400 cuvette. Measurements were taken considering the natural fluctuation of environmental elements.

Chl fluorescence was measured with a pulse amplitude modulation fluorometer (PAM-2000, Heinz Walz GmbH, Effeltrich, Germany). As leaf gas exchange measurements, Chl fluorescence was evaluated under natural air CO_2 concentration, i.e., 365 ± 12 μmol(CO_2) mol^{-1}. Leafclips were used for measurements of the minimum (F_O) and maximum (F_M) fluorescence yield in dark-adapted (30 min) leaf tissues. In light-adapted

leaves, steady-state (F) and maximum (F_M') fluorescence yields were assessed throughout the diurnal period. These measurements were taken under natural variation of PPFD (Fig. 1), with leaf tissues being subjected to each PPFD intensity for at least 5 min.

The variable fluorescence yield in both dark- (F_V = F_M − F_0) and light-adapted (ΔF = F_M' − F') leaves was calculated (Roháček 2002). F_M and F_M' were measured after a light saturation pulse [λ < 710 nm, PPFD ~ 10.000 μmol(photon) m^{-2} s^{-1}, 0.8 s]. These Chl fluorescence parameters were used to estimate the maximum (F_V/F_M) and effective ($\Delta F/F_M$') photosystem II (PSII) quantum yield, the apparent electron transport rate (ETR = ($\Delta F/F_M$' × PPFD × 0.5 × 0.84), the photochemical (q_P) and the non-photochemical [NPQ = (F_M − F_M') / F_M'] quenching (Roháček 2002). For ETR calculation, it was assumed that quanta were evenly distributed between PSII and PSI (0.5), and leaf light absorption was considered to be 0.84 (Schreiber *et al.* 1998). F_0' was measured using far-red light (λ = 735 nm, PPFD ~ 50 μmol(photon) m^{-2} s^{-1}, 3.0 s).

Leaf water potential (Ψ) was measured in leaves similar to those used for leaf gas exchange and Chl fluorescence measurements in both the winter and summer seasons. At pre-dawn (Ψ_W, at 6:00 h) and afternoon (Ψ_{W14}, at 14:30 h), leaf discs (diameter of 0.6 cm) were detached and immediately placed into sample chambers (*C-52, Wescor,* Logan/UT, USA) for 2 h. After the equilibrium time, Ψ was evaluated by the psychrometric method, using a microvoltmeter (*HR-33T*, Wescor, Logan UT, USA) operating in the hygrometric dew-point mode. All sample chambers were calibrated before the experimental period using NaCl solutions ranging from 0.1 to 1.2 M. According to Hubbard *et al.* (2001), the whole-plant leaf specific hydraulic conductance (K_L) was calculated as $K_L = E_{14} / \Delta\Psi$, where E_{14} is transpiration evaluated at 14:30 h and $\Delta\Psi = \Psi_W - \Psi_{W14}$.

Data were subjected to analysis of variance (ANOVA) and mean values were compared by the *Tukey* test (p<0.05) when a significant difference was detected. Mean values were calculated from five replications from different plants.

Results

Environmental conditions: At the experimental site, maximum PPFD was found around midday in both seasons (Fig. 1). However, this environmental variable was significantly higher in summer [*ca.* 2300 μmol(photon) m^{-2} s^{-1}] than in winter [*ca.* 1300 μmol(photon) m^{-2} s^{-1}]. The photoperiod was also increased in summer, being 2 h longer than in winter. Accordingly, a higher T_{AIR} was found in summer, with the highest values around 31 ºC being observed around 15:00 h (Fig. 1). At about this time, maximum T_{AIR} was around 27 ºC in winter. The minimum diurnal T_{AIR} was also higher in summer (21 ºC) than in winter (17 ºC), with a difference of around 4 ºC in the early morning (Fig. 1). T_{LEAF} reached 32 ºC in exposed leaves under summer conditions, while these leaves had T_{LEAF} around 28.5 ºC in winter (Fig. 1). The plant-related environmental variable VPD was higher in summer. There was a peak in VPD between 14:00 and 15:00 h in winter (*ca.* 2.4 kPa), while VPD remained around 2.7 kPa from 13:00 to 17:00 h in the summer season (Fig. 1).

Diurnal course of leaf gas exchange and plant water status: Leaf CO_2 assimilation was higher in summer than in winter (p<0.01), regardless of the time of day (Fig. 1). The highest P_N were found around 9:00 h in both seasons, reaching 11.0 and 5.9 μmol(CO_2) m^{-2} s^{-1} in summer and winter, respectively. Maximum P_N values in summer were almost two-fold as high as those found in winter, with the diurnal-integrated P_N being around 2.9 times higher in summer. Beginning at 9:00 h, we observed a decreasing pattern for P_N during both seasons (Fig. 1). However, the P_N decrease in winter was smoother throughout the daytime compared to the sharp decrease found around midday in summer. In general, the diurnal course of P_N followed the same diurnal pattern of g_S (Fig. 1). Maximum g_S occurred during the morning in both seasons and it was two-fold higher in summer [0.139 mol(H_2O) m^{-2} s^{-1}] compared to winter [0.065 mol(H_2O) m^{-2} s^{-1}]. A sharp reduction in g_S was recorded at midday under summer conditions, with g_S being reduced by 44 % around 13:00 h relative to early morning (Fig. 1). Although low g_S occurred in winter, a significant reduction in g_S (p<0.01) was also noticed when comparing values observed in early morning [0.065 mol(H_2O) m^{-2} s^{-1}] to those obtained around 15:00 h [0.027 mol(H_2O) m^{-2} s^{-1}], the time at which the highest VPD occurred (Fig. 1). Leaf transpiration was higher in summer than in winter (p<0.01), with the diurnal-integrated E being almost four times higher in summer (Fig. 1). The diurnal course of E was not similar to the courses of P_N and g_S. The highest E values were found around midday in both seasons; however, those values were lower than 1.0 mmol(H_2O) m^{-2} s^{-1} in winter and higher than 2.7 mmol(H_2O) m^{-2} s^{-1} in summer.

The relationship between P_N and E, *i.e.*, the actual leaf water use efficiency WUE, showed similar diurnal patterns under both winter and summer conditions (Fig. 1). However, WUE was higher in the winter than in the summer season (p<0.01) throughout the diurnal period (Fig. 1). Considering the diurnal-integrated P_N and E values (WUE$_i$), our data revealed an increase of around 27 % in water use efficiency under winter conditions when there were lower T_{AIR} and VPD when compared to the summer season (Fig. 1).

Even in well–watered plants, significant differences in Ψ between seasons were observed in the afternoon measurements (Fig. 2). Ψ showed a significant decrease at 14:30 h ($p<0.01$), with plants showing more negative values in winter. At approximately the same Ψ_W (predawn), a higher maximum g_S was observed in summer ($p<0.01$). When considering the response of g_S to decreasing Ψ in the afternoon, it was verified that a smaller decrease in water potential led to a larger decrease in g_S under summer conditions (Figs. 1,2).
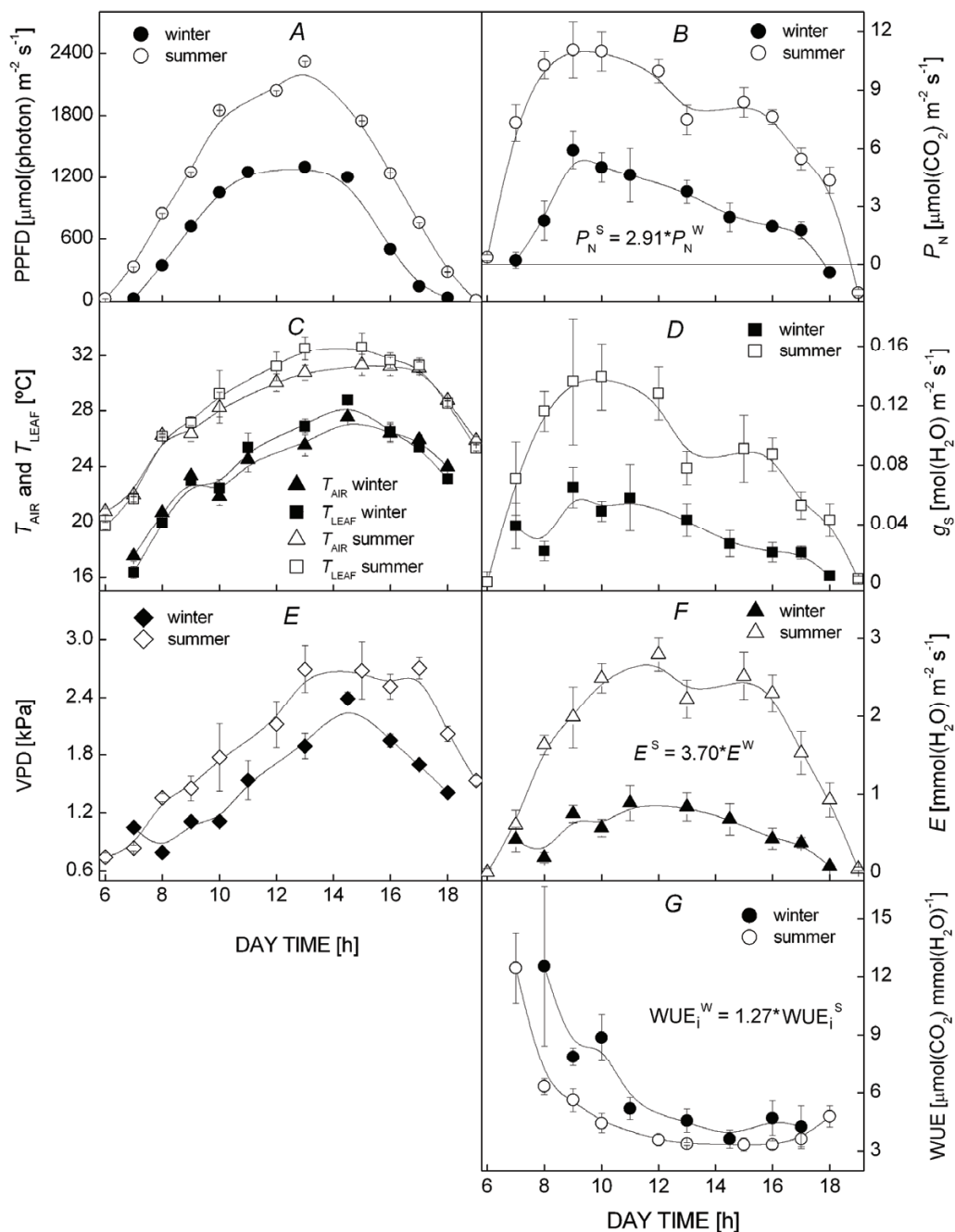
Fig. 1. Diurnal changes in photosynthetic photon flux density (PPFD, *A*), leaf CO_2 assimilation (P_N, *B*), air (T_{AIR}) and leaf (T_{LEAF}) temperatures (*C*), stomatal conductance (g_S, *D*), leaf-to-air vapor pressure difference (VPD, *E*), transpiration (*E*, F) and water use efficiency (WUE, *G*) in 'Valencia' sweet orange plants during winter and summer days (Piracicaba, SP, Brazil). Each symbol represents the mean value of five replications (± SD). The equations shown for P_N (in *B*), *E* (in *F*) and WUE (in *G*) refer to the relationships between the diurnal-integrated values of those physiological variables sampled in winter (index W) and summer (index S) seasons.

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The whole-plant leaf specific hydraulic conductance was also affected by the seasonal changes in environmental conditions, even in well-hydrated plants. When compared to winter, citrus plants showed an increase ($p<0.01$) of 17.9 times in K_L during the summer season [10.6 *vs.* 0.6 mmol(H_2O) m^{-2} s^{-1} MPa^{-1}].

Diurnal course of photochemical activity: The diurnal courses of the maximum PSII quantum yield (F_V/F_M) were quite similar between summer and winter (Fig. 3), seasons with contrasting solar energy availability (Fig. 1). In both seasons, minimum F_V/F_M values were found around 15:00 h ($F_V/F_M \sim 0.7$), with plants showing recovery trends beginning at this time (Fig. 3). The effective PSII quantum yield ($\Delta F/F_M'$) was similar in both winter and summer during the early morning (Fig. 3). At midday, $\Delta F/F_M'$ was around 0.21 in winter and 0.13 in the summer season, representing a reduction of around 38% due to summer conditions. The diurnal dynamic of $\Delta F/F_M'$ led to higher apparent electron transport rates (ETR) in summer compared to winter (Fig. 3).

The photochemical quenching (q_P) showed a diurnal pattern similar to that found for $\Delta F/F_M'$, with a decreasing trend as PPFD increased in the early morning (Fig. 3). Significant differences between seasons were only noticed in the afternoon ($p<0.01$). Non-photochemical quenching (NPQ) was also affected by season ($p<0.01$), with plants showing higher NPQ in summer than

in winter (Fig. 3). The highest NPQ values were found between 9:00 and 12:00 h in summer (NPQ ~ 6.5) and between 8:00 and 15:00 h in winter (NPQ ~ 4.0).
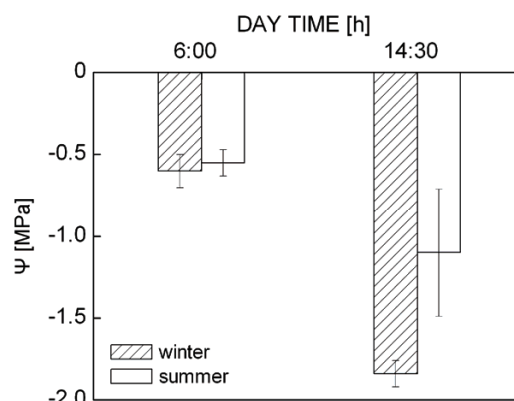

Fig. 2. Leaf water potential (Ψ) at 6:00 and 14:30 h in 'Valencia' sweet orange plants as affected by winter and summer conditions (Piracicaba, SP, Brazil). Each bar is the mean value of four replications ($\pm$ SD).

A higher ETR/P_N was noticed in the winter than in the summer season. The ETR/P_N values found in summer were almost two times smaller ($p<0.01$) than in winter [27.4 *vs.* 14.0 µmol(electron) µmol(CO_2)$^{-1}$], indicating that fewer electrons were used for each CO_2 molecule assimilated.

Discussion

Seasonal changes in environmental conditions caused significant reductions in stomatal aperture during the winter season, without changing the diurnal g_S pattern. The diurnal decrease of g_S in both seasons was promoted by increases of VPD (Fig. 1), being a common feature to prevent excessive shoot dehydration in citrus plants (Syvertsen and Lloyd 1994; Machado *et al.* 2002; Jifon and Syvertsen 2003). The higher VPD found in the afternoon also caused a reduction in Ψ measured at 14:30 h (Fig. 2). This decrease in Ψ_{W14} was probably related to an imbalance between transpiration and water uptake in both seasons.

Leaf transpiration (E) remained high during midday, even with plants showing low g_S (Fig. 1). This transpiration pattern was a result of concomitant changes in g_S and VPD and may play an important role in T_{LEAF} regulation (Nobel 1999) and in shoot water status, as suggested by the decrease in Ψ_{W14} (Fig. 2). Our results also demonstrated a reduction in g_S as VPD increased from 1.5 kPa in both seasons (Fig. 1). Some reports have argued that high transpiration, rather than VPD, regulates g_S through changes in Ψ within the mesophyll/epidermis and guard cells (Eamus 1999). However, our results indicate a stomatal regulation by VPD as the highest E values were found in summer and plants in this season

had higher Ψ_{W14} (Figs. 1,2). In addition to VPD regulation, stomata were more sensitive to Ψ changes in the summer than in the winter season (Figs. 1,2).

Curiously, the lower g_S observed during winter occurred despite of optimum diurnal environmental conditions to stomatal aperture (Ribeiro *et al.* 2004; Machado *et al.* 2005). In fact, T_{LEAF} and VPD were around 22.5 °C and 1.1 kPa when maximum g_S [around 0.06 mol (H_2O) m^{-2} s^{-1}] was reached in winter (Fig. 1). This seasonal reduction of g_S was responsible for the lower photosynthetic rates during winter, causing reduced CO_2 availability to be fixed into carbohydrate molecules. However, this seasonal response of stomata also promoted increases in WUE (Fig. 1), reducing the water cost for each CO_2 molecule fixed. Regardless season, it is important to consider that light availability was not limiting for both g_S and P_N, which have low light saturation points (Vu 1999; Ribeiro *et al.* 2003; Machado *et al.* 2005).

As low temperature is an environmental characteristic of winter (Ribeiro *et al.* 2006), we may suppose some influence on citrus physiology. Such influence may be caused by low air temperature (T_{AIR}) and/or low soil temperature, affecting both the plant shoot hydration and the photosynthetic metabolism (Allen and Ort 2001; Wan
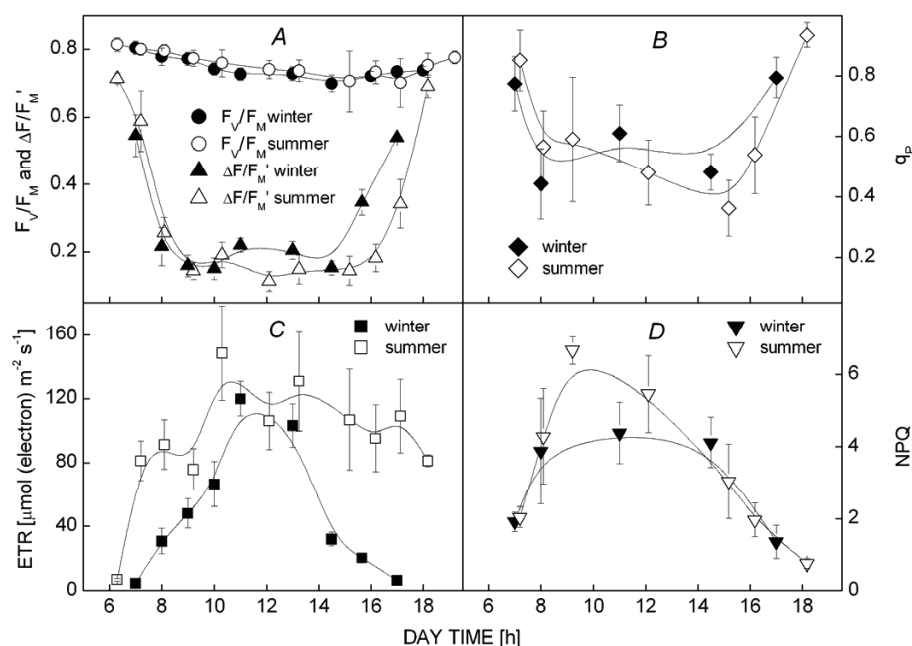
Fig. 3. Diurnal changes in the maximum (F_V/F_M, *A*) and effective ($\Delta F/F_M$', *A*) PSII quantum yield, photochemical quenching (q_P, *B*), apparent electron transport rate (ETR, *C*) and non-photochemical quenching (NPQ, *D*) in 'Valencia' sweet orange plants during winter and summer days (Piracicaba, SP, Brazil). Each symbol is the mean value of five replications (± SD).

et al. 2004; Norisada *et al.* 2005; Veselova *et al.* 2005). In fact, minimum nocturnal T_{AIR} was around 10 °C in winter, whereas it varied around 18 °C during the summer season (data not shown). Citrus plants have a low temperature threshold of around 13 °C, with plant metabolism being severely reduced at lower temperatures (Davies and Albrigo 1994). Low night T_{AIR} has been reported to cause stomatal closure due to a dysfunction of guard cells or due to an indirect effect of increasing C_i caused by reduced RuBP carboxylation (Allen *et al.* 2000). In fact, both alternatives may explain the lower g_S found in winter, when mean C_i values were around 27 % higher compared to summer values [227.2 *vs.* 179.0 $\mu mol(CO_2)$ mol^{-1} in winter and summer, respectively]. In addition, low soil temperatures disrupt root functionality and decrease shoot hydration due to an increase in plant hydraulic resistance (Syvertsen *et al.* 1983; Moreshet and Green 1984; Norisada *et al.* 2005). Soil temperature reached around 9.4 °C at 10 cm depth during the winter season (data not shown), causing significant differences in whole-plant leaf specific hydraulic conductance between seasons.

Therefore, we may argue that the low g_S and Ψ_{W14} found in winter were also consequences of low K_L in well-watered citrus plants, being this dysfunction in plant water relations caused by low night temperature (Figs. 1,2). In addition, stomatal closure and reduced transpiration during winter may also be consequences of modified sap composition and pH and/or of hormonal imbalances caused by low soil temperature such as increases in ABA and decreases in cytokinin content in shoots (Wan *et al.* 2004; Veselova *et al.* 2005).

Our data did not reveal any significant change in photochemical performance between seasons that would induce low P_N during winter (Fig. 3). The diurnal dynamics of $\Delta F/F_M$' were very responsive to the diurnal changes in PPFD, maintaining ETR values compatible with the leaf CO_2 assimilation rates in both seasons (Figs. 1,3). Even with a reduction in q_P due to the closure of PSII centers under high light pressure, high ETR values were found around midday (Fig. 3). Regarding light availability, high PPFD could lead to photoinhibition of photosynthesis in citrus plants (Medina *et al.* 2002). Such a situation of excessive PPFD would be found in both seasons, since plants had low P_N during winter and there was high PPFD during summer. However, plants seem to have been well adapted to the contrasting light regimes of winter and summer (Figs. 1,3), as dynamic photoinhibition of the PSII was observed in both seasons – given by the diurnal reduction and recovery of F_V/F_M (Osmond 1994). This photoprotective mechanism is well correlated with the increase in NPQ. NPQ is an important mechanism to avoid light-induced damage in plant tissues, being related to the xanthophyll cycle and the development of trans-thylakoidal ΔpH (Horton *et al.* 1996; Schreiber *et al.* 1998).

It is noteworthy that the amount of light energy during summer was significantly higher than in winter (almost two-fold), and that citrus plants did not show signs of light-induced damage, such as chronic photoinhibition. While citrus plants showed higher NPQ under summer conditions, the use of electrons in metabolic reactions other than photosynthesis was more active during the winter season. This phenomenon is also indicated by higher q_P and lower P_N in winter than in summer, regarding measurements taken in the afternoon (Figs. 1,3). Under stressful conditions, more electrons are driven to nitrogen metabolism, photorespiration, and the water-water cycle rather than to photosynthesis (Osmond *et al.*

1997; Baker *et al.* 2007). This excess electron fate and the increases of NPQ were effective for the avoidance of over-excitation of PSII and consequent photoinhibition of photosynthesis in citrus plants (Fig. 3).

In conclusion, the diurnal pattern of photosynthesis in young citrus trees was not significantly affected by the environmental changes when compared the summer and winter seasons in subtropical climate. However, citrus plants showed higher photosynthetic performance in the summer season than in the winter one. Since diurnal environmental conditions were more favorable to the citrus photosynthesis in winter, we may conclude that the

low night temperature was the main environmental element limiting the photosynthesis of well-watered plants. Low temperature caused dysfunctions in plant water status (decreased whole-plant hydraulic conductance, low shoot hydration, stomatal closure) and increase in the electron use by metabolic processes other than photosynthesis (given by increase in ETR/P_N). Regarding the seasonal energy availability, the high radiation loading in the summer season did not impair the citrus photochemistry, since photoprotective mechanisms were active in both seasons.

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