Seasonal effects on the relationship between photosynthesis and leaf carbohydrates in orange trees

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Abstract. To understand the effect of summer and winter on the relationships between leaf carbohydrate and photosynthesis in citrus trees growing in subtropical conditions, ‘Valencia’ orange trees were subjected to external manipulation of their carbohydrate concentration by exposing them to darkness and evaluating the maximal photosynthetic capacity. In addition, the relationships between carbohydrate and photosynthesis in the citrus leaves were studied under natural conditions. Exposing the leaves to dark conditions decreased the carbohydrate concentration and increased photosynthesis in both seasons, which is in accordance with the current model of carbohydrate regulation. Significant negative correlations were found between total non-structural carbohydrates and photosynthesis in both seasons. However, non-reducing sugars were the most important carbohydrate that apparently regulated photosynthesis on a typical summer day, whereas starch was important on a typical winter day. As a novelty, photosynthesis stimulation by carbohydrate consumption was approximately three times higher during the summer, i.e. the growing season. Under subtropical conditions, citrus leaves exhibited relatively high photosynthesis and high carbohydrate levels on the summer day, as well as a high nocturnal consumption of starch and soluble sugars. A positive association was determined between photosynthesis and photoassimilate consumption/exportation, even in leaves showing a high carbohydrate concentration. This paper provides evidence that photosynthesis in citrus leaves is regulated by an increase in sink demand rather than by the absolute carbohydrate concentration in leaves.

Additional keywords: Citrus sinensis, gas exchange, growth, seasonality, source–sink.

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Introduction

Under field conditions, citrus leaves show the highest photosynthetic rates during the spring when trees are flowering and fruiting (Ribeiro and Machado 2007). These phenological phases are characterised by a high demand of photoassimilate, with developing tissues causing a reduction in the starch concentration in leaves, stems and roots (Goldschmidt and Golomb 1982; Prado et al. 2007; Ramos 2009). Although high sink demand occurs during the spring, citrus leaves maintain high sugar concentration due to continuous photosynthetic activity throughout the year.

The non-structural carbohydrate availability in leaf tissues reaches more than 200 mg g\textsuperscript{-1} during early spring when leaves show the highest CO\textsubscript{2} uptake (Ribeiro and Machado 2007). According to the current model of photosynthetic inhibition by high leaf carbohydrate content (Iglesias \textit{et al.} 2002) we would expect a downregulation of photosynthesis in citrus plants during the spring/summer season as a response to high sugar availability. However, this is not observed in citrus leaves under subtropical conditions (Ribeiro and Machado 2007).

Recently, Nebauer \textit{et al.} (2011) have reported that citrus photosynthesis is regulated by the turnover of starch and soluble carbohydrates rather than by their absolute concentration, adding more complexity to this interesting theme.

Artificial manipulation of the source–sink relationship, such as girdling (Iglesias \textit{et al.} 2002), sucrose injection (Goldschmidt and Huber 1992), shoot removal (Syvertsen 1994; Iglesias \textit{et al.} 2002) and de-fruiting (Syvertsen \textit{et al.} 2003), supports the inhibition of photosynthesis by high concentration of carbohydrates in citrus leaves. However, plant
girdling may disturb the tree carbon status, affecting the carbon metabolism and water relations (De Schepper et al. 2010), which could influence the growth pattern as well as the relationship between photosynthesis and carbohydrates.

The role of sink demand in controlling the photosynthesis of citrus plants is not clear (Goldschmidt and Koch 1996), especially when plants are studied under natural conditions. Therefore, it would be innovative and less metabolically disturbing to study the source–sink relationships in citrus plants growing under natural conditions, without unnatural carbohydrate injections and without removing the fruit. In subtropical areas, it is well established that citrus trees show a high sink demand during the spring/summer season and an apparent reduction in growth during the winter season (Davies and Albrigo 1994; Ramos et al. 2010). Thus, a higher carbohydrate demand would occur during the spring/summer season than in the winter season, when growth is restrained.

Using young ‘Valencia’ citrus trees growing under subtropical conditions, we measured leaf carbohydrate concentration, photosynthesis and total leaf area during the summer growing season and also during winter when there was no apparent growth. We tested the hypotheses that (i) artificially-induced reductions in leaf carbohydrate concentrations would stimulate the maximal photosynthetic capacity and that (ii) the relationship between carbohydrates and photosynthesis in citrus leaves is influenced by the growing season. In addition, we discuss the relationship between photoassimilates usage and photosynthesis in citrus leaves in subtropical conditions.

Materials and methods

Plant material and experimental set-up

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), which were fertilised according to van Raij et al. (1996). Measurements and samplings were taken in a group of four trees. The plants did not exhibit any symptom of nutritional deficiency in the summer or in the winter. Plants were grown in an experimental garden, in Piracicaba, SP, Brazil (22°42’S, 47°30’W, 576 m of altitude). The region has a Cwa-type climate, according to the Köppen classification, with rainy summers and dry winters and a mean air temperature higher than 22°C in the warmest month.

Aluminium envelopes were used to impose darkness for periods of 12 (overnight), 48 and 96 h over attached leaves in both winter and summer seasons. Afterwards, excised leaf discs (10 cm²) were used to evaluate photosynthetic rates as well as chlorophyll and carbohydrate concentrations. The dark period of 12 h was considered to be the reference because it represented the natural night-time period. Maximum photosynthetic capacity (measured under saturated light and air CO₂ concentration and also under optimum temperature) and chlorophyll a fluorescence were simultaneously measured in leaf discs, which were enclosed in an LD2/3 leaf chamber (Hansatech, King’s Lynn, UK). Inside the leaf chamber, each leaf disc was placed on a wet felt disc during the photosynthetic measurements. We used three replications (discs) from different trees to evaluate each of the three darkness periods, which were imposed in July (winter) and February (summer). Samples were taken only on one day in February, a typical summer day and on one day in July, a typical winter day. The same trees were used for evaluation of the diurnal patterns of carbohydrates and actual photosynthesis of untreated leaves under natural conditions in both seasons. Four measurements for each evaluation time were taken in different trees to assess the diurnal patterns of the parameters mentioned above in 1 day.

Microclimate

The environmental conditions during the experimental period were monitored with an automatic weather station, equipped with sensors to measure the air temperature (minimum, mean and maximum values), relative humidity (probe model HMP45C, Campbell Scientific, Logan, UT, USA) and solar radiation (pyranometer model LI-200, Li-Cor, Lincoln, NE, USA).

Maximum photosynthetic capacity

Maximum photosynthetic capacity (Ap) was estimated by measuring the photosynthetic O₂ evolution on excised leaf discs with a leaf disc oxygen electrode (Hansatech). Measurements were taken at 35°C (Ribeiro et al. 2006) and the photosynthetic photon flux density (PPFD) of 1120 μmol m⁻² s⁻¹ was provided by an external light source (LS3, Hansatech). The Ap values were recorded using the Oxygraph measurement system (ver. 2.22, Hansatech). The saturated CO₂ concentration inside the chamber was generated by 0.2 cm³ of a carbonate/bicarbonate buffer solution (1 M, 1:19 v/v), according to Ribeiro et al. (2003).

Chlorophyll a fluorescence

A modulated fluorometer (FMS1, Hansatech) was used to evaluate the following photochemical variables: the maximum (Fm/Fm') and the actual (ΔF/ΔFm') quantum yield of primary photochemistry; the apparent electron transport rate (ETR0 = ΔF/ΔFm' × PPFD × 0.5 × 0.84); and the non-photochemical [NPQ = (Fm/Fm')/Fm'] and photochemical (qP = (Fm/Fm')/(Fm/Fm')) quenchings (Rohácek 2002). For the calculation of ETR0', 0.5 was used as the fraction of the excitation energy distributed to PSII and 0.84 was used as the fractional light absorbance (Björkman and Demmig 1987). Measurements of Ap and photochemistry were recorded after the full induction of photosynthesis, which occurred after 10 min of illumination.

Chlorophyll concentrations

The leaf chlorophyll concentrations were evaluated in the dark exposed leaves. The pigments were extracted by grinding leaf discs in 6 mL of acetone solution (80%, v/v) under dark conditions. The solution was centrifuged at 2000g for 5 min and the supernatant absorbance was measured with a spectrophotometer set to 646 and 663 nm. Chlorophylls a (chl a) and b (chl b) as well as the total chlorophyll (chl a + b) were determined according to the equations proposed by Lichtenhailer and Wellburn (1983).
Carbohydrates concentration

Leaf carbohydrate concentrations were measured after the evaluation of $A_D$ in the leaf discs excised from the leaves that had been exposed to each of the three darkness periods (12, 48 and 96 h). For this, leaf tissues were rapidly frozen in liquid nitrogen, dried and then ground. Soluble sugars were extracted from samples of 75 mg of ground tissue after three cycles of extraction in an ethanol solution (80%, v/v) at 55°C. The reducing sugars (RS) were quantified by the Somogyi-Nelson method (Somogyi 1952) and the total soluble sugars (SS) were determined by the Anthrone method (Yemm and Willis 1954). After the extraction of ethanol-soluble sugars, the starch concentrations (St) were quantified in the insoluble fraction by the Anthrone method. The fraction of the total non-structural carbohydrates (TS) was expressed as the sum of SS and St (Syvertsen et al. 2003). The non-reducing sugars (NRS) concentration was calculated as NRS = SS – RS.

Using the same analytical methods described above, we also evaluated the diurnal dynamics of carbohydrates concentrations in leaves under natural conditions that were collected at sunrise (0600 or 0700 hours), 1000 hours, 1400 hours and at sunset (1800 or 1900 hours) in July (winter) and February (summer), from the same potted plants. An additional leaf sample was collected at sunrise of the following day to estimate the daily changes of leaf carbohydrates. The diurnal change was calculated as the difference between carbohydrate concentrations measured at sunset of day 1 and at sunrise of day 1, whereas the nocturnal change was the difference between carbohydrate concentrations measured at sunrise of day 2 and at sunset of day 1.

Actual photosynthesis

Additionally, the diurnal course of CO$_2$ assimilation ($A_N$) in sun-exposed and fully expanded leaves (~6 months old) was measured with an infrared gas analyser (LI-6400, Li-Cor). Measurements were taken in four leaves of four different potted trees and such data were recorded when the total coefficient of variation (CV) inside the leaf chamber was less than 0.5%, which happened after 3 min. The CV was calculated by the Open software of the LI-6400 system and it considered the variations of air temperature and solar radiation concentrations of CO$_2$ and water vapour. The CO$_2$ concentration inside the leaf chamber was around 380 μmol mol$^{-1}$. The air pumped into the IR gas analyser was passed through a buffering gallon (5 L) to reduce the time for measurement stabilisation. Measurements were taken under the natural fluctuation of air temperature and vapour pressure deficit (VPD). Measurements were taken on clear days and the PPFD used for each assessment was provided by an artificial light source (LI-6400–02 LED, Li-Cor), which was set to provide the same PPFD that was tracked by an external light sensor (LI-250, Li-Cor) installed on the leaf cuvette. Only data on the diurnal-integrated CO$_2$ assimilation ($A_D$) are shown, see the ‘Results’ section.

The leaf photoassimilate exportation/consumption (PEC) was calculated as PEC = $A_D$ + $D_D$ − $A_D$. When $A_D$ and $TS_{d2}$ are the leaf TS concentrations at sunset of day 1 and day 2, respectively, and $A_D$ is the diurnal-integrated CO$_2$ assimilation on day 1 (g CH$_2$O m$^{-2}$). The $A_D$ value in g CO$_2$ m$^{-2}$ was converted to g CH$_2$O m$^{-2}$ using a factor of 0.68 as a multiplier (Kalt-Torres et al. 1987). The total non-structural carbohydrates (TS) on a mass basis were converted to a leaf area basis using a specific leaf mass, which was evaluated in both seasons. The PEC was calculated using the diurnal CO$_2$ assimilation reported in a previous paper (Ribeiro et al. 2009a), which is shown in fig. 1.

The total leaf area (LA) of three plants was measured in both seasons using a digital planimeter (LI-3100, Li-Cor).

Data analysis

The data were subjected to the analysis of variance procedure either as a factorial design (season vs dark period) or as a random block design when comparing seasons. When significant differences were detected, mean values were compared by the Tukey’s test ($P < 0.05$). The relationships between leaf sugar concentrations and photosynthesis were evaluated by using the Pearson’s correlation coefficient ($r$) when statistical significance was detected ($P < 0.05$).

Results

Microclimate

As expected, air temperature and incoming solar radiation were significantly different between the winter and summer (Table 1). The cumulative rainfall during the summer and winter seasons was 487 and 43 mm respectively. This large difference is expected to affect the relative humidity, which was more elevated during the summer season. As citrus trees were potted and irrigated, the influence of water availability from rainfall was not considered in this study. In general, the summer was warmer than the winter season and the evaluation days were representative of such contrasting seasons. On the days measurements were performed, the minimum and mean air temperatures and solar radiation were higher (+7.4°C, +6.0°C and +8.8 MJ m$^{-2}$ day$^{-1}$ respectively) in the summer than in the winter.

Relationships between carbohydrates and maximum photosynthetic capacity in dark exposed leaves

Prolonged exposure to dark conditions led to increased $A_P$ and the highest rates were observed after 96 h, regardless of the season (Fig. 1a). Relative to the 12 h of darkness (overnight), the $A_P$ increased by ~1.6- and 1.3-times after 96 h of darkness on both the measured summer and winter days. On the typical

Table 1. Environmental conditions during the experimental period: entire seasons and evaluation dates

<table>
<thead>
<tr>
<th>Variables</th>
<th>Winter</th>
<th>Day</th>
<th>Summer</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{average}$ (°C)</td>
<td>18.4 ± 0.2</td>
<td>17.8</td>
<td>22.7 ± 0.2</td>
<td>23.8</td>
</tr>
<tr>
<td>$T_{maximum}$ (°C)</td>
<td>26.5 ± 0.3</td>
<td>25.3</td>
<td>29.1 ± 0.3</td>
<td>30.9</td>
</tr>
<tr>
<td>$T_{minimum}$ (°C)</td>
<td>11.3 ± 0.3</td>
<td>11.1</td>
<td>18.1 ± 0.2</td>
<td>18.5</td>
</tr>
<tr>
<td>RH$_{average}$ (%)</td>
<td>68.5 ± 1.2</td>
<td>72.3</td>
<td>79.9 ± 0.9</td>
<td>78.6</td>
</tr>
<tr>
<td>RH$_{minimum}$ (%)</td>
<td>35.8 ± 1.5</td>
<td>40.3</td>
<td>50.9 ± 1.5</td>
<td>37.4</td>
</tr>
<tr>
<td>Qg (MJ m$^{-2}$ day$^{-1}$)</td>
<td>13.5 ± 0.3</td>
<td>13.2</td>
<td>18.6 ± 0.6</td>
<td>22.0</td>
</tr>
</tbody>
</table>
summer day, exposure to darkness reduced the concentration of TS, SS and NRS, whereas on the measured winter day significant decreases in TS and St were observed (Fig. 1).

The photochemical activity was higher on the summer day than on the winter day (Fig. 2). The highest values of $\Delta F'/F'_m$ and $q_P$ were found on the summer day, regardless of the period under darkness. The $\Delta F'/F'_m$, $q_P$ and ETR$_0$ tended to decrease with the increase of the period leaves remained in darkness only on the winter day. The largest differences in $\Delta F'/F'_m$, $q_P$ and ETR$_0$ values between seasons were observed after 96 h under dark conditions. However, the darkness and seasons did not affect NPQ or $F_v/F_m$. Similarly, neither the dark periods nor the seasons cause changes in chl $a$, chl $b$ or chl $a + b$ (Table 2).

Negative relationships were observed between leaf carbohydrates and maximum photosynthetic capacity in both seasons (Table 3), with the highest photosynthetic rates in leaves with reduced carbohydrate concentration. On the measured winter day, a significant negative correlation was determined between St concentration and $A_P$. Since starch is the main carbohydrate in leaf tissues, the negative relationship between TS and $A_P$ was also significant. On the measured summer day, significant correlations were found between NRS and $A_P$, SS and $A_P$ and between TS and $A_P$. The highest slopes from the correlations were noticed in the summer season, i.e. 0.724 (summer) vs 0.218 (winter), for the relationship between TS and $A_P$. This pattern suggests high responsiveness of photosynthesis to changes in leaf carbohydrate concentration during the growing season (summer).

**Carbohydrates and actual photosynthesis under natural conditions**

The leaves exhibited higher concentrations of St, SS, NRS and TS on the summer day as compared with the winter day (Fig. 3a, b, d, e), whereas RS was similar in both seasons (Fig. 3c). In general, there was a large daily change in leaf carbohydrates, increasing from sunrise and reaching the highest values around midday in both seasons, regardless of the sugar type. The diurnal increase rate of St in leaves was higher on the winter day than on the summer day.

In the diurnal period, a higher accumulation of St was found on the winter day (Fig. 4a), whereas a higher increase in SS and
RS was observed on the summer day (Fig. 4b, c). In the nocturnal period, the pattern observed for St was inverted, with leaves presenting higher St consumption on the summer day. Both diurnal and nocturnal changes of SS and RS were higher on the summer than on the winter day. Diurnal and nocturnal changes of NRS were the same for both seasons (Fig. 4d). The largest difference between seasons was observed in the nocturnal consumption of St, which was approximately four times higher on the summer day than the winter day.

The high daily changes in the carbohydrate concentrations observed in the summer were in accordance with the leaf photoassimilate exportation/consumption. The amount of carbohydrates exported or consumed in leaves was approximately three times higher on the measured summer day than on the winter day (Fig. 5a). Accordingly, high AD was observed on the summer day (Fig. 5b), which was ~2.9-times higher than on the winter day. Regarding the vegetative growth, plants showed 5.8-times more LA in the summer than in the winter (Fig. 5c). The specific leaf mass was not affected by season, varying from 77.2 ± 3.7 g DM m⁻² in the winter to 75.7 ± 4.6 g DM m⁻² in the summer.

**Discussion**

*Maximum photosynthetic capacity as affected by leaf carbohydrates*

There was a negative correlation between AP and leaf carbohydrate concentrations on both the summer and...
winter days (Table 2). This relationship is well documented in tree species in which an external treatment artificially induces increases in leaf carbohydrate concentrations and reductions in photosynthesis (Goldschmidt and Huber 1992; Syvertsen 1994; Iglesias et al. 2002; Syvertsen et al. 2003; De Schepper et al. 2010). We observed the lowest \( A_P \) in leaves that exhibited the highest sugar concentrations (Table 2; Fig. 1). Low photosynthetic rates in leaves showing high carbohydrate concentrations may be ascribed to impaired CO\(_2\) diffusion, decreased biochemical activity of photosynthesis and/or the downregulation of photosynthetic genes (Goldschmidt and Huber 1992; Koch 1996; Nakano et al. 2000; Paul and Foyer 2001; Paul and Pellny 2003).

Citrus shoot growth has a close relationship with the consumption of carbohydrates stored in mature leaves and roots (Bueno et al. 2011), so we may argue that the higher LA observed in the summer, in contrast with LA found in the winter

<table>
<thead>
<tr>
<th>Season</th>
<th>Relationship</th>
<th>Probability</th>
<th>Linear regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>RS vs ( A_P )</td>
<td>( P &gt; 0.05 )</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>NRS vs ( A_P )</td>
<td>( P &gt; 0.05 )</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>SS vs ( A_P )</td>
<td>( P &gt; 0.05 )</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>St vs ( A_P )</td>
<td>( P \leq 0.05 )</td>
<td>( A_P = (-0.249 \times \text{St}) + 43.22 )</td>
</tr>
<tr>
<td></td>
<td>TS vs ( A_P )</td>
<td>( P \leq 0.05 )</td>
<td>( A_P = (-0.218 \times \text{TS}) + 50.81 )</td>
</tr>
<tr>
<td>Summer</td>
<td>RS vs ( A_P )</td>
<td>( P &gt; 0.05 )</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>NRS vs ( A_P )</td>
<td>( P \leq 0.05 )</td>
<td>( A_P = (-0.912 \times \text{NRS}) + 47.78 )</td>
</tr>
<tr>
<td></td>
<td>SS vs ( A_P )</td>
<td>( P \leq 0.05 )</td>
<td>( A_P = (-0.901 \times \text{SS}) + 67.76 )</td>
</tr>
<tr>
<td></td>
<td>St vs ( A_P )</td>
<td>( P &gt; 0.05 )</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>TS vs ( A_P )</td>
<td>( P \leq 0.05 )</td>
<td>( A_P = (-0.724 \times \text{TS}) + 89.58 )</td>
</tr>
</tbody>
</table>

Fig. 3. Daily changes in (a) starch (St), (b) soluble sugars (SS), (c) reducing sugars (RS), (d) non-reducing sugars (NRS) and (e) total non-structural carbohydrates (TS) in leaves of sweet orange trees on typical winter (closed circles) and summer (open circles) days. Each symbol represents the mean value of four replications (±s.e.). White bar in the bottom position in (d) and (e) refers to the diurnal period and the black bar refers to the nocturnal period.
The strength of sinks regulates photosynthesis through the use of Calvin cycle end products, which represents a mechanism to ensure the appropriate balance between carbon uptake and consumption (Paul and Pellny 2003; Smith and Stitt 2007). In this way, the maximum photosynthetic capacity of citrus leaves may be stimulated by increases in inorganic phosphorus ($P_i$) availability due to carbohydrate turnover (Bush 1999). Indeed, increases in $A_P$ due to darkness cannot be ascribed to photochemical activity or chlorophyll concentrations because stimulation of $A_P$ (Fig. 1a) occurred even with decreases in $\Delta F'/F_m'$, $q_F$ and $ETR_s$ (Fig. 2) and no changes in leaf chlorophyll concentration (Table 2). Although the decreased photochemical activity during the winter may be justified by the low air temperature during the nocturnal period (Table 1), the low $\Delta F'/F_m'$, $q_F$ and $ETR_s$ after 96 h of darkness are probable consequences of photochemistry-biochemistry uncoupling due to excessive darkness. Therefore, our first hypothesis was confirmed; with citrus leaves presenting a large stimulation of maximum photosynthetic capacity when carbohydrate concentration was artificially reduced.

The relationship between carbohydrates and photosynthesis being affected by growing season

The dark conditions applied in the present study confirmed the relationship between carbohydrates and photosynthesis established in citrus species (Iglesias et al. 2002). However, we observed new relationships between distinct carbohydrate types and $A_P$ values, which varied according to the season. Changes in St concentrations affected $A_P$ on the measured winter day, whereas the changes in SS and NRS were more correlated with the changes of $A_P$ on the measured summer day (Table 3).

As a remarkable increase in LA was observed from the winter to the summer (Fig. 5c) and as most of vegetative growth is well established to occur during the warmer months (Ramos et al. 2010), we argue that the sink activity was higher in February (summer) than in July (winter). During the summer (growing season), the NRS, sucrose among them, was the main carbohydrate type that was negatively related to photosynthesis (Table 3; Fig. 1c). Further, sucrose is the main sugar exported from leaves to other organs, such as roots, stems and young leaves of tree species during the growing season (Dickson 1991). Therefore, one would expect a significant influence of sucrose concentrations on photosynthesis, as determined in this study (Table 3). The high sucrose concentration in the phloem of mature leaves potentially inhibits the activity of proton-sucrose symporters, reducing the phloem loading (Bush 1999). As a consequence, leaves accumulate sucrose, the antiport transport of triose-phosphate and $P_i$ through chloroplast membrane is reduced and then photosynthesis is impaired due to low $P_i$ availability (Paul and Foyer 2001; Paul and Pellny 2003). Moreover, sucrose has been described as a potential signalling molecule regulating genes from carbohydrate metabolism and photosynthesis (Koch 1996; Li et al. 2003; Paul and Pellny 2003; Rolland et al. 2006; McCormick et al. 2006; Smith and Stitt 2007). High sucrose concentration in leaf tissues induces the expression of genes responsible for carbohydrate storage and usage, whereas it downregulates genes that are responsible for the photosynthetic activities (Ainsworth and Bush 2011).
Starch decreased by 20% on the winter day and non-reducing sugars decreased by 53% on the summer day and these events were apparently responsible for photosynthesis stimulation in citrus leaves after 96 h under dark conditions (Fig. 1). On average, we found consumption rates of 0.16 mg g⁻¹ h⁻¹ for NRS on the summer day and 0.13 mg g⁻¹ h⁻¹ for St on the winter day. These reduction rates were higher than previously reported rates for citrus plants (Iglesias et al. 2002). Therefore, maximum photosynthetic capacities may show associations with specific carbohydrates depending on season.

The photosynthetic responses obtained herein revealed how specific carbohydrates may influence photosynthesis of citrus leaves in different seasons. Rather, the negative correlations between leaf carbohydrates and maximum photosynthetic capacity (Figs 3, 5) do not determine what regulates photosynthesis in citrus leaves. Besides the probable regulation imposed by absolute contents of leaf carbohydrates, it is important to comment that the actual photosynthesis of citrus leaves is higher in the summer than in the winter due to increased ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) activity, augmented regeneration of RuBP dependent on electron transport and also due to elevated stomatal conductance (Habermann and Rodrigues 2009; Ribeiro et al. 2009a, 2009b). Such photosynthetic performance in summer conditions are clearly related to significant vegetative growth in subtropical conditions, as observed in leaf area of citrus trees (Fig. 5b, c).

A positive association was determined between the actual photosynthesis and the photoassimilate exportation/consumption, which occurred in parallel to a large increase in vegetative growth, given by the total leaf area in plants without external manipulation of the source-sink relationship (Fig. 5). Under subtropical conditions we know that citrus vegetative growth is intense during the spring/summer season (Ramos et al. 2010) and the demand of growing shoots cause significant impact on carbohydrate concentration of mature leaves, reducing the amount of carbohydrate reserves (Bueno et al. 2011).

According to Geiger et al. (2000) and Smith and Stitt (2007), there was no net accumulation of St during the diurnal period on the summer day. In this evaluation, however, we detected a large consumption of St during the night (Figs 3a, 4a). In addition, the nocturnal consumption of soluble sugars was higher on the warmer summer day than on the winter day (Figs 3b, 4b). These carbohydrate dynamics strongly agreed with the high demand for photoassimilates in the summer caused by intense vegetative growth during this season (Fig. 5).

The adjustment of carbon supply for plant growth is accomplished by the complex control of photoassimilate partitioning between sucrose and starch; thus, the plants have enough sucrose to support the immediate diurnal demand and sufficient starch to supply the nocturnal carbon demand (Smith and Stitt 2007). This pattern for the source–sink interaction was also detected in our data, with plants showing high sucrose concentrations in the diurnal period and high starch concentrations in the late evening on a typical summer day.

The occurrence of high actual photosynthesis and high photoassimilate usage in citrus trees suggests a probable upregulation of photosynthesis through increase in sink demand. This happens with leaf tissues showing high carbohydrate concentration (Fig. 3) coexisting with maximum photosynthetic capacity (Table 3; Fig. 1). This is a novel result and it is in agreement with a recent paper by Nebauer et al. (2011), in which citrus photosynthesis was demonstrated to be regulated by carbohydrate turnover and not by its absolute concentration. When there is no strong sink demand, genes related to sucrose synthesis and exportation were repressed in citrus leaves before any significant change in carbohydrate concentration (Nebauer et al. 2011), which happened in parallel with photosynthesis reduction.

A comparison between the coefficients from equations established by the linear correlations between TS and AP (Table 3) indicated that the effects from changes in TS on AP were approximately three times higher in the summer than in the winter (i.e. 0.724 vs 0.218). Therefore, our second hypothesis was also confirmed, with the relationship between
leaf carbohydrates and photosynthesis being affected by season. Such stimulatory effect was related to the vegetative growth of citrus trees, which was higher during the spring-summer season in comparison with the fall-winter.

Our data suggest that the pattern of photassimilate consumption/production is an important element affecting the photosynthesis in broad leaf evergreen species growing in subtropical conditions. The carbon metabolism change between the summer and winter days could well represent what is importantly affecting the plant growth and the physiological performance of orange trees; not only when considering those selected days in both seasons, but over time.

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