

## Nitrogen use efficiency in lemon and orange trees: a growth and resources allocation analysis

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### SUMMARY

The economical and environmental success of the citrus industry requires improved efficiency of the use of the agricultural inputs. Among those, nitrogen (N) fertilization greatly impacts fruit yield and potentially affects environmental quality. Therefore, N use efficiency by citrus groves has been increasingly required worldwide. Among the *Citrus* species, lemon trees are more vigorous and show superior nitrogen-use-efficiency (NUE), which highest fruit yields are achieved with 20-24 g kg<sup>-1</sup> of N in the leaves, compared to 24-30 g kg<sup>-1</sup> of N in the case of orange trees. To access the physiological aspects that explain greater vigor and growth efficiency of the former species, experiments were carried out in 42 L containers with lemon and orange young trees (seven-month-old) supplied with sufficient N for maximum growth. The CO<sub>2</sub> assimilation, dry mass partitioning and total soluble carbohydrates and starch in plants were evaluated in autumn and summer. Lemon trees presented higher photosynthetic nitrogen use efficiency (Pn/N), invested relatively more N and biomass in leaf production whereas oranges trees invested more in root-biomass production. This preferential investment in leaf area in lemon trees suggests that the greatest vigor is associated with the fate of assimilates produced by trees.

**Index terms:** carbohydrates reserves, photosynthesis, lemon, sweet orange.

### Eficiência de uso do nitrogênio em *C. limon* e *C. sinensis*: análise do crescimento e alocação dos recursos

### RESUMO

O sucesso econômico e ambiental da indústria citrícola requer de uma melhora constante na eficiência de uso dos insumos agrícolas, dentre eles a fertilização com nitrogênio (N) impacta não apenas no potencial produtivo como também afeta a qualidade do meio ambiente. Assim o incremento na eficiência de uso do N (EUN) torna-se necessária. Dentre as espécies cítricas, o limoeiro é mais vigoroso e apresenta maior EUN que plantas de laranja, desde que a maior produtividade é alcançada com 20-24 g kg<sup>-1</sup> de N na massa seca das folhas, enquanto são necessários 25-30 g kg<sup>-1</sup> de N para laranja. A fim de avaliar aspectos fisiológicos que permitam explicar o maior vigor e EUN, foram conduzidos experimentos com plantas de limoeiro e laranja de sete meses de

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idade, em vasos de 42 L supridas com dose suficiente de N. No outono e no verão foram avaliadas: assimilação de  $\text{CO}_2$ , acúmulo e partição de biomassa e concentração de carboidratos metabolizáveis. Foi observado que o limoeiro apresentou maior eficiência fotossintética de uso do N ( $\text{Pn}/\text{N}$ ) e maior investimento de biomassa em produção de folhas, no entanto a laranjeira investe maior proporção na produção de raízes. Esta preferência pelo investimento em área foliar sugere uma associação entre maior vigor e o destino dos assimilados produzidos.

**Termos de indexação:** carboidratos de reserva, fotossíntese, limão, laranja doce.

## INTRODUCTION

*Citrus* trees, are subject to variations in environmental conditions what affect carbon (C) demand of growing plant parts (sinks) throughout the year. Thereafter, production of C assimilates via photosynthesis and assimilation of nitrogen (N), associated to nutrient consumption are seasonally regulated (Legaz et al., 1995; Machado et al., 2002) and balanced with accumulation of starch and N in plant organs. These reserves, especially in the leaves and roots, are mobilized and redistributed in the plant during flowering and the fruit set (Ruiz et al., 2001; Dovis et al., 2014).

Sweet orange [*Citrus sinensis* (L.) Osbeck] and lemon trees [*C. limon* (L.) Burm. f.] present great difference in the efficiency of use of N absorbed. In orange trees, fruit yield is maximized with 28 g kg<sup>-1</sup> of N in the leaf dry mass (Quaggio et al., 1998), while in lemon ones, fruit yield is maximized with 20 g kg<sup>-1</sup> of N (Quaggio et al., 2002). These two species also differ in specific leaf area (m<sup>2</sup> kg<sup>-1</sup>) and foliar anatomy, as well as  $\text{CO}_2$  assimilation, growth rate and fruit production per unit of N absorbed (Romero-Aranda et al., 1997; Boaretto et al., 2010) what affect the N use efficiency (NUE) by each one.

Higher vigor and NUE are observed in lemons from the first months after tree planting, however little is known about the dynamics of the accumulation of C and N in young plants of this species compared to sweet orange trees. In addition, few efforts have been addressed to elucidate the relationship between the initial development of the leaf area and the accumulation of tree reserves, as well as how these may affect the productive capacity of the plant.

Therefore, based on the hypothesis that lemon trees allocated greater proportion of assimilates and N absorbed to the vegetative growth than buildup of reserves compared to orange trees, the objective of this study was to evaluate the initial growth and the accumulation of C and N reserves in different plant parts during two distinct growth periods (autumn and summer), as well as the efficiency of biomass production of trees considering the same N fertilization regime.

## MATERIAL AND METHODS

The experiment was carried out in a screenhouse covered with transparent plastic film, with Lisbon Limonera 8A lemon [*Citrus limon* (L.) Burm. f.] and Valencia sweet orange trees [*C. sinensis* (L.) Osbeck], at nine-month-old and transplanted into 42 L plastic pots filled with a mixture of sand and pine bark substrate, both grafted on Swingle citrumelo [*C. paradisi* Macfad. x *Poncirus trifoliata* (L.) Raf.]. The plants were fertilized with a complete nutrient solution at annual doses per plant of: 28 g of N, 16.8 g of Ca, 5.5 g of P, 6.6 g of K, 2.0 g of Mg and 2.4 g of S. Micronutrients were regularly applied via foliar according to Quaggio et al. (2003). Plants were drip irrigated four times a day to maintain substrate at maximum water storage capacity.

Environmental conditions inside the screenhouse were automatically monitored by an automatic weather station Vantage Pro2 (Davies Instruments, California, USA). Maximum, minimum and mean temperature, total radiation and air humidity were registered every 30 min during the experimental period.

Plant evaluations started 8 months after transplantation, which were done in two periods: (i) during typical days of autumn (cold nights) and (ii) typical days of summer (hot and dry days) in the morning (between 08:30 to 09:30 h) and at midday (between 12:30 to 13:30 h). Gas exchange measurements were conducted using an integrated infrared gas exchange analyzer (IRGA, LI-6400, Li-Cor, Inc. Lincoln, EUA). The measurements were taken from one mature leaf per replication, completely exposed, between the third and fifth position from the end of the youngest branches. Measurement conditions were 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of PAR in the morning and 1400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of PAR at midday, 380  $\mu\text{mol CO}_2 \text{mol}^{-1}$  was used for all measurement and temperature in leaf chamber was not controlled. Air ( $T_a$ ) and leaf ( $T_l$ ) temperatures, vapor pressure difference between leaf and air ( $\text{VPD}_{l-a}$ ), relative humidity in the chamber (% RH),  $\text{CO}_2$  assimilation ( $\text{Pn}$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mol m}^{-2} \text{s}^{-1}$ ), transpiration ( $E$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ) and intercellular  $\text{CO}_2$  concentration ( $\text{Ci}$ ,  $\mu\text{mol mol}^{-1}$ ) were measured. The apparent carboxylation efficiency ( $\text{Pn}/\text{Ci}$ )

was calculated as the relation:  $P_n (\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}) / C_i (\text{Pa})$ ; water use efficiency (WUE) was calculated as the relation:  $P_n (\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}) / E (\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1})$  and photosynthetic nitrogen use efficiency ( $P_n/N$ ) was calculated as the relation:  $P_n (\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}) / N (\text{g m}^{-2} \text{ leaf})$ .

After the gas exchange measurements in each evaluation period, plants were harvested for evaluation of leaf area and dry mass of different parts. Total N (Malavolta et al., 1997) and soluble carbohydrates extracted by the MCW method (methanol:chloroform:water) and determined by phenol-sulfuric method (Dubois et al., 1956; Bialeski & Turner, 1966) were measured in dry mass of branches, leaves and roots. Starch was determined according to Amaral et al. (2007). The experiment was set up in a complete randomized design, with five replicates, and the results were submitted to the analysis of variance (ANOVA) with two factors and two levels each (day time x specie), independent analysis were performed for autumn and summer period. Tukey test was used for pairwise comparisons of measured variables.

## RESULTS AND DISCUSSION

### Environmental conditions during experimental period

The air temperature measured in the screenhouse during the two periods (i) between transplant and the first plant harvest (autumn) showed daily averages similar to those observed during the period (ii) between

the first and second harvest (summer), although the daily minimum temperature was approximately 2°C higher and the maximum was 1°C lower in the autumn compared to the summer (Table 1). Thus, the thermal conditions were more favorable for plant growth in the first period (autumn) given the less occurrence of days with temperatures outside the optimal range that limit photosynthesis (Machado et al., 2005; Pimentel et al., 2007; Ribeiro et al., 2009a, 2009b, 2012b; Santos et al., 2011; Dóvis et al., 2014).

Furthermore, environmental conditions during the days (between 12:00 h and 13:00 h) that gas exchange measures were taken were also limiting in the summer, when maximum temperature recorded was above the optimum range (25-30 °C) for both orange and lemon trees (Machado et al., 2005; Pimentel et al., 2007), with maximum temperature above 40 °C. Minimum relative ambient humidity, recorded around 13:00 h, was lower (21%) and *VPD* (vapor pressure deficit) was higher (6.2 kPa) in summer compared to winter (Table 1) what favored stomatal closure.

### CO<sub>2</sub> assimilation and photosynthetic nitrogen use efficiency

CO<sub>2</sub> assimilation ( $P_n$ ) and stomatal conductance ( $g_s$ ) was higher in the morning and lower in the midday both in the autumn and in the summer periods (Figure 1), without differences between species. Lower  $g_s$  was observed in Valencia sweet orange trees as a consequence

**Table 1.** Environmental conditions inside the screenhouse during experimental periods: from transplanting to autumn and from winter to summer; and environmental conditions during measurement days in autumn and summer

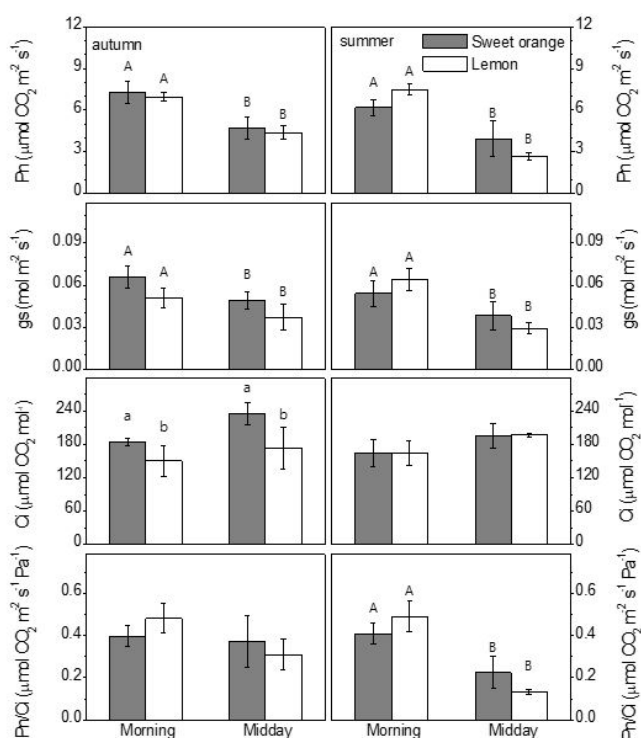
Characteristic Period	Mean environmental values during period of evaluation	
	from transplanting to autumn	from winter to summer
Daily T (°C)	23.7 ± 2.6	22.7 ± 2.5
Tmax (°C)	31.4 ± 3.1	32.2 ± 2.6
Tmin (°C)	18.5 ± 3.6	16.1 ± 2.2
Period	Mean environmental values during measurement days	
	autumn	summer
Tmax (°C)	27.8	41.2
Tmin (°C)	13.6	22.9
Min RH (%)	51	21
Qg (MJ m <sup>-2</sup> day <sup>-1</sup> )	12.9	26.0
Max Rad (W m <sup>-2</sup> )	326	647

Daily temperature (Daily T, °C); maximum temperature (Tmax, °C); minimum temperature (Tmin, °C); minimum relative humidity (Min RH, %); total radiation (Qg, MJ m<sup>-2</sup> day<sup>-1</sup>) and maximum radiation (Max Rad, W m<sup>-2</sup>).

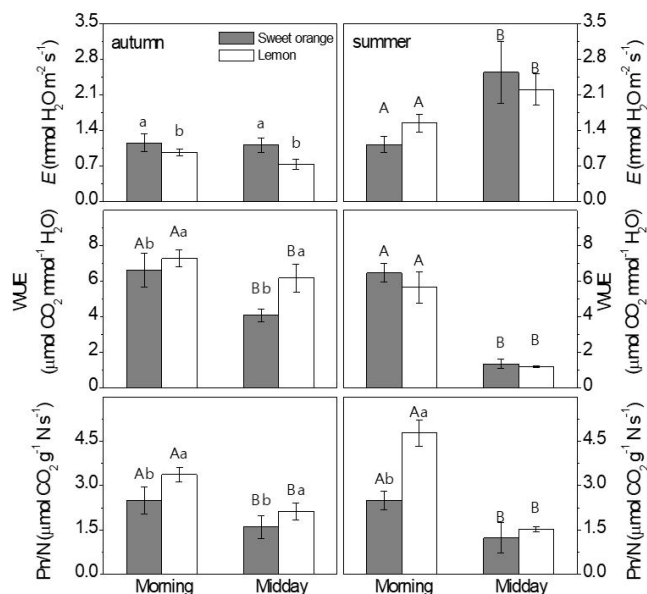
of high  $VPD$ , with low temperature and with seasonal variation or thermal regimes, without water deficit (Machado et al., 2002, 2005, 2010; Ribeiro et al., 2012a). The lemon trees presented lower  $CO_2$  concentration in the substomatal chamber ( $C_i$ ), and since photosynthesis did not differ, apparent carboxylation efficiency ( $Pn/C_i$ ) would likely be greater in lemon than in orange trees. However, no differences were observed in such variable (Figure 1), probably because differences were small and were covered up by high variability between replications. The  $g_s$  and  $Pn$  were significantly lower at midday in both autumn and summer. Low  $g_s$  in autumn could be caused by low night temperature and by endogenous regulation of  $Pn$  (Ribeiro et al., 2009b), without difference in  $Pn/C_i$ . In summer, lower  $Pn$  at midday was likely a consequence of high air temperature and  $VPD$  (Table 1). These are associated to low biochemical activity, reducing the  $Pn/C_i$

recorded in summer ( $Pn/C_i < 0.4 \mu mol CO_2 m^{-1} s^{-1} Pa^{-1}$ ) as well reducing the  $g_s$ , caused by limiting ambient conditions (higher  $VPD$  and temperature) with no difference between species. These diurnal and seasonal responses to environmental factors were reported for orange trees (Machado et al., 2002; Ribeiro et al., 2009a, 2009b; Santos et al., 2011), although responses of lemon trees to these factors are still poorly understood (Pimentel et al., 2007).

Plant transpiration was lower in autumn than in summer in both species what markedly affected the water use efficiency (WUE) in these two periods (Figure 2). This was probably associated with the midday  $VPD$  and the maximum temperature, higher in summer (6.2 kPa and 43.1 °C, respectively) than in autumn (2.1 kPa and 27.6 °C, respectively) what likely resulted in the reduction of the carboxylation rate by Rubisco and of the regeneration of RuBP (Machado et al., 2005; Ribeiro et al., 2009a, 2009b). Furthermore, lemon trees transpired less in autumn and thus were more efficient in water use.



**Figure 1.** Leaf  $CO_2$  assimilation ( $Pn$ ), stomatal conductance ( $g_s$ ), concentration of  $CO_2$  in intercellular spaces ( $C_i$ ) and apparent carboxylation efficiency ( $Pn/C_i$ ) in sweet orange (grey bars) and lemon trees (white bars) in autumn and summer, measured in the morning and midday. Each symbol represents the mean value of five replications ( $\pm$  s.e). Uppercase letters indicate significant differences between diurnal periods and lowercase indicate differences between species, Tukey test ( $p < 0.10$ ).



**Figure 2.** Leaf transpiration ( $E$ ), Water use efficiency (WUE) and Photosynthetic nitrogen use efficiency ( $Pn/N$ ) in sweet orange (grey bars) and lemon trees (white bars) in autumn and summer, measured in the morning and midday. Each symbol represents the mean value of five replications ( $\pm$  s.e). Uppercase letters indicate significant mean differences between diurnal periods and lowercase indicate mean differences between species, Tukey test ( $p < 0.10$ ).



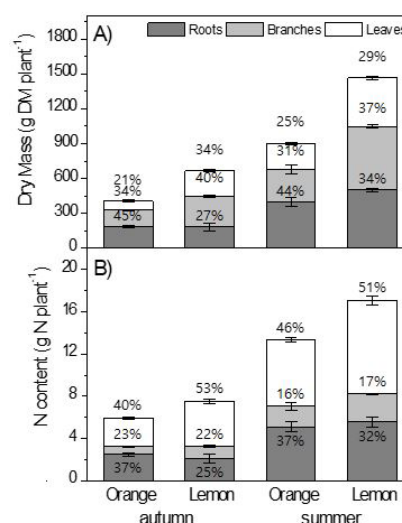
The photosynthetic nitrogen use efficiency (Pn/N) was markedly different between the studied species with high values found in lemons in all measurements (Figure 2). Correspondingly, fruit and dry biomass produced by unit of N in the plant tissue ( $\text{g DM g}^{-1} \text{N}$ ) was reported to be greater in lemon compared to orange trees (Boaretto et al., 2010). However the characteristic that determines this higher  $\text{g DM g}^{-1} \text{N}$  are still under study by our research group. Thus, in spite of the fact that no higher instantaneous Pn in lemons compared to orange trees was observed (Figure 1), this former species presents higher efficiency in the use of water and (Pn/N) (Figure 2).

### Biomass and nitrogen partitioning in plants

Lemon trees produced twice as much leaf area compared to orange ones with greater specific leaf area (SLA,  $\text{m}^2 \text{kg}^{-1} \text{DM}$ ) (Table 2). In contrast, the concentration of N in the leaves was 20% to 35% lower in the lemon trees than the orange. Based on this, we estimated that lemons produced 40% to 70% more leaf area per gram of N compared to orange trees (Table 2). Characteristics of higher biomass production per unit of N in lemons as well differences in leaf anatomy between those two species are described in the literature (Romero-Aranda et al., 1997; Boaretto et al., 2010). According to Romero-Aranda et al. (1997), lemon trees present smaller number of cells in the leaf mesophyll with a smaller volume, the intercellular spaces are bigger and there is less concentration of chlorophyll by area. Other studies indicate that leaf thickness is negatively related to photosynthesis, as a consequence of the effect on the conductance of the mesophyll and the difficulties of

$\text{CO}_2$  diffusion to the catalytic site (Syvertsen et al., 1995; Tomás et al., 2013). Thus, the larger SLA in lemon trees could explain, in part, the higher Pn/N observed in this species.

Compare to the lemons, the orange plants invested proportionally more in root growth than in the canopy (Figure 3), which biomass allocation values are comparable to those reported by Morgan et al. (2006). Therefore, the greatest investment in leaf production observed with lemon trees would result in an initial advantage in the ability of those to produce assimilates; however, on the other hand this larger canopy to root ratio compared to oranges could



**Figure 3.** (A) Biomass partitioning and (B) nitrogen content partitioning in lemon and sweet-orange trees growing during transplanting-autumn period (15 month-old) and autumn-summer period.

**Table 2.** Total leaf area, specific leaf area, N concentration in leaves and leaf area per leaf N in sweet orange and lemon trees growing during autumn and summer period

	Transplanting to autumn						
	Sweet Orange tree			Lemon tree			Significance <sup>1</sup>
Total leaf area (m <sup>2</sup> )	0.92	±	0.12	2.46	±	0.12	**
Specific leaf area (m <sup>2</sup> kg <sup>-1</sup> DM)	9.65	±	0.08	11.21	±	0.24	**
N concentration (g kg <sup>-1</sup> DM)	34.52	±	2.21	22.26	±	0.95	**
Leaf area/N (m <sup>2</sup> g <sup>-1</sup> N)	0.34	±	0.04	0.48	±	0.01	**
	autumn to summer						
Total leaf area (m <sup>2</sup> )	2.32	±	0.11	5.98	±	0.62	**
Specific leaf area (m <sup>2</sup> kg <sup>-1</sup> DM)	10.89	±	0.50	14.00	±	1.42	**
N concentration (g kg <sup>-1</sup> DM)	28.66	±	1.84	22.70	±	0.52	**
Leaf area/N (m <sup>2</sup> g <sup>-1</sup> N)	0.37	±	0.03	0.62	±	0.05	**

<sup>1</sup>\*\*Significant differences between treatments, ANAVA  $p < 0.01$ .

more severely expose lemon trees to abiotic stresses, particularly in the case of water deficit.

Lemon trees also showed N concentrations from 15% to 30% lower than orange ones in all organs of the plant (data not shown). Nevertheless, the total N content was higher in lemon trees than orange trees, both in autumn as in summer (Figure 3). The orange trees allocated more N in the roots and less in the leaves, as observed by Mattos-Junior et al. (2003). Such larger pool of root N could contribute to the maintenance of the production in situations of restriction of nutrients achieved by N remobilization.

### Carbohydrates concentration and distribution

Soluble sugar concentrations and starch in the leaf dry mass of lemon trees as well as in the roots were higher than orange ones in the autumn (Figures 4A and C). This could be correlated with the higher growth observed in these former plants immediately after transplant, since the sugar concentration in the plants varies according to the presence and strength of sinks (Goldschmidt & Golomb, 1982; Ribeiro et al., 2012b; Dovis et al., 2014). However, this higher growth was not correlated with higher photosynthetic activity in these plants, as observed by Bueno et al. (2012). These authors point out that such effect is due to the greater efficiency of use of photoassimilates for biomass production when conditions of greater thermal amplitude prevail. In this condition, greater differences

are observed in plant respiration but not for assimilation of CO<sub>2</sub>. This difference could be associated with reduced growth respiration or lower construction and maintenance costs of biomass. A higher concentration of soluble sugars in the roots of orange trees and in the leaves of lemon trees was observed in the summer, what was associated with the greater allocation of C in these organs, since orange trees invested proportionally more resources in root growth (Figures 3, 4B and 4D).

### CONCLUSIONS

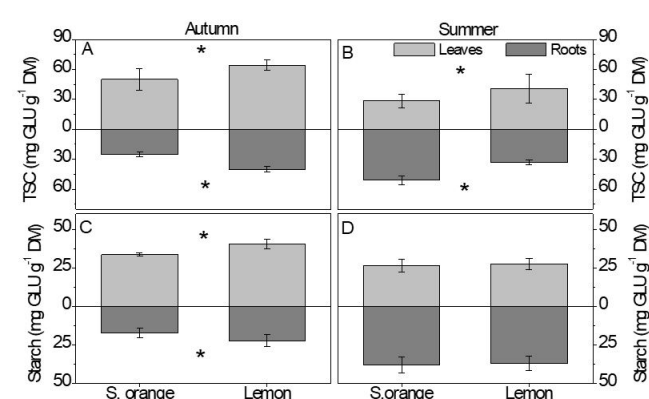
The Pn/N was higher in lemon trees than orange ones. However this difference was not related to the biochemical aspects of CO<sub>2</sub> assimilation per leaf area, but with higher investment in leaf area rather than roots. Other biochemical plant characteristics still need to be explained, such as the partition of leaf N among different structural components or the capacity of N assimilation per unit of chlorophyll. Lemon trees showed a small advantage in the assimilation of CO<sub>2</sub> than oranges only in the summer evaluation, indicating that the greatest vigor of this species is associated with the fate of assimilates produced in the increase of leaf area. Therefore, we might consider that lemon trees preferably invests a greater proportion of the biomass in the production of leaves than orange ones, since the initial advantage given by the highest leaf production increases exponentially the growth by greatest production of assimilates in the total of the leaf area and not necessarily by greatest production per leaf area unit.

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**Figure 4.** (A-B) Total soluble carbohydrates concentration (TSC) (mg GLU g<sup>-1</sup> DM) and (C-D) starch concentration (mg GLU g<sup>-1</sup> DM) in leaves of sweet-orange and lemon trees. (A-C) autumn (15 month-old) and B-D) summer (19 month-old). Each symbol represents the mean value of five replications ( $\pm$  s.e). \*Indicate significant differences between species, ANAVA ( $p < 0.10$ ).

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