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Abstract: The objective of this work was to study the influence of fruit load on CO2 assimilation in the leaves of citrus trees presenting alternate bearing habits, and the importance of this factor on photosynthetic rate variability throughout the year and under regular cropping conditions. The photosynthetic rate was measured on 60 days throughout the year on field-grown sweet orange plants under natural conditions in the Valencian Community, the most important citrus-producing area of Spain. The experiments were performed on the 'on' (high crop) and 'off' (low crop) bearing 40-year-old Salustiana sweet orange trees growing in the same orchard. Gas exchange and fluorescence parameters were measured during the year in young and old leaves on sun-exposed branches with and without fruit in the 'on' trees, and in fruitless branches of the 'off' trees. In non-manipulated Citrus trees, fruit load has no significant effect in any season on the photosynthetic rate in the leaves from branches without fruit. However, in high crop trees, the leaves of branches bearing fruit present a slightly lower photosynthetic rates (approx. 10%) than those of fruitless branches. Variations in mineral content (N, K and P) might explain not only these differences, but also the lower photosynthesis rate observed in old leaves (13 to 24 month-old leaves). Environmental conditions were the main factor for the variation of the photosynthetic rate, with variability of the monthly mean photosynthetic rate being much lower than that between days in the same month.

Crop load does not increase the photosynthetic rate in *Citrus* leaves under regular cropping conditions. A study throughout the year

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ABSTRACT

The objective of this work was to study the influence of fruit load on CO_2 assimilation in the leaves of citrus trees presenting alternate bearing habits, and the importance of this factor on photosynthetic rate variability throughout the year and under regular cropping conditions. The photosynthetic rate was measured on 60 days throughout the year on field-grown sweet orange plants under natural conditions in the Valencian Community, the most important citrus-producing area of Spain. The experiments were performed on the ‘on’ (high crop) and ‘off’ (low crop) bearing 40-year-old Salustiana sweet orange trees growing in the same orchard. Gas exchange and fluorescence parameters were measured during the year in young and old leaves on sun-exposed branches with and without fruit in the ‘on’ trees, and in fruitless branches of the ‘off’ trees. In non-manipulated Citrus trees, fruit load has no significant effect in any season on the photosynthetic rate in the leaves from branches without fruit. However, in high crop trees, the leaves of branches bearing fruit present a slightly lower photosynthetic rates (approx. 10%) than those of fruitless branches. Variations in mineral content (N, K and P) might explain not only these differences, but also the lower photosynthesis rate observed in old leaves (13 to 24 month-old leaves). Environmental conditions were the main factor for the variation of the photosynthetic rate, with variability of the monthly mean photosynthetic rate being much lower than that between days in the same month.

Keywords: *Citrus sinensis*; photosynthesis; environmental conditions; fruit load; leaf age

1. Introduction

The *citrus* fruit set depends on the supply of carbohydrates that the fruit receives (Guardiola, 1988; Duarte et al., 1996). Post-anthesis abscission frequently coincides with starvation reserves and, from this time onwards, the carbohydrates supply depends only on the photosynthesis rate, which limits fruit set (González-Ferrer et al., 1995). The relatively low maximum CO_2 assimilation rates of citrus (Kriedemann, 1971; Syvertsen and Lloyd, 1994) are one of the main factors limiting the growth and productivity of citrus trees (Goldschmidt, 1999). In order to improve photosynthetic efficiency, it is essential to identify the relative importance of the processes limiting CO_2 assimilation rates, such as light, temperature, CO_2 supply, carbon source-sink balance (Jifon and Syvertsen, 2003b; Nebauer et al., 2011) and leaf characteristics (age, position, orientation, etc.).

Maximum efforts have been made to clarify the effect of high temperature and high irradiance conditions on the photosynthesis of citrus leaves (Vu and Yelenosky, 1988; Brakke and Allen, 1995; Jifon and Syvertsen, 2003a,b; Guo et al., 2006; Hu et al., 2007; Otero et al., 2011). Temperatures of 25-30ºC are optimal for photosynthetic activity, unlike temperatures of 35ºC and above, which reduce photosynthesis (Vu and Yelenosky, 1988; Brakke and Allen, 1995; Guo et al., 2006; Hu et al., 2007). The maximum net CO_2 assimilation rate (A_c) in sun-acclimated leaves on the outer portions of citrus canopies is light-saturated at about one third of full sunlight (600 to 700 μmol m^{-2} s^{-1}; Sinclair and Allen, 1982; Syvertsen, 1984; Vu and Yelenosky, 1988). Excess radiant energy may affect photochemical reactions. Photoinhibition has been found to occur in citrus plants (Jifon and Syvertsen, 2003a; Hu et al., 2007; Ribeiro and Machado, 2007). Ribeiro and Machado (2007) pointed out that the influence of low temperature has been largely placed aside and very few reports on this important environmental constraint are available (Syvertsen et al., 1983; Vu and Yelenosky, 1987; Ribeiro et al., 2009a,b). Most of the above-mentioned results have been obtained under controlled or semi-controlled conditions. Some works which aimed to study the effect of midday depression on the net photosynthetic rate have studied CO_2 assimilation under field conditions, but only on certain representative days (Jifon and Syvertsen, 2003b; Hu et al., 2007). Nevertheless, there are few reports that deal with routine experimentations of field-grown citrus plants throughout the year (Ribero and Machado, 2007), and diverse ecophysiological patterns under natural conditions need to be revealed. Photosynthetic efficiency variability throughout the year and the importance of seasonal variation are unknown. Ribeiro et al. (2009a,b; 2012) reported seasonal changes in the photosynthetic rate, but these authors studied the photosynthesis process only on one day in summer and on one day in winter. One of the aims of this work was to evaluate the relative importance of seasonal environmental changes on the photosynthetic rate in relationship with other internal factors (crop load and leaf characteristics) in the Valencian Community, the most important citrus-producing area of Spain. This area is characterised by a hot, dry summer season. Precipitations are scarce and take place mainly in autumn when rainfall is heavy, and a medium winter temperature is moderate. These environmental conditions are very different to those studied in previous works.

There are few reports about the influence of leaf age on photosynthetic rates. Variation in CO_2 assimilation rates according to leaf development has been reported only in Satsuma mandarin trees (Kubota and Motoyama, 1972; Iglesias et al., 2002). The results of these works show that leaf activity peaks throughout the first year. During the second year, however, the photosynthetic rate decreased to 60-70% of previous season values. If we consider that sweet orange leaf duration lasts more than 24 months (Spiegel-Roy and Goldschmidt, 1996), and that the percentage of leaves per tree aged over one year is about 30-50% (Monerri et al., 2011), knowledge of photosynthetic rate variation with leaf age on sweet orange trees is of much interest.

The influence of fruit load on, and the role of sink demand in, controlling the photosynthesis of citrus plants remain unclear, especially when plants are studied under natural and regular cropping conditions (Goldschmidt and Koch, 1996; Ribeiro et al., 2012). Artificial manipulation of the source-sink relationship, such as girdled leaf systems (Goldschmidt and Huber, 1992; Iglesias et al., 2002), sucrose injection (Iglesias et al., 2002) and de-fruiting trees (Syvertsen et al., 2003), supports an inhibitory effect due to the accumulation of photosynthetic products in citrus leaves. Although a feedback inhibition of photosynthesis has been proposed due to starch accumulation, the role of soluble sugars and starch has not been well-established (Iglesias et al., 2002; Syvertsen et al., 2003; Nebauer et al., 2011). Excised discs from leaves exposed to dark conditions have also been used to show that maximum photosynthetic capacity and photoassimilate consumption are positively associated, even in leaves with a high carbohydrate concentration (Ribeiro et al., 2012). Seasonal effects on the relationship between photosynthesis and leaf carbohydrates have also been noted. However, girdling and other artificial manipulations used in the above-mentioned works to study the control of photosynthesis by carbohydrate levels may disturb the tree carbon status (De Schepper et al., 2010). As pointed out by Ribeiro et al. (2012), the study of source-sink relationships under natural conditions would prove less metabolically disturbing. Under regular cropping conditions and with fully grown trees, such inhibition would not be apparent because of the presence of alternative sinks, which remove photosynthates from source leaves. The root system seems to be a particularly strong, unsaturable sink (Goldschmidt and Koch, 1996), but it is not clear to what extent sink demand controls citrus photosynthetic rates under regular field conditions.

The objective of this work was to measure the net photosynthetic rate in mature field-grown sweet orange trees under cropping conditions and throughout the year to study photosynthetic process variability and the relative importance of variation due to fruit load, seasonal changes and leaf age in the most important citrus-producing area of Spain, the Valencian Community.

2. Materials and methods

2.1. Plant material

Experiments were performed on 40-year-old Salustiana sweet orange trees (*Citrus sinensis* [L.] Osbeck.) grafted on Troyer citrange (*C. sinensis* [L.] Osb. x *Poncirus trifoliata* Raf.) rootstocks. Trees were drip-irrigated, and mineral elements were supplied in irrigation water from February to September. Fertilisation was decided depending on the leaf analysis performed the previous year. Trees present alternate bearing habits. Flowering intensity depends on the fruit load of the previous year. Trees alternated between years of abundant flowering and fruit set (‘on’ year) and years of almost no flowering (‘off’ year). During each year, the ‘on’ and ‘off’ trees were found in the same orchard, and experiments were performed on both ‘off’ and ‘on’ trees. The orchard, located in Museros (Valencia, Spain), displayed a north-south (NS) row orientation. Trees for the study were selected in accordance with their behaviour in previous years and confirmed from sprouting characteristics at the beginning of the experiment. In the year prior to the study, the 'on' trees, which entered an 'off' year, averaged 1,632 fruits/tree, whereas the 'off' trees averaged 68 fruits/tree.

In Salustiana trees, the spring flush is the most important. Mainly inflorescences, but also vegetative sprouts, are formed in the 'on' trees, whereas vegetative sprouts are mainly formed in the 'off' trees (Monerri et al., 2011). The midsummer flush is much smaller and vegetative shoots are almost exclusively formed during this period.

2.2. Gas exchange and fluorescence measurements

Photosynthetic rate (A_N), stomatal conductance (g_s), transpiration (E) and substomatal CO_2 concentration (C_i) were measured at the steady state under conditions of ambient light, temperature, relative humidity and CO_2 concentration with an LCi Portable Photosynthesis System (ADC, Herts, UK). Air (T_{air}) and leaf (T_{leaf}) temperatures, photosynthetic photon flux density (PPFD), atmospheric pressure (P_{atm}), air vapour pressure (VP) and ambient CO_2 partial pressure (Ca) were provided by the LCi. Water use efficiency (WUE) was estimated by the A_N/E ratio and VPD (leaf-to-air vapour pressure difference) was calculated according to Buck (1981). The maximum quantum yield of PSII (F_v/F_m) was measured on leaves after 30 min in the darkness using a portable pulse amplitude modulation fluorometer (MINI PAM, Walz, Effeltrich, Germany). The background fluorescence signal in the dark-adapted leaves (F_o) was determined with a 0.5 μmol photon m^{-2} s^{-1} by measuring light at a frequency of 600 Hz. The application of a saturating flash of 10,000 μmol photon m^{-2} s^{-1} allowed maximum fluorescence (F_m) estimations. Gas exchange and fluorescence measurements were taken from 9:00 h to 12:00 h (local time). One measurement per tree was taken on a fully expanded mature leaf (third or fourth leaf from the shoot apex). Measurements were taken from 10 trees for each condition. The monthly averages of mean diurnal photosynthetic photon flux density, air temperature and leaf-to-air vapour pressure difference during the experiments are provided in Figure 1. Atmospheric CO_2 content, as indicated by the LCi device during the experiment, varied between 366 and 383 ppm.

2.3. Seasonal variation of photosynthesis and effect of crop load

Gas exchange and fluorescence parameters were measured during the year (4 to 10 determinations per month) in sun-exposed (SE orientation) fruit and fruitless (vegetative) branches in the ‘on’ trees, and on the fruitless branches in the ‘off’ trees.

2.4. Influence of leaf position on photosynthesis in branches with fruit

The effect of leaf position along the branch was assessed by measuring photosynthesis in the leaves located at positions 1, 3-4 and 7 from the apex. Measurements were taken in the mature leaves of the branches which formed during the last spring flush and on selected cloudless days.

2.5. Effect of leaf age on photosynthesis

The photosynthetic characteristics of young (1 to 12-month-old) and old (> 12 months) leaves on fruitless branches, which developed during the spring flush of consecutive years, were compared.

2.6. Mineral content analysis

Mineral analyses were carried out as described in Ruiz et al. (2001). Leaf samples were dried at 65ºC in a forced air oven. Three independent extracts, obtained from nine trees (five leaves per tree and three trees per extract), were used in all the determinations.

Total nitrogen content was determined by the micro-Kjeldahl method. After the digestion of samples with an acid mixture, phosphorus content was determined colorimetrically and potassium content was established by atomic-absorption spectrophotometry using an AA 100 (Perkin Elmer, Shelton, USA) (Ruiz et al., 2001).

2.7. Statistical analyses

An analysis of treatment comparisons was performed by ANOVA (Statgraphics Plus 5.1 for Windows, Statistical Graphics Corp.). Mean separations were performed with the LSD multiple range test. A regression analysis ($P < 0.05$) was used to evaluate the relationships between parameters.

3. Results

3.1. Seasonal variation of the photosynthesis rate in the sun-exposed leaves of vegetative sprouts and the relative importance of crop load

The development of most leaves in Salustiana sweet orange initiated during the spring flush at the beginning of March, which fully expanded and matured in May after 2 months. The seasonal photosynthetic rate variation of sun-exposed leaves under the Valencian Community environmental conditions from that time onwards (and until the next spring flush) during their first year of life was measured. The mean photosynthetic rates for the 60 days measured throughout the year in the ‘on’ and ‘off’ trees is shown in Figure 2. No differences were observed in the maximum quantum yield of PSII (F_v/F_m) between both types of trees during the year (Mean $F_v/F_m = 0.794$; $P < 0.05$). The analysis of variance components (Table 1) showed no significant differences in the photosynthetic rate between the trees with low and high crop loads in any month of the year. Environmental conditions were the main factor for the variability in this parameter, but no significant seasonal variation was observed. The variability of the monthly mean photosynthetic rate was much lower than that between the days within a month (1% *vs.* 99% of the variance components) due to the change in environmental conditions among days. The lowest values (< 1µmol m^{-2} s^{-1}) were reached in summer months and on winter days with low temperatures. The highest values (> 10 µmol m^{-2} s^{-1}) were obtained in autumn and also in spring. The leaf photosynthetic capacity did not vary during the first year, and thus, maximum photosynthetic rates could be observed throughout the first year of the leaf.

The relationships between photosynthetic rate and the photosynthetic photon flux density (PPFD), air temperature (T_{air}) and leaf-to-air vapour pressure difference (VPD) followed optimisation curves. The maximum photosynthetic rate values were reached for the PPFD values of 550 - 600 µmol m^{-2} s^{-1}, the temperature values of 25-30ºC and the VPD values close to 24 mbar. As expected, a close relationship between stomatal conductance and the photosynthetic rate was observed (A_N = 4.3 ln(g_s) + 16.6; R^2 = 0.64). Stomatal conductance depends on PPFD, VPD (data not shown) and temperature. The results we obtained for the relationship between conductance and temperature are of much interest given the wide range of temperatures tested throughout the year, including low winter temperatures, which have been studied in very few works. For the T_{leaf} values above 25ºC, we found that the higher the T_{leaf}, the lower the g_s, and that the effect was more prominent when there was a high air vapour pressure (VP, Fig 3A). For VP values under 20 mbar, temperature explained only 4% of the observed variability. Conversely in T_{leaf} values below 25ºC, a positive correlation between leaf temperature and stomatal conductance was found (Fig. 3B).

3.2. Photosynthesis on the leaves from fruiting and non-fruiting branches from the 'on' crop trees. Effect of distance to fruit and changes in mineral elements

When the photosynthetic rate was measured from June to January on the leaves from both fruiting and non-fruiting branches in the 'on' crop trees, slightly higher A_N, g_s and E were observed in the leaves on non-fruiting branches (Table 2). Adjacent fruit had no effect on C_i/C_a. N, P, and K concentration was lower in the leaves from fruiting branches (Fig. 4). The distance from the leaf to the fruit along the branch influenced the photosynthetic rate as well as g_s and E (Table 3).

3.3. Effect of leaf age on the photosynthetic rate. Changes in mineral elements

Throughout the first year of life, the photosynthetic rate of leaves remained stable and a constant F_v/F_m value was observed (Mean $F_v/F_m = 0.798 \pm 0.003$). Nevertheless during the second year, this capacity diminished and the leaves of the new spring flush of growth showed a higher photosynthetic rate than the older ones (Table 4). Old leaves presented significantly lower N, P, and K concentrations (Fig. 4). However, leaf age had no effect on F_v/F_m ($P < 0.05$).

Leaf age had no significant effect on the relationship between photosynthesis and the parameters PPFD, T_{leaf}, and VPD, or on the relationship between photosynthesis and the related parameters (g_s, E, WUE)(data not shown).

4. Discussion

4.1. Crop load does not increase the photosynthesis rate in the leaves of vegetative sprouts under regular cropping conditions.

Photosynthesis regulation (A_N) by sink demand has been demonstrated in *Citrus* using potted trees (Syvertsen and Lloyd, 1994; Goldschmidt and Koch, 1996; Iglesias et al., 2002) and by altering the source-sink balance in the tree by means of girdling (Li et al., 2003), de-fruiting trees (Syvertsen et al., 2003), sucrose injection (Iglesias et al., 2002) or by exposing leaf discs to dark conditions using aluminium envelopes (Ribeiro et al., 2012). However, the extent to which sink demand controls photosynthetic rates in *Citrus* under regular cropping conditions, or the exact regulation mechanism involved, remains unclear (Goldschmidt and Koch, 1996; Nebauer et al., 2011; Ribeiro et al., 2012). We addressed this issue by measuring A_N in trees aged around 40 years under orchard conditions in diverse developmental stages over the year. Our data support the view that photosynthesis in non-manipulated *Citrus* trees is source-limited rather than sink-regulated throughout the year. No significant differences in the photosynthetic rate were found between trees with high and low crop loads, irrespectively of the developmental stage. As previously shown for deciduous trees, like apple (Lakso et al., 1998) and cherry (Roper et al., 1988; Flore and Layne, 1999), fruit load had no significant effect on photosynthesis as carbohydrates may be translocated to alternative sinks. Our findings are in accordance with Goldschmidt and Koch (1996) and with Monerri et al. (2011), who stated that the root system could be a non-saturable sink under regular cropping conditions and with fully grown trees, and that the CO_2 assimilation rate of fruitless trees is similar to that of trees with high fruit loads. Furthermore, when considering the seasonal changes of leaf carbohydrate concentration in citrus leaves (Monerri et al., 2011), our results support the view that citrus photosynthesis is not regulated by the absolute concentration of soluble carbohydrates (Nebauer et al., 2011; Ribeiro et al., 2012).

Syvertsen et al. (2003) found that in winter (late June in the southern hemisphere, the equivalent to late December in the northern hemisphere), de-fruited “Spring” navel orange trees had a 40% lower A_N value than fruiting (and already harvested) trees. De-fruiting may bring about a transient change in A_N (Gucci et al., 1991), while the trees reported by Syvertsen et al. (2003) were smaller in size (5 years old) than those employed in our experiments. Thus, the root sink strength of younger trees can be much lower. Artificial manipulation of the source-sink relationship may disturb the tree carbon status which, in turn, can influence the relationship between photosynthesis and carbohydrates in such a way that the results may not be comparable with regular growing conditions.

Although no differences in the photosynthetic rate on the leaves from vegetative branches between the ‘on’ and ‘off’ trees were observed, it is interesting to point out that in the same high crop tree, the leaves of those branches bearing fruit gave a slightly lower photosynthetic rate (approx. 10%) than those of branches without fruit, and in all the fruit developmental stages. Similar results have been reported by Syvertsen et al. (2003). These authors pointed out that low nitrogen content in the leaves of fruiting branches (Sanz et al., 1987) can explain these results. Our data confirm this statement; furthermore, a decrease in other mineral elements that influence photosynthetic rate, such as P and K levels, is observed. Phosphorus plays a key role in the storage and use of energy from photosynthesis, while potassium plays a vital role in the osmotic mechanism of stomata aperture modulation (Epstein, 2005). A deficiency in either of them can explain the low photosynthetic rate. Monerri et al. (2011) reported that, after the fruit abscission period in June, 13% of sprouts with leaves bear fruit in the ‘on’ Salustiana trees, whereas only 1% of sprouts with leaves bear fruit in the ‘off’ trees. In view of these data, the reduced photosynthetic rate on the leaves of branches bearing fruit has to be taken into account when estimating the diurnal CO_2 assimilation in trees with different crop loads.

On the branches with fruit, the photosynthetic rate, stomatal conductance and transpiration rate were also affected by the leaf position along the branch. The mineral element remobilization from leaves to the fruit, stimulated under conditions of limitation in the supply of mineral elements, may start from the older leaves on the branch.

4.2. Seasonal variation of the photosynthetic rate in the leaves of vegetative sprouts

The interesting result that this study produces in terms of seasonal photosynthetic rate variation and the relative importance of environmental and physiological parameters (crop load) is that no significant seasonal trend was found. More than 90% of variability was explained by variation in environmental conditions on different days in a given month, and the variability for the monthly mean photosynthetic rate was low. Hence the seasonal changes of environmental conditions were less important than the daily changes when considering citrus photosynthesis.

The diurnal mean minimal values (near 1 μmol m^{-2} s^{-1}) and the maximum values (near 10-13μmol m^{-2} s^{-1}) coincide with those described by Syvertsen and Lloyd (1994), Spiegel-Roy and Goldschmidt (1996) and Ribeiro and Machado (2007, 2009a,b). Ribeiro et al. (2009a) reported a higher photosynthetic rate in summer as compared to winter. However in this work, leaf gas exchange was evaluated on a clear day for each season, and intra-seasonal variability was not considered. Furthermore, the environmental conditions in the work of Ribeiro et al. (2009a,b), which was carried out in Piracicaba (Brazil), was marked by rainy summers and dry winters. This fact can also explain the differences observed. There are no works available on seasonal photosynthetic rate variation in the Valencian Community, and any knowledge of this issue in field-grown citrus trees under regular cropping conditions in this major citrus-producing area under Mediterranean conditions is of great interest.

The main factors influencing the photosynthetic rate are photosynthetic photon flux density (PPFD), leaf temperature (T_{leaf}) and leaf-to-air vapour pressure difference (VPD) (Vu and Yelenosky, 1988; Brakke and Allen, 1995; Jifon and Syvertsen, 2003b; Guo et al., 2006; Hu et al., 2007), all of which intercorrelate. The optimum conditions found in the present work are similar to those observed in other works on citrus (Khairi and Hall, 1976; Sinclair and Allen, 1982; Brakke and Allen, 1995; Jifon and Syvertsen, 2003b). A lot of work has been done to clarify the effect of high temperature and high irradiance conditions on the photosynthesis of citrus leaves (Vu and Yelenosky, 1988; Brakke and Allen, 1995; Jifon and Syvertsen, 2003a,b; Guo et al., 2006; Hu et al., 2007; Otero et al., 2011; Santini et al., 2012), but insisting on these aspects is not our objective. Our work confirms and reinforces the reliability of previous studies. Most former results have been obtained by studying the physiological response of citrus to a certain factor under controlled conditions and during a short period. We noted how the photosynthetic rate evolved throughout the year by observing trees under different physiological conditions.

Very few reports on the influence of low temperature on citrus physiology are available. Vu (1999), Ribeiro and Machado (2007) and Ribeiro et al. (2009a) reported a reduced maximum rate of Rubisco carboxylation ($V_{c\ max}$), electron transport driving RuBP regeneration (J_{max}) and stomatal conductance in winter. In the present study, we observe a positive correlation between leaf temperature and photosynthesis for temperatures below 25ºC. Stomatal conductance (g_s) also positively correlates with temperature for this temperature range. Our findings agree with those of Ribeiro and Machado (2007), who state that stomata sensitivity is low to temperatures at between 25-40ºC, but beyond this range, stomata aperture is probably affected, as occurs in Brazil in winter. However, it is necessary to point out that this statement is correct only for VP lower than 20 mbar.

4.3. Leaf photosynthesis rate remains stable throughout the first year of life of leaves, but decreases 20% during the second year

The variation of CO_2 assimilation rates according to leaf age has been studied only in Satsuma mandarin trees (Kubota and Motoyama, 1972; Iglesias et al., 2002). Photosynthate export and source activity were maximum throughout the first year. During the second year, the photosynthetic rate of overwintering citrus leaves recovered 60-70% of previous season values. Our findings in Salustiana sweet orange confirm that leaf photosynthetic capacity remains stable throughout the first year of life of leaves, but lowers during the second year. In contrast to previous results, this capacity decreased by 20% in both ‘on’ and ‘off’ trees. The number of leaves of the fully grown trees of the Salustiana sweet orange cultivar is near 93,000 (Monerri et al., 2011). However, the young leaf percentage is 64% in ‘off’ trees and 53% in ‘on’ trees. It is necessary to bear these data in mind when estimating the diurnal CO_2 assimilation in trees with differences in crop load.

To conclude, the results of this work indicate that the sun-exposed leaves of Salustiana sweet orange trees show a slight seasonal variation in the mean photosynthetic rate under the Valencian Community environmental conditions. Most variability observed is explained by variation in daily environmental conditions. Leaf photosynthetic capacity remains stable throughout the first year of life of leaves. Nevertheless during the second year, photosynthesis diminishes and the leaves from the new spring flush of growth present a higher photosynthetic rate than older ones. Another issue of special interest is that in the mature Salustiana trees aged around 40 years, and in any season, fruit load has no significant effect on photosynthesis as carbohydrates may be translocated from alternative sources. In non-manipulated *Citrus* trees, photosynthesis appears to be source-limited rather than sink-regulated. However, in the same high crop tree, the leaves of branches bearing fruit present a slightly lower photosynthetic rate (approx. 10%) than those of fruitless branches. Variations in mineral content (N, K and P) might explain not only these differences, but also the lower photosynthesis rate in old leaves.

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Legends for Figures

Fig. 1. Mean photosynthetic photon flux density (PPFD, A), air temperature (T_{air}, B) and leaf-to-air vapour pressure difference (VPD, C) values throughout the year in the studied citrus orchard. Values are the mean of 20 diurnal determinations per day in different trees ('on' and 'off' trees). Bars indicate LSD.

Fig. 2. Mean photosynthetic rate (A_N) values during the year in 'Salustiana' sweet orange in the 'on' (black circles) and the 'off' (white circles) trees. Values are the mean of 10 determinations in sun-exposed leaves from different trees. Bars indicate LSD.

Fig. 3. Relationship between stomatal conductance (g_s) and leaf temperature. (A) Relationship at temperatures above 25ºC depending on air vapour pressure (VP). (B) Relationship at temperatures below 25ºC. Linear fits ($P < 0.05$) with their determination coefficients (R^2) are shown in the figures.

Fig. 4. Mean values (±SE) for nitrogen (A), phosphorus (B) and potassium (C) content in Salustiana sweet orange leaves. Determinations were made on young leaves from non-fruiting (white circles) and fruiting (black circles) branches, and on old leaves (white triangles).

Table 1 Effect of crop load and time of year on the photosynthetic rate. Summary of the ANOVA analysis. Statistics include: sum of squares (SSC), degrees of freedom (df), mean square (CM) and F ratio (F).

Source of variation	SSC	df	CM	F
‘on’-‘off’ trees	0.79	1	0.79	0.10 NS
Month	123.2	11	11.2	1.42 NS
‘on’-‘off’ trees x Month	36.7	11	3.34	0.42 NS
Error	806.4	102	7.91	

NS = not significant

Table 2

Table 2 Effect of being adjacent to fruit versus non-fruit branches on the photosynthetic rate and related parameters in the 'on' trees. Photosynthetic rate (A_N; µmol m^{-2} s^{-1}), stomatal conductance (g_s; mol m^{-2} s^{-1}), transpiration rate (E; mmol m^{-2} s^{-1}), substomatal CO_2 concentration to ambient CO_2 concentration (C_i/C_a), water use efficiency (WUE; µmol $mmol^{-1}$), photosynthetic photon flux density (PPFD; µmol m^{-2} s^{-1}), leaf temperature (T_{leaf}; ºC) and leaf-to-air vapour pressure difference (VPD; mbar). Measurements were taken from June to September.

	A_N	gs	E	C_i/C_a	WUE	PPFD	T_{leaf}	VPD
Leaves of branches with fruit	5.9 b	0.10 b	2.2 b	0.66 NS	3.02 NS	1193 NS	34 NS	37.8 NS
Leaves of branches without fruit	6.5 a	0.12 a	2.4 a	0.66	3.01	1207	34	38.1
Leaf type x date	NS	NS	NS	NS	NS	NS	NS	NS

Within each column, different letters indicate significant differences ($P < 0.05$). NS = not significant.

Table 3

Table 3 Effect of leaf position (distance to the fruit) along the branch on the photosynthetic rate and related parameters in ‘on’ trees. Measurements were taken on four days in May, 2013. Photosynthetic rate (A_N; µmol m^{-2} s^{-1}), stomatal conductance (g_s; mol m^{-2} s^{-1}), transpiration rate (E; mmol m^{-2} s^{-1}), substomatal CO_2 concentration (C_i; µmol mol^{-1}), water use efficiency (WUE; µmol $mmol^{-1}$), maximum efficiency of photosystem II (Fv/Fm), photosynthetic photon flux density (PPFD; µmol m^{-2} s^{-1}), leaf temperature (T_{leaf}; ºC) and leaf-to-air vapour pressure difference (VPD, mbar).

Leaf position	A_N		gs		E		C_i		WUE		Fv/Fm		PPFD		T_{leaf}		VPD	
1st	7.2	a	0.09	a	2.2	a	187	NS	3.3	NS	0.81	NS	1430	NS	31	NS	34.5	NS
3rd	6.3	ab	0.08	a	1.9	b	171		3.3		0.81		1320		32		33.9	
7th	4.7	b	0.06	b	1.4	c	168		3.4		0.82		1410		31		35.3	

Within a column, different letters indicate significant differences ($P < 0.05$). NS: not significant

Table 4

Table 4 Effect of leaf age and crop load on the photosynthetic rate and related parameters. Measurements were taken on 24 days from the beginning of May to the end of July. Values are the means of 10 leaves from 10 trees for each age and crop load. Photosynthetic rate (A_N; µmol m^{-2} s^{-1}), stomatal conductance (g_s; mol m^{-2} s^{-1}), transpiration rate (E; mmol m^{-2} s^{-1}), substomatal CO_2 concentration (C_i; µmol mol^{-1}), water use efficiency (WUE; µmol $mmol^{-1}$), maximum efficiency of photosystem II (Fv/Fm), photosynthetic photon flux density (PPFD; µmol m^{-2} s^{-1}), leaf temperature (T_{leaf}; ºC) and leaf-to-air vapour pressure difference (VPD; mbar)

Crop load and leaf age	A_N		gs		E		C_i		WUE		Fv/Fm		PPFD		T_{leaf}		VPD	
‘on’																		
3-6 months	8.6	a	0.17	a	2.6	a	268	NS	3.6	NS	0.794	NS	1147	NS	34	NS	35.8	NS
13-15 months	7.1	b	0.11	b	2.1	b	273		3.7		0.795		1019		33		34.2	
‘off’																		
3-6 months	8.7	a	0.16	a	2.4	a	271	NS	3.9	NS	0.776	NS	1051	NS	33	NS	34.9	NS
13-15 months	7.0	b	0.10	b	1.9	b	274		4.1		0.787		971		33		33.1	

Within a column and for each crop load, different letters indicate significant differences ($P < 0.05$). NS = not significant

Figure 1

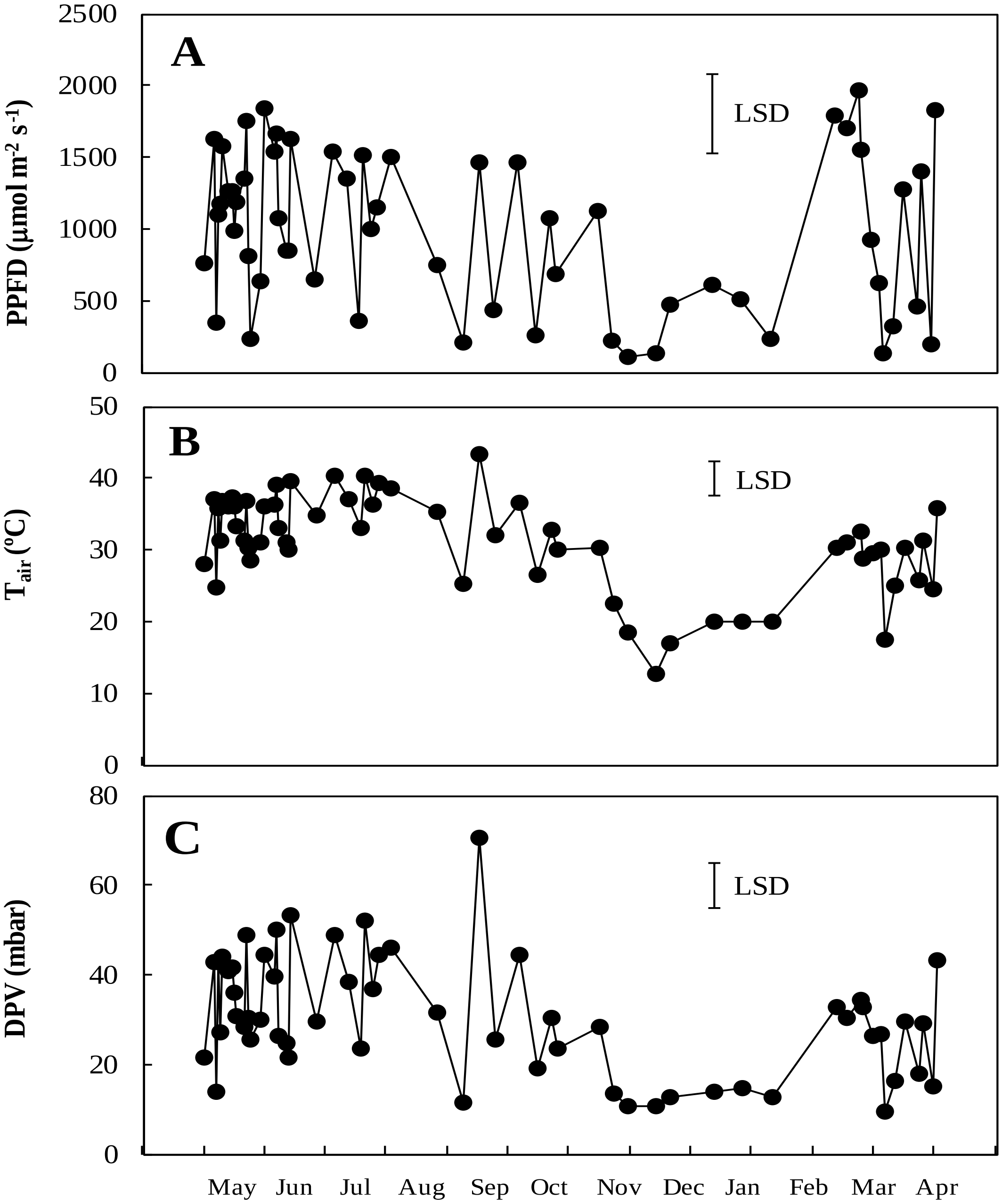
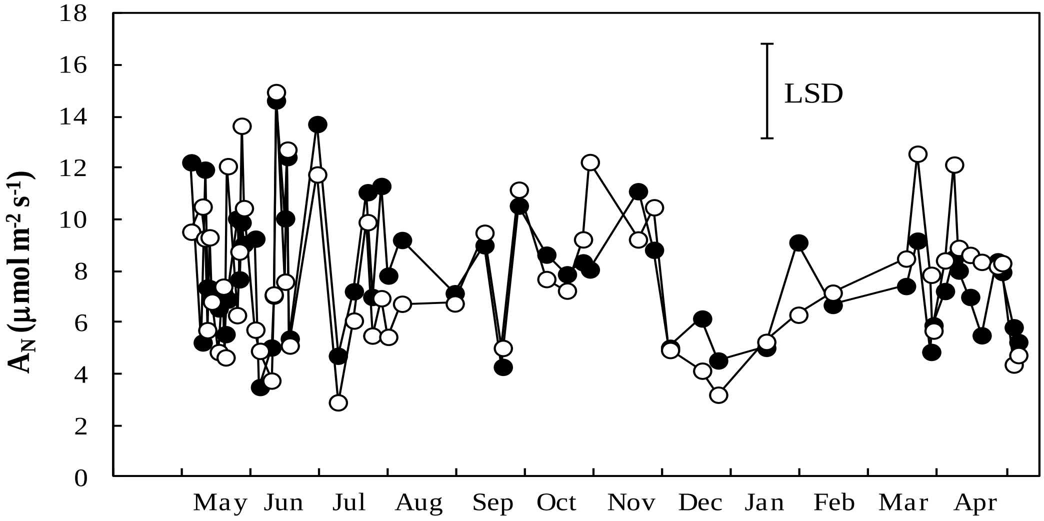

A
B
C
LSD
LSD
LSD
PPFD (µmol m-2 s-1)
Tair (ºC)
DPV (mbar)
0
500
1000
1500
2000
2500
0
10
20
30
40
50
0
20
40
60
80
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr

Figure 2



A_N (µmol m^{-2} s^{-1})
0
2
4
6
8
10
12
14
16
18
LSD
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr

Figure 3

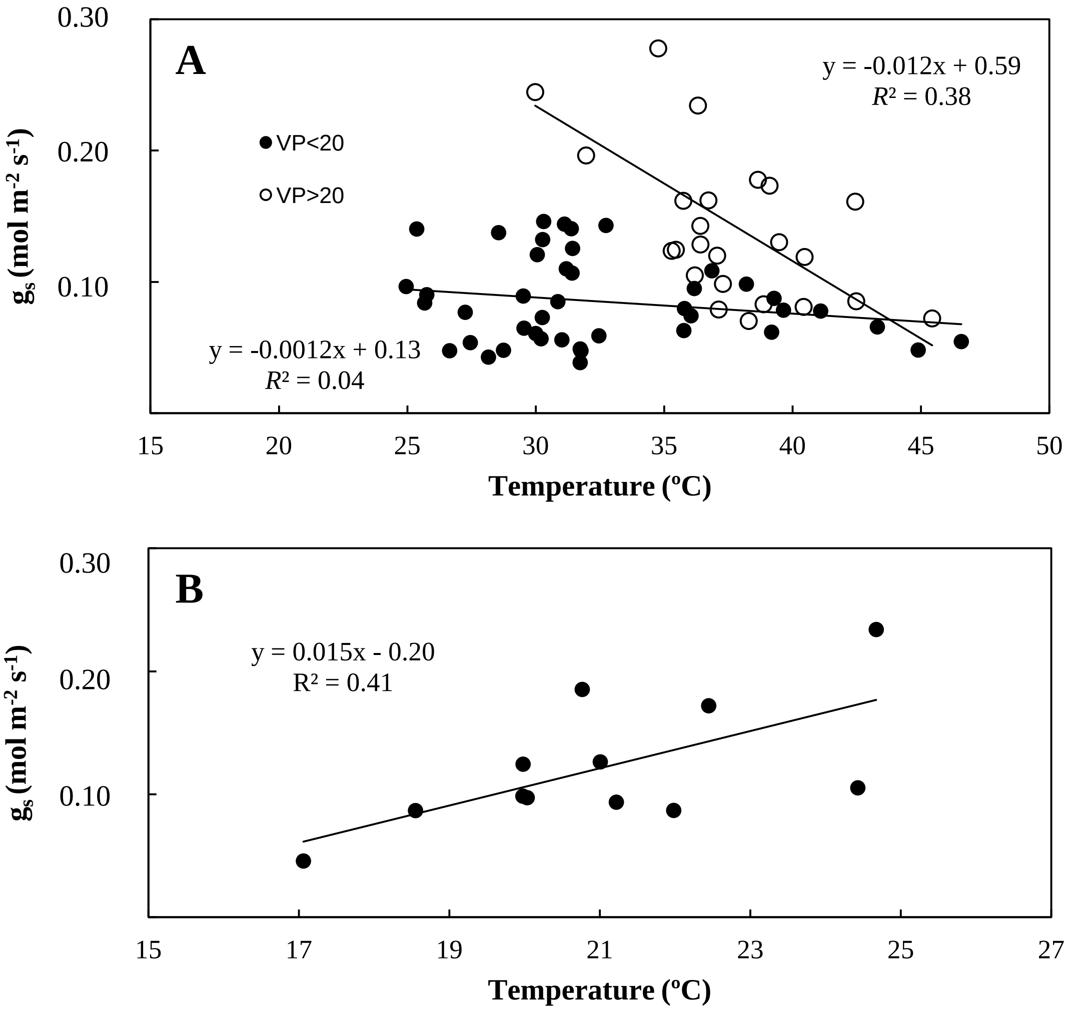
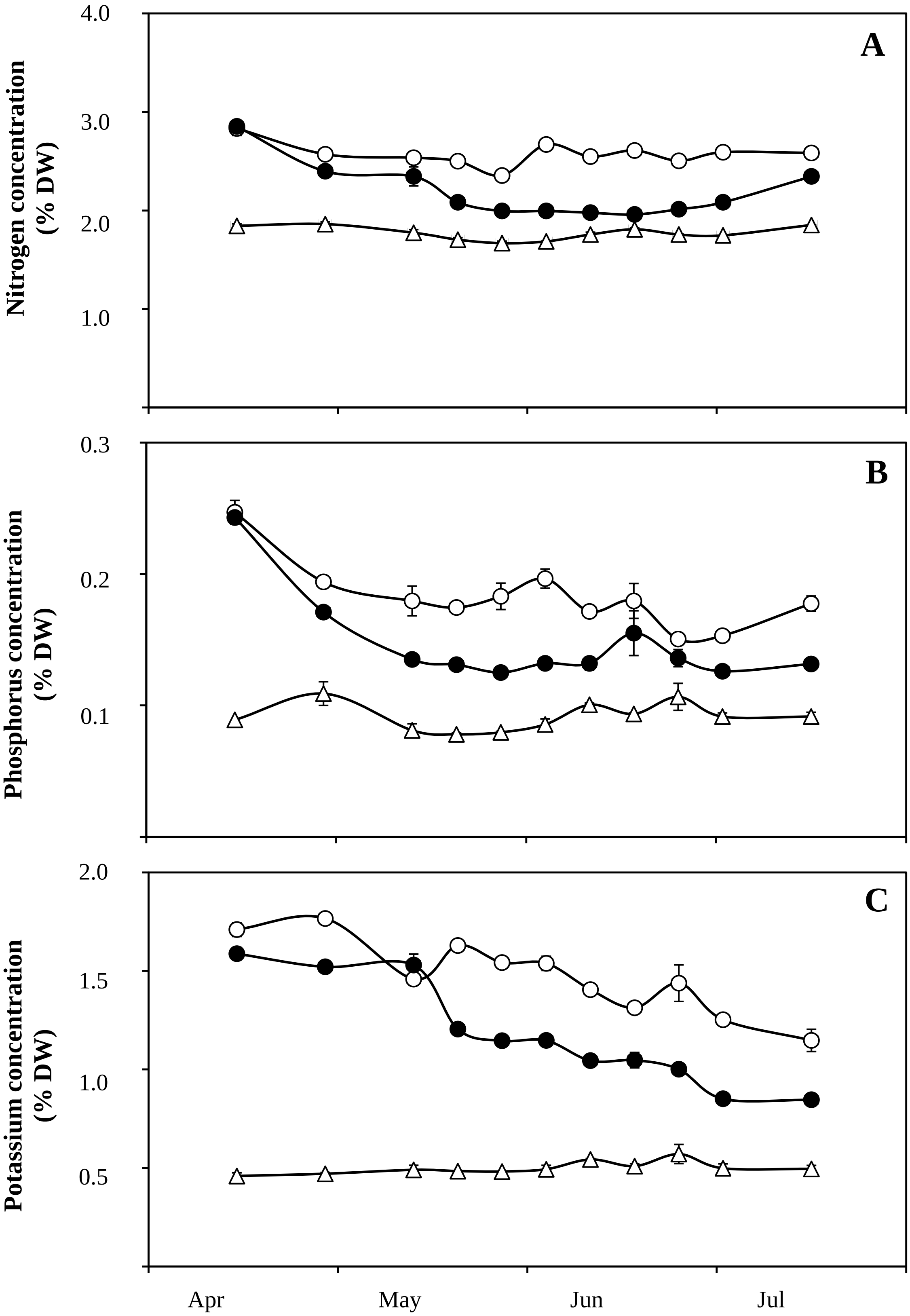

0.30
0.20
0.10
A
y = -0.012x + 0.59
R^2 = 0.38
● VP<20
○ VP>20
y = -0.0012x + 0.13
R^2 = 0.04
g_s (mol m^{-2} s^{-1})
15
20
25
30
35
40
45
50
Temperature (ºC)
0.30
0.20
0.10
B
y = 0.015x - 0.20
R² = 0.41
g_s (mol m^{-2} s^{-1})
15
17
19
21
23
25
27
Temperature (ºC)

Figure 4



4.0
3.0
2.0
1.0
A
Nitrogen concentration
(% DW)
0.3
0.2
0.1
B
Phosphorus concentration
(% DW)
2.0
1.5
1.0
0.5
0.5
C
Potassium concentration
(% DW)
Apr
May
Jun
Jul