Relation of carbohydrate reserves with the forthcoming crop, flower formation and photosynthetic rate, in the alternate bearing Salustiana sweet orange (*Citrus sinensis* L.).


Departamento de Producción Vegetal. Universidad Politécnica de Valencia. Camino de vera s.n. 46022-Valencia (Spain).

* Corresponding author: Tel: +34-963877410; Fax: +34-963877419. E-mail address: sergonne@bvg.upv.es (S.G. Nebauer).
ABSTRACT

The influence of crop load on photosynthetic CO\textsubscript{2} fixation and the accumulation and mobilization of carbohydrate reserves in leaves, twigs and roots, was determined in the alternate bearing Salustiana cultivar of sweet orange (*Citrus sinensis* [L.] Osbeck) in order to assess the significance of the carbohydrate reserves in relation to flower formation and fruit set, and the effect of carbohydrate use in fructification on CO\textsubscript{2} fixation. A heavy crop load failed to increase leaf photosynthesis as compared to non fruiting trees. In fruiting trees most of the fixed carbon accumulated in the mature fruit, and no accumulation of reserve carbohydrates occurred in the roots before harvest. In the non fruiting trees, part of the fixed carbon was transported to the root and utilized in growth processes and, after December, stored as reserves. Reserve carbohydrate accumulation in the leaves started by early December, and the levels in the leaves were, until bud sprouting, the same in on and off trees. The heavy flower formation which followed an off year caused the rapid mobilization of the stored reserves, which were exhausted at full bloom. We could not find evidence for carbon fixation regulation by fruit demand or by the carbohydrate levels in the leaves. The carbohydrate reserves played no role in fruit set, which relied on current photosynthesis.

Keywords: Carbohydrate reserves, *Citrus*, Flowering, Fruit growth, Fruit set, Photosynthesis
1. Introduction

Under tropical climate conditions, Citrus trees accumulate carbohydrate reserves during the winter rest and mobilise them during the spring flush of growth (Goldschmidt and Koch, 1996). This behaviour is similar to that described in deciduous fruit trees, which accumulate carbohydrate reserves before leaf fall and utilise them during the dormant season and the spring growth (Schaffer et al., 1999), except for some differences in the partitioning of the reserves, and their importance in plant growth regulation and survival. In deciduous trees, the root system is the major storage organ for carbohydrates (Loescher et al., 1990). In Citrus, the root system may still be the major storage organ for carbohydrates, but carbohydrates also accumulate in the leaves at a high concentration (Goldschmidt and Golomb, 1982). The importance of reserve carbohydrates in deciduous trees seems evident. Winter respiration and the beginning of both vegetative and, in some species, reproductive growth, occur in the absence of photosynthesing leaves, and must be totally dependent on reserves (Loescher et al., 1990). On the contrary, photosynthesis proceeds in Citrus during winter at a rate high enough to affect growth significantly (Syvertsen et al., 1997; Goldschmidt, 1999).

Therefore, the reserves should not be as critical for winter and spring growth as in deciduous trees, yet a role for carbohydrate reserves in some aspects of development has been postulated.

The accumulation of reserves is inversely related to crop load (Goldschmidt and Golomb, 1982), and a depletion of them under heavy crop load has been related to tree collapse (Smith, 1976) and the triggering of an alternate bearing habit (Monselise and Goldschmidt, 1982; Guardiola, 1992; Syvertsen and Lloyd, 1994). Although flower formation could be correlated in some experiments with the accumulation of
carbohydrates (Smith, 1976; Goldschmidt and Golomb, 1982), carbohydrate levels are not the sole factor regulating flower formation (Goldschmidt, 1999; García-Luis and Guardiola, 2000). During flower formation and fruit set, part of the reserves are translocated to the reproductive organs (Akao et al., 1981), but the contribution of the reserves to these processes must vary widely as indicated by the rate of their depletion. This rate of depletion may vary among cultivars (Borrás et al., 1984; González-Ferrer et al., 1984), but differences in the rate of depletion within a cultivar have also been reported (García-Luis et al., 1988; Ruiz and Guardiola, 1994; Ruiz et al., 2001). The rate of depletion has been related to flower number (García-Luis et al., 1988).

There are some studies about the significance of reserves in alternate bearing citrus trees (Goldschmidt and Golomb, 1982; Borrás et al., 1984). However, the research has only been performed on one day during the flowering period. In the present report, we have studied the seasonal variation of carbohydrate reserves in the leaves, in the twigs (both in the bark and in the wood) and in the roots, as well as the rate of mobilization during the spring flush of growth, in relation to crop load.

It has been suggested that the carbohydrate accumulation may interfere with photosynthesis by way of product inhibition (Goldschmidt and Koch, 1996), a suggestion supported by the inhibition demonstrated in girdled branches in the absence of a sink outlet (Schaffer et al., 1986). However, this effect is controversial under regular cropping conditions (Goldschmidt and Koch, 1996).

The main objectives of the present work were: i) to assess the importance of reserves for the development of the forthcoming crop, and their role, if any, in the initiation of an alternate bearing cycle; ii) to analyse the regulatory role of carbohydrates in flowering and how the changes in carbohydrate reserves during the year reflect the variations in supply and demand on the diverse organs; and iii) to study the effect of crop load and
leaf carbohydrate levels on CO$_2$ fixation rate. The measurements were performed in the 
Salustiana cultivar of sweet orange. This cultivar has a strong alternate bearing 
behaviour, which allowed us to study the above-mentioned parameters both in trees 
without fruit and in trees with a heavy crop load.

2. Materials and methods

2.1. Plant material

The experiments were performed on 35-year-old trees of the “Salustiana” cultivar of 
sweet orange (Citrus sinensis [L.] Osbeck) grafted onto Troyer citrange (Citrus sinensis 
The trees of this parthenocarpic (seedless) cultivar presented an intense alternate 
bearing habit. In the year of beginning our experiments (2001; year 1), some of the trees 
(on trees), formed a huge number of flowers, resulting in a heavy crop load, which was 
followed by a year (2002; year 2) with very few flowers formed or none at all. Some of 
the trees (off trees) presented the same fruiting habit but shifted by one year. They 
formed almost no flowers, and had a low yield, the year we started the experiment, but 
formed many flowers the following year. Under the conditions of our experiments, bud 
sprouting of the spring flush of growth occurred from mid February (fruitless trees) to 
early-mid March (fruiting trees). Vegetative shoots (bearing only leaves), leafy 
inflorescences (bearing flowers and leaves) and leafless inflorescences (bearing only 
flowers) were formed. Flower opening started by April, 1. Two waves of fruit 
abscission were produced from April to June. The fruit was harvested by early February 
the following year.

2.2. Experimental design
Shortly before flower opening, **on** and **off** trees were chosen. For the sake of clarity, the
trees are named in the text as **on** or **off** according to the number of flowers they formed
in the first year of the experiment. In these two groups of trees we determined the
photosynthetic CO$_2$ fixation rate and carbohydrate accumulation in the leaves, the bark
and the wood of the vegetative sprouts formed during the spring flush of growth, and in
the roots. These changes were related to the use of dry matter (=carbohydrates) in
fructification, which was calculated for the **on** trees during the first year of the
experiment.

2.3. **The characteristics of the spring flush of growth and dry matter use in fructification**

A sufficient number of vegetative shoots formed during the preceding year and totalling
at least 1,000 axillary buds, were selected before flower opening in each one of six trees
of each group on year 1. The numbers of vegetative shoots, leafy inflorescences, and
leafless inflorescences, started during the spring flush of growth, as well as their
characteristics (number of leaves and of flowers), were measured. The number of
abscised flowers and fruits was measured periodically (at 10-12 days intervals)
collecting the abscised organs with a plastic net placed under the tree canopy. Adding to
this value the number of mature fruit at harvest, we obtained the total number of flowers
formed. From these figures, the number of mature fruit present on the tree at any time
from flower initiation until harvest, and the number of shoots and of leaves formed
during the spring flush of growth, were calculated. Values are averages of six trees.
The use of dry matter in fructification was calculated from the number of mature fruit at
the end of each one of the periods considered and the amount of dry matter accumulated
by the mature fruit in that period. The increase in weight of the fruit was calculated as
the difference in dry weight of the fruit at the end and the beginning of each time
interval. In the first two samplings, the values thus calculated were corrected for respiratory losses (Bustan and Goldschmidt, 1998). The weights were calculated by measuring the diameter of 200 ovaries/fruits selected at random. The relationship between fruit diameter and weight was determined in random samplings performed on each date on non-measuring trees of the same characteristics, in order to avoid any alteration in fruit number.

2.4. Carbohydrate contents in the leaves, twigs and roots

Soluble sugars and starch in the leaves from vegetative shoots were determined from June of year 1, to the end of June of year 2. From June to September of the first year, three independent samples of 50 leaves each (two different trees per sample) were sampled from the mid portion of vegetative sprouts, both from off and on trees. From October onwards, and until late June on year 2, each sample consisted of the apical portion (having the five most apical phytomers) of ten twigs (from two different trees per sample). These twigs were separated into their component parts (leaves, bark and wood), which were analysed separately. The sampling procedure, the handling of the samples, and carbohydrate determinations were performed as described by Ruiz and Guardiola (1994).

Roots were sampled at a depth of 10 to 25 cm from positions close to a drip emitter (five positions per tree). On each tree, ten fibrous roots, with a thickness of 1.5 to 3 mm, were sampled. After careful washing to remove the soil, the fibrous roots were separated from the recently formed feeder roots. These two root samples were analysed separately. The analytical procedure was the same as for the leaves.

2.5. Determination of leaf gas exchange
The rate of net CO$_2$ fixation ($A_N$) was measured from June to October of the year 1, and from March to May during the year 2, in leaves of vegetative shoots of the spring flush of growth formed the first year of the experiment. The measurements were performed in attached leaves exposed to the sunlight in ambient conditions, with an LCi Portable Photosynthesis System (ADC, Herst, UK). During the measurements, the leaves were held perpendicularly to sunlight. On each day, three repeated measurements were performed on ten leaves from different on trees, and ten leaves from off trees. In each of the periods indicated, the measurements were performed on fifteen to twenty different days, and the values were averaged. The measurements were performed from 10 a.m. until 12 p.m., to avoid the midday drop in photosynthesis. The measurements from the leaves of the two tree types were intercalated, to ensure the similarity of the environmental conditions.

2.6. Tree girdling

To determine the influence of the transport to the root system on carbohydrate distribution within the plant, on and off trees were girdled at the base of the scaffold branches, cutting the bark with a single edge knife. This procedure removed a 2 mm thick ring of bark. Carbohydrate accumulation in the leaves and in the bark from vegetative sprouts, were determined 6-9 weeks after girdling. No girdled trees served as controls. Each treatment consisted of three one-tree replicates. The experiment, with the same lay-out, was performed on different trees in June, September, October and November.

2.7. Statistical analyses
Results were subjected to an analysis of variance (SPSS for Windows version 12.0.1, Illinois, USA). Mean comparisons were performed with Tukey’s test.

3. Results

3.1. The characteristics of the spring flush of growth

The characteristics of the spring flush of growth are presented in Table 1. During the first year, the percentage of bud sprouting was much higher ($P \leq 0.01$) in the on trees (56.3 ± 2.7 %) than in the off trees (22.4 ± 0.29 %). This resulted in a much higher number of inflorescences initiated (20.6 vs. 0.8 thousands per tree respectively; $P \leq 0.01$) and of flowers formed (75 vs. 2 thousands per tree; $P \leq 0.01$). On the contrary, the number of vegetative sprouts initiated was 5-fold higher (8.2 vs. 1.6 thousands per tree) in the off trees. The number of leaves initiated in spring was similar in both tree groups.

In the off trees, most of the 60 thousand new leaves were on vegetative sprouts; in the on trees, about 80% of the 49 thousand leaves formed were located in leafy inflorescences. Individual leaf area was higher ($P \leq 0.05$) in the off trees (30 ± 1 cm$^2$ per leaf) than in the on trees. In the on trees, the individual leaf area was similar in the vegetative sprouts (26 ± 1 cm$^2$) and in the inflorescences (23 ± 1 cm$^2$).

The number of old leaves (up to 1-year-old at flowering time), could also be calculated from our flower counts. As the on trees formed 75 thousand flowers, and the flower count (in thousands of flowers per 100 nodes) was 0.17 ± 0.025 (average value of six trees), the number of nodes (= old leaves) present was close to 44 thousand per tree ($\lfloor 75/0.17\rfloor \times 100$). A similar calculation for the off trees yielded 33 thousand old leaves (calculated from a flower count of 0.006 flowers per 100 nodes and a total flower number of 2 thousand per tree).
During the second year of the experiment, the number of flowers formed was high in
the off trees (which were in an on year), and low, although somewhat higher than
expected, in the on trees (which were in an off year; Table 1). These figures were
obtained from the counts of abscised flowers and fruits, and of mature fruit. The
characteristics of the spring flush of growth were not measured.

3.2. Use of dry matter in fructification

In the on trees, fruit abscission occurred from anthesis until the end of June, with two
distinct peak values: shortly after anthesis (during April), and by the end of May. The
fruit dropped during the first wave of abscission had shown little growth (data not
shown). During the second wave of abscission, the rate of accumulation of dry matter in
the fruit increased gradually, reaching a value close to the maximum by mid June,
shortly before abscission ceased (Fig. 1).

The rate of utilization of dry matter in flower formation and fruit growth was high from
bud sprouting to anthesis, reflecting the cost of flower formation (Fig. 1). Then it fell to
a low value, around a fifth of the previous one, coinciding with the first peak of
abscission, to increase gradually to reach a highest value shortly before the end of
abscission, as the rate of accumulation of dry matter in the mature fruit increased (Fig.
1). After the period of abscission, dry matter use in fruit formation paralleled individual
fruit growth, decreasing gradually from September onwards.

During the first year of the experiment, the rate of dry matter utilization in the off trees
was in the range 3-5 % of the values presented for the on trees during flower formation
and fruit abscission and of 11 % after fruit abscission (Table 2). The amount of dry
matter recovered in the fruit at harvest ranged between 64% (on trees) and 74% (off
trees) of the total dry matter use. The rest was lost through flower and fruit abscission, and respiritory losses.

During the second year, the off trees entered an on year and formed a huge number of flowers (Table 1). This year, the rate of dry matter use from flower initiation to anthesis was 256 g tree\(^{-1}\) day\(^{-1}\), rising at the end of June to 415 g tree\(^{-1}\) day\(^{-1}\). These values are similar to the ones reported above for the on trees during the first year.

3.3. Carbohydrate content in leaves, bark and wood

The carbohydrate content in the leaves from the vegetative sprouts formed during the spring flush of growth of the first year is presented in Fig. 2. During the first year, the total carbohydrate content in the leaves during the final stages of fruit abscission (June) was 2.5-fold higher in the off than in the on trees. Afterwards, there was a gradual loss of carbohydrates from the leaves of the off trees, and from September to December the carbohydrate content was lowest, and similar in both tree classes. Carbohydrate accumulation in the leaves started by early December and proceeded at a similar rate in both tree classes until the start of bud sprouting in the off trees by the end of February. In these trees, which entered an on year, the level of carbohydrates fell to a low value at anthesis (early April), recovered partially at the beginning of fruit abscission, to fell to a lowest value close to 10% (on dry matter basis) by the end of abscission (end of June; Fig. 2). In the on trees, which entered an off year, carbohydrate content in the leaves was maximal during the period April to May, decreasing by the end of June (Fig. 2).

The changes in starch content accounted for most of the changes in total carbohydrates in the leaves (Fig. 2), and the two parameters were closely related (\(r^2 = 0.94; n = 30, P \leq 0.001\)). The changes in soluble sugar content were much smaller, and in most of the samples no differences were found between the two tree classes. At flowering, the sugar
concentration in the leaves was higher in the trees entering an off year than in those entering an on year (Fig. 2).

The pattern of the changes in carbohydrates in the bark and in the wood was similar to that described for the leaves (Fig. 3). Accumulation in the bark started after November, and no significant differences were found between the on and the off trees until sprouting. At this time, there was a drastic reduction in carbohydrates, both in the bark and in the wood, in those trees entering an on year. As for the leaves, the changes in starch contents accounted for most of these changes (evidence not presented). The $r^2$ value between these two parameters was 0.94 (bark) and 0.80 (wood).

3.4. The effect of girdling on leaf carbohydrates

Girdling the scaffold branches caused the accumulation of carbohydrates in the leaves of the off trees. This accumulation was much greater during June, October and November, above 30% of the content in the leaves of the ungirdled controls, than in September, when it was only 14% of the value of the controls (Fig. 4).

In the on trees, girdling during September and October did not affect the carbohydrate concentration in the leaves (Fig. 4). When performed in June or in November, girdling increased carbohydrate contents by 10% above the controls (Fig. 4).

Both in the on and the off trees, the effect of girdling (performed in October or in November) on carbohydrate contents in the bark of the twigs was similar to that described for the leaves.

3.5. Carbohydrate content in the roots

From October to early December, carbohydrate content in the roots was the same in the off and in the on trees (Fig. 5). At this date, a gradual accumulation of carbohydrates
started in the roots of the off trees, both in the fibrous and, in a lesser amount, in the
feeder roots (Fig. 5). This accumulation ceased at the time of bud sprouting, when a
significant part of the accumulated reserves were utilised in 20 days (Fig. 5). At this
time, there was a significant increase in the proportion of feeder roots (Fig. 6).

In the on trees, the accumulation of carbohydrates started after harvest, and ended by
bud sprouting (Fig. 5). The maximum concentration of carbohydrates in the roots of the
on trees was much smaller than in the off trees. After sprouting, the concentration of
carbohydrates in the roots was identical in the two tree classes.

Most of the changes in carbohydrate contents reflected the accumulation and the
mobilization of starch, whose concentration was closely related to the concentration of
total metabolizable carbohydrates ($r^2 = 0.98$ and 0.90 for the feeder and the fibrous
roots, respectively; $n = 20$; $P \leq 0.001$).

3.6. The effect of fruit load on photosynthesis

Leaf photosynthesis was largely determined by the environmental conditions, which
were the main factor in the variability of this parameter. During the periods with a
highest rate of dry matter utilization in the on tress, that is to say, by March-April when
flowering is going on, and June-July when the fruit growth rate is the highest (Fig. 2),
there were no significant differences in photosynthetic rates between the trees with a
low and a high crop (Table 3). It is important to point out that differences in
carbohydrates between tree groups are highest during these periods (Fig. 3).

4. Discussion

It is a well established fact that the fruit is a major and priority sink in Citrus, and that a
heavy fruit load reduces both the diversion of carbohydrates towards the root system
and the accumulation of carbohydrate reserves (Goldschmidt and Koch, 1996). The amount of the mid-winter carbohydrate reserves in an off year in an adult tree of the alternate bearer Wilking mandarin has been estimated at ca. 24 Kg (Goldschmidt and Golomb, 1982). These reserves are mobilized during the next on year (Syvertsen and Lloyd, 1994; Goldschmidt and Koch, 1996), and it has been speculated they could satisfy a considerable portion of the dry matter requirements of the following crop (Goldschmidt and Golomb, 1982). The aim of the present study was to assess the importance of these reserves for the development of the forthcoming crop, and their role, if any, in the initiation of an alternate bearing cycle.

4.1. Use of dry matter in fructification

In our experiments, the concentration of reserve carbohydrates in the off trees, which entered an on year, at the onset of bud sprouting was of the same order of magnitude as reported for other alternate Citrus cultivars at the end of an off year (Goldschmidt and Koch, 1996). These reserves were used-up during bud sprouting, and at the time of full bloom (early April) the carbohydrate concentration in the leaves and in the twigs had fallen to a minimum value (Fig. 2 and 3). During the period of fruit abscission (May and June), in which competition for carbohydrates is considered to be a limiting factor for fruit retention (Goldschmidt, 1999), fruit nutrition was supported by current photosynthesis and the carbohydrates stored after anthesis (during April; Fig. 2 and 3). The relatively high carbohydrate contents in the leaves and in the bark at full bloom, around 10 % on a dry matter basis, may represent a non-utilizable fraction (Goldschmidt and Koch, 1996; Ruiz et al., 2001).

As reported for other woody species (Loescher et al., 1990), the root system was the main storage organ for carbohydrates. These reserves fell dramatically at the onset of
bud sprouting, in particular in the fibrous roots (Fig. 5), coinciding with the resumption
of root growth (Fig. 6). This fall in carbohydrate reserves was relatively smaller than in
the leaves and in the twigs, and the fibrous roots of the trees which entered an on year,
had during anthesis a carbohydrate concentration twice the amount found in early
October. These reserves could potentially be utilized, but the net change in these
reserves during the period of fruit abscission was very small (Fig. 5), and the girdling
experiments demonstrated that at this time the root system competed with the fruit for
the available carbohydrates (Fig. 4). Although some transport of carbohydrates from the
root system to the shoot was demonstrated in labelling experiments (Kubota and
Motoyama, 1972; Goldschmidt and Koch, 1996), we found no evidence for the
contribution of the root reserves to fruit set. A similar conclusion was reached by
Loescher et al. (1990) in their review on root reserves in deciduous trees, as these
authors stated that evidence that the roots play a special role on fruiting behaviour is
unclear.

While the reserve carbohydrates may have supported the initial stages of vegetative
growth and reproductive development (Akao et al., 1981), they played no significant
role in fruit set. Most of the reserves stored during the off year may have gone in
surplus flower formation, whose cost in term of carbohydrates was about 60 % of the
total reserves (Table 2). This surplus flower formation had little or no effect on fruit set
(Becerra and Guardiola, 1984), but in some conditions may impair it (Becerra and
Guardiola, 1984; Guardiola et al., 1984).

4.2. The role of carbohydrate reserves in alternate bearing habit

It is generally accepted that the cycles of alternate bearing are caused by the inhibition
of flower formation after a heavy fruit load (Guardiola, 1992), and flower formation has
been related to carbohydrate levels (Goldschmidt and Golomb, 1982; García Luis et al., 1988, 1995). This role of flower inhibition by the fruit was supported by our results (Table 1), but we could not find evidence for a regulatory role of carbohydrates. Most alternate *Citrus* cultivars are late maturing, and the presence of developing fruit prevents the accumulation of carbohydrates in all tree organs during an on cycle (Syvertsen and Lloyd, 1994). In the early maturing Salustiana orange used in this study, the low fruit strength of the fruit after November (Fig. 1) allowed the accumulation of reserves during winter in the leaves and the twigs of the on trees (Figures 2 and 3), and during the period of flower induction and initiation (from early November until bud sprouting) the carbohydrate levels in these organs were identical in the off and the on trees. We may conclude therefore that reserve carbohydrates do not play a regulatory role in flower formation, a role whose importance was questioned by Goldschmidt (1999) and García-Luis and Guardiola (2000). As most of the flowers formed in the axillary buds of the vegetative shoots initiated during the spring flush of growth of the preceding year, the 5-fold higher number of these buds in the off trees than in the on trees (8.2 vs. 1.6 thousand vegetative shoots per tree, respectively; Table 1) would explain only in part the 20 to 35-fold differences in flower formation between the off and the on trees (Table 1). A direct effect of the fruit on flower initiation in the buds also seems evident.

4.3. **Source-sink effects on seasonal carbohydrate reserves**

The changes in carbohydrate reserves during the year reflect the variations in supply and demand. The carbohydrate demand during the initial stages of vegetative growth and reproductive development in trees which entered in an on year determined an initial drop in the leaf and twig reserves which was followed by a transient recovery and a
further drop at the end of fruit abscission (Fig. 2 and 3). In trees which entered an off year with a low carbohydrate demand for fructification, carbohydrate accumulation continued until early April and remained high until the end of May (Figures 2 and 3). The carbohydrate levels in the leaves during spring in trees with a low crop were twice as much the levels in the high crop trees (Fig. 2). The differences in the pattern of the changes in carbohydrate reserves reported by different authors (see references in Introduction), which lead sometimes to high starch levels during May in the northern hemisphere (Borrás et al. 1984), might be the result of differences in flower formation rather than a varietal characteristic.

After fruit abscission, carbohydrate levels in the leaves declined and stayed low during summer and autumn. As no effect of the fruit on photosynthesis (Table 3) or on leaf carbohydrates (Fig. 2) was found during those months, most of the carbon fixed by the off trees must have been transported to the root system, a conclusion supported by the girdling experiments (Fig. 4). The smaller retention caused by girdling in the off trees during September, may reflect competition from the summer flush of growth. This higher transport of carbohydrates to the root system in the off trees did not increase the root reserves (Fig. 5) nor root growth as assessed by the proportion of feeder roots to the total root sample (Fig. 6), and was probably used in the thickening of the major roots. Interestingly, Goldschmidt ad Golomb (1982) demonstrated in Wilking mandarin a 2-fold higher weight of the major roots after an off year than after an on year.

Despite the low sink strength of the fruit from December onwards, it prevented the accumulation of carbohydrate reserves in the root, which only occurred after harvest, an effect previously observed in potted trees (García-Luis et al., 1995). This fruit effect, whose regulation was not investigated, did not affect the accumulation of carbohydrates in the leaves (Fig. 2). During this period of time, fruit load affected the carbohydrate
reserves in the roots but had no effect on their concentration in the leaves. As pointed out by Goldschmidt and Golomb (1982), the leaves are not always the most sensitive indicator organs. In this study we explained how, depending on the time of sampling, the carbohydrate concentration in the leaves of the trees with a high crop was lower (March-July) or the same (autumn and winter months) than in the trees with a low crop.

4.4. Photosynthesis regulation

The regulation of photosynthesis ($A_N$) by sink demand has been demonstrated in *Citrus* either using potted trees (Syvertsen and Lloyd, 1994; Goldschmidt and Koch, 1996; Iglesias et al. 2002), or altering the source-sink balance in the tree (Li et al., 2003), and a regulatory role for the carbohydrate level in the leaves has been suggested (Iglesias et al., 2002). However, to what extent sink demand controls photosynthetic rates in *Citrus* under regular cropping conditions is not clear, nor is the mechanism of regulation (Goldschmidt and Koch, 1996). We addressed this issue by measuring $A_N$ under orchard conditions at developmental stages in which the differences in the sink strength of the developing flowers and fruits between the high and low crop trees were highest; the flower formation period (March-April), and the main fruit growth stage (June-July). The highest differences in leaf carbohydrate contents happened during these periods. In both of them, the environmental conditions were the main determinant of $A_N$. No enhancement of $A_N$ by a high sink strength related to fruiting was found in our experiments. A delay of leaf senescence caused by fruiting, and an enhancement of autumn photosynthesis, has been demonstrated in apple (Tartachynk and Blake, 2004), but is doubtful whether a similar regulation may occur in *Citrus*, whose leaves show a high photosynthetic efficiency during 2 years (Kubota and Motoyama, 1972). Our data supported the view that photosynthesis in unmanipulated *Citrus* trees was source rather
than sink limited during most of the year. As shown previously for deciduous trees like apple (Lakso et al., 1899) and cherry (Roper et al., 1988), fruit load had no significant effect on photosynthesis as carbohydrates may be translocated to alternate sinks. Nor could we find a depressing effect of leaf carbohydrates on $A_N$, as this parameter was similar in trees with low and high crop when differences in carbohydrate content were highest, and the 13 to 15-month-old leaves of the low crop trees (Table 3) had a very high carbohydrate concentration (Fig. 2). Interestingly, the carbohydrate concentration in these leaves (25% on a dry matter basis) was higher than the concentration that Iglesias et al. (2002) reported as inhibitory for manipulated Satsuma mandarin trees (Citrus unshiu) (ca. 18% on dry matter basis). These conclusions may not extend to the full year, as Syvertsen et al. (2003) found that during winter (late June in the southern hemisphere, equivalent to late December in the northern hemisphere) defruited “Spring” navel orange trees had a 40% lower $A_N$ value than fruiting (and already harvested) trees. Although defruiting may cause a transient change in $A_N$ (Gucci et al., 1991) and the trees used by Syvertsen et al. (2003) were smaller in size (5 years old) than the ones we used in our experiments, the possibility that tree behaviour during the winter months may be different as described in this report cannot be dismissed.

In conclusion, the changes in carbohydrate reserves during the year reflected the variations in supply and demand. Carbohydrates were stored in shoots and roots by winter in non-fruiting trees, and mobilised during the spring flush of growth. Shoot reserves were used-up during bud sprouting and flowering, and at the time of full bloom carbohydrate concentration had fallen to a minimum value. We did not find evidence for the contribution of the root reserves to fruit set. Thus, from the period of fruit abscission, fruit nutrition was supported by current photosynthesis. Fruit load caused the inhibition of flowering after heavy crop load in alternate bearing trees, but a regulatory
role of the carbohydrates could not be observed. Nor could be found an effect of leaf carbohydrates or fruit load on photosynthesis. Thus, fixation rates were mainly modulated by environmental conditions in Salustiana sweet orange.

Acknowledgements

We thank Ing. Agr. J.M. Torres (ANECOOP, Valencia, Spain) for providing the orchard facilities and logistic help, the R+D+i Linguistic Assistance Office at the Universidad Politécnica de Valencia for their help in revising this paper and Y. Bordón for her cooperation in some experiments. This Research was funded by grants from the Consellería de Agricultura, Pesca y Alimentación (GV-CAPA00-11) and the Conselleria d’Empresa, Universitat i Ciència, Generalitat Valenciana (Grupos 04/059).

References


Legends for figures

Fig. 1. Number of surviving fruit (●), and rate of dry matter (DM) utilization in fructification by the on trees.

Fig. 2. Changes in total metabolizable carbohydrates, starch and sugars in the leaves of vegetative sprouts formed during the spring flush in on (●) and off (○) trees. The dotted vertical lines indicate the time of harvest. The beginning of bud sprouting during the second year is indicated by arrows in the axis (S). It occurred earlier in the off trees, which entered an on year, than in the on trees, which entered an off year. Results expressed as percentages on dry weight (DW) basis.

Fig. 3. Total reserve carbohydrates content in the bark (A) and wood (B) of vegetative twigs formed during the spring flush in year 1, in on (●) and off (○) trees. On year 2, the off trees formed 63 thousands of flowers whilst the on tree formed 3.5 thousands of flowers. Results expressed as percentages on dry weight (DW) basis. Values are averages of three independent samples ± SE. The dotted line indicates the time of harvest; the arrows bud sprouting.

Fig. 4. The effect of girdling on the accumulation of carbohydrates in the leaves in on (open bars) and off (hatched bars) trees. Results expressed as a percentage of the carbohydrate contents in the leaves of non girdled (control) trees ± SE (n = 3). The asterisks indicate statistically significant differences to the controls at $P \leq 0.05$ (*) and $P \leq 0.01$ (**).
Fig. 5. Total reserve carbohydrates content in the thin (A) and the fibrous (B) roots in on (●) and off (○) trees. On year 2, the off tree formed 63 thousand flowers whilst the on tree formed 3.5 thousand flowers. Values are averages of three independent samples ± SE. The dotted line indicates the time of harvest; the arrows bud sprouting.

Fig. 6. Feeder roots weight, expressed as a percentage of the total weight of the sample, in the root samples from on (●) and off (○) trees.
Table 1. Flowering and yield parameters of the trees used in this study.

<table>
<thead>
<tr>
<th>Parameter and year</th>
<th>On trees</th>
<th>Off trees</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First year</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bud sprouting (%)</td>
<td>56.3 a</td>
<td>22.4 b</td>
</tr>
<tr>
<td>Shoots initiated in spring</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Vegetative</td>
<td>1.6 b</td>
<td>8.23 a</td>
</tr>
<tr>
<td>- Leafy inflorescences</td>
<td>6.4 a</td>
<td>0.48 b</td>
</tr>
<tr>
<td>- Leafless inflorescences</td>
<td>14.2 a</td>
<td>0.34 b</td>
</tr>
<tr>
<td>Number of flowers (thousands tree(^{-1}))</td>
<td>75 a</td>
<td>2.0 b</td>
</tr>
<tr>
<td>Number of leaves (thousands tree(^{-1}))</td>
<td>49 a</td>
<td>60 a</td>
</tr>
<tr>
<td>Fruit set (units tree(^{-1}))</td>
<td>1,790 a</td>
<td>124 b</td>
</tr>
<tr>
<td><strong>Second year</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of flowers (thousands tree(^{-1}))</td>
<td>3.5 b</td>
<td>63 a</td>
</tr>
</tbody>
</table>

Values within a line with different letters are statistically different \((P < 0.05)\).
Table 2. Dry matter used in fructification (Kg tree\(^{-1}\)).

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>on trees (75,000 flowers)</th>
<th>off trees (2,000 flowers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flower formation</td>
<td>14.6 a</td>
<td>0.41 b</td>
</tr>
<tr>
<td>From flowering to the end of drop</td>
<td>15.0 a</td>
<td>0.80 b</td>
</tr>
<tr>
<td>From the end of drop to harvest</td>
<td>26.1 a</td>
<td>3.1 b</td>
</tr>
<tr>
<td>Total</td>
<td>55.7 a</td>
<td>4.3 b</td>
</tr>
<tr>
<td>Dry weight in mature fruit at harvest</td>
<td>35.4 a</td>
<td>3.2 b</td>
</tr>
</tbody>
</table>

Values within a line with different letters are statistically different ($P < 0.05$).
Table 3. Photosynthetic rates at ambient conditions in the leaves of vegetative sprouts from the spring flush of growth in on and off trees. The values are average of 15 determinations (10 replicates each time) performed from June to July (4 to 5 month-old leaves), and 20 determinations (10 replicates each time) performed from March to April (13 to 14 month-old leaves).

<table>
<thead>
<tr>
<th>Tree characteristics</th>
<th>Leaf age and photosynthetic rate (CO$_2$ m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 to 5 months</td>
</tr>
<tr>
<td>On trees</td>
<td>7.0 a</td>
</tr>
<tr>
<td>Off trees</td>
<td>8.2 a</td>
</tr>
</tbody>
</table>

Values within a column with different letters are statistically different ($P < 0.05$).
Figure 2
Click here to download high resolution image
Figure 4

[Bar chart showing total carbohydrates (% over controls) for different dates of girdling: 2 Jun, 8 Sept, 6 Oct, 17 Nov. The chart includes error bars indicating variability.]