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Roots are important sources of carbohydrates during flowering and fruiting in 'Valencia' sweet orange trees with varying fruit load

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ABSTRACT

The influence of fruit loading on flowering and fruiting, CO_2 assimilation, and non-structural carbohydrates (NSC) in citrus trees was evaluated in four-year-old 'Valencia' sweet orange grafted onto 'Rangpur' lime. One group of trees was completely de-fruited (DFT) on May 14 (autumn), whereas fruit were left on the remaining trees (FT). The seasonal variation in photosynthesis and stomatal conductance was unaffected by fruit loading, with the minimum diurnal-integrated CO_2 assimilation (P_{NI}) occurring in July (98.3 mmol m^{-2} day^{-1}) and the maximum P_{NI} occurring in November (199.5 mmol m^{-2} day^{-1}). Fruit loading inhibited sprouting and flowering in the citrus trees, but this effect was not correlated with NSC in the leaves, branches, or roots. The DFT trees exhibited nearly four times as many reproductive structures as the FT trees, with a high remobilization of reserves. Our data showed that flowers are stronger sinks than fruit and that flowering is the most expensive phenological stage. In the DFT trees, approximately 80% of NSC were consumed prior to the end of fruit drop, primarily until flowering. NSC reserves from leaves, branches, and roots were remobilized. Between the stages of de-fruiting and the end of physiological fruit drop, the plants remobilized approximately 312 g NSC, with the roots contributing more than 230 g NSC per plant.

1. Introduction

Fruit yield in *Citrus* trees depends on a complex chain of events that includes vegetative growth, bud induction and differentiation, flowering, fruiting, and fruit ripening (Davies and Albrigo, 1994; Spiegel-Roy and Goldschmidt, 1996; Goldschmidt, 1999; Agustí, 2003). Mature 'Valencia' sweet orange trees can produce more than 80,000 flowers per plant in temperate climates, with only 2% of produced flowers being ultimately harvested as fruit (Monselise, 1985). In the São Paulo State, Brazil, in subtropical conditions the same cultivar produces many fewer flowers, between 170 and 450 flowers m^{-3} of canopy, setting between 3% and 18% of these flowers as fruit (Ribeiro et al., 2008; Sekita, 2008). Independent of the growing region, the large production of flowers represents a great investment of energy, typically concentrated in a brief period of time, and such investment is even higher in late cultivars due to the presence of fruit load. In particular, late cultivars present an inhibition of bud induction and differentiation by the presence of fruit (García-Luis et al., 1995; Yahata et al., 2004; Prado et al., 2007), a phenomenon associated with alternate bearing (Monselise and Goldschmidt, 1982; Guardiola and García-Luis, 2000; Agustí, 2003).

Competition for carbohydrates and hormonal imbalance are the main factors leading to alternate bearing. The use of growth regulators, girdling, and fruit thinning has demonstrated that bud induction and flowering intensity in alternate-bearing trees are affected by endogenous hormones produced by fruit (García-Luis et al., 1988; Yamanishi, 1994; García-Luis et al., 1995; Yahata et al., 2004; Prado et al., 2007; Martínez-Fuentes et al., 2010; Monerri et al., 2011). In fact, gibberellins have been hypothesized to regulate the expression of genes associated with floral induction (Muñoz-Fambuena et al., 2012; Goldberg-Moeller et al., 2013). On the other hand, there is an intense remobilization of carbohydrates from leaves during bud development, which is accompanied by starch degradation (Jones and Steinacker, 1951; Sanz et al., 1987;

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García-Luis et al., 1988; Spiegel-Roy and Goldschmidt, 1996; Prado et al., 2007; Monerri et al., 2011; Park, 2011). Goldschmidt et al. (1985) have reported a high intensity of flowering associated with high starch content in leaves. However, other researchers have observed intense flower production independently of starch status in leaves (García-Luis et al., 1995; Yahata et al., 2004; Prado et al., 2007; Monerri et al., 2011).

Regardless of their role in bud induction and flowering, carbohydrates are required during the phenological phases when citrus trees have increased demand. In grapefruit, carbon investment in flower production represents up to 14% of the annual production of assimilates and may be higher than 27% when considering the abscission of fruitlets (Bustan and Goldschmidt, 1998). In 'Salustiana' sweet orange trees, around 36% of the total dry matter used in fruit production may be lost from flowering to physiological fruit drop (Monerri et al., 2011). Between the stages of fruiting and physiological fruit drop, there is a linear increase in fruit diameter, suggesting an increased demand for assimilates due to sink strength. If the demand for assimilates exceeds the supply capacity, a self-regulatory mechanism adjusts the fruit load to carbon availability (García-Luis et al., 1988; Guardiola and García-Luis, 2000; Iglesias et al., 2003). Several authors have studied the consumption of carbohydrate reserves from leaves during flowering and fruiting (Sanz et al., 1987; Ruiz et al., 2001; Iglesias et al., 2003; Léchaudel et al., 2005); however, only a few studies have evaluated the consumption of reserves from branches during the same period (Jones and Steinacker, 1951; Sanz et al., 1987) and even less have studied the root reserves (Li et al., 2003a,b; Monerri et al., 2011).

As evergreen species, citrus trees assimilate and consume carbon throughout the year. The seasonal variation in photosynthetic activity has been associated with environmental conditions, mainly temperature (Ribeiro and Machado, 2007; Ribeiro et al., 2009; Monerri et al., 2011; Nebauer et al., 2013). Although photosynthesis has been associated with sink demand (Iglesias et al., 2002; Sekita, 2008; Dovis et al., 2009; Ribeiro et al., 2012), a sink effect on photosynthesis is not always evident. Our aim was to study the influence of carbohydrate status on flowering and fruiting of citrus trees with varying fruit load. Under such a scenario, we hypothesized that the energy and carbon invested in flowering and fruiting are supplied by photosynthesis and carbohydrate reserves from all plant organs, even under non-extreme conditions of sink demand. We also hypothesized that the contribution from the roots and branches is more significant than that from the leaves during reserve remobilization. These hypotheses were tested by studying the entire carbon balance in citrus trees between the bud induction period and the end of physiological fruit drop.

2. Materials and methods

2.1. Plant material and growth conditions

Four-year-old trees of 'Valencia' sweet orange scion grafted onto 'Rangpur' lime rootstock were grown in pots (100 L) filled with a substrate composed of a mixture of sand:organic compound:soil (1:2:1, v/v/v). The trees were grown at a spacing of 3 m × 2 m in a greenhouse located in Cordeirópolis, SP, Brazil (22°27′40″S; 47°24′4″W; 639 m elevation). The trees were fertilized with 230 g N $year^{-1}$ as $Ca(NO_3)_2$ or $Mg(NO_3)_2$ in eight equal applications at regular intervals between August 2009 and April 2010. Every 60 days, micronutrients were applied using 3.5 g $plant^{-1}$ of a fertilizer containing 2.0% B, 0.8% Cu-EDTA, 5.6% Fe-EDTA, 0.3% Mo, 3.2% Mn-EDTA and 2% Zn-EDTA. The trees were watered daily with an automatic drip irrigation system (2 L h^{-1}) to maintain the leaf water potential between −0.3 and −0.4 MPa at predawn. When the fruit diameter was around 35 mm, one group of trees was completely de-fruited (DFT), whereas a second group was maintained with fruit (FT). The trees were evaluated at the time of de-fruiting and at the phenological stages 00 (rest), 65 (full bloom), 71 (full fruiting), and 74 (end of physiological fruit drop), according to the BBCH scale (Agustí et al., 1995).

The air temperature (maximum, minimum, and average) and solar radiation were monitored inside the greenhouse using a maximum/minimum thermometer and an LI-200 light sensor (Li-Cor Inc., Lincoln, NE, USA).

2.2. Sprouting and flowering evaluation

Prior to sprouting, five branches per tree (20–50 nodes on young branches and 10–20 nodes on mature branches) were labeled in 15 trees per treatment. The number of sprouted nodes, the number and types of shoots, and the number of flowers and fruits were counted weekly. The structures were classified as vegetative (leaves) or reproductive, and the reproductive structures were reclassified as flower buds (stages 56–59 on the BBCH scale), flowers, and fruitlets. The proportion of sprouted nodes (PSn) was calculated as PSn = [(Sn/Tn)*100], where Sn is the number of sprouted nodes and Tn is the total number of nodes. The number of nodes was counted in a sample of 30 young branches and then the total number of young nodes per tree was estimated. The reproductive sprouting rate (RSR) was calculated as RSR = [(Rb/Tb)*100], where Rb is the number of reproductive buds and Tb is the total number of buds. The number of reproductive structures per 100 nodes was also calculated.

2.3. Photosynthesis

Net photosynthesis (P_N) and stomatal conductance (g_s) were measured under natural conditions of light, temperature, relative humidity and air CO_2 concentration using a portable infrared gas analyzer (LI-6400 Li-Cor Inc., Lincoln, NE, USA) equipped with an LI-6400-02B LED light source (Li-Cor Inc., Lincoln, NE, USA). The diurnal course of P_N and g_s was evaluated every 2 h from 7:00 to 17:00 h in three leaves per plant and four plants per treatment. Fully expanded and sunlight-exposed leaves at the middle third of tree canopy were evaluated. The diurnal-integrated CO_2 assimilation (P_{NI}) was calculated by integrating P_N between 7:00 and 17:00 h.

2.4. Leaf, stem, and root carbohydrates

Twelve leaves per plant, similar to those used to evaluate photosynthesis, were sampled at each evaluation time (Section 2.1). Mature (>6 months old) and young (<6 months old) branches, as well as fibrous (<0.5 mm in diameter) and large (>0.5 cm in diameter) roots were also sampled. All plant material was immediately frozen (−70 °C) and then dried at 52 °C in a forced-air circulation drying oven.

The total soluble carbohydrates (TSC) were extracted in methanol:chloroform:water solution (Bieleski and Turner, 1966) and quantified by using the phenol–sulfuric acid method (Dubois et al., 1956). Starch (Sta) was determined by the enzymatic method proposed by Amaral et al. (2007). The concentration of nonstructural carbohydrates (NSC) was calculated as NSC = TSC + Sta.

The leaf photoassimilate exportation/consumption (PEC) between de-fruiting and full bloom (flowering), full bloom and full fruiting (fruiting), and full fruiting and the end of physiological fruit drop (fruit drop) was calculated according to Ribeiro et al. (2012): $PEC = [(NSC_b + P_{NIint}) - NSC_e]$, where NSC_b and NSC_e are the concentrations of NSC at the beginning and end of each period and P_{NIint} is the period-integrated CO_2 assimilation (mol CO_2 m^{-2}). P_{NIint} in mol CO_2 was first converted to g CO_2 and then to g CH_2O