Total Soluble Sugars Dynamics in Coffee Fruits Under Development

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Abstract

Coffee (Coffea arabica L.) fruits are stronger sinks and is known its development may be more than four times higher than that allocated to branch growth during the annual production cycle. However, the origin and carbohydrates distribution used during the fruiting development are not yet known. Four assimilates sources are potentially available for fruit growth: (i) the photoassimilates produced by the fruit itself, (ii) branch reserves, (iii) leaf reserves and (iv) the current photosynthesis that occurs during fruit growth. To better understand the carbohydrate dynamics, its allocation in coffee fruits and to evaluate fruit dependence on other tree parts at the bean-filling stage, four carbohydrates sources were imposed in fully mature trees in Northeast of Sao Paulo State, Brazil. Variables such as total sugar and dry mass were analyzed. We observed that leaves from the nodes are very important for fruit development. Comparison between fruits with leaves and fruits without leaves clearly revealed its influence on dry matter and total sugars accumulation in the fruits. The removal or covering of leaves near fruits limits the development of fruit.

Keywords: Coffea arabica L., dry mass, carbohydrates, source-sink

1. Introduction

In perennial plants such as Coffea arabica L. the active vegetative and reproductive growth in field-grown occur concurrently, what causes a competition between these simultaneous sinks by carbohydrates synthesized by plant. Coffee fruits are strong sinks (Cannell, 1985) and, therefore, directly impact in the carbohydrates assimilation to other plant organs, a process called carbohydrate partitioning (Taiz & Zeiger, 2013) that results in a differential carbohydrate distribution that depends on the dynamic demand of each of these organs (Bihmidine, Iii, Johns, Koch, & Braun, 2013; Marcelis, 1996).

The carbohydrates, produced in leaves of the same season or stored in the plant reserves, are transported to the organs of the plant that cannot satisfy their energy requirement by themselves. These organs, like leaves, roots, branches and fruits, require carbohydrates to achieve their development potential (Erel et al., 2016; Haouari, Van Labeke, Steppe, & Mariem, 2013). However, if the carbohydrates supply is limited or demand is excessive (i.e., high fruit demand) there is a hierarchy of preferential supply to developing parts that is spatially and temporally dynamic among sinks (Reyes, DeJong, Franceschi, Tagliavini, & Gianelle, 2016). The coffee dynamic demand depends on the fruit load and its growth mobilize large quantities of carbohydrates in the rapid expansion phase. Developing fruits themselves can be a carbohydrates source, as they have stomata and chlorophyll. However, photosynthesis performed by the pericarp is estimated to account only 30% of the total carbon allocated to coffee fruits (Geromel et al., 2006; Vaast, Angrand, Franck, Dauzat, & Génard, 2005).

The mobilization carbohydrate dynamics by the coffee flower buds has already been studied and clarified by Melloto, Barros, and Maestri (1993). However, the origin and carbohydrates distribution used during the fruiting development are not yet known. What is known so far is just the fruit is a preferential sink and due to great demand, photoassimilates allocated to attend fruit development may be more than four times higher than that allocated to branch growth during the annual production cycle (DaMatta, 2004; Vaast et al., 2005).

Four assimilates sources are potentially available for fruit growth: (i) the photoassimilates produced by the fruit itself, (ii) branch reserves, (iii) leaf reserves and (iv) the current photosynthesis that occurs during fruit growth. Knowledge of carbohydrate mobilization by developing fruits can provide practical subsidies for coffee cultivation, because coffee tree seems not to exert a fine control on the fruit sink strength in the balance with
carbohydrates and mineral resources (Cannell, 1985). Then, both resources might be diverted to the fruit at the expenses of the shoot apices and root-trunk system, sometimes to the point of causing overbearing dieback (Cannell & Huxley, 1969). However, if the supply carbohydrates are suitable, then the imbalance between vegetative and reproductive growth will be smaller, consequently, less “biennial effect” over the years.

From the scientific standpoint, the elucidation of some aspects about coffee fruiting physiology is very important, due to the complexity of the subject. Knowledge of this information may also indicate the most important carbohydrate source for fruiting and through its protection and provide conditions for the plant to improve production with larger, heavier and higher quality fruits. Therefore, the main objective of this research was to gain deeper insight into carbohydrate dynamics, its allocation in coffee fruits and to evaluate fruit dependence on other tree parts at the bean-filling stage.

2. Method

2.1 Plant Material and Growth Conditions

The study was carried out from November 2016 to May 2017 on Arabica (Coffea arabica L.) trees from a orchard at the research station of the Procafé Foundation in Franca, Brazil (20°28′19″S; 47°24′33″W and 1025 m above sea level), on Oxixoil soil (US Taxonomy), with local climate classified as Cwb—Humid subtropical with dry winters and temperate summers (Köppen). The mean annual rainfall in the experimental area is 1637 mm and the mean annual temperature 20 °C. The coffee plants, cv. Yellow Bourbon, were in their eighth production cycle and 3.8 × 0.7 m spacing. The crop was unshedded and clean-weeded. The plants were submitted to best agricultural practices for commercial coffee bean production, including integrated pest management.

2.2 Experimental Design

The experimental design was randomized blocks with two factors—(I) carbohydrate source, four levels and (II) time (40, 81, 158 and 182 days after marking) with four replicates (4 × 4 × 4). The carbohydrates sources levels were (i) leaves associated with fruits, which the node with the fruits was isolated by ringing the branch below and above the node, (ii) pre-existing carbohydrates in leaves associated with fruits, which the leaves were covered in double-sided paper packages and annealed above and below the node, (iii) fruits, which the leaves were removed and annealed above and below the node and (iv) leaves and part of the branch, annealing a section containing a pair of leaves in the distal position and a region of approximately 0.20 m internodes. Marking was performed at the beginning of fruiting, after flowering. To determine the contribution of photoassimilates from the branch, the value found in leaves and part of the branch (iv) was subtracted by leaves associated with fruits (i).

2.3 Sampling Procedures and Plant Analyzes

At beginning of the study (day 0—31/10/2016) an initial sample of two thousand fruits was collected to determine total sugars and dry mass. After, fruits samples were collected at 40 (10/12/2016), 81 (20/01/2017), 158 (04/04/2017) and 182 (01/05/2017) days after marking. All samples were lyophilized at -60 °C until constant weight in the Lirotop 1101 equipment. Subsequently, the materials were finely milled using the crucible. 40 mg of each sample was placed in threaded tubes and added 5 ml of 70% ethanol. Then, were taken into the water bath for 30 minutes at 60 °C and stirred every 10 minutes. Later, the tubes were centrifuged at 5000 RPM with a temperature of 18 °C for 5 minutes. These procedures were performed three times using the same volume of the 70% ethanol solution and removing the supernatant at each centrifugation. The evaluations of total soluble sugar contents were performed by the Antrona method (Umbreit, Burris, & Stauffer, 1957).

2.4 Statistical Procedure

The datasets were subjected to residual normality and variance homogeneity tests. The Analysis of Variance F-test was performed considering < 0.05 of probability through SAS software version 9.4 (SAS Institute Inc, 2014). When F probability was significant, the means were fitted to linear and polynomial regression using Origin software version 9.60 (OriginLab Corporation, 2019).

3. Results

Total soluble sugars accumulation in coffee fruits in the growth beginning was 44.57 mg g⁻¹ dry mass (DM). The plant fruits that were removed and/or covered the leaves of the nodes (ring-barking without leaves and ring-barking + covered leaf) did not survive after 40 days (Figure 1). They reached this stage with a total solubles sugars mean 49.49 and 48.51 mg g⁻¹ MS (Figure 2).
Plant fruits which the leaves of the nodes were exposed (ring-barking + leaves and distant ring-barking + leaves) developed normally and with similar tendency. However, leaves and part of the branch provided the largest accumulation of sugars at 81 days after marking with 94.31 mg g⁻¹ MS. After, total solubles sugars decrease for 37.93 mg g⁻¹ MS at 158 days and for 41.51 mg g⁻¹ at 182 days after marking (Figure 2).

Figure 2. Total sugars (mg per gram of coffee fruits) measured before (0 day) and after marking the branches (40, 81, 158 and 182 days) for different ring-barking types: ring-barking only, ring-barking plus covered leaf, distant ring-barking + leaves (y = 5E-05x³ − 0.0199x² + 1.7953x + 42.318 with R² = 0.93) and ring-barking + leaves (y = 5E-05x³ − 0.0157x² + 1.1561x + 45.434 with R² = 0.96). Vertical bars represent the standard error.

Fruit dry matter accumulation of the plants with leaves removed and/or covered (ring-barking without leaves and ring-barking + covered leaf) was lower compared to fruits with leaves at 40 days after marking. They presented mean 7.15 mg per fruit while the fruits with leaves 18.68 and with leaves plus part of the branch with 28.04 mg per fruit. Fruits with leaves and leaves plus part of the branch, which developed normally, presented similar tendency until 158 days after marking. This time meaning the maturation phase. After, 182 days, the fruits with leaves only continued increasing the dry mass and achieved 211.3 mg per fruit. Instead, the fruits plus part of the branch decrease in this time with 159.5 mg per fruit (Figure 3).
Figure 3. Dry matter accumulation (mg per coffee fruit) after marking the branches (40, 81, 158 and 182 days) for different ring-barking types: ring-barking only, ring-barking plus covered leaf, distant ring-barking + leaves ($y = 0.9451x - 1.3694$ with $R^2 = 0.98$) and ring-barking + leaves ($y = 0.0046x^2 + 0.3358x + 2.0555$ with $R^2 = 0.99$). Vertical bars represent the standard error.

4. Discussion

This study illustrates the effect of different carbohydrate sources such as leaves in activity, pre-existing carbohydrates in leaves, own fruit and part of the branch on fruit growth. Leaves from the nodes are very important for fruit development. Comparison between fruits with leaves and fruits without leaves clearly revealed its influence on dry matter and total sugars accumulation in the fruits. The removal or covering of leaves near fruits limits the development of fruit from 40 days after removal or coverage (Figures 1-3). These results indicate coffee tree source-sink interactions, which are modulated by carbon assimilation and partitioning during growth and development. Moreover, the trend of the plant to develop larger sink demand by fruits and how much this depends on the vegetative part of the plant. Like Vaast et al. (2005) and Geromel et al. (2006) our results showed that green coffee berries can not provide 100% of the growth requirements through their own photosynthetic activity. Therefore, we conclude that coffee leaf photosynthesis is important, especially in view of the large fruit sink strength and because the berry photosynthetic area represents low total tree photosynthetic area.

The dead fruits showed aspects of attack by pathogens such as Phoma tarda and Pseudomonas syringae pv. Garcae. It is suggested the absence of the adjacent leaves or their activity exposes the fruits to diseases due to the lack of secondary metabolites produced by the leaves (Salgado & Favarin, 2004; Taiz & Zeiger, 2013). In addition, it has been shown the photosynthesis produced by the fruit itself is not enough for its development (Geromel et al., 2006). The primary and secondary metabolism of carbon are dependent on photosynthesis and carbohydrate production (Salgado & Favarin, 2004), so it is possible to infer when the leaves adjacent to the fruits were removed or covered the primary metabolism was compromised by the absence of photosynthesis (in leaves covered due to the lack of light and the removal of the leaf itself). Consequently, compounds necessary to get secondary metabolites were not produced, showing fruits with adjacent leaves unable to produce carbohydrates usually can not develop until the maturation stage. It is important to point out that the possibility of leaf removal to be a gateway to pathogens was excluded, since the presence of the covered leaf, i.e., without the “gateway” to pathogens, presented the same disease attack. In addition, fruiting points near the attacked fruits were healthy (Figures 1a and 1b).

The branch contributed to the greater accumulation of total soluble sugar in the fruits. There was a difference of 34.46 with part of the branch plus the leaves at 81 days compared to the ringing of the branch below and above the node with leaves. It is suggested the coffee fruits used the reserves that are stored in the lateral branches for their growth (Chaves Filho & Oliveira, 2008). These reserves are carbohydrates stored as starch and is the main reserve in vascular plants (Bahaji et al., 2014). Previous studies have shown the contribution of starch as an
energy source during flowering, budding, pollination, and fruit setting in both perennial and deciduous species (Boldingh et al., 2016; Guerra & Rodrigo, 2015; Klein, Vitasse, & Hoch, 2016; Tixier et al., 2017). Similarly, starch hydrolysis provides soluble carbohydrates that allow the survival of perennial structures (Dietze et al., 2014), or support periods of stress (Martínez-Vilalta et al., 2016).

Fruits presented low accumulation of dry matter and total soluble sugar at 40 days after the marking as well as the results found by (Laviola et al., 2007). According to Leon and Fournier (1962), and Cannell (1971) coffee fruits at this stage show a high rate of division and cellular respiration, which would prevent the accumulation of total soluble sugars.

High content of total soluble sugars at the end of the expansion phase (81 days) may be due to the presence of sucrose, a fundamental compound in biochemical routes such as respiration and biosynthesis of wall polysaccharides or storage (Geromel et al., 2006). It is known at the initial stage that the fruit is basically formed by perisperm which is a transitory tissue (De Castro & Marraccini, 2006) and that it has high contents of total soluble sugars in the initial stages (about 60 days after flowering) followed of a fall, as soon as this tissue begins to disappear (Geromel et al., 2006). With the disappearance of the perisperm, the endosperm originates, representing 95% of the mass of the mature seed (Geromel et al., 2006). Therefore, it is assumed that the total soluble sugars in the fruits observed at 158 days are due to the disappearance of the perisperm (tissue rich in the sucrose disaccharide) giving rise to the endosperm, which is 44% formed by polysaccharides (Trugo, 2003), 50% of these polysaccharides represented by galactomannans (Geromel et al., 2006). In green coffee beans, this high content of polysaccharides is associated with the cell wall (Wolf, Plunkett, & Laver, 1960).

In the maturation phase (182 days) the contents rose slightly (Figure 2). This may have happened due to the total soluble sugars accumulation at the end of the reproductive cycle, mainly in the fruit pulp (Geromel et al., 2006). It is also associated with morphophysiological changes related to maturation (Amaral, Da Matta, & Rena, 2001; Puschmann, 1975), as the gradual increase of non-reducing sugars and total sugars in fruits as the harvest is delayed (Pimenta & Vilela, 2002). This increase can be attributed to the intensification of maturation due to the lower presence of green fruits, which present significantly lower non-reducing sugars than cherry fruits (Pimenta & Carvalho, 1995).

The dry matter accumulation is mainly related to deposition of wall material (Coombe, 1976), essential for the process of cell stretching (Marenco & Lopes, 2005). Therefore, it is suggested that this accumulation of dry mass during fruit development was mainly due to the formation of the endosperm, since it represents 95% of the mass of the mature seed (Geromel et al., 2006).

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