# SPATIO-TEMPORAL VARIABILITY OF CARBOHYDRATE AND CHLOROPHYLL CONTENT IN THE COFFEE CANOPY

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**ABSTRACT**: The spatial variability of the total chlorophyll content and carotenoids content, starch and soluble sugars of coffee canopy were mapped throughout the day. Therefore, evaluations were carried out in a 'Catuaí Vermelho' coffee plant with 1.7 meters height. A vertical gradient (from the apex to the base of the plant canopy) and a horizontal gradient (plagiotropic branches) were established to analyze different positions of the canopy. Thus, in the vertical direction, four heights were analyzed in the plant: top, upper, middle and lower regions. In the horizontal gradient, the plagiotropic branches were divided into three parts: basal, median and apical. Collection of leaf samples was performed on the east and west sides of the canopy, at 9 a.m., totaling 24 collection points at each time. Higher content of photosynthetic pigments and concentration of sugars were observed in the western face and in the inner parts of the coffee tree. The content of chloroplast pigments and sugars of an individual coffee leaf diverge considerably from other leaves, which requires caution when scaling estimates at the global canopy level. The analysis of some punctual leaves does not serve to discriminate the overall dynamics of a canopy.

Index terms: Coffea arabica, sugars, plant pigments.

# VARIABILIDADE ESPAÇO-TEMPORAL DO TEOR DE CARBOIDRATO E DE CLOROFILA NA COPA DO CAFEEIRO

**RESUMO**: A variabilidade espacial do teor de clorofila total e dos carotenoides, amido e açúcares solúveis da copa de cafeeiro foi mapeada ao longo do dia. Portanto, as avaliações foram realizadas em uma planta de cafeeiro 'Catuaí Vermelho', medindo 1,7 metros de altura. Foi estabelecido um gradiente vertical (do ápice à base da copa) e um gradiente horizontal (ramos plagiotrópicos) para analisar diferentes posições dentro da copa. Assim, na direção vertical, foram analisadas quatro alturas na planta: topo, superior, média e inferior. No gradiente horizontal, os ramos plagiotrópicos foram divididos em três partes: basal, mediana e apical. A coleta foi realizada nos lados leste e oeste do dossel, às 9h, totalizando 24 pontos de coleta. Foi visto maior teor de pigmentos fotossintéticos e concentração de açúcares na face oeste e nas partes internas do cafeeiro. As características de conteúdo dos pigmentos fotossintéticos e açúcares de uma folha de café individual divergem consideravelmente de outras folhas, o que requer cautela ao dimensionar as estimativas no nível global de dossel. Sendo assim, a análise de algumas folhas pontuais não serve para discriminar a dinâmica da copa do cafeeiro.

Termos para indexação: Coffea arabica, açúcares, pigmentos vegetais.

## **1 INTRODUCTION**

The coffee tree is native from the African continent, grown in shady environments, but it has been usually cultivated in sunny environments, with high production (POMPELLI et al., 2010). The behavior of a coffee plant is a result of the integration among canopy dynamism and the environmental conditions. However, different parts of the plant canopy can respond differently to environmental variations, once there are variations in the physical structure of the canopy, the age of the leaves and the distribution and translocation of the photo assimilates among the different parts and heights in the canopy of the plant (RODRÍGUEZ et al., 2011). These changes in canopy position may lead to different responses, such as photosynthetic, anatomical modifications, pigment content and biochemical changes in coffee metabolism (CHAVES et al., 2012).

The usual indication for analysis of the coffee canopy is the collection of the 3rd or 4th pair of leaves from the apex of productive branches, from each side of the plant, at the medium height of the plant (GUIMARÃES et al., 1999). On the other hand, Raij (2011) indicates the sampling of leaves, branches and fruit (from four leaves with petiole per plant), in the four cardinal points, being the third pair from the apex of the branches, in the medium height of the plant. However, there are still differences in literature between authors regarding the number of leaves, branches and fruits to be collected per sampling unit for diagnosis purposes.

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These differences, together with the lack of information on the variation of the error in the estimation of the sample mean for the coffee tree, are objects of concern (CINTRA et al., 2015). It is suggested that, throughout the canopy, the different parts have discriminable values for different variables, due to biotic and abiotic factors. The determination of carbohydrate content in coffee fruits and leaves demonstrate a reallocation difference between the different levels of the coffee plant (CHAVES et al., 2012). Field observations show that the magnitude of fruit production can be differentiated between opposite faces of coffee trees grown in lines (CHAVES et al., 2012). Also, it was observed that the production of the western face, which received the most total solar radiation due to the slope of the terrain, was larger than the eastern face (ALVES, 2005).

Thus, it was hypothesized that there would be a spatial and temporal difference in the content of chlorophyll and carbohydrates in the coffee tree under field conditions due to the considerable autonomy of the plagiotropic (lateral) branches of a coffee tree (CARVALHO et al., 2010). Therefore, the objective in this work was to evaluate the spatial variability of the total chlorophyll content and carotenoids, starch and soluble sugars in each canopy of the coffee tree.

#### **2 MATERIAL AND METHODS**

The experiment was carried out in the middle of January 2015, in the Experimental Field at the Coffee Section of the Department of Agriculture (DAG), Federal University of Lavras (UFLA) (21 ° 14 'S and 45 ° 00' W), at 918 m altitude. The average temperature was around 27 ° C and RH was around 60% (INMET, 2015). Aiming to create a map of the ecophysiological responses of a coffee tree, one 'Catuaí Vermelho' coffee tree (*Coffea arabica* L.), measuring 1.7 meters in height, was evaluated in 24 different parts at four different times along the day, allowing to build a map of coffee ecophysiological behavior throughout the day.

The coffee canopy was divided into a vertical (from the apex to the base of the canopy) and a horizontal gradient (plagiotropic branches). Therefore, in the vertical gradient, four heights were analyzed in the plant: top of the canopy, upper region, middle and lower region. For the horizontal gradient, plagiotropic branches were subdivided into three parts: basal (next to the orthotropic branch), median and apical (external part of the plagiotropic branch). Sampling was performed on the east and west sides of the canopy, at 9 am, totaling 24 collection points. Coffee fruits and fully expanded leaves of each canopy part were collected and properly stored until the biochemical analyses. The draft containing all the parts that plant canopy was divided is represented in Figure 1.



**FIGURE 1** - Representative draft of the 24 parts of the coffee canopy sampled at 9 a.m. The vertical dashed gray line represents the orthotropic branch and the gray horizontal line represents the plagiotropic branches. The vertical gradient consisted of top, upper, middle and lower branches (represented by the gray lines) and the horizontal gradient consisted of basal, median and apical portions (represented by the black dots) of the plagiotropic branches. Samples were collected from both eastern and western faces of the canopy (SANTINI et al., 2019).

The quantification of chloroplast pigments was performed according to Lichtenthaler and Buschman (2011). Plant material (0.1g of fresh matter) was grinded in 80% (w/v) acetone and the volume was filled up to 10 mL. The spectrophotometric readings were performed at 470, 646.3 and 663.2 nm. Total chlorophyll and carotenoids content was expressed in  $\mu$ g.g<sup>-1</sup> of fresh matter.

For carbohydrate quantification, fully expanded leaves and fruits were dried in a forced circulation oven, at 65 °C, until constant weight. The extraction was performed according to Zanandrea et al. (2010) and the supernatant was used for reducing sugars (MILLER, 1959), total soluble sugars and starch (DISCHE, 1962) quantification.

The data were submitted to geostatistical analysis, with the SURFER 8.04 - Surface Mapping System software (GOLDEN SOFTWARE, 2004), using the kriging method. The semivariograms were calculated from the sampling data, so that a theoretical model was adjusted.

### **3 RESULTS AND DISCUSSION**

Chlorophyll and carotenoids content were higher in the western face of the coffee tree (Figure 2). Chlorophyll content was more concentrated in leaves from the middle and the lower regions of the canopy, mainly in the apical parts of the branches (Figure 2A). Carotenoids had higher content in the leaves from the top and upper regions of the canopy, mainly in the basal portion of the plagiotropic branches (Figure 2B). In the eastern face, higher content of both pigments was found at the inner portion of the canopy.

Fruits from the western face of the canopy showed the highest carbohydrate content (Figure 3). However, total soluble and reducing sugars were more concentrated in the middle region of the western face (Figure 3 B and C), while the amount of starch was higher in fruits located at middle and lower regions of the both sides of the canopy (Figure 3 A). Considering the horizontal gradient, fruits from the apical region of the plagiotropic branch had the highest starch content.

Considering the levels of carbohydrate in coffee leaves, the amount of starch was higher at the western face of canopy (Figure 4A), while total soluble sugars were more concentrated in leaves of the eastern side, mainly at the top and upper regions of the canopy (Figure 4B). Reducing sugars showed the highest amounts at the top at the middle regions of the western face of the canopy (Figure 4C). The leaves from the basal part of the plagiotropic branches showed lower amounts of carbohydrates than the leaves from the apical part.

The eastern face is the most productive face of the coffee tree, where there is the highest photosynthetically active radiation (CHAVES et al., 2012) and high carbohydrate assimilation (DaMATTA, 2004).



**FIGURE 2** - Graphic of isolines of total chlorophyll concentration (A) ( $\mu$ g of chlorophyll g<sup>-1</sup> of fresh matter) and carotenoids (B) ( $\mu$ g carotenoids g<sup>-1</sup> of fresh matter) in different positions of coffee canopy at 9 a.m. The numbers in the ordinates indicate the height of the plant (m) and the abscissa the length of the plagiotropic branches (cm) considering the 0/0 coordinates as the starting point of the stem system. The vertical line represents the orthotropic branch.



**FIGURE 3** - Graph of isolines of starch (A), total soluble sugars (B), and reducing sugars (C) content ( $\mu$ mol of glucose g<sup>-1</sup> dry mass) in coffee fruits from different positions of the coffee canopy. The numbers in the ordinates indicate the height of the plant (m) and the abscissa the length of the plagiotropic branches (cm), considering the 0/0 coordinates as the starting point of the stem system. The vertical line represents the orthotropic branch.

Due to the elevated incident radiation, leaves from the east side of the canopy showed lower chlorophyll content than the ones from the west side. However, inside the coffee canopy, the leaves that are self-shaded retain high levels of chlorophyll compared to leaves exposed to the full sunlight. Thus, the low incident radiation can be related to the dark green color (high chlorophyll concentration) of the leaves, which is an adaptation to the shadow condition (BONFIM et al., 2010).

Despite the small variations in carotenoids content throughout the coffee canopy, the higher concentration of this protective pigment was observed at the top and the upper region of the canopy. It is well known that carotenoids are important pigments that act in preventing the induction of oxidative damage caused by the excessive photosynthetically active radiation (PELOSO et al., 2017). In this way, it can be suggested that the accumulation of carotenoids is also a mechanism of protection to maintain the photochemical apparatus protected from the excessive solar radiation, since it was observed that in this region of the canopy there is a continuous incident radiation throughout the day (SANTINI et al., 2019).

The highest total soluble sugars contents in the leaves from the eastern face of the canopy, in a descending gradient from the sunny outer top to the rest of the canopy can be justified by the photoassimilated balance. Soluble sugars are the main transporters in the plant (DURÁN et al., 2016), thus the products of photosynthesis are rapidly reduced to soluble sugars, which are transported to the sink regions and can be rapidly used or stored as starch (KLUGE, TEZOTTO-ULIANA, SILVA, 2015). In this way, at the right side of the canopy, where the photosynthesis is more effective at 9 a.m. (SANTINI et al., 2019), there is very high production of sugars to maintain canopy demand (other leaves and fruits).



**FIGURE 4** - Graph of isolines of starch (A), total soluble sugars (B), and reducing sugars (C) content ( $\mu$ mol of glucose g<sup>-1</sup> dry mass) in coffee leaves from different positions of the coffee canopy. The numbers in the ordinates indicate the height of the plant (m) and the abscissa the length of the plagiotropic branches (cm), considering the 0/0 coordinates as the starting point of the stem system. The vertical line represents the orthotropic branch.

Also, it is suggested that the right side of the canopy, mainly near the lower portion, the leaves behaved as drains, since they generally show low photosynthesis when compared to the right face and the top of the canopy in general (BALIZA et al., 2014; SANTINI et al., 2019). On the other hand, the lower demand of photo assimilates by the fruits from the top and upper portions of the canopy may have been compensated by the increase in the energy spent in the higher vegetative growth rate (CHAVES et al., 2012).

Carbohydrate content was higher in the fruits located on the western face and in the inner portions of the plant. In field conditions, the regulation of photosynthesis is associated with an accumulation of soluble sugars in coffee leaves during the afternoon (BATISTA et al., 2011). Since the eastern face of the canopy is the most productive, it can be assumed that there are more fruits produced in each branch than in the western face. So, it can be suggested that probably there are more carbohydrates stored in fruits from the western face because the partitioning of the carbohydrate is performed to a reduced number of fruits, when compared to the eastern face. Also, in the dynamism of the coffee canopy is assumed that the elevated productivity is related to the leaf area production in the branch due to the high amount of space demanded by the fruits (CHAVES et al. 2012). In this way, there is a reduced leaf area to produce the carbohydrates needed for fruit filling.

The development of the coffee canopy is related to the acquisition of a refined dynamism. Not only are the environmental conditions modified, but also the physical structure of the canopy, the age of the leaves and the distribution and translocation of the photo assimilates between the different parts and heights of the plant (RODRÍGUEZ et al., 2011). The coffee canopy is dynamic as a whole, and the sugars are produced and exported throughout the plant according to the needs.

#### **4 CONCLUSION**

In general, a higher content of photosynthetic pigments and concentration of sugars were observed in the western face and in the inner parts of the coffee tree. The content of chloroplast pigments and sugars of an individual coffee leaf diverge considerably from other leaves of the whole plant, which requires caution when scaling to estimate at the canopy level. Thus, it is necessary to understand the variable parameters inside the canopy of the coffee tree to perform a mapping of the biochemical responses of the plant, since the change in metabolism varies throughout the canopy. The analysis of some leaves and fruits of coffee in a punctual way do not serve to discriminate the global dynamics of a canopy.

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