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Varying leaf-to-fruit ratios affect branch growth and dieback, with little to no effect on photosynthesis, carbohydrate or mineral pools, in different canopy positions of field-grown coffee trees

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ABSTRACT

Heavy bearing is a typical phenomenon on unshaded coffee (*Coffea arabica* L.) trees and limits both the production and retention of leaves, leading to branch dieback, and, thus, results in a strong biennial bearing pattern. The major goals of this study were to investigate the physiological mechanisms that may be associated with the leaf-to-fruit ratio (LFR), branch dieback, biennial production and the relationships between carbohydrate and mineral fluctuations and branch dieback in coffee plants. The trees were grown in north–south-oriented hedgerows under conditions of full sunlight. Leaves and plagiotropic branches from the upper and lower strata of the east- and west-facing sides of the hedgerow were examined. A strong biennial pattern of coffee production was observed over three harvests. Overall, the east face of the hedgerow produced a more sellable crop than the west face, and this was associated with more light availability for the east-facing branches. The branch growth rate was higher with an increasing LFR during 2006–2007, regardless of the canopy position, and no compensatory increase in the photosynthetic rate was found in response to a decreasing LFR. Due to a relatively low fruit yield in 2007–2008, there was no branch dieback. The extent of branch dieback increased dramatically with decreasing LFR and was probably not closely related to changes in the concentrations of carbohydrates, amino acids and minerals. The extent of branch dieback was apparently unrelated to the differences in the photosynthetic rates per unit leaf area, carbon isotope composition, or oxidative stress, as was assessed by the electrolyte leakage from the leaf tissues. We discuss these responses in terms of the relative lack of branch autonomy in coffee trees.

1. Introduction

The coffee tree tends to flower heavily, particularly in non-shaded plantations, producing a high crop load without a concomitant balance in leaf area formation. Because the coffee berries act as priority sinks, the photoassimilates allocated to fruit development may be more than four times that allocated to branch growth over the annual production cycle (Vaast et al., 2005). A remarkable limitation on shoot growth is, therefore, commonly observed in heavy-bearing coffee trees. Overbearing limits both the production and retention of leaves and leads to branch dieback (descending branch death), thus, resulting in a strong biennial bearing pattern, which ultimately leads to tree degeneracy (Cannell, 1985; DaMatta et al., 2010). These responses have traditionally been associated with strong decreases in the carbohydrate and mineral contents in the root-trunk system (Cannell, 1985, and references therein). However, studies by Carvalho et al. (1993) have found no consistent relationship between the depression of shoot growth or branch dieback and the exhaustion of stored carbohydrates and minerals.

In addition to the large quantities of carbohydrates required during the expansion phase, the fruits of coffee trees also require substantial amounts of both potassium and nitrogen (N) and have been reported to capture 95% of the current total uptake of N during the endosperm-filling stage, which can lead to symptoms of N deficiency in the leaves (Cannell, 1985). Decreases in the N content may impair photosynthesis, thereby contributing to the creation of excess energy in the photosynthetic apparatus

Abbreviations: A, net carbon assimilation rate; Chl, chlorophyll; C_i/C_a, internal-to-ambient CO_2 concentration ratio; DW, dry weight; FW, fresh weight; g_s, stomatal conductance; LE, Lower East; LFR, leaf area-to-fruit ratio; LW, Lower West; R1, LFR below 6 cm^2 $fruit^{-1}$; R2, LFR between 6.1 and 14 cm^2 $fruit^{-1}$; R3, LFR greater than 14 cm^2 $fruit^{-1}$; Q_A, incident photosynthetically active radiation; r, Pearson correlation coefficient; UE, Upper East; UW, Upper West; δ_e, leaf-to-air vapor pressure deficit; $\delta^{13}C$, carbon isotope composition ratio; ρ, Spearman correlation coefficient.

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(Ramalho et al., 1998; DaMatta et al., 2002), which may ultimately bring about oxidative stress and cell damage (Ramalho et al., 1998, 2000; Fortunato et al., 2010; Pompelli et al., 2010) in the coffee trees. In fact, N deficiency can lead to considerable increases in branch dieback (DaMatta, 2004), especially in trees grown under full sun. However, the links between N deficiency, branch dieback and oxidative stress have not yet been established in coffee trees.

Field observations show that the magnitude of fruit production can vary between the opposing faces of coffee trees grown in rows. Alves (2005) has shown that the production of the west face, which received more total solar radiation due to the slope of the terrain, was 40% greater than that of the east face. Coffee yields may increase with increasing light availability because of the higher whole-tree carbon assimilation, greater stimulation of flower, rather than vegetative buds, more nodes formed per branch and more flower buds at existing nodes (DaMatta, 2004). However, it is not known whether production differences within the canopy are consistent among harvests or if there are biennial differences in the crop yield within the same plant between the different faces of the hedgerow. It is also not known whether these anticipated differences in crop production could be reflected in varying patterns of carbohydrate and mineral contents and branch dieback within the canopy of the coffee trees.

A leaf area of approximately 20 cm^2 is needed to support each fruit to avoid severely restraining the vegetative growth of the coffee plant (Cannell, 1985). This area, however, can be considerably smaller in heavily bearing coffee trees (DaMatta et al., 2008). An increased sink strength that parallels decreases in the leaf area-to-fruit ratio (LFR) could be partially compensated for by an increased rate of photosynthesis to sustain the fruit burden, as has been reported in some woody species, such as apple (Gucci et al., 1994), chestnut (Proietti et al., 2000), mango (Urban and Léchaudel, 2005) and peach (Li et al., 2007), but not in other perennials, such as olive (Proietti, 2000) and oil palm (Legros et al., 2009). In coffee, relatively higher photosynthetic rates have been observed in trees with lower LFR values (Vaast et al., 2005; Franck et al., 2006; DaMatta et al., 2008). However, these results have been found when the source/sink relationship was manipulated by defoliation and/or defruiting or even the girdling of branches. Furthermore, DaMatta et al. (2008) have only observed stimulation of photosynthesis associated with an increased sink strength when comparing two extreme treatments, namely, by comparing completely defruited coffee plants with those with a full crop load and the leaf area reduced by half. Therefore, it remains to be demonstrated whether the increased fruit demand for assimilates under a situation of a decreased LFR could be met by an anticipated increase in photosynthetic rates in intact coffee trees growing under real field conditions.

The major goals of this study were to investigate the physiological mechanisms that may be associated with the LFRs, branch dieback and biennial production, as well as the relationships of carbohydrate and mineral fluctuations with branch dieback in coffee plants. Given the considerable autonomy of the plagiotropic (lateral) branches of a coffee tree (Cannell, 1971), it was hypothesized that (i) there could be a biennial production between the different solar exposure faces of the same plant, (ii) the extent of branch dieback and photosynthetic rates should increase with decreasing LFR, and (iii) an expected oxidative damage associated with an N deficiency due to a high sink demand could trigger the process leading to branch dieback. To test these hypotheses, the vegetative growth and production, branch dieback, photosynthesis, carbohydrate and mineral pools and cell damage in branches with different LFRs and in various canopy positions were analyzed in unmanaged coffee trees growing under plantation conditions. Traits were assessed over two agricultural years; crop production was further assessed on a third agricultural year.

2. Material and methods

2.1. Plant material and growth conditions

The experiment, carried out under field conditions, began in 2006 with seven-year-old, approximately 2-m tall coffee trees (*Coffea arabica* L. 'Catuaí Vermelho IAC 99′) growing on a red-yellowish podzol (13% terrain slope) in Coimbra (720 m a.s.l., 20°51′24″S, 42°48′10″W), Southeastern Brazil. The site is characterized by a subtropical climate, with a mean annual temperature of 20 °C, and receives an average rainfall of 1300 mm, mainly distributed from September to March (growing season). The trees had been cultivated in full sunlight and were planted with 2.0×1.0 m spacing in north–south-oriented hedgerows. The plants were submitted to routine agricultural practices for commercial coffee bean production, including hoeing and the chemical control of insect and pathogen attacks. Each tree was fertilized with 0.167 kg of N, supplied as ammonium sulfate, and 0.167 kg of K, as KCl, per year. The fertilizer was applied three times during the growing season, coinciding approximately with periods of supplemental fertilization for most commercial coffee crops in Brazilian Arabica coffee-producing regions. In addition, 0.15 kg of dolomitic limestone was applied per tree in December 2006, and 0.5 kg of manure was supplied per tree in August of each year. No supplemental irrigation was provided, but there were abundant rains during the growing season.

In Southeastern Brazil, the shoot growth of a coffee tree is slow during the dry, cool season (April to early September) and rapid in the rainy, warm season (September to late March), though growth rates are particularly maximal between September and November (Silva et al., 2004) when the competition between vegetative and reproductive growth is at a minimum. Flowering is gregarious in coffee: it usually occurs in September after the first rains. The entire fruit development period lasts, on average, 34 weeks, and the maximum assimilate demand for the developing fruits occurs during the *endosperm-filling stage*, from approximately early January to late March. Subsequently, the fruits enter the *ripening stage* (with changes occurring mostly in the pericarp, with only slight increases in dry mass), which is distributed over a period of up to 10 weeks, after which, the fruits are harvested (often from late May to June/July; DaMatta et al., 2010).

2.2. Experimental design

In 2006, 30 trees were selected based on their uniformity and vigor. Twenty-four useful plagiotropic branches were identified per tree and divided into four groups of six branches according to their positions in the canopy: Upper East (UE), Lower East (LE), Upper West (UW) and Lower West (LW). The upper and lower positions refer to the upper third and lower third of the plant canopies, respectively. For all of the branches analyzed, the fruit number was counted, and the total leaf area was estimated using the maximum leaf widths and lengths and the equations described by Antunes et al. (2008). Based on these data, branches from the different canopy positions described above were selected (in early November 2006) with the three following distinct LFRs: R1, with an LFR lower than 6 (3.5 ± 1.8) cm^2 $fruit^{-1}$; R2, with an LFR between 6.1 and 14 (9.4 ± 2.2) cm^2 $fruit^{-1}$; and R3, with an LFR greater than 14 (23.0 ± 7.1) cm^2 $fruit^{-1}$. It should be emphasized that a remarkable variability among branches with regard to LFRs was noted, particularly in the two upper thirds of the plant canopies. In total, 432 branches were analyzed, 360 of which were used for the assessments of growth, production quantification and branch dieback. Growth rates were assessed with a tape measure (Silva et al., 2004) over the course of the growing season; assessments of the crop yield and branch dieback were performed in early June. The remaining 72 branches were used for physiological assessments,

including gas exchange, the carbon isotope composition ratio, the concentrations of carbohydrates, chlorophylls, carotenoids, minerals, and electrolyte leakage. The evaluations for the 2006–2007 growing season were performed in March 2007 during the final phase of fruit filling, a period that is characterized by the greatest demand for assimilates by the fruits. These evaluations were conducted on the leaves (one leaf per selected branch) of the third pair from the apex of plagiotropic branches; carbohydrates and amino acids were also evaluated in the central part of the plagiotropic branches, where bearing nodes are concentrated, by collecting a 2-cm length branch fragment (about 300 mg fresh mass). Gas-exchange parameters were measured three times on the same leaf from March 12 to March 25, giving similar results. The plant material was collected at approximately 14:30 h, transported from the field to the laboratory in liquid nitrogen, and stored at −80 °C until required.

For the 2007–2008 season, we analyzed the same 30 plants selected for the 2006–2007 season. In early November 2007, 384 branches were selected by measuring leaf area and the number of fruits on each branch as described above. However, due to the low fruit yield during this season, it was not possible to classify the branches into the three LFR categories described for the 2006–2007 season. In this case, only the UE, LE, UW and LW branches with high LFRs (>20 cm^2 $fruit^{-1}$) were compared. Of the 384 plagiotropic branches analyzed, 360 were used for the evaluations of growth, quantification of production and branch dieback, and the remaining 24 were used for the physiological evaluations. All of the evaluations in 2007–2008 were made in the same months as described for the 2006–2007 period.

For branch growth, branch dieback and crop production, 30 replicates per canopy position per LFR condition were analyzed, whereas for physiological traits, a total of six replicates were considered.

2.3. Crop yield

For the 2006–2007 and 2007–2008 agricultural years, all of the fruits from the selected branches were harvested and classified as normal (full) or buoyant (malformed fruits with a lower density than water) fruits. Additionally, total fruit production was evaluated for the 2008–2009 period. The dry fruit mass per branch and per plant was determined according to usual agronomic practices after drying to a standardization of the moisture content at 13%.

2.4. Branch dieback

After harvesting, the number of healthy and dry branches (branch dieback) in each group and in the plant as a whole was quantified. Branches with at least 10 cm of dead apical tissue were considered “dry”.

2.5. Agrometeorological parameters

The incident photosynthetically active radiation (Q_A) intercepted by the leaf was measured using a photometer/radiometer (LI-185, LI-COR, Lincoln, USA). The leaf-to-air vapor pressure deficit (δ_e) was estimated as described in Chaves et al. (2008). These measurements were made concomitantly with the gas exchange assessments (see below). We also measured the total daily Q_A over the 2008–2009 growing season using LI-190SA quantum sensors (LI-COR) positioned 1 m above the canopy and at the east and west (mid-canopy height) sides of the hedgerows. All of the sensors were connected to an LI-1400 data logger (LI-COR), which acquired data from the sensors every minute and stored them as 5-min averages.

2.6. Gas exchange and carbon isotope composition ratio

Gas exchange was measured at four different time points throughout the day: 7:00–9:00 h, 9:30–11:30 h, 12:00–14:00 h and 15:00–17:00 h (solar time). The net rate of carbon assimilation (A), stomatal conductance (g_s) and the internal-to-ambient CO_2 concentration ratio (C_i/C_a) were measured in an open system under both ambient light and CO_2 concentration using an infrared gas analyzer (LI-6400, LI-COR, Lincoln, USA). After fitting the leaf tissue in the leaf chamber, the rates of gas exchange were typically settled within 3–4 min, nearly paralleling the stabilization for internal CO_2 values. The measurements were repeated on three separate days (for each leaf within each time point), such that the gas-exchange parameters for each replicate were computed as the average values obtained over the measurement days.

Leaves were collected, oven-dried for 72 h at 60 °C and ground before combustion at 950 °C under continuous O_2 flux. The relative abundances of ^{13}C and ^{12}C were measured relative to the international Pee Dee Belemnite standard using a mass spectrometer (ANCA-GSL 20-20, Sercon, Crewe, UK), from which the leaf carbon isotope composition ratio ($\delta^{13}C$) was estimated. Further details have been provided previously (DaMatta et al., 2003).

2.7. Other assays

Branch concentrations of hexoses (glucose + fructose), sucrose, starch and free amino acids were colorimetrically assayed using an ELISA reader (Tunable Microplate Reader, VERSAmax, Sunnyvale, USA), as described previously (Praxedes et al., 2006; Ronchi et al., 2006). Leaf mineral concentrations were estimated in oven-dried leaf tissues at 70 °C for 72 h. Total N was determined as the sum of ammonium–N (determined using the Nessler reagent at 440 nm) and nitrate–N (extracted from tissues during 4 h at 45 °C and colorimetrically determined at 410 nm) pools, as described in DaMatta et al. (1999). The P, S, K, Ca and Mg concentrations were determined in the leaf samples (after being submitted to nitroperchloric digestion) by routine methods: visible spectrophotometry for P and S, flame photometry for K, and atomic absorption spectrometry for Ca and Mg. The chlorophylls and total carotenoids were extracted using 80% (v/v) aqueous acetone and quantified according to Lichtenthaler (1987). Electrolyte leakage was used as a proxy for oxidative stress/cell damage and was analyzed immediately after leaf sampling using a conductivity meter (DM31, Digimed, Santo Amaro, Brazil), as has been reported previously (Lima et al., 2002).

2.8. Statistics

The experiments were conducted according to a randomized design. For the period of 2006–2007, the experiments were analyzed based on a split-plot design. The plots were composed of the four positions of the plants, namely UE, LE, UW and LW, and the subplots were the three classes of LFRs. For the 2007–2008 period, the experiment was evaluated in a factorial 2 × 2 (two exposure faces, east and west, and two strata, upper and lower, for each plant). The evaluations of the production, Q_A and δ_e, in the four canopy positions were performed in a factorial 2 × 2 (two faces and two strata for each plant) when comparisons were made within the same agricultural year. When the production was compared among the three harvest periods, the data were evaluated using a split-plot analysis, with the plots consisting of the canopy positions and subplots in the agricultural years. The comparison of the production of the east and west faces among the three agricultural years was performed using split-plot analysis, with the plots consisting of the canopy faces and the subplots in the three agricultural years.

The data was analyzed by ANOVA, and the means were compared using the Newman–Keuls test at $P \leq 0.05$. Correlation

analyses were made using Pearson's (r) parametric and Spearman's (ρ) non-parametric methods using different variables, where Student's t-tests and z-tests were used, respectively. All of the statistical analyses were performed using the SAEG System version 9.1 (SAEG, 2007).

3. Results

3.1. Branch growth rate

Overall, the growth rates of plagiotropic branches in 2006–2007 increased with the increasing LFR, regardless of the canopy position. The greatest differences in growth rates among the LFRs were observed between 12/21/2006 and 02/24/2007. The R3 group (LFR > 14 cm^2 $fruit^{-1}$) showed the highest ($P = 0.0001$) growth rate, followed by the R2 (6.1 < LFR < 14 cm^2 $fruit^{-1}$) and R1 (LFR < 6.0 cm^2 $fruit^{-1}$) groups (Fig. 1). After 02/24/2007, a decrease in the growth rate for all of the LFRs was evident; from 03/10/2007 to the end of the study, the growth rates for all of the LFRs treatments were similar, regardless of the canopy position (Fig. 1).

The growth rates of the branches varied significantly among the canopy positions during 2007–2008 (Fig. 2). In general, from 12/01/2007 to the end of the study, both the UE and UW branches showed greater growth than the LE and LW branches. Beginning on 02/06/2008 the growth rate of the branches decreased for all of the positions, but, in contrast to the observations made during the 2006–2007 growing season, there were no similarities in the growth rates among the different canopy positions. The single exception was found on 05/08/2008, when the growth rates of the branches from the LE and LW positions were similar. As a whole, the growth rate was higher in the UW and UE branches than in their LW and LE counterparts.

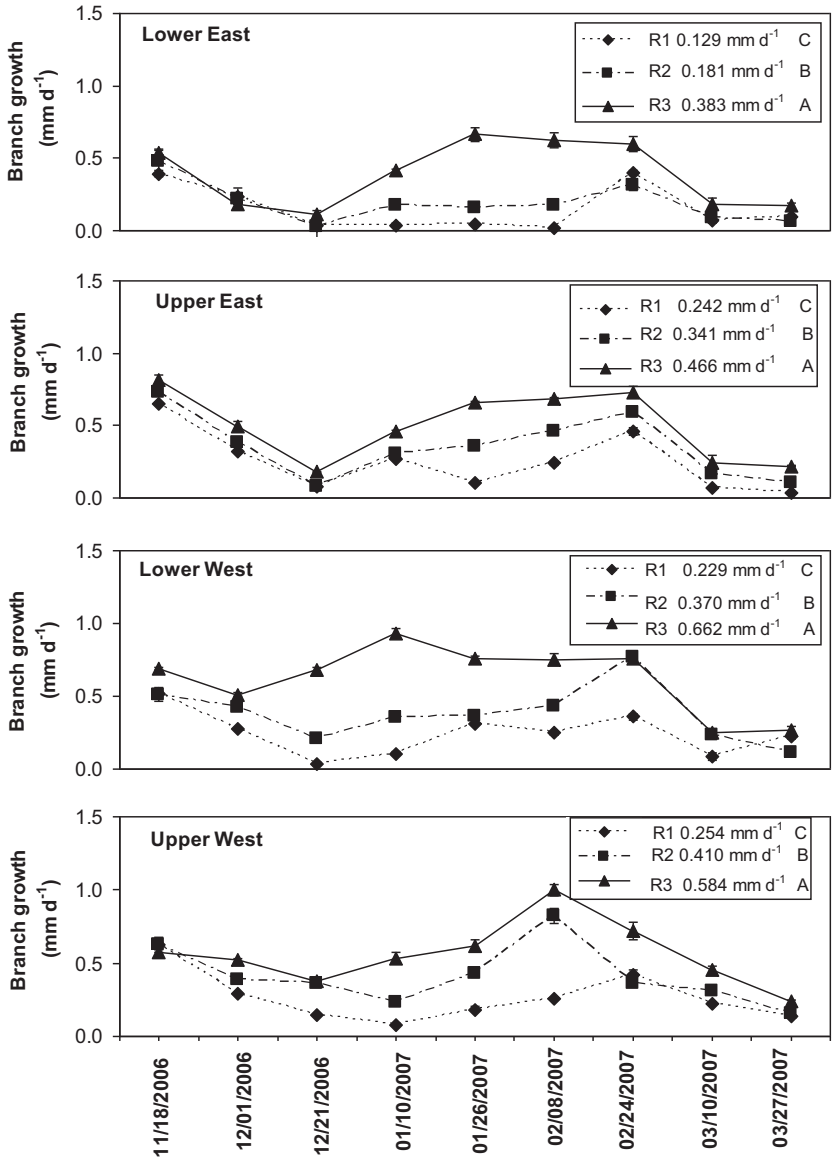

Fig. 1. Branch growth in different canopy positions (Upper East, Lower East, Upper West and Lower West) of field-grown coffee trees. Branches with different leaf area-to-fruit ratios (LFRs) were evaluated between November 2006 and March 2007 and were defined as follows: R1, <6 cm^2 $fruit^{-1}$; R2, from 6.1 to 14 cm^2 $fruit^{-1}$; and R3, >14 cm^2 $fruit^{-1}$. Each point represents the mean of 30 replicates. Vertical bars denote SE; when not shown, the SE was smaller than the symbols. In the insets, the average branch growth rates (from November to March) for the three LFRs are shown. Values followed by distinct letters within each canopy position differed significantly from each other (Newman–Keuls, $P \leq 0.05$).

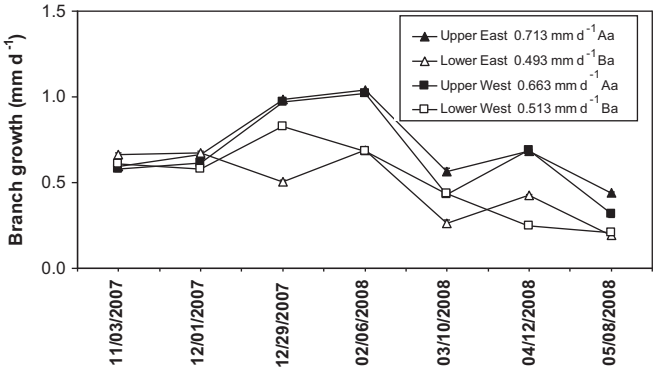

Fig. 2. Branch growth in different canopy positions (Upper East, Lower East, Upper West and Lower West) of field-grown coffee trees. Branches with leaf area-to-fruit ratios greater than 20 cm^2 $fruit^{-1}$ were analyzed between November 2007 and May 2008. Each point represents the mean of 30 replicates. Vertical bars denote SE; when not shown, the SE was smaller than the symbols. In the insets, the average branch growth rates (from November to May) for the four canopy positions are shown. Uppercase letters indicate significant differences between the averages of the two strata in each face, and lowercase letters indicate significant differences among the averages of each stratum between the two faces (Newman–Keuls, $P \leq 0.05$).

3.2. Crop yields and branch dieback in relation to leaf-to-fruit ratios

For 2007, the total, normal and buoyant fruit production for the LE, UE and UW positions decreased significantly with the increasing LFR, although no significant difference between the R2 and R3 groups was found for the LE position (Fig. 3a–c). In the LW branches, the production of both the total and normal fruit was statistically different among the three LFRs, with higher production in R2 and lower production in R3 (Fig. 3a and b). The production of buoyant fruits on the LW branches was higher in the R1 and R2 groups than in the R3 group (Fig. 3c).

The comparison of 2007 fruit production within each LFR showed that, for R1, the total and normal fruit production was higher on the UE branches than in the other branches, followed by production in the UW and LE/LW branches (Fig. 3a and b); the buoyant fruit production decreased in the following order: UE > UW > LE > LW (Fig. 3c). For R2, the total fruit production was similar among the UE, UW and LW branches and, on average, higher than the LE branches. However, the production of normal fruits was lower on LE branches, whereas the buoyant fruit production was higher on UE branches, than for any of the other positions (Fig. 3b and c). For R3, the total and normal fruit production was similar for all of the positions evaluated (Fig. 3a and b), whereas the production of buoyant fruits was significantly higher on the branches facing east than the ones facing west (Fig. 3c).

The percentage of branch dieback within each LFR was similar for all four of the positions in 2007 (Fig. 3d). The percentage of branch dieback was approximately 78% in R1, 50% in R2 and 20% in R3 (Fig. 3d), regardless of the canopy position.

For 2008, we did not observe the production of buoyant fruit or branch dieback, independent of the face or stratum analyzed (data not shown).

3.3. Crop yield in relation to the branch position

The data shown in Fig. 4 illustrate the strong biennial production pattern of coffee plants, regardless of the canopy position analyzed. For 2007, the total fruit production was higher on the branches from the UE position, followed by the UW, LE and LW positions, whereas, for 2008, the production on the UE branches tended to be superior to the production at the other positions. For 2009, the production was higher for the UE and UW positions than for the LE and LW positions (Fig. 4a). In general, the total production of the branches on the east face (UE + LE) was higher than that of the branches on the west face (Fig. 4b), which could be associated with the greater availability of light on the east side of the canopy, as found in 2008–2009 growing season (Table 1). Overall, our data illustrate the absence of a biennial production pattern within each face of the hedgerow over the three harvests assessed.

Table 1
Mean daily values of the photosynthetically active radiation (Q_A) intercepted by the leaf and the leaf-to-air vapor pressure deficit (δ_e) of field-grown coffee trees. Measurements were taken at approximately 8:00, 10:30, 13:00 and 16:00 h; the results of these measurements are expressed as daily averages. The assessments were carried out in different canopy positions (upper and lower strata in both east and west faces) in March 2007 and March 2008. Uppercase letters indicate differences between the averages of the two strata in each face. Lowercase letters indicate the difference between the averages between the two canopy faces in each stratum (Newman–Keuls, $P \leq 0.05$; $n = 6 \pm$ SE). Measurements of the total daily Q_A over the 2008–2009 growing season were also performed at a mid-canopy height of the east and west sides of the hedgerows; values are shown as the means ± SE. The total daily Q_A above (1 m) the canopy was 19.6 ± 1.3 mol m^{-2} d^{-1}.

Parameters	Stratum	Canopy position	
		East	West
2007			
Q_A (μmol m^{-2} s^{-1})	Lower	581 Ba	636 Aa
	Upper	1358 Aa	685 Ab
δ_e (kPa)	Lower	4.11 Aa	3.81 Aa
	Upper	4.17 Aa	3.99 Aa
2008			
Q_A (μmol m^{-2} s^{-1})	Lower	87 Ba	143 Ba
	Upper	322 Aa	292 Aa
δ_e (kPa)	Lower	1.32 Aa	1.25 Aa
	Upper	1.37 Aa	1.42 Aa
2008–2009			
Q_A (mol m^{-2} d^{-1})	–	9.8 ± 0.9	4.1 ± 0.1

3.4. Environment and gas exchanges

Overall, the total incident daily Q_A, expressed as the percentage of incident irradiance reaching the top canopies, was higher (50%) for the east face than for the west face (21%), as deduced from Table 1. In March 2007, the incident daily Q_A intercepted by the leaf was higher in the UE branches than in the branches from the remaining positions of the canopy (Table 1). There was no difference in the δ_e among the branch groups (Table 1), with average daily values of approximately 4 kPa. For the evaluations during March 2008, which were conducted on days that were partially cloudy (at this location, there were no cloudless days in March 2008, in contrast to March 2007), the Q_A intercepted by the leaf was higher in the upper stratum than in the lower one, whereas the δ_e values were similar (~1.3 kPa) among the groups (Table 1).

In 2007, the maximum A was found in the early morning and was quite low after 10:30 h, tracking the pattern of the g_s (Fig. 5). The C_i/C_a ratio was quite low (0.4 on average; data not shown) and similar in all of the groups. Overall, there were only small variations in the A, g_s and $\delta^{13}C$ values in response to the various treatments (Figs. 5 and 7), with no consistent pattern observed among the branch groups. In 2007, the average daily maxima for A (2.4 μmol m^{-2} s^{-1}) and g_s (60 mmol m^{-2} s^{-1}) (Fig. 5) were lower than those exhibited in 2008, ~6.7 μmol m^{-2} s^{-1} and ~187 mmol m^{-2} s^{-1}, respectively (Fig. 6). This was likely due to the lower δ_e in 2008 than in 2007 (Table 1). In 2008, the A was significantly higher in the upper stratum than in the lower stratum, with the smallest values found in the leaves from the LE branches (Fig. 6a). The g_s values were similar between the two strata for both faces; however, it was higher in the LW than in UW position (Fig. 6b). For both faces, the C_i/C_a ratio was slightly higher in the lower stratum than in the upper stratum (about 0.83 against about

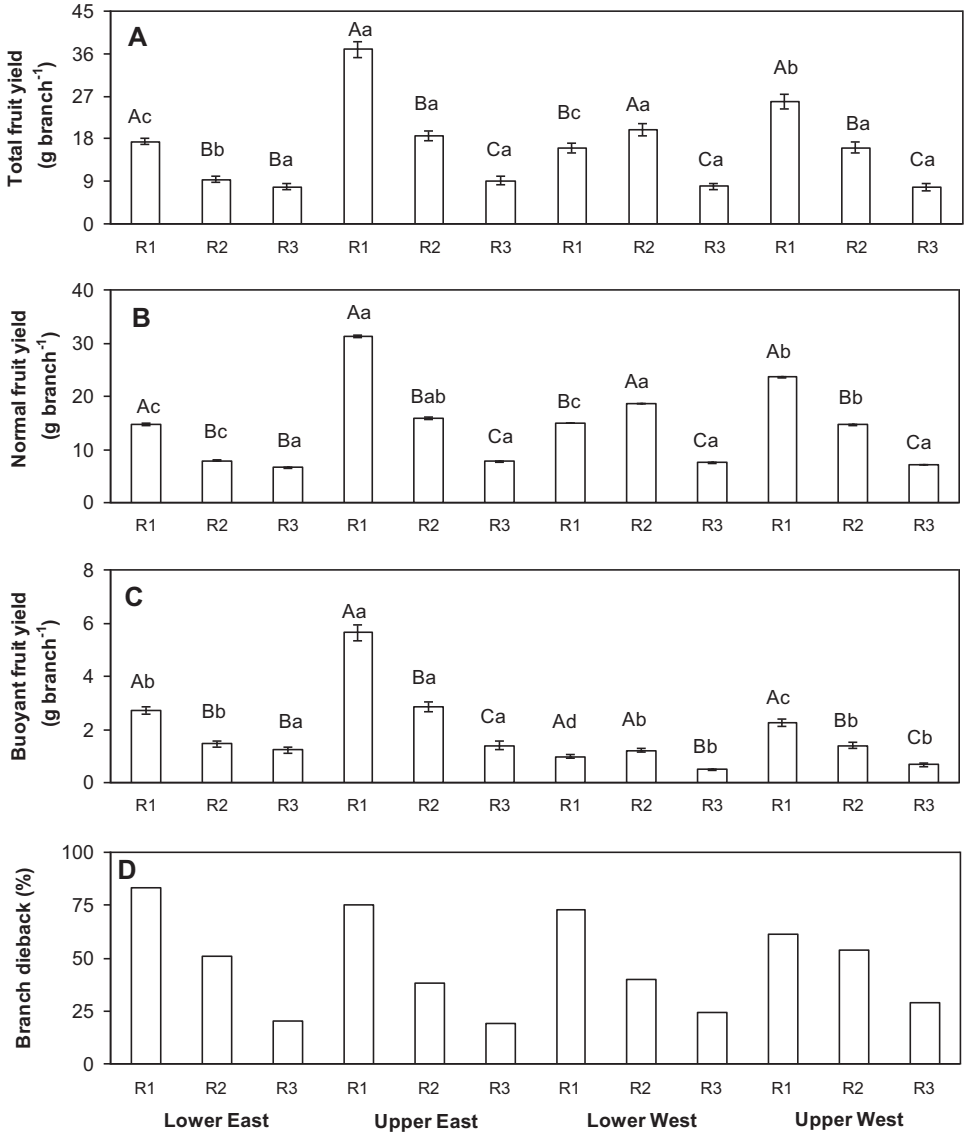

Fig. 3. Total (A), normal (B) and buoyant (C) fruit production and the percentage of branch dieback (D) of field-grown coffee trees evaluated in 2007. The analysis included branches with leaf area-to-fruit ratios (LFRs) <6 cm^2 $fruit^{-1}$ (R1), from 6.1 to 14 cm^2 $fruit^{-1}$ (R2) and >14 cm^2 $fruit^{-1}$ (R3). The branches analyzed were from Lower East, Upper East, Lower West and Upper West positions in the plant. Note the differences in the y-scale. Uppercase letters indicate significant differences among the averages of the three LFRs within each canopy position. Vertical bars denote SE. Lowercase letters indicate significant differences among the means for each LFR in the four canopy positions (Newman–Keuls, $P \leq 0.05$; $n = 30$).

0.74; data not shown), whereas an opposite response was found for the $\delta^{13}C$ (Table 2).

3.5. Branch concentrations of carbohydrates and total amino acids

Because growth rates may be much more dependent on the branch, rather than the leaf, concentrations of carbohydrates and amino acids (DaMatta et al., 2010), analyses were performed in the central part of the plagiotropic branches. Overall, the trends for the carbohydrate and amino acid pools described for the branches (see below) were also similar for the leaf tissues (data not shown). In 2007, a clear tendency toward lower concentrations of hexoses in the LE and LW branches was observed, as compared to the UE and UW branches (Fig. 8a). There was little or no alteration in the sucrose or starch concentrations for any of the positions (Fig. 8b and c). The total amino acid concentration was higher in R3, tending to increase with the increasing LFR, regardless of the branch position in the canopy (Fig. 8d).

For 2008, the concentration of hexoses was similar among the different groups (Table 2). The sucrose concentration was similar in both the upper and lower strata within each canopy face; however, in the lower stratum, the sucrose concentration was higher in the branches from the east face than that from the west face (Table 2). The starch concentrations were similar between the upper and lower strata for both faces, but they were higher in the east than in the west face within each stratum. The total amino acid concentration was similar among all of the groups (Table 2). Overall, similar trends for carbohydrates and amino acids pools were found in leaf tissues (data not shown).

3.6. Leaf concentrations of chlorophylls, carotenoids and minerals and electrolyte leakage

In March 2007, the concentration of total chlorophylls (Chl) was similar in the LE branches among the three LFRs and higher in the R3 group than in the R1 and R2 groups in the branches of the remaining positions (Fig. 9a). The Chl concentrations in R1 and R2 of the

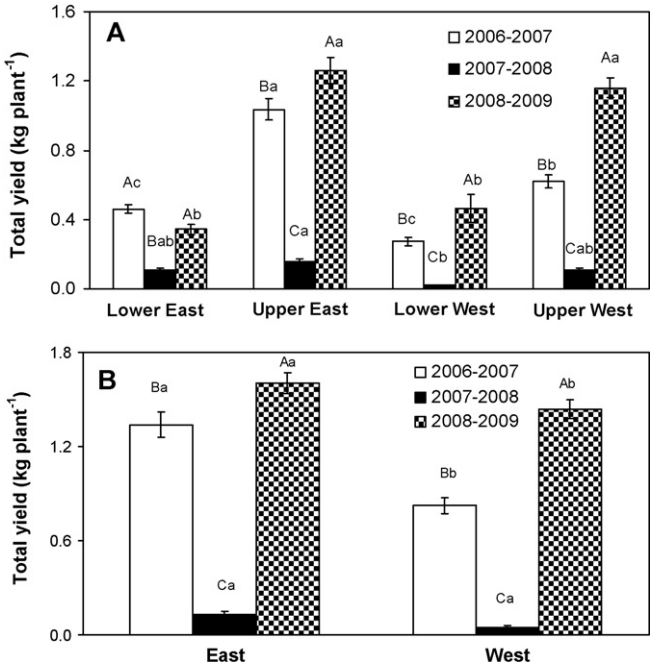

Fig. 4. Total coffee fruit production in different canopy positions (Upper East, Lower East, Upper West and Lower West) of field-grown coffee trees (A) and total coffee fruit production in the east and west faces of the coffee hedgerows (B) during the 2006–2007, 2007–2008 and 2008–2009 harvests. Vertical bars denote SE. Uppercase letters indicate significant differences among the averages of each canopy position (A) and between the east and west faces (B) for the three agricultural years. Lowercase letters indicate significant differences among the averages from the four canopy positions (A) and between the two faces (B) within each agricultural year (Newman–Keuls, $P \leq 0.05$; $n = 30$).

LE and LW branches were higher than those observed for the same LFRs in the UE and UW branches, whereas the Chl concentration in the R3 group was lower in the UE branches than in the other canopy positions (Fig. 9a). The concentrations of total carotenoids were similar in the LE and LW branches for all three of the LFRs, whereas, in the UE branches, the total carotenoid concentration was higher in the R3 group than in the R1 and R2 groups (Fig. 9b). In the UW branches, the total carotenoid concentration increased with the increasing LFR (Fig. 9b). The nitrogen concentration was higher than 23 g kg^{-1} dry weight (DW), with the exception of the R1 group in the UE and UW branches, where the N concentrations were 21.5 and 18.3 g kg^{-1} DW, respectively (Fig. 9c). These lower values are indicative of an N deficiency, as concentrations below 23 g N kg^{-1} DW have been shown to induce visual symptoms of N deficiency in coffee (Moraes, 1981). The Chl/N ratio observed was similar for all of the groups, with the exception of R2 in the UE and UW positions, which was significantly lower than in the other canopy positions (Fig. 9d). Deficiency in any of the remaining minerals analyzed, namely P, K, S, Ca and Mg, was not found (data not shown). As was observed for electrolyte leakage (Fig. 9e), the levels of these minerals did not differ statistically among the different groups (data not shown). In March 2008, the values of all of the above variables were similar, regardless of the canopy position (Table 2). It should be noted that the leaf N concentration was higher in 2008 (approximately 30 g N kg^{-1} DW, irrespective of the group) than in 2007, probably due to the lower N requirements associated with the decreased sink demand in 2008.

3.7. Correlations among variables

The correlations among variables in 2007 are shown in Table 3. The growth rates of plagiotropic branches increased significantly with increasing LFR, regardless of the canopy position ($r = 0.678$), whereas the percentage of branch dieback decreased significantly with increasing LFR ($\rho = -0.416$). Weak negative, though significant, relationships between branch dieback and starch ($\rho = -0.289$) and N ($\rho = -0.372$) were observed, whereas branch dieback was unrelated to changes in A ($\rho = 0.067$), hexoses ($\rho = 0.110$), sucrose ($\rho = 0.024$), Chl ($\rho = 0.114$) and electrolyte leakage ($\rho = 0.069$). No significant correlations between the LFR and A ($r = 0.032$) or between the LFR and $\delta^{13}C$ ($r = 0.131$) were found. Despite the slight variations in the carbohydrate and amino acid levels among the three LFRs within the canopy positions, significant positive correlations between the LFRs and hexoses ($r = 0.344$), sucrose ($r = 0.325$), starch ($r = 0.410$), amino acids ($r = 0.409$), total

Table 2
Carbon isotope composition ratio ($\delta^{13}C$), concentrations of hexoses, sucrose, starch, total amino acids, total chlorophylls, total carotenoids and nitrogen, total chlorophyll-to-nitrogen ratio, and electrolyte leakage as assessed in different canopy positions (upper and lower strata in both east and west faces) of field-grown coffee trees in March 2008. Analyses of carbohydrates and amino acids were performed using segments of branches, and the remaining analyses were made on leaf material. Uppercase letters indicate differences between the averages of the two strata in each face. Lowercase letters indicate the difference between the averages between the two canopy faces in each stratum (Newman–Keuls, $P \leq 0.05$; $n = 6 \pm$ SE).

Parameters	Stratum	Canopy position	
		East	West
$\delta^{13}C$ (‰)	Lower	29.50 ± 0.348 Aa	29.03 ± 0.265 Aa
	Upper	27.35 ± 0.221 Ba	27.61 ± 0.183 Ba
Hexoses (mmol kg^{-1} FW)	Lower	1.850 ± 0.140 Aa	1.623 ± 0.128 Aa
	Upper	1.697 ± 0.137 Aa	1.662 ± 0.095 Aa
Sucrose (mmol kg^{-1} FW)	Lower	3.067 ± 0.365 Aa	2.046 ± 0.192 Ab
	Upper	2.414 ± 0.170 Aa	2.330 ± 0.174 Aa
Starch (mmol kg^{-1} FW)	Lower	14.77 ± 1.25 Aa	11.82 ± 0.41 Ab
	Upper	15.73 ± 1.01Aa	12.77 ± 0.70 Ab
Amino acids (mmol kg^{-1} FW)	Lower	15.71 ± 1.10 Aa	13.74 ± 0.19 Aa
	Upper	14.642 ± 0.793 Aa	13.31 ± 0.57 Aa
Chlorophyll $a+b$ (g kg^{-1} FW)	Lower	1.876 ± 0.097 Ab	2.142 ± 0.123 Aa
	Upper	2.165 ± 0.096 Aa	2.021 ± 0.155 Aa
Carotenoids (g kg^{-1} FW)	Lower	0.359 ± 0.035 Aa	0.420 ± 0.032 Aa
	Upper	0.445 ± 0.038 Aa	0.378 ± 0.033 Aa
Nitrogen (g kg^{-1} DW)	Lower	31.47 ± 0.26 Aa	29.62 ± 0.88 Aa
	Upper	29.67 ± 0.64 Aa	29.33 ± 0.55 Aa
Chlorophyll/nitrogen	Lower	0.205 ± 0.015 Aa	0.218 ± 0.015 Aa
	Upper	0.206 ± 0.008 Aa	0.194 ± 0.015 Aa
Electrolyte leakage (%)	Lower	2.380 ± 0.290 Aa	2.155 ± 0.048 Aa
	Upper	2.390 ± 0.145 Aa	2.307 ± 0.037 Aa

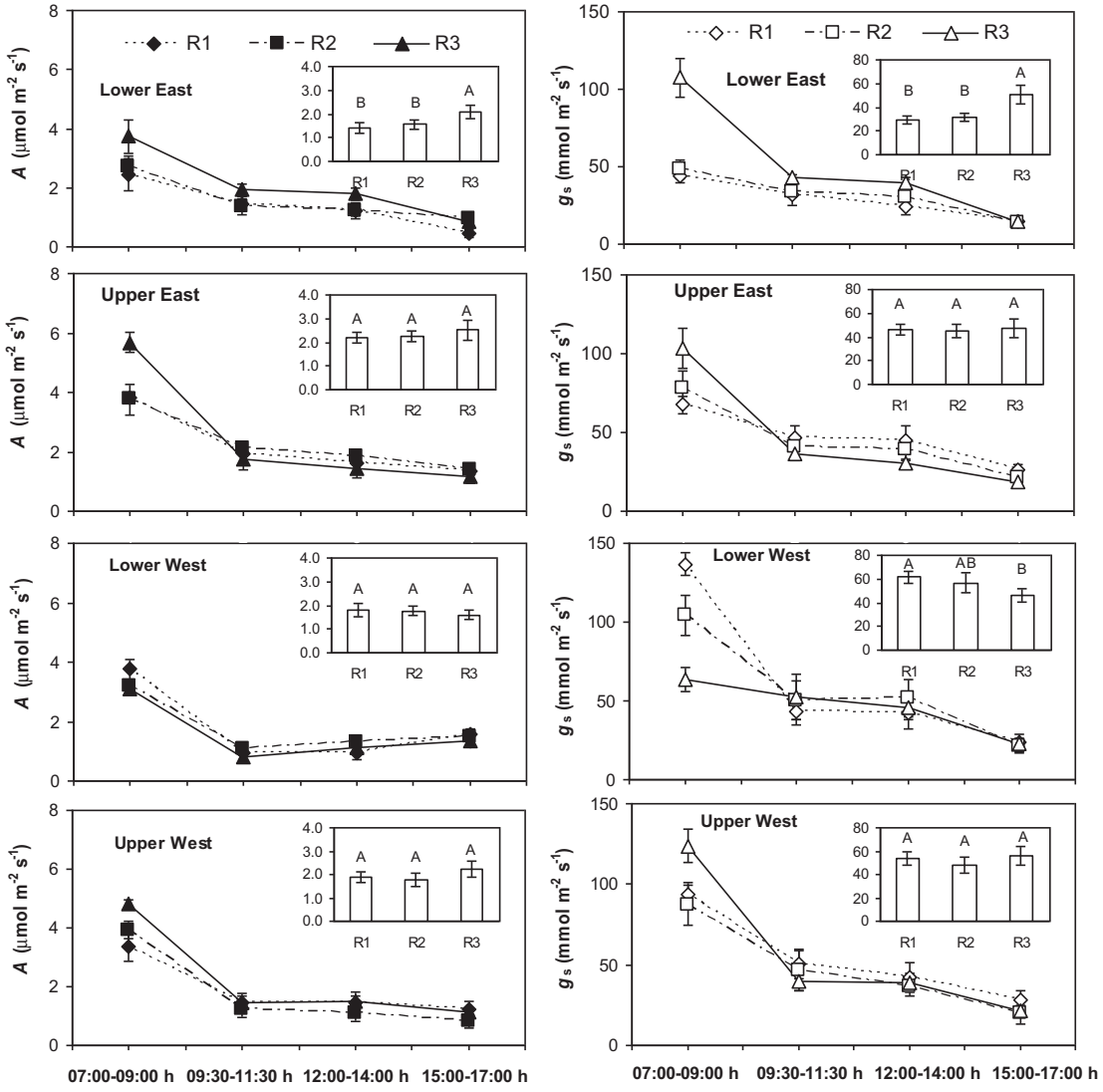

Fig. 5. Time-course study of the net carbon assimilation rate (A) and stomatal conductance (g_s) of field-grown coffee trees in March 2007. The analysis included branches with leaf area-to-fruit ratios <6 cm^2 $fruit^{-1}$ (R1), from 6.1 to 14 cm^2 $fruit^{-1}$ (R2) and >14 cm^2 $fruit^{-1}$ (R3). The branches analyzed were from Lower East, Upper East, Lower West and Upper West positions in the plant. Each point represents the mean ± SE of six replicates. In the insets, average diurnal values of the A and g_s are shown; uppercase letters indicate significant differences among the averages (Newman–Keuls, $P \leq 0.05$).

Table 3
Correlation coefficients (and their respective P values within parenthesis) of a range of variables with branch dieback (Spearman's non-parametric method) and leaf-to-fruit-ratios (Pearson's parametric method). $n = 360$ for the correlation coefficients shown in the first two lines; $n = 72$ for the other correlation coefficients.

	Branch dieback	Leaf-to-fruit ratio
Branch dieback	–	−0.416 (0.0001)
Growth rate	0.438 (0.0001)	0.678 (0.0001)
Net CO_2 assimilation rate	0.067 (0.256)	0.032 (0.748)
Carbon isotope composition	−0.378 (0.0011)	0.132 (0.270)
Starch	0.289 (0.0139)	0.410 (0.0003)
Hexoses	0.110 (0.358)	0.344 (0.0031)
Sucrose	0.024 (0.844)	0.325 (0.0053)
Amino acids	0.097 (0.415)	0.409 (0.0004)
Nitrogen	0.372 (0.0114)	0.474 (0.0001)
Chlorophylls	0.114 (0.340)	0.598 (0.0001)
Carotenoids	0.113 (0.346)	0.445 (0.0002)
Chlorophylls/carotenoids	−0.208 (0.798)	0.048 (0.685)
Electrolyte leakage	0.069 (0.564)	0.072 (0.544)

chlorophylls ($r = 0.598$), total carotenoids ($r = 0.446$) and total N ($r = 0.474$) were found overall, whereas the correlations between the LFRs and the Chl/N ratio ($r = 0.048$) and between the LFRs and electrolyte leakage ($r = 0.072$) were not significant.

All of the above correlations were found to be not significant for the data collected in 2008 (data not shown).

4. Discussion

The decrease in the growth rates of plagiotropic branches with decreasing LFR was consistent with the higher average rate of vegetative growth in the R3 group of the four evaluated canopy positions in 2006–2007 and with the higher production of buoyant fruit in the R1 group. Therefore, the overall lower demand of assimilates by the fruits in R3 may have, to some extent, been offset by the increased energy expenditure associated with the higher rate of vegetative growth. In part, this would explain the similarity in the carbohydrate concentrations and gas exchange rates among the different LFRs. However, in this study, the carbohydrate pools were analyzed during a fruit stage with a great demand for

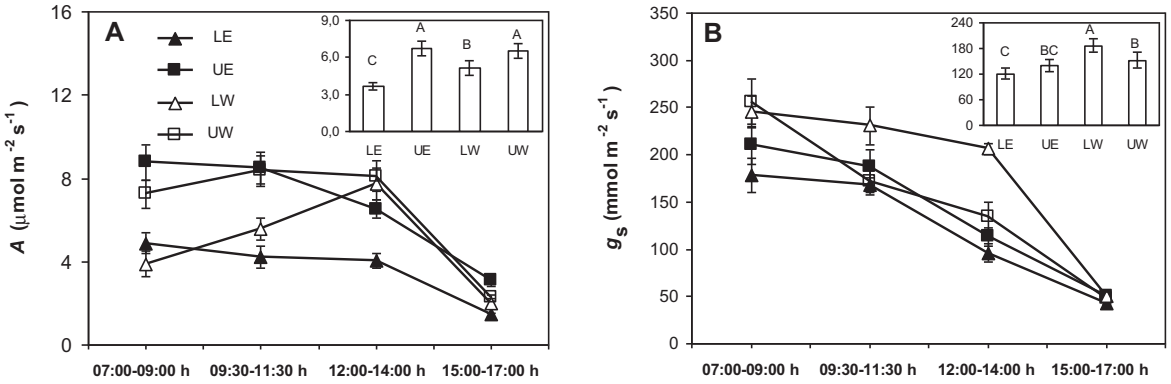

Fig. 6. Time-course study of the net carbon assimilation rate (A) and stomatal conductance (g_s) of field-grown coffee trees in March 2008. The branches analyzed were from Lower East (LE), Upper East (UE), Lower West (LW) and Upper West (UW) positions in the plant. In the insets, the mean diurnal values of the A and g_s are shown. The statistics are provided as in Fig. 5.

assimilate, a period during which the differences in the vegetative growth rates among the treatments were minimal, particularly for the 2006–2007 season. Cannell (1971) has previously reported the existence of a rather discrete movement of assimilates in coffee trees from the branches with a lower fruit load to those with a higher fruit burden during the period of high demand for assimilates by the fruit; therefore, we a priori assumed a considerable autonomy of the plagiotropic branches of a coffee tree. Our results suggest that this mobilization could be significant, which would suggest a low autonomy in coffee branches, at least during periods of high requirements for assimilates. Such a low autonomy might, to a certain extent, obstruct the potential relationships between vegetative and reproductive growth with carbohydrate and mineral pools when considering individual branches with variant LFRs. In any case, the movement of carbon from the branches with a lower fruit load to those with a higher fruit load, but not among branches with a low fruit load or during the phase of slow reproductive growth, has been reported for other trees, such as apple (Palmer, 1991) and peach (Walcroft et al., 2004; Nicolás et al., 2006; Volpe et al., 2008). Together, these results suggest that endogenous mechanisms can reduce the autonomy of branches.

Despite the suggestion of a relative lack of autonomy by the branches, it is important to emphasize that this lack of autonomy would be limited, especially because the higher percentage of buoyant fruit and higher extension of branch dieback were observed precisely in the R1 group. Further evidence that branch autonomy was only partially lost is supported by the positive Pearson correlations of the LRFs with the carbohydrate, amino acid and total N pools. Despite the negative (weak) Spearman correlations between branch dieback and starch and N, the concentrations of carbohydrates and nutrients, as well as the extent of electrolyte leakage, varied little, if at all, among the different conditions. Thus, we suggest that the increase in branch dieback with decreasing LFR was probably not closely related to the depletion of the carbohydrate and mineral reserves or to oxidative stress. It is noteworthy, however, that, in this study, both the electrolyte leakage and the carbohydrate and mineral pools were evaluated in late March, whereas branch dieback was assessed in early June, although the first symptoms of branch dieback were apparent in late March and progressively increased thereafter. Nevertheless, Carvalho et al. (1993) have also found no relationship between branch dieback, as evaluated in June, and the content of carbohydrates and minerals evaluated in February, May or June.

In some cases, a direct relationship between the concentrations of N, amino acids and photosynthetic pigments was observed, especially for the Chl with the LFR. This information, taken together with the lower N concentration in 2007 than in 2008, could suggest a greater remobilization of N with increasing sink strength in 2007. However, given that the Chl/N ratio remained virtually unchanged among the different conditions and was not correlated with the LFRs, it is likely that this relationship is a reflection of the N redistribution among the branches of the different LFR groups. The constancy of this ratio also suggests that there was no degradation of pigments, a typical symptom of oxidative stress (Krause, 1988), with the reduction of the LFR, which supports the data for the electrolyte leakage. Therefore, at least during the phase of high demand for assimilates, it is unlikely that oxidative stress was triggered, which could have led to any observed branch dieback.

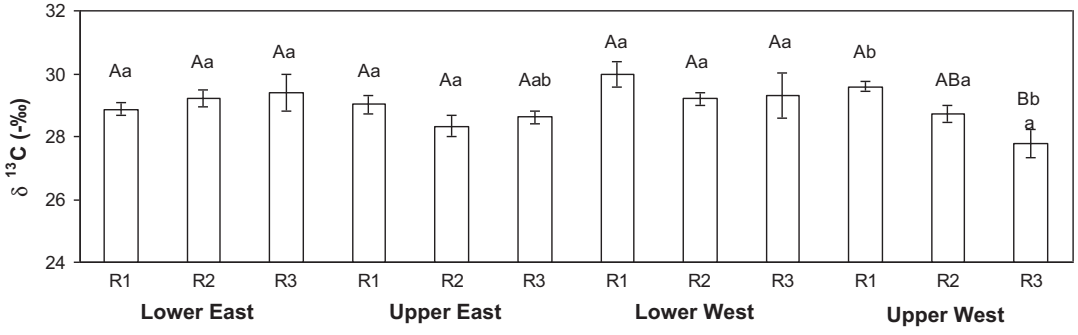

Fig. 7. The carbon isotope composition ratio ($\delta^{13}C$) of field-grown coffee trees in March 2007. The analysis included branches with leaf area-to-fruit ratios <6 cm^2 $fruit^{-1}$ (R1), from 6.1 to 14 cm^2 $fruit^{-1}$ (R2) and >14 cm^2 $fruit^{-1}$ (R3). The branches analyzed were from Lower East, Upper East, Lower West and Upper West positions in the plant. Vertical bars denote SE. Uppercase letters indicate significant differences among the averages of the three LFRs within each canopy position. Lowercase letters indicate significant differences among the means for each LFR in the four canopy positions (Newman–Keuls, $P \leq 0.05$; $n = 6$).

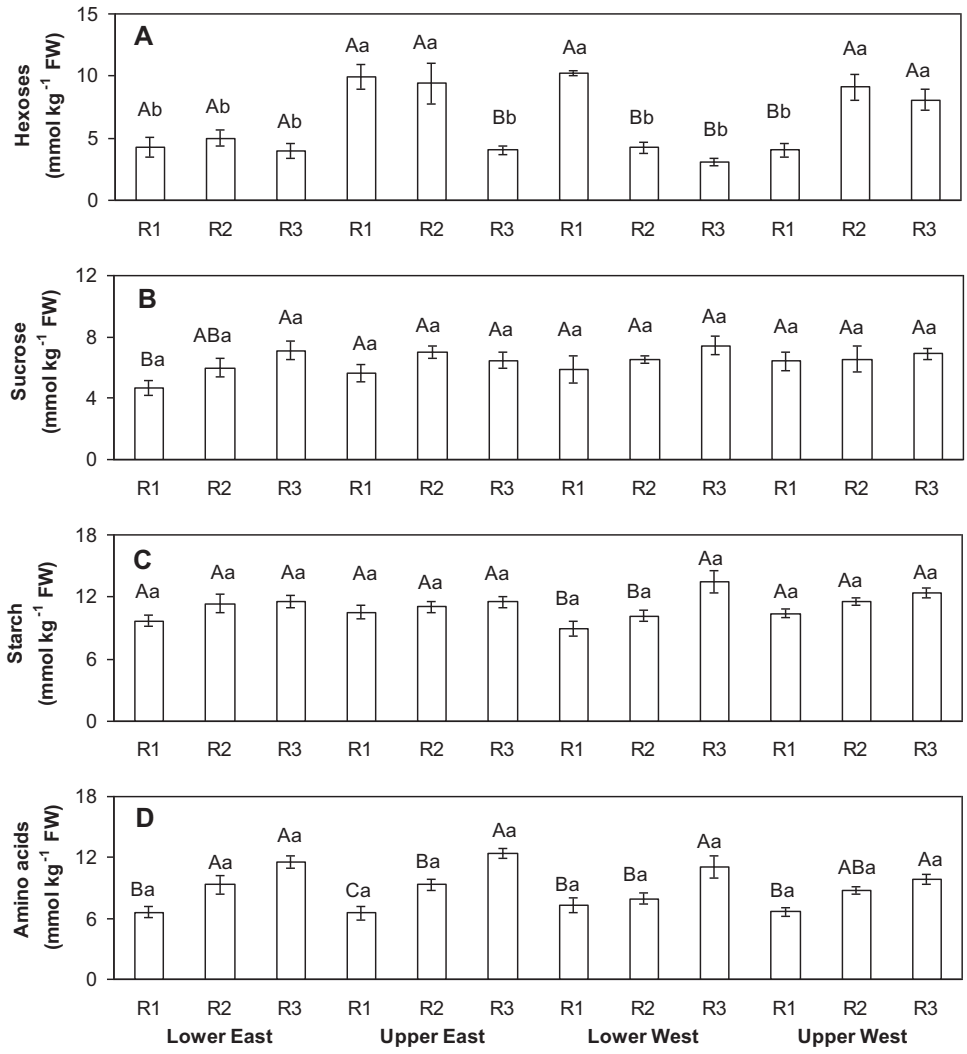

Fig. 8. Branch concentrations of hexoses (A), sucrose (B), starch (expressed as glucose equivalents) (C) and total amino acids (D) of field-grown coffee trees in March 2007. The analysis included branches with leaf area-to-fruit ratios (LFRs) <6 cm^2 $fruit^{-1}$ (R1), from 6.1 to 14 cm^2 $fruit^{-1}$ (R2) and >14 cm^2 $fruit^{-1}$ (R3). The branches analyzed were from Lower East, Upper East, Lower West and Upper West positions in the plant. The statistics are provided as in Fig. 7.

The low *A* values exhibited in 2007 were similar to those described previously in studies with field-grown coffee (Araújo et al., 2008; Chaves et al., 2008; DaMatta et al., 2008), paralleling quite low values of both g_s and C_i/C_a ratio; taken together, these data suggest strong stomatal limitations to photosynthesis. In contrast, relatively higher *A* values were found in 2008, paralleling the higher g_s and C_i/C_a values. To a large extent, the differences in the δ_e for 2007 and 2008 should explain the magnitude of the gas exchanges, in addition to illustrating the strong stomatal sensitivity to the δ_e of coffee (Barros et al., 1997; Silva et al., 2004; Ronquim et al., 2006; Franck and Vaast, 2009). The similarity reported here for the values of the *A*, g_s and C_i/C_a ratio among the different LFRs is not consistent with the data of Vaast et al. (2005), Franck et al. (2006) or DaMatta et al. (2008). However, sink-related stimulation of *A* rates have been observed in coffee trees subjected to manipulation through defoliation and/or defruiting or girdling (Vaast et al., 2005; Franck et al., 2006; DaMatta et al., 2008), and this response might be completely different in unmanaged plants growing under real field conditions. Taken together, these considerations may lend some support to the explanation of why the effects of an increase in the demand of sinks on photosynthesis are not a universal phenomenon (see Section 1). However, taking into consideration that the branch autonomy was partially lost, it could be suggested that the balance between the requirements for vegetative and reproductive growth would result in a stimulation of photosynthesis that would be difficult to quantify in an unmanaged plant. It should be kept in mind, however, that even the $\delta^{13}C$, which expresses the magnitude of gas exchange over time instead of a discrete measurement (Farquhar et al., 1989), also did not change among the different groups. Because differences in the $\delta^{13}C$ can arise due to changes in either the *A* or g_s or both (Farquhar et al., 1989), the lack of significant changes in the $\delta^{13}C$ suggest that there were no long-term compensations in terms of an increase in the *A* in response to lower LFRs.

Contrary to the proposed working hypothesis, we did not observe any biennial production pattern within each face of the coffee hedgerows. The west face, which showed increased vegetative growth and lower branch dieback relative to the east face in 2007, systematically showed lower fruit production over the three harvests (although it was not significant in 2007–2008). It is noteworthy that this pattern was followed in 2008 by similar growth rates between the west and east faces of the hedgerow, with higher concentrations of starch and amino acids in March 2008 than in March 2007 but with concentrations of soluble sugars (hexoses + sucrose) markedly lower than in 2007. Higher starch concentrations can indicate a lower requirement for assimilates

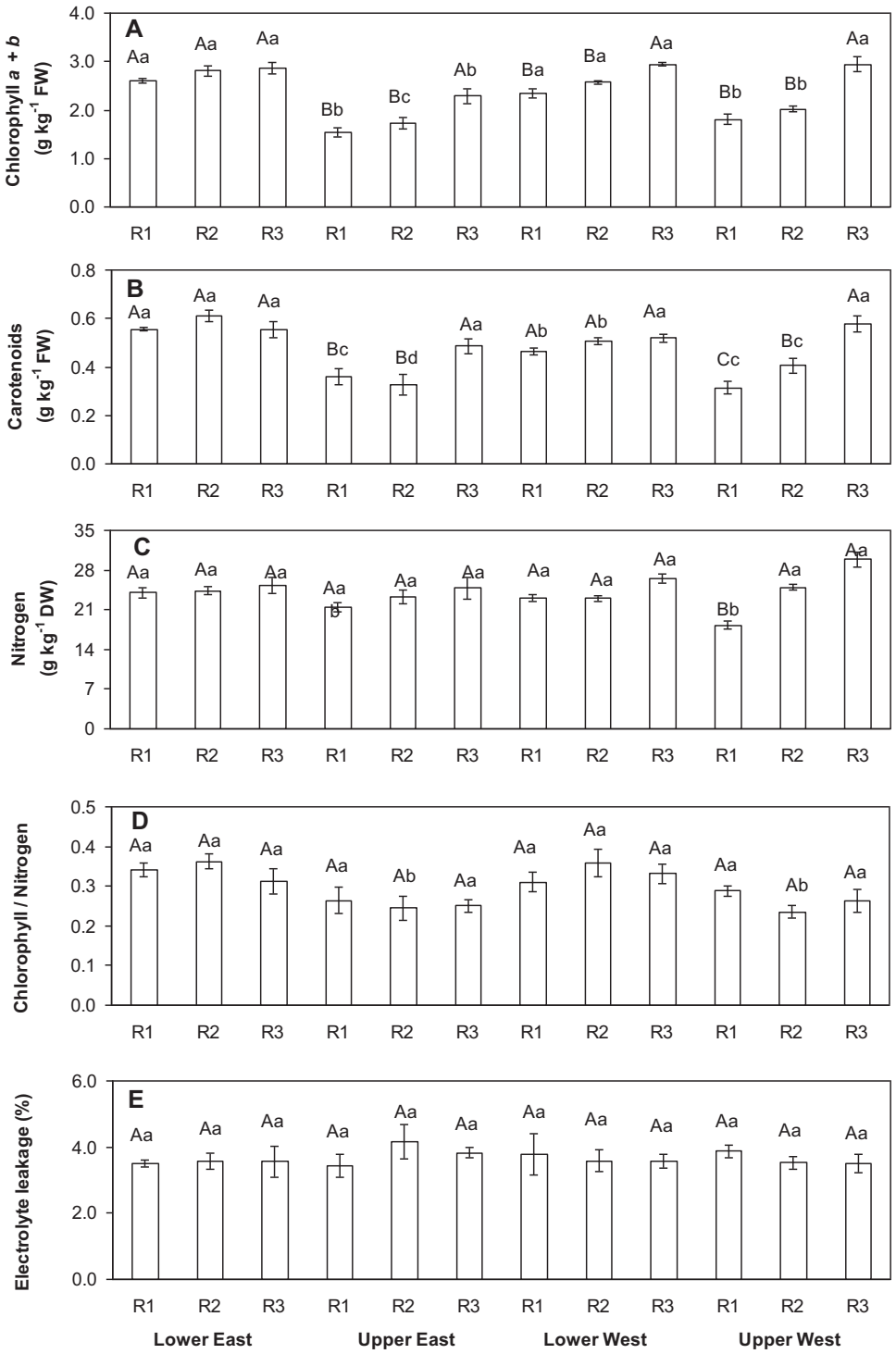

Fig. 9. Leaf concentrations of total chlorophyll (A), total carotenoids (B), total nitrogen (C), total chlorophyll-to-nitrogen ratio (D) and electrolyte leakage (E) of field-grown coffee trees in March 2007. The analysis included branches with leaf area-to-fruit ratios (LFRs) <6 cm^2 $fruit^{-1}$ (R1), from 6.1 to 14 cm^2 $fruit^{-1}$ (R2) and >14 cm^2 $fruit^{-1}$ (R3). The branches analyzed were from Lower East, Upper East, Lower West and Upper West positions in the plant. The statistics are provided as in Fig. 7.

(a lower demand from the fruits), whereas a higher concentration of amino acids could reflect the greater availability of N (Sulpice et al., 2009). Finally, the lower concentration of sugars, especially sucrose, may suggest lower rates of assimilate export (Praxedes et al., 2006). Nonetheless, when concomitantly comparing data from 2006–2007 and 2007–2008, no obvious indication of a compensation of higher vegetative growth and/or higher concentrations of carbohydrates due to the low load of fruit in 2007–2008 was found.

In summary, the higher sugar pools in 2007 than in 2008 paralleled an opposite response for starch concentrations and seemed to illustrate the varying patterns of carbohydrate movement or storage in response to a changing sink demand. Furthermore, even when comparing quite contrasting LFRs, as for the R1 and R3 conditions, the similarity in the sucrose and starch pools (but not hexoses), regardless of the branch position and LFRs, reinforces the suggestion that branch autonomy was, in fact, partially lost during the

period of a high requirement for assimilates that is associated with an increased sink strength.

5. Conclusion

The results presented here unequivocally demonstrate the existence of a strong biennial pattern in coffee production. We were unable to associate the differences in production observed between the groups with any differences in photosynthetic rates or carbohydrate and mineral availability in the coffee trees. This could be explained by a relative lack of branch autonomy, especially during the periods of high demand for assimilates by the fruit. Furthermore, no changes in the biennial production pattern between the canopy faces were observed. The higher production found in the east face could be associated with the greater availability of light on that side of the canopy. The exact mechanism by which the decreased LFRs induced branch dieback remains unknown; however, it is reasonable to hypothesize that, under our experimental conditions, this branch dieback was probably not closely related to either mineral and carbohydrate availability or oxidative stress.

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