

THE EFFECTS OF PRUNING AT DIFFERENT TIMES ON THE GROWTH, PHOTOSYNTHESIS AND YIELD OF CONILON COFFEE (*COFFEA CANEPHORA*) CLONES WITH VARYING PATTERNS OF FRUIT MATURATION IN SOUTHEASTERN BRAZIL

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SUMMARY

The economics of coffee plantations is intrinsically linked to pruning, which can improve the canopy architecture and thereby increase productivity. However, recommended pruning times on conilon coffee plantations have been made on an entirely empirical basis. In this study, by evaluating growth, photosynthetic gas exchanges, starch accumulation and crop productivity, the effects of pruning at different times between harvest and flowering were investigated for six conilon coffee clones with distinct stages of fruit maturation (early, intermediate and late). Clones with an early maturation stage were pruned at four different times: 0, 30, 60 and 90 days after harvest (DAH). Intermediate clones were pruned at 0, 30 and 60 DAH, and late clones were pruned at 0 and 30 DAH. Overall, the rates of shoot growth and net photosynthesis, the stomatal conductance and the crop yield were not affected by the pruning treatments in any of the clones. In addition, pruning times did not affect the concentrations of starch or the photochemical efficiency of photosystem II. The carbon isotope composition ratio was marginally affected by the treatments. These results suggest that the pruning time after harvests is relatively unimportant and pruning operations can be scheduled to optimise the use of labour, which directly impacts the production costs of coffee.

INTRODUCTION

After oil products, coffee is the second most commonly traded commodity worldwide. On a broader scale, when all steps from cultivation to the sale of final consumable products are considered, the international coffee trade involves approximately 500 million people (DaMatta *et al.*, 2010). Of the approximately 100 species of the genus *Coffea* (Davis *et al.*, 2006), only *C. arabica* L. (arabica coffee) and *C. canephora* Pierre ex A. Froehner (robusta coffee) are economically important worldwide; these two species are responsible for 99% of world bean production. Currently, arabica

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coffee accounts for approximately 62% of the consumed coffee, and robusta coffee accounts for the rest. Robusta is the most widely cropped cultivar of *C. canephora* in the world, and the name of this cultivar designates the common name of the species. In Brazil, kouillou (also known as conilon) is practically the sole cropped cultivar of *C. canephora* (DaMatta and Ramalho, 2006).

Coffea canephora displays a multi-stem architecture. It has multiple vertical, or orthotropic, branches from which the productive (horizontal) plagiotropic branches emerge. Until recently, conilon plants on Brazilian coffee plantations were allowed to grow freely. This leads to a high degree of self-shading and a consequent reduction in the penetration of sunlight into the canopy. Moreover, the free growth of conilon coffee accelerates the aging of plantations because the leaf area associated with photosynthesis decreases as the total dry mass of the plant increases. This is a result of an excess of orthotropic branches, which increase the demand for photoassimilates. The small leaf area cannot adequately supply this demand, so the vitality and, consequently, the productivity of the crop decline (Ronchi and DaMatta, 2007). Thus, the economical operation of coffee plantations is intrinsically linked with the efficiency of the pruning system, which can improve yields and ensure the longevity of the plantation.

The formation of new shoots after pruning produces changes in the plant, especially in the stores of carbohydrate and nitrogen. The quantity of nutrients stored and the plant's ability to govern their translocation largely determine the success with which new branches are formed (Berninger *et al.*, 2000; Ourry *et al.*, 1994). The partial removal of foliage can improve the net carbon assimilation rate (A) in the remaining foliage due to changes in the source/sink relationship (DaMatta *et al.*, 2008) and increase the amount of light that penetrates into the canopy. This can partially offset the reduction in photosynthesis on a whole plant basis associated with a decreased leaf area after pruning.

The period between harvest and flowering in Brazil (usually in mid-September) can last for more than three months, as observed in early maturing conilon coffee clones. Based on empirical observations, pruning during this period has been recommended (Ferrão *et al.*, 2004; Silveira and Rocha, 1995). Recently, Fonseca *et al.* (2007) suggested that pruning immediately after harvest could be advantageous because it would allow faster recovery from the damage and stress caused by harvesting and pruning activities. However, at the farm level, pruning is commonly performed in July and August for both early and intermediate maturing crops, which are harvested in April and May, and late maturing crops, which are harvested just before pruning. Thus, pruning occurs when the entire harvest is finished, weeks or even days before flowering begins.

No scientific studies have documented the effects of pruning at different times on the physiology and yield of coffee trees. This lack of understanding obstructs the issuance of recommendations on pruning times for clones with different fruit maturation periods and has confounded answers to the following questions. When, during the period between harvest and flowering, can the coffee tree be pruned without interfering with its growth and production? What are the effects of pruning at different times on the growth, carbon economy and production capacity of clones with different patterns of fruit maturation?

Cultivation conditions and treatments

Experiments were conducted at the experimental farm of the Institute for Research and Rural Assistance of Espírito Santo State (INCAPER) in Marilândia (19°407'S, 40°539'W, 110 m a.s.l.), northwestern region of Espírito Santo, Brazil. The soil at the location is classified as a strongly undulated, dystrophic red Latosol. The site receives an average annual rainfall of 1200 mm, with a marked dry season from March or April to September or October. The average annual temperature is 24.0 °C. Experiments were conducted on a mature conilon coffee crop planted in rows with a spacing of 2.5 × 1.0 m. Each row consisted of a single clone and was oriented from north to south. There were 16,000 productive stems (orthotropic heads) per hectare and shoots of different ages. The crop was grown without irrigation, and the recommended cultural practices (e.g. weeding, fertilising and pest control) for conilon coffee plantations were applied.

Beginning in May 2006, farm renewal was initiated using the renewal pruning methods described by Ronchi (2009). Approximately 50% of older orthotropic branches, plagiotropic branches in the lower part of the canopy that had no production potential and all existing shoots were removed; only 8000 productive orthotropic stems per hectare (two stems per plant) remained. Three thinnings were performed in October and December 2006 and February 2007, leaving, in addition to two productive stems, three buds per plant to renew the crop the following year. The early, intermediate and late clones were harvested in May, June and July 2007, respectively. In addition, after harvesting in 2007, old stems were removed at the precise pruning times specified for the treatments for each group of clones (see below). In August 2007, the crop was completely renewed, with 12,000 one-year-old stems per hectare. In subsequent years, while following the pruning and harvesting regimens for each clone, production pruning was conducted to remove all shoots and the plagiotropic branches that had reached 70% of their production capacity (Fonseca *et al.*, 2007). Therefore, 12,000 productive stems per hectare were preserved during the evaluated crop seasons (i.e. 2008, 2009 and 2010).

In total, 18 treatments arranged in incomplete blocks were tested, with four replicates of each treatment. Each experimental plot consisted of rows of coffee trees with 10 plants, and the eight central plants were used in the experiments. Six conilon coffee clones with distinct stages of fruit maturation (03 and 67, early; 16 and 120, intermediate; and 19 and 76, late maturation stages) were evaluated. The entire fruit development (from bloom to full ripening) period lasts, on average, for 34, 41 and 45 weeks for early, intermediate and late clones, respectively (Ronchi and DaMatta, 2007). Depending on the maturation times of the clones, the treatments consisted of different pruning times after harvest. The early maturation clones had less time for fruit formation and were harvested on May 15. Because this harvest time was the furthest from the time of flowering, which usually occurs in September, these clones were pruned at four different times, i.e. 0 (May 15), 30 (June 15), 60 (July 15) and 90 (August 15) DAH. For the intermediate maturation clones, the harvest was performed

on June 15 and three pruning treatments were applied: 0 (June 15), 30 (July 15) and 60 (August 15) DAH. In the late clones, the fruits required more time to reach maturity; the crop was harvested on July 15, and because the bloom occurs soon after harvest, only two prunings were performed: 0 (July 15) and 30 (August 15) DAH.

Growth and productivity

Plant growth in the upper third of the plant canopy was evaluated. From June 2008 to April 2010, the length of each branch (six primary plagiotropic branches per replicate) was measured with a tape measure. The absolute growth rate (AGR) of branches was also estimated. In January 2010, plagiotropic branches located in the middle third of the plant were collected from six plants per treatment (three branches per plant). The leaf area was determined from these branches by measuring the maximum width and length of each leaf and using the equations described by Antunes *et al.* (2008). The fruits and leaves were then dried at 70 °C and weighed. With this information, the ratios between the dry mass of leaves and the fruit dry mass (LMFR) and between the leaf area and the fruit dry mass (LAFR) were calculated.

In 2008, 2009 and 2010, fruit from the early, intermediate and late clones were harvested in May, June and July, respectively. Harvests were performed when more than 50% of fruits were ripe (red colour). The fruits were dried and weighed according to the standard procedures for coffee. The yield data, expressed in kilograms of processed coffee per hectare, refer to the average of three harvests.

Physiological evaluations

Physiological evaluations were performed at two distinct phenological times: before flowering (27–29 August 2009) and post-anthesis, in the ‘pinhead’ fruit phase (25–28 September 2009). For six plants from each treatment, three leaves from the east side and three from the west side of the orchard rows were assessed. All measurements and leaf samples were taken from the third or fourth leaf pair from the apex of the plagiotropic branches in the middle third of the plants. The stomatal conductance (g_s) and net carbon assimilation rate (A) were assessed with a portable infrared LI-6400 gas analyser (LI-COR Biosciences Inc., NE, USA) equipped with a blue/red light source, model LI-02B-6400 (LI-COR). Measurements were performed in the morning under ambient CO₂, temperature and humidity conditions with a photosynthetic photon flux density of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

The leaf carbon isotope composition ratio ($\delta^{13}\text{C}$), which provides an integrated measurement of internal plant physiological and external environmental properties influencing photosynthetic gas exchange over time (Farquhar *et al.*, 1989), was measured relative to the international Pee Dee Belemnite (PDB) standard with a mass spectrometer (Delta-S Finnigan MAT, Bremen, Germany) as described previously (DaMatta *et al.*, 2002).

Chlorophyll *a* fluorescence parameters were measured immediately after gas-exchange measurements with a portable pulse amplitude modulation fluorometer (MINI-PAM, Walz, Effeltrich, Germany). The quantum yield of photosystem II

(PSII) electron transport (Φ_{PSII}) and the apparent electron transport rate (ETR) under ambient light conditions were estimated as described previously (Chaves *et al.*, 2008; DaMatta *et al.*, 2002). In addition, the minimum (F_0) and maximum (F_m) dark-adapted (30 min) fluorescence values were measured using a dark-adaptation leaf clip. From these values, the F_v/F_m ratio, where $F_v = F_m - F_0$, was calculated. This ratio has been used as a measure of the potential photochemical efficiency of PSII.

In order to quantify the amount of starch in leaves, leaf tissues were collected around midday and frozen immediately in liquid nitrogen. The samples were lyophilized at -48°C and crushed in a cell disruptor with 3.2-mm metal beads (Mini-BeadBeater-96, BioSpec Products, Bartlesville, OK, USA). A 10-mg sample of ground tissue was added to pure methanol, and the mixture was incubated at 70°C for 30 min. After centrifugation (13,000 *g*, 5 min), the supernatant was discarded, and the starch in the pellet was quantified according to Praxedes *et al.* (2006).

Statistical analysis

The experimental design included incomplete blocks in a split plot design. In the primary plot, six clones (03, 16, 19, 67, 76 and 120) with different times of fruit maturation (two clones per fruit maturation time) were distributed, and within each clone group, different pruning times (0, 30, 60 and 90 DAH, depending on the clone) were applied to form subplots. The data were subjected to an analysis of variance, and the averages were compared using the Newman–Keuls test at a 5% probability with the program Statistical and Genetic Analysis, UFV 5.0 (Sistemas de Análises Estatísticas e Genéticas (SAEG), 1993). The results are expressed as averages \pm standard error (SE).

RESULTS

There was no significant difference in the AGR among treatments during the experiment, except for clone 120. For this particular clone, the plants pruned immediately after harvest had the AGR (1.24 mm d^{-1}) that was approximately 25% and 20% higher than the plants pruned 30 and 60 DAH, respectively (Figure 1). No significant difference was observed in the LMFR and LAFR values among treatments (Figure 1). Yields, which ranged from 2082 kg ha^{-1} (clone 19) to 3984 kg ha^{-1} (clone 76), were not significantly affected by the pruning treatments (Figure 1).

In August 2009, the average values of g_s differed only in clone 76 and were 54% higher in plants pruned immediately after harvest compared with those pruned 30 DAH (Figure 2). In September, the pruning time did not affect g_s (Figure 3) in any of the clones. The A value was similar for all pruning times and clones in August and September (Figures 2 and 3). In August, significant variations in $\delta^{13}\text{C}$ were observed in clones 67 and 120; however, they were inconsistent among treatments (Figure 2). In September, $\delta^{13}\text{C}$ was not affected by the pruning treatment (Figure 3).

In August, the maximum photochemical efficiency of PSII (F_v/F_m) was similar in all treatments. For each clone, ETR did not vary consistently in response to pruning times, with the exception that ETR was generally lower in clones pruned at 0 DAH

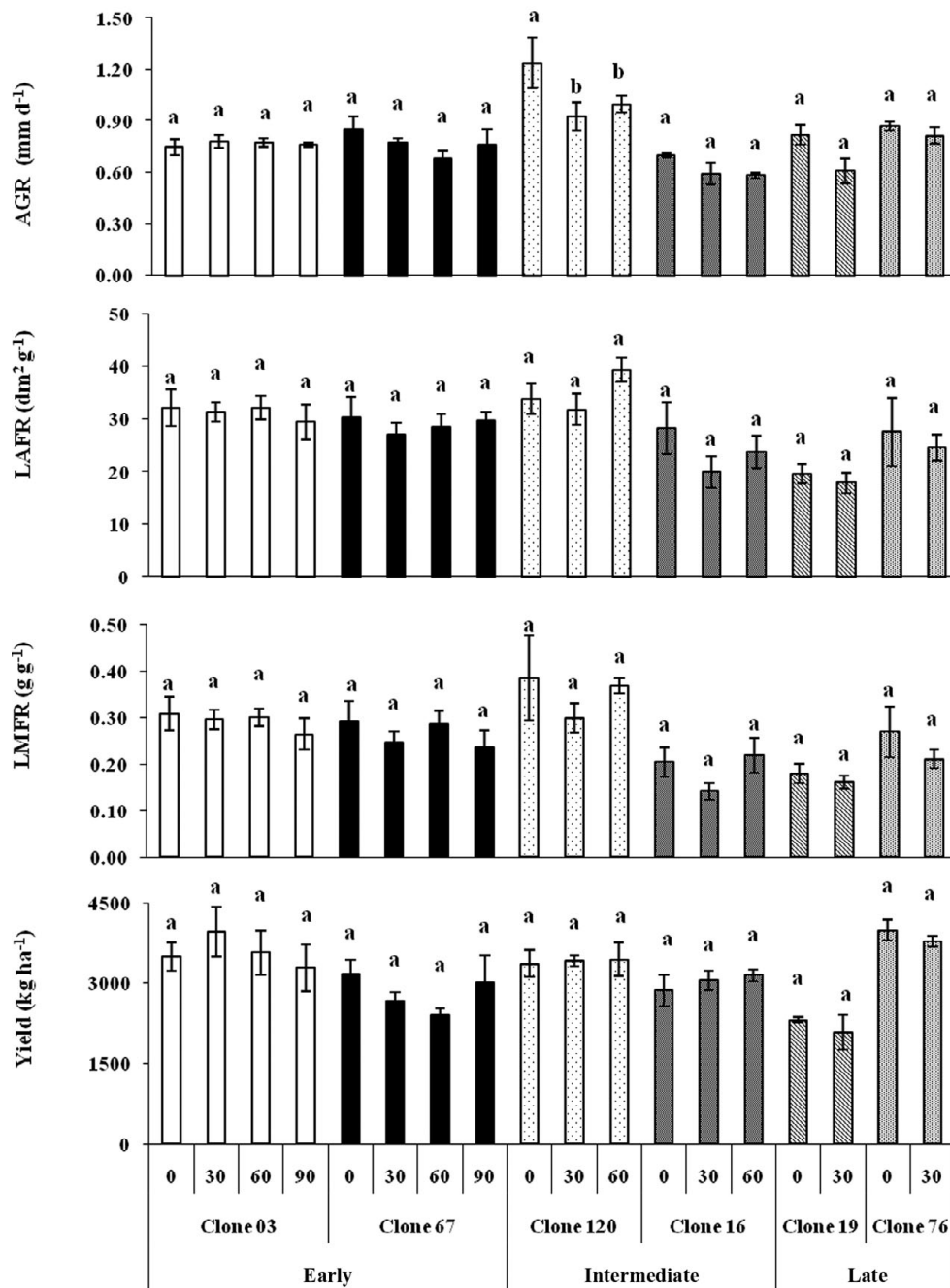


Figure 1. Effect of different pruning times (0, 30, 60 and 90 days after harvest (DAH)) in conilon coffee clones (03, 67, 120, 16, 19 and 76) on the absolute growth rate (AGR) of branches, the leaf area to fruit mass ratio (LAFR), the leaf mass to fruit mass ratio (LMFR) and crop yield. Values followed by the same letter do not significantly differ within each clone (the Newman-Keuls test, $P > 0.05$).

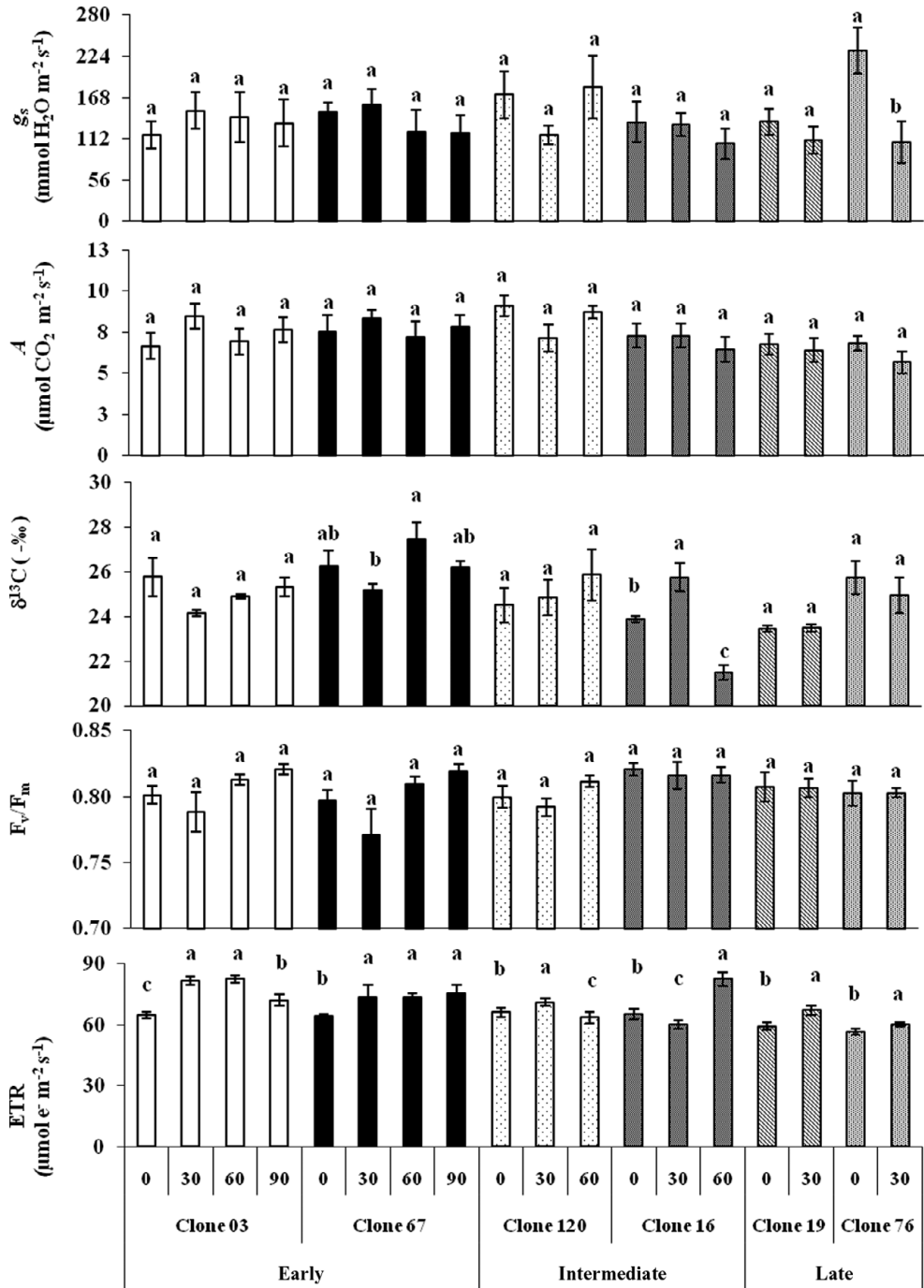


Figure 2. Effect of different pruning times (0, 30, 60 and 90 DAH) in conilon coffee clones (03, 67, 120, 16, 19 and 76) on the stomatal conductance (g_s), net carbon assimilation rate (A), carbon isotope composition ratio ($\delta^{13}C$), maximum photochemical efficiency of PSII (F_v/F_m) and electron transport rate (ETR) in August 2009. Values followed by the same letter do not significantly differ within each clone (the Newman-Keuls test, $P > 0.05$).

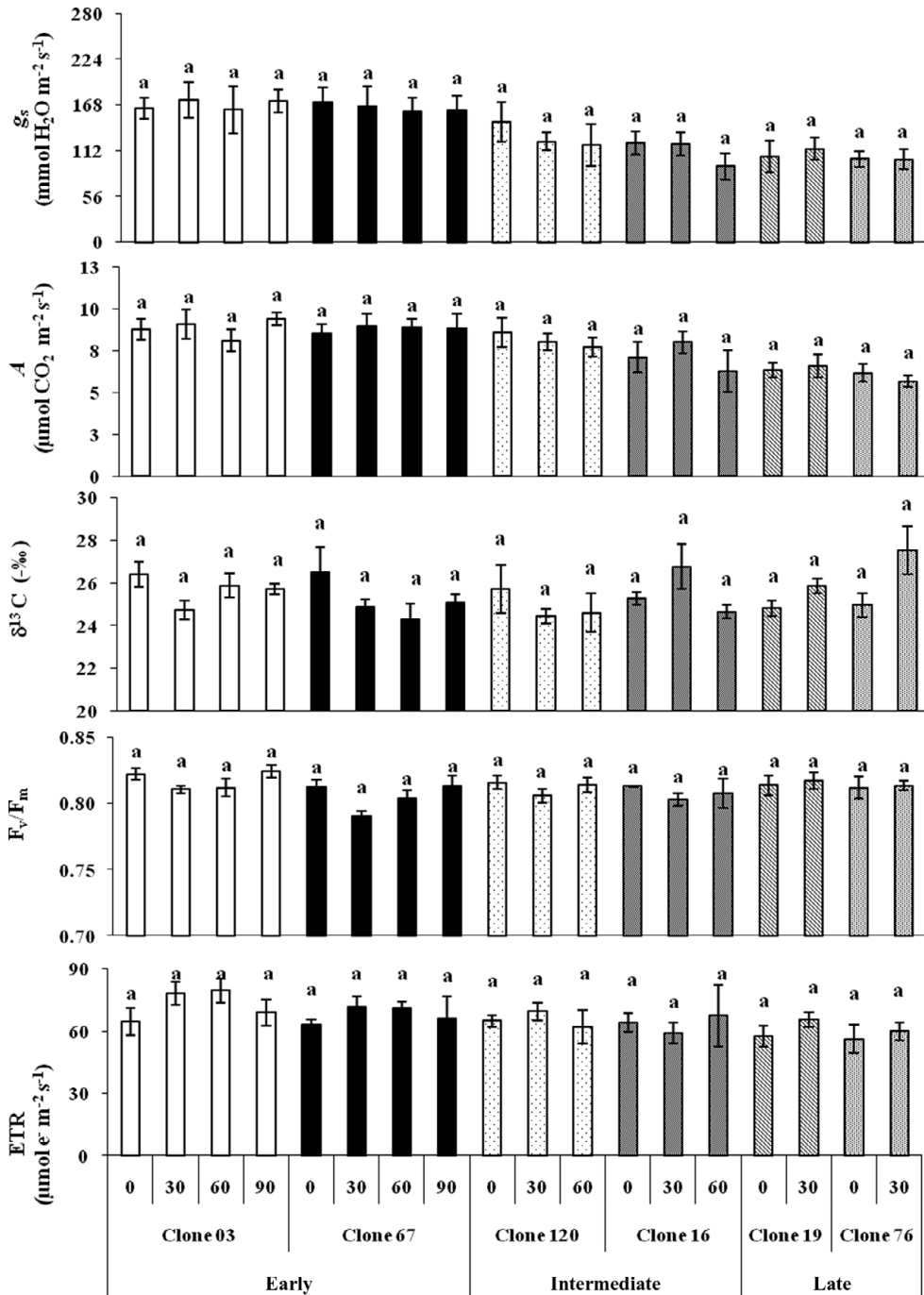


Figure 3. Effect of different pruning times (0, 30, 60 and 90 DAH) in conilon coffee clones (03, 67, 120, 16, 19 and 76) on the stomatal conductance (g_s), net carbon assimilation rate (A), carbon isotope composition ratio ($\delta^{13}C$), maximum photochemical efficiency of PSII (F_v/F_m) and electron transport rate (ETR) in September 2009. Values followed by the same letter do not significantly differ within each clone (the Newman–Keuls test, $P > 0.05$).

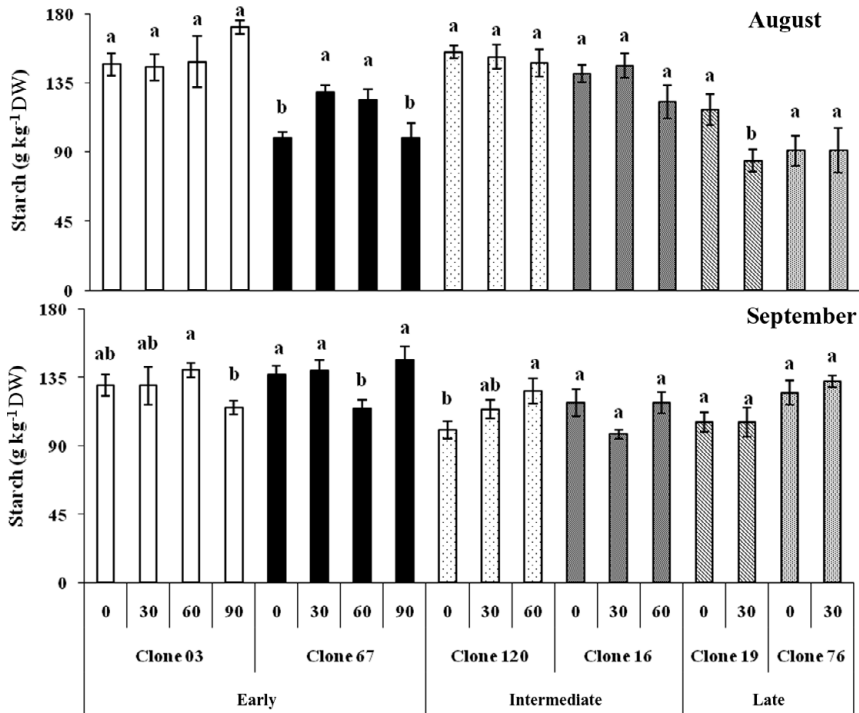


Figure 4. Effect of different pruning times (0, 30, 60 and 90 DAH) in conilon coffee clones (03, 67, 120, 16, 19 and 76) on the foliar starch concentration in August and September 2009. Values followed by the same letter do not significantly differ within each clone (the Newman–Keuls test, $P > 0.05$).

compared with later prunings (Figure 2). In September, no significant variations in the fluorescence parameters were observed, irrespective of the pruning time, within each clone group (Figure 3).

In August, in clone 67, lower starch concentrations were recorded in the leaves of plants pruned at 0 and 90 DAH, while in clone 19, the starch concentration was significantly lower (28%) in plants pruned at 30 DAH compared with plants pruned immediately after harvest. In other clones, no significant changes in the concentrations of starch as a function of pruning time were observed (Figure 4). The highest absolute concentrations of starch were observed in clone 03 (171 g starch kg^{-1} dry weight (DW)). In September, there were no consistent changes in starch concentrations in response to different pruning times (Figure 4). The early clones had higher concentrations, reaching 146 g starch kg^{-1} DW in clone 67 at 90 DAH. The lowest absolute concentration of starch (98 g starch kg^{-1} DW) was observed in clone 16 at 30 DAH.

DISCUSSION

Overall, the growth rates of the plagiotropic branches were not affected by the pruning treatments. Although the number of nodes was not quantified, the average

length between nodes was not significantly affected by the pruning treatments (data not shown). This suggests that the number of nodes was also not affected, and therefore, the number of potentially productive nodes should not be affected during the following growing season. Indeed, the yield of the clones was not affected by the pruning treatments. Therefore, from a scientific point of view, pruning can be performed immediately after harvest, as proposed by Fonseca *et al.* (2007), or later, before flowering, without affecting yields. These results suggest that pruning operations between the harvest and subsequent flowering can be staggered without adversely affecting the physiology of the coffee tree or the plant production. This would help to optimise the use of labour and, thus, result in lower production costs.

The pruning treatments did not affect the magnitude of gas exchange (g_s and A). Moreover, $\delta^{13}\text{C}$, which expresses the magnitude of gas exchange over time instead of a discrete measurement (Farquhar *et al.*, 1989), showed little to no response to the treatments. These results suggest that there was no long-term variation in the photosynthetic rate or the efficiency of water use within each clone in response to pruning at different times. The photosynthetic rates were similar to average values ($8.3 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) observed in *C. canephora* plants under appropriate cultivation conditions, as reported by DaMatta *et al.* (2010). In some cases, the ETR exceeded $80 \mu\text{mol m}^{-2} \text{ s}^{-1}$, a value that is higher than the photochemical needs required to support the observed rates of photosynthesis. A high ETR with a relatively low A usually leads to excess reducing power, which can be used for the production of reactive oxygen species that can trigger a range of photoinhibitory and photooxidative effects (Lima *et al.*, 2002). However, the maximum photochemical efficiency of PSII, estimated by the F_v/F_m ratio, did not vary in the treatments, indicating that the plants did not suffer photoinhibitory damage.

Starch concentrations were little affected in the treatments probably because the sink demand was low when leaf samplings were performed (Cannell, 1970; Wormer and Ebagole 1965). Although only leaf concentrations were evaluated, the starch concentration in the leaves was considered to vary in parallel with that in the branches, a phenomenon that has been documented in arabica coffee (e.g., Chaves, 2009; Patel, 1970). The starch content was estimated at the end of the slow growth phase (August) and at the beginning of the growth recovery phase (September). In all cases, the observed starch content was well above the maximum content observed in arabica coffee (approximately $100 \text{ g starch kg}^{-1} \text{ DW}$; Amaral, 1991) during the slow growth phase. The highest starch concentrations should reflect the maintenance of photosynthesis at relatively high rates, even during slow vegetative growth, as shown by DaMatta *et al.* (2003) and also confirmed in this study. For example, the maximum values of A reported by Silva *et al.* (2004) under field conditions were approximately $3 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ during the slow growth phase of arabica coffee in Viçosa, southeastern Brazil. Therefore, a steady A value in the slow growth phase should allow for greater accumulation of starch in conilon coffee. This could partially explain the greater potential yield of this species compared with arabica coffee (DaMatta *et al.*, 2010).

CONCLUSION

Pruning at different times after harvest, irrespective of the fruit maturation time, has little or no influence on the rate of branch growth, photosynthesis, starch content or yield of conilon coffee. The data suggest that, even in the early maturation clones, there is neither an advantage nor a disadvantage to pruning immediately after harvest or later, before flowering. Therefore, because crop productivity is not compromised, pruning operations between harvest and flowering can be scaled up to optimise the use of labour, which directly impacts the production costs of conilon coffee.

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