

## GRAFTING FOR IMPROVING NET PHOTOSYNTHESIS OF *COFFEA ARABICA* IN FIELD IN SOUTHEAST OF BRAZIL

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### SUMMARY

Leaf gas exchange and leaf water potential ( $\Psi_{\text{leaf}}$ ) were measured seasonally on non-grafted and grafted *Coffea arabica* on *Coffea canephora* in the field to investigate whether grafting would be able to protect the carbon balance against the rise of in vapour pressure deficit (VPD) and air temperature ( $T_{\text{air}}$ ) under future climate change. The net maximum photosynthetic rate obtained from the net photosynthesis ( $P_N$ ) curve as a function of photosynthetic photon flux density (PPFD) in wet and dry periods was used to estimate the integrated potential diurnal net  $\text{CO}_2$  assimilation (IPP<sub>N</sub>) around midday. The difference between IPP<sub>N</sub> and the integrated values of  $P_N$  during diurnal courses (IP<sub>N</sub>) was measured to test grafting as suitable practice for minimizing midday depression of  $P_N$ . Higher values of  $P_N$  in grafted plants around midday showed that grafting was important even when environmental conditions were favourable in field conditions. Reduced susceptibility of grafted plants to midday depression was revealed by lower values of  $\Psi_{\text{leaf}}$  associated with higher values of  $P_N$  and leaf transpiration (E) on sunny days in summer and spring, and by higher values of stomatal conductance ( $g_s$ ) around midday in autumn, winter and spring. The differences of E,  $g_s$ ,  $P_N$  and  $\Psi_{\text{leaf}}$  between non-grafted and grafted plants were higher in dry periods in winter and spring. In addition, the ratio IP<sub>N</sub>/IPP<sub>N</sub> in grafted was double that in non-grafted plants around midday in sunny summer and in spring. Indeed,  $P_N$  and  $g_s$  of non-grafted plants showed higher dependence on VPD than grafted ones. The lower susceptibility of grafted plants to water stress demonstrated the graft efficiency for increasing positive components of leaf carbon balance of *C. arabica* in the field, especially under high VPD in projected future climate conditions.

### INTRODUCTION

*Coffea arabica* came originally from Ethiopia, where it grows naturally in shade (Marin *et al.*, 2005). In Brazil, *C. arabica* is cultivated mainly in open areas to increase grain production (DaMatta, 2004), but full sun irradiance in open field areas has been related to high leaf temperature, stomatal pore narrowing and decrease in net photosynthesis (Barros *et al.*, 1997; Camargo-Bortolin *et al.*, 2008; Ronquim *et al.*, 2006). On the other hand, sun leaves of *C. arabica* have shown high tolerance to excessive irradiance (Chaves *et al.*, 2007) and the ability to prevent photoinhibition of photosynthesis (Araujo *et al.*, 2008). Low water availability in the rhizosphere during seasonal drought could aggravate the drop of net assimilation during the diurnal course by extending

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the midday depression of leaf gas exchange in *C. arabica*. Grafting is a simple and low cost practice that could improve development of coffee plants in the field. Fahl *et al.* (2001) pointed out that using *C. canephora* as rootstock to graft *C. arabica* could alleviate water stress because of the greater ability of *C. canephora* to acquire water and nutrients. *Coffea canephora* progeny were less susceptible than *C. arabica* to attack by soil nematoids (Silvarolla *et al.*, 1998) and to the fungus *Hemileia vastatrix* (Fazuoli *et al.*, 1983). Indeed, grafted plants of *C. arabica* under field conditions were taller, had more numerous plagiotropic branches and higher grain production than non-grafted plants (Fahl *et al.*, 2001). Therefore, grafting could be an appropriate practice to maintain the carbon balance of *C. arabica* as positive as possible during drought.

The maximum net photosynthetic rate of *Coffea* usually occurs in early morning in the wet season (Fahl *et al.*, 2001). This maximum CO<sub>2</sub> net assimilation could be used to estimate the potential diurnal net photosynthesis unaffected by midday depression. The difference between potential and actual diurnal net photosynthesis is an estimate of CO<sub>2</sub> that was not assimilated as a result of environmental and plant impediments to the photosynthetic process (Kikuzawa *et al.*, 2004; Ronquim *et al.*, 2006). Therefore, the difference between actual and potential diurnal net photosynthesis strongly depends on the intensity and the extension of midday depression (Camargo-Bortolin *et al.*, 2008).

In the present study, the responses of leaf gas exchange and leaf water potential ( $\Psi_{\text{leaf}}$ ) of grafted and non-grafted *C. arabica* were compared seasonally in plants under field conditions. We expected that grafted plants would be able to maintain greater intensity of leaf-to-atmosphere gas exchange than non-grafted plants, especially under water stress at midday due to the ability of *C. canephora* to acquire water. Grafted individuals could tolerate lower values of  $\Psi_{\text{leaf}}$  under water stress because of their capacity to restore leaf water status more rapidly than non-grafted plants. The difference between actual and potential net carbon assimilation around midday was used to check if grafting is suitable to minimize the effects of high seasonal drought under future climate change when evaporative demand and air temperature will increase simultaneously.

#### MATERIAL AND METHODS

##### *Study area, plant material, meteorological determinations and periods of study*

The experiment was carried out in southeast Brazil (22°02'15''S; 47°46'57''W). The natural vegetation of the area is a mosaic of Cerrado vegetation (Brazilian neotropical savanna) and semi-deciduous forests. The area has a podzol soil with slightly undulating topography at 845 m asl. The climate is Cwa according to Köppen's classification with dry winters (April to September) and wet summers (October to March). Historical annual average  $\pm$  *s.d.* values of rainfall, relative humidity, temperature and vapour pressure deficit (VPD) are 1506  $\pm$  26 mm, 71  $\pm$  5%, 21.0  $\pm$  0.5 and 0.72  $\pm$  0.13 kPa respectively (Damascos *et al.*, 2005).

The non-grafted genotype Obatã IAC 1669–20 (*C. arabica*) and the same genotype grafted on Apoatã IAC 2258 (*C. canephora*) were used. The plantations of non-grafted and grafted individuals were established in 1997 by the Brazilian Agronomic Institute

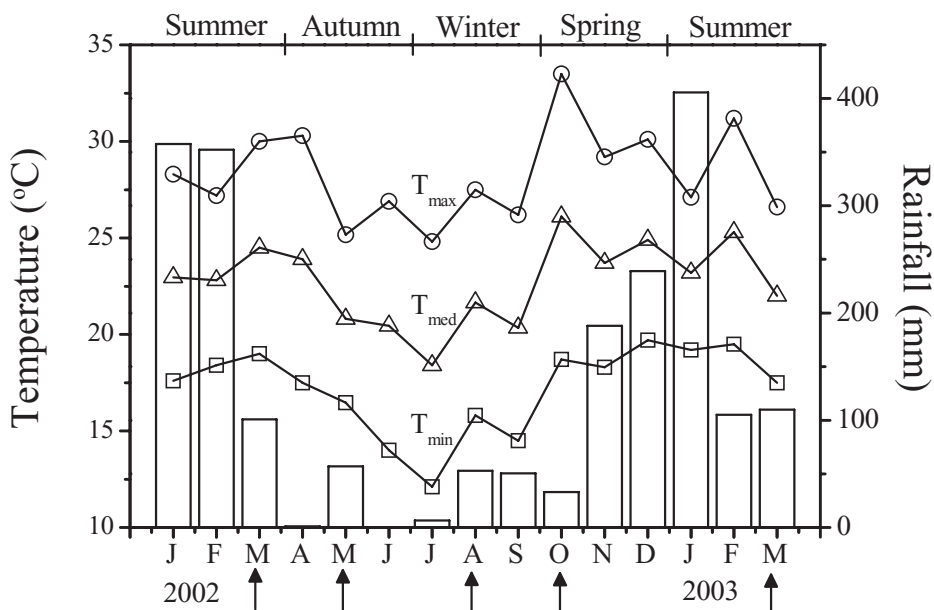


Figure 1. Monthly average values of maximum ( $T_{\max}$ ), medium ( $T_{\text{med}}$ ) and minimum ( $T_{\min}$ ) air temperature (symbols), and monthly total rainfall (columns) from January 2002 to March 2003. Sunny diurnal courses of leaf gas exchange and leaf water potential were measured in summer (March), autumn (May), winter (August) and spring (October). The cloudy diurnal course was measured in summer 2003 (March). Arrows indicate the diurnal course dates.

(IAC). The experimental design of the *Coffea* plots was completely randomized. An experimental *Coffea* plot consisted of six rows of plants, with 1.0 m between plants and 3.0 m between rows. There were three sets of 10 individuals of non-grafted Obatã intercalated with three sets of 10 individuals of grafted Obatã in each line. Phytosanitary and nutritional care was the same for non-grafted and grafted plants, following the commercial practices determined by the IAC for *Coffea* plantations in the Cerrado area.

The non-grafted and grafted Obatã individuals were four years old when the diurnal course of leaf gas exchange and  $\Psi_{\text{leaf}}$  was measured. The experiment was carried out from March 2002 to March 2003. Monthly meteorological data such as rainfall, maximum, medium and minimum air temperature, and the total diurnal hours of sunshine during the experimental period were obtained from national meteorological station number 83726, 12 km from the experimental area. High rainfall occurred in summer (January to March) when the sunny (2002) and cloudy (2003) diurnal course of leaf gas exchange and  $\Psi_{\text{leaf}}$  was measured (Figure 1). Coffee plants were growing under minimum soil water stress in summer because monthly rainfall was between 100 and 300 mm. Measurements were made in autumn and winter when the monthly rainfall was 50 mm, and in spring, at the peak of dry season, when monthly rainfall was below 33 mm.

The diurnal course in summer was measured on sunny (8 March 2002) and cloudy (18 March 2003) days. The autumn sunny diurnal course was measured on 3 May

2002, at the transition from rainy to dry period. During dry periods, the sunny diurnal course was measured in winter on 27 August 2002 and in spring on 18 October 2002. The predawn ( $\Psi_{pd}$ ) leaf water potential was determined at 05:00 hours in the wet period (8 March 2002) and at the peak of the dry period (18 October 2002).

#### *Micrometeorological, leaf gas exchange, and water potential determinations*

A portable infra red gas analyser (IRGA) model LCA-4 (ADC, Hoddesdon, UK) connected to a Parkinson narrow leaf chamber PLCN-4 (ADC) was used to obtain leaf-to-atmosphere gas exchange. Microclimate data during diurnal courses such as air temperature and relative humidity at the site of the experiment were obtained using the PLCN-4 opened in shade and free of leaves. The PLCN-4 had a quanta sensor on the top to determine the photosynthetic photon flux density (PPFD). Ambient air temperature during the diurnal courses was monitored with a regular thermometer placed in shade. Leaf temperature was determined by a copper-constantan thermocouple connected to PLCN-4. The leaf chamber temperature was maintained equal to the air temperature during the day by a Peltier system (ADC) attached to PLCN-4.

The gas exchange parameters obtained during the diurnal courses were net photosynthesis ( $P_N$ ), transpiration ( $E$ ) and stomatal conductance of water vapour ( $g_s$ ). The LCA-4 worked as an open system during gas exchange measurements comparing  $CO_2$  and  $H_2O$  molar fractions before and after passing through PLCN-4. The instantaneous water use efficiency (WUE) was determined as  $P_N/E$  (Nogueira *et al.*, 2004). The values of  $\Psi_{leaf}$  and  $\Psi_{pd}$  during diurnal courses were obtained by pressure chamber model 3005 (Santa Barbara Soil Moisture, Santa Barbara, USA).  $\Psi_{leaf}$  was measured immediately after leaf gas exchange determinations during the day. The values of VPD during diurnal courses were calculated using the equation described by Jones (1992).

The leaf gas exchange,  $\Psi_{leaf}$ , PPFD and VPD measurements were carried out at two-hourly intervals from 06:00 to 18:00 hours. Two non-grafted and two grafted individuals growing in different lines under field conditions were used in every determination of leaf gas exchange and  $\Psi_{leaf}$ . On each plant two branches totally exposed to solar irradiance were chosen and two completely expanded and healthy illuminated leaves were selected. These leaves were usually the third leaf pair from the apex on a plagiotropic branch in the upper third of plant canopy.

#### *Net photosynthesis as a function of PPFD*

The  $P_N$ -PPFD curves were obtained using IRGA LCA-4 and PLCN-4 described previously. The net maximum photosynthetic rates ( $P_{Nmax}$ ) obtained from the  $P_N$ -PPFD curves for wet and dry periods were used to estimate the integrated potential diurnal net  $CO_2$  assimilation ( $IPP_N$ ) around midday as described below. The PLCN-4 was connected to a PLU-002 light source with a halogen 12 V 20 W dichroic lamp (ADC, Hoddesdon, UK). The leaf temperature ( $T_{leaf}$ ) was maintained with a Peltier system at  $25 \pm 0.5^\circ C$  during  $P_N$  measurements. The PPFD intensity was

attenuated by reducing the voltage on PLU-002 and by fitting neutral glass filters (Comar Instruments, Cambridge, UK) in the PLCN-4 chamber between leaf and light source. Measurements from two grafted and two non-grafted plants were used to produce the  $P_N$ -PPFD curves. For each plant two completely expanded and healthy sun-leaves were chosen on plagiotropic branches in the upper third of the plant canopy. The  $P_N$ -PPFD curves were carried out at a favourable time for plant  $CO_2$  assimilation (07:00–09:00 hours) in the wet period on 1 and 26 March 2002, and in the dry period on 8 August 2002. Two  $P_N$ -PPFD curves were obtained for each treatment (non-grafted and grafted plants) in wet and dry periods. The two  $P_N$ -PPFD curves for each treatment were merged. The values of merged  $P_N$ -PPFD curves were adjusted using the equation described by Prado and Moraes (1997):

$$P_N = P_{N_{\max}}(1 - \exp^{-k(\text{PPFD} - L_c)}) \quad (1)$$

where  $P_N$  = net photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ );  $P_{N_{\max}}$  = net maximum photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ );  $e$  = natural logarithmic base;  $k$  = constant of proportionality; PPFD = photosynthetic photon flux density ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ); and  $L_c$  = light compensation point ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ).

The PPFD value when  $P_N$  achieved 90% of  $P_{N_{\max}}$  (equation 1) was termed the light saturation point ( $L_s$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) as described by Prado and Moraes (1997).

#### *Midday depression of diurnal photosynthesis*

The integrated values of  $P_N$  during diurnal courses ( $IP_N$ ) were calculated on sunny days in summer and in spring, because the average values of  $P_N$  around midday (10:00 to 16:00 hours) were significantly different ( $p < 0.05$ ) between grafted and non-grafted treatments.  $IP_N$  was determined from 10:00 to 16:00 hours on sunny days of summer and spring using equation 2 (Kikuzawa *et al.*, 2004; Prado *et al.*, 2001; Ronquim *et al.*, 2006):

$$I(y) = \int f(x) dx \quad (2)$$

where  $x$  = independent variable, time interval in seconds from 10:00 to 16:00 hours during diurnal courses;  $I(y)$  = integrated  $y$  value; and  $f(x)$  = the dependent variable ( $P_N$ ).

$IP_N$  expresses the net  $CO_2$  assimilation in leaves limited by ambient and internal plant impediments such as high VPD and  $\Psi_{\text{leaf}}$ , respectively (Prado *et al.*, 2001). Integrated potential  $CO_2$  assimilation ( $IPP_N$ ) represents the diurnal assimilation in leaves limited occasionally only by PPFD (Kikuzawa *et al.*, 2004).  $IPP_N$  around midday (10:00 to 16:00 hours) was calculated in two steps. First, the  $P_{N_{\max}}$  obtained from the  $P_N$ -PPFD curve and the values of PPFD determined during the diurnal courses were applied in equation 1 to determine the expected net  $CO_2$  assimilation at every measurement between 10:00 and 16:00 hours. These predictable  $P_N$  values around midday were subsequently integrated using equation 2 resulting in  $IPP_N$ . From the contrast between  $IP_N$  and  $IPP_N$  it was possible to estimate how much non-grafted and

grafted plants reduced the net CO<sub>2</sub> assimilation as a function of environmental and internal impediments around midday (10:00 to 16:00 hours).

### *Statistical analysis*

Differences in average values of PPFD, E, g<sub>s</sub>, Ψ<sub>pd</sub>, Ψ<sub>leaf</sub>, WUE and P<sub>N</sub> at each time of the diurnal courses, and P<sub>Nmax</sub>, and Ls from the P<sub>N</sub>-PPFD curves between grafted and non-grafted treatments were tested considering significant those differences with decision level (α) at *p* < 0.05. Significant differences were tested by Student's *t*-test when the data showed normal distributions or by non-parametric analysis (Mann-Whitney) when they showed abnormal distributions.

## RESULTS

Values of P<sub>Nmax</sub> and Ls were higher in wet than dry periods in both treatments (Figure 2). The values of P<sub>Nmax</sub> in non-grafted and grafted treatments were, respectively, 9.8 and 8.4 μmol m<sup>-2</sup> s<sup>-1</sup> in the wet period and 7.2 and 5.2 μmol m<sup>-2</sup> s<sup>-1</sup> in the dry period. These values were significantly higher in non-grafted than grafted plants in both periods. The values of Ls in non-grafted and grafted treatments were, respectively, 832 and 789 μmol m<sup>-2</sup> s<sup>-1</sup> in the wet period, and 591 and 394 μmol m<sup>-2</sup> s<sup>-1</sup> in the dry period (Figure 2). Ls was significantly higher in non-grafted than in grafted plants only in the dry period. Some values of PPFD were not similar (*p* < 0.05) between non-grafted and grafted plants during the diurnal courses (Figure 3A–E), although they were higher than Ls (Figure 2) in the majority of daytimes in both treatments. The most favourable condition for leaf gas exchange occurred on summer cloudy days (wet period) because PPFD was not excessive (500–1000 μmol m<sup>-2</sup> s<sup>-1</sup>, Figure 3A), VPD was lower than 3 kPa around midday (Figure 3F), and there was more water available in soil from rainfall (Figure 1). Despite low PPFD on cloudy days, irradiance intensity was enough to maintain g<sub>s</sub> and P<sub>N</sub> at high rates (Figures 4F and 5F) but under leaf transpiration lower than 2.1 mmol m<sup>-2</sup> s<sup>-1</sup> (Figure 4A) and Ψ<sub>leaf</sub> higher than –1.0 MPa (Figure 5A) during the cloudy diurnal course in both treatments. Grafted plants showed significantly higher values of P<sub>N</sub> than non-grafted ones around midday on cloudy days (Figure 5F). Despite the greater values of P<sub>N</sub> around midday, grafted plants showed significant lower values of WUE in almost the entire cloudy diurnal course (Figure 6A).

Excessive values of PPFD around midday occurred in sunny diurnal courses (around 1500 μmol m<sup>-2</sup> s<sup>-1</sup>, Figure 3B–E). Besides, VPD values were higher than 3 kPa around midday in sunny days of summer and spring (Figure 3G and 3J). Under these stressful environmental conditions grafted plants showed significantly (*p* < 0.05) higher values of g<sub>s</sub> around midday in autumn, winter and spring (Figure 4H–J), higher values of E around midday in sunny daily courses in all seasons (Figure 4B–E), and greater values of P<sub>N</sub> around midday during summer, winter and spring (Figure 5). When VPD was greater than 3 kPa around midday in summer and spring (Figure 3J and 3J, respectively), there were lower values of Ψ<sub>leaf</sub> (Figure 5B and 5E) in grafted than non-grafted plants. Grafted plants showed significantly lower values of WUE around

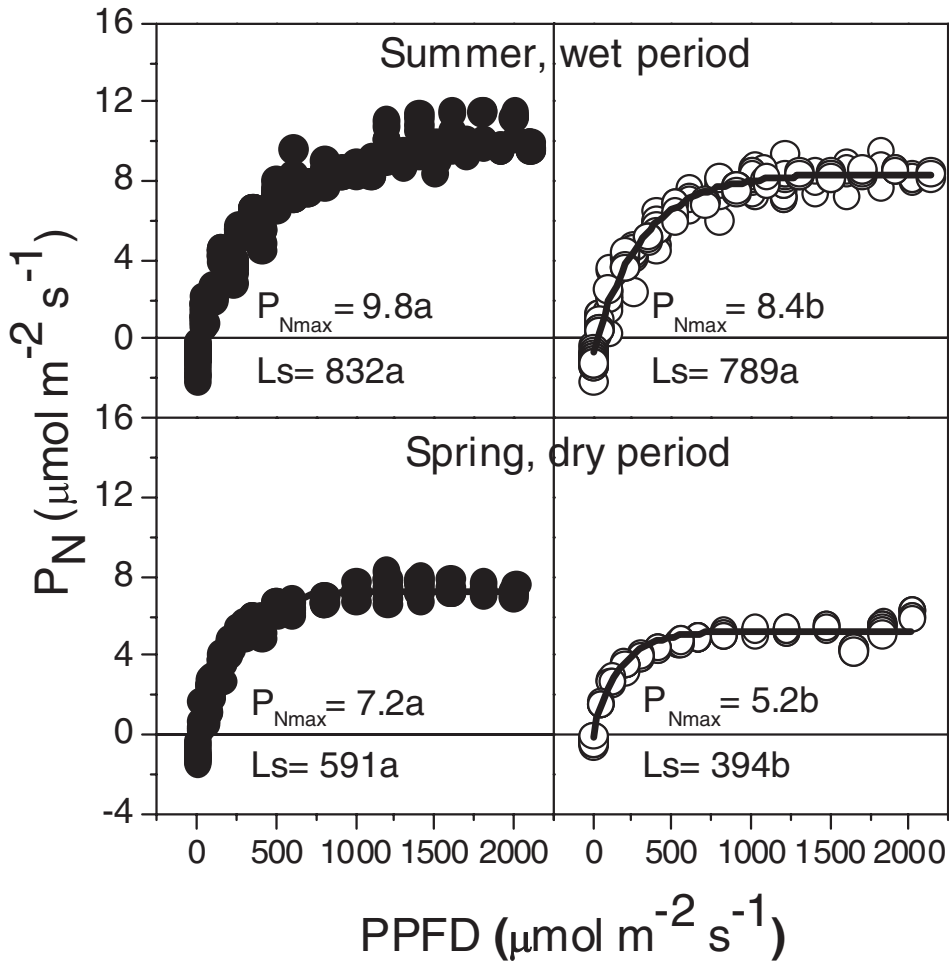


Figure 2. Net photosynthesis ( $P_N$ ) as a function of photosynthetic photon flux density (PPFD) on leaves of non-grafted (●) and grafted (○) *C. arabica* over *C. canephora* during early morning in summer (March 2002, wet period) and spring (October 2002, dry period). The values of maximum net photosynthesis ( $P_{Nmax}$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and light saturation point ( $L_s$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) are shown at the bottom in each panel. Two curves were merged in each panel before adjustment. Significant differences ( $p < 0.05$ ) in  $P_N$  and  $L_s$  values between treatments in the same period are indicated by different letters.

midday in summer (Figure 6B), but the differences between treatments during the daily courses was reduced towards spring. Significantly higher values of WUE in grafted than in non-grafted plants during the entire diurnal course occurred only at the peak of the dry season in spring (Figure 6J). Average ( $\pm s.e.$ ) values of  $\Psi_{pd}$  of non-grafted and grafted plants were, respectively,  $-0.60 \pm 0.90$  and  $-0.52 \pm 0.17$  MPa in the wet and  $-1.41 \pm 0.32$  and  $-0.53 \pm 0.28$  MPa in the dry period. There was a significant difference ( $p < 0.05$ ) between treatments about  $\Psi_{pd}$  only in the dry period. The mean values of  $P_N$  were significantly different between grafted and non-grafted plants during the stressful daytimes from 10:00 to 16:00 hours in summer and spring sunny daily courses

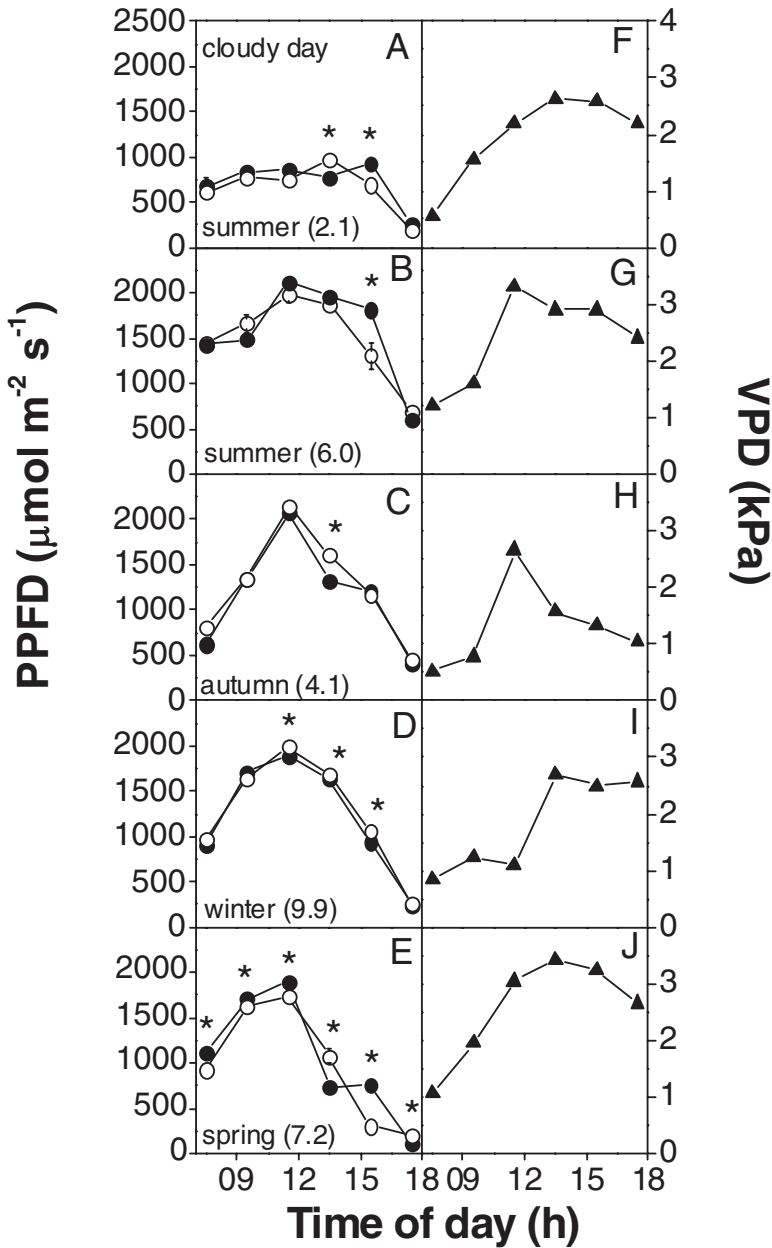


Figure 3. Diurnal courses of photosynthetic photon flux density (PPFD) and vapour pressure deficit (VPD) on leaves of non-grafted (●) and grafted (○) individuals of *C. arabica* on cloudy (March 2003, A and F) and sunny (March 2002, B and G) summer days, and during sunny days in autumn (May 2002, C and H), winter (August 2002, D and I), and in spring (October 2002, E and J). The numbers in brackets represent the total hours of sunshine in each day. Circles represent average values, bars denote *s.e.* and asterisks indicate significant differences ( $p < 0.05$ ) between non-grafted and grafted treatments at each time of the diurnal courses.



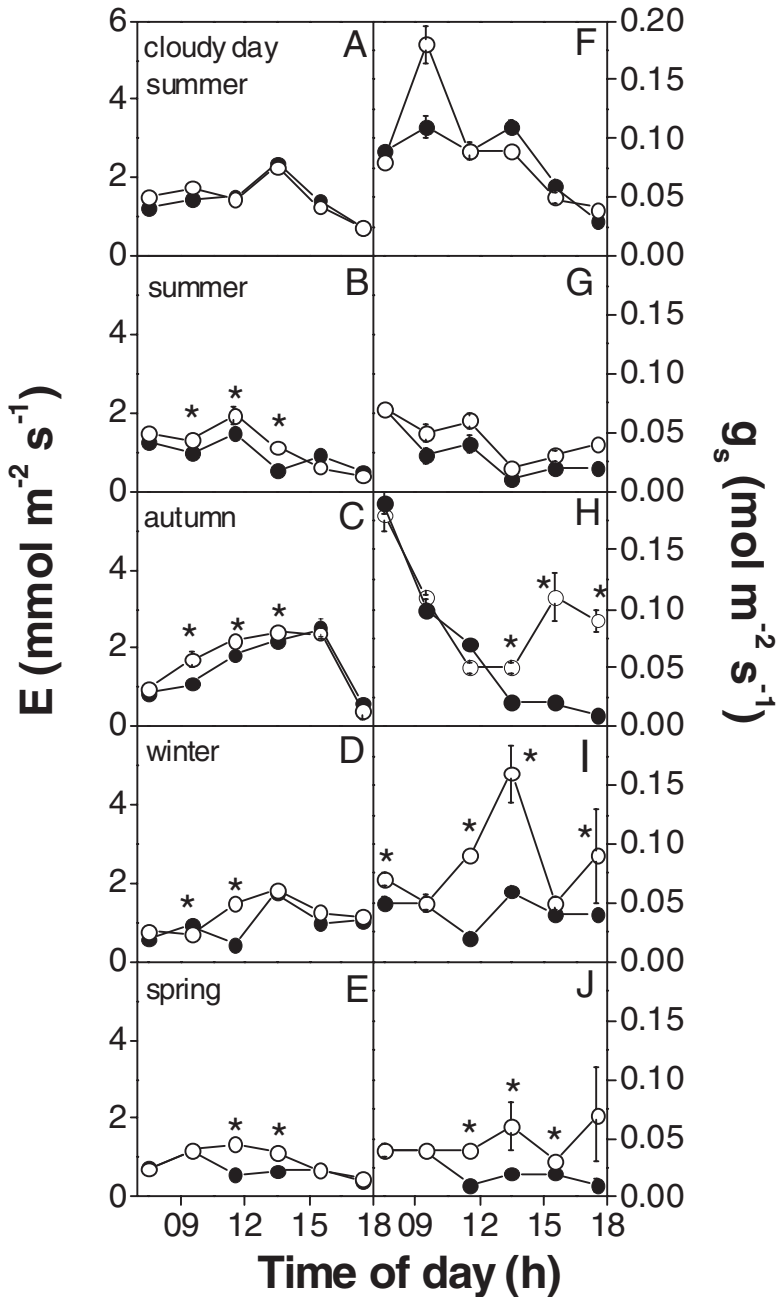


Figure 4. Diurnal courses of leaf transpiration ( $E$ ) and stomatal conductance ( $g_s$ ) in leaves of non-grafted ( $\bullet$ ) and grafted ( $\circ$ ) individuals of *Coffea arabica* on cloudy (March 2003, A and F) and sunny (March 2002, B and G) summer days, and during sunny days in autumn (May 2002, C and H), winter (August 2002, D and I), and in spring (October 2002, E and J). Circles represent average values, bars denote *s.e.* and asterisks indicate significant differences ( $p < 0.05$ ) between non-grafted and grafted treatments at each time of the diurnal courses.

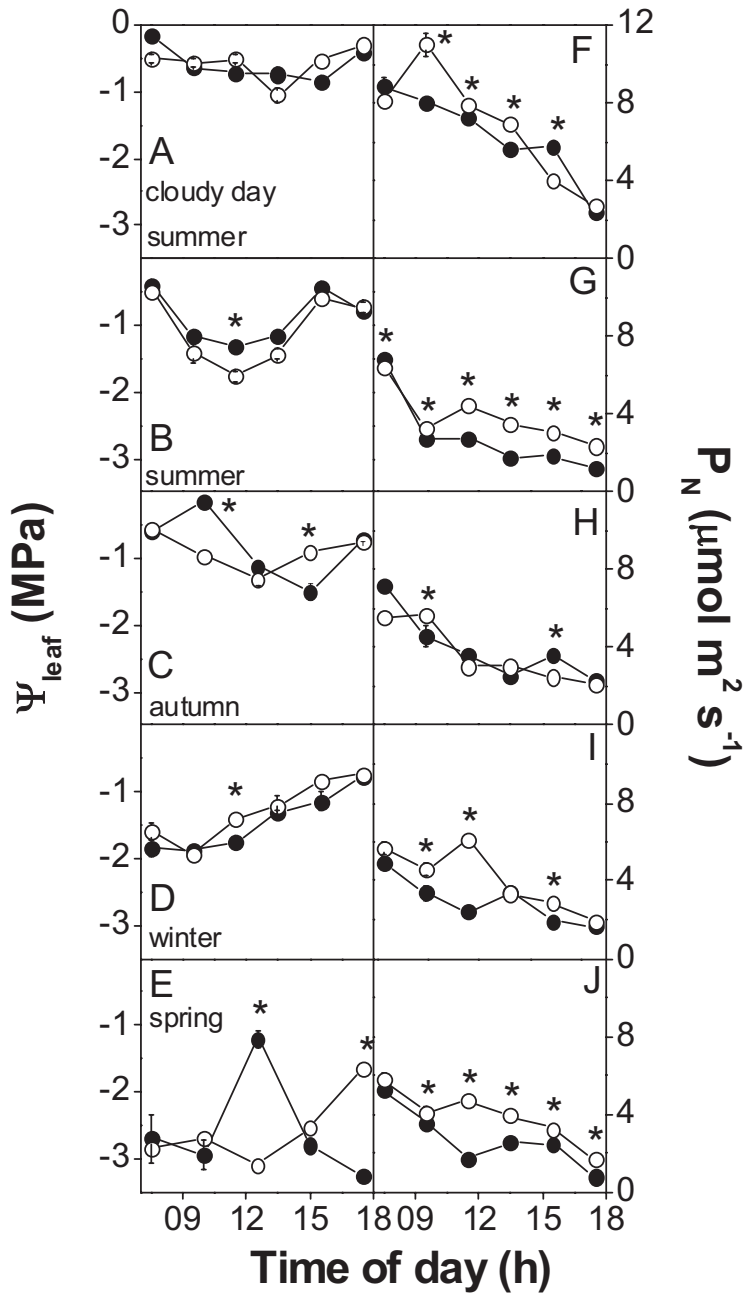


Figure 5. Diurnal courses of leaf water potential ( $\Psi_{\text{leaf}}$ ) and net photosynthesis ( $P_N$ ) in leaves of non-grafted (●) and grafted (○) individuals of *C. arabica* on cloudy (March 2003, A and F) and sunny (March 2002, B and G) summer days, and during sunny days of autumn (May 2002, C and H), winter (August 2002, D and I) and spring (October 2002, E and J). Symbols represent average values, bars denote *s.e.* and asterisks indicate significant differences ( $p < 0.05$ ) between non-grafted and grafted treatments at each time of the diurnal courses.

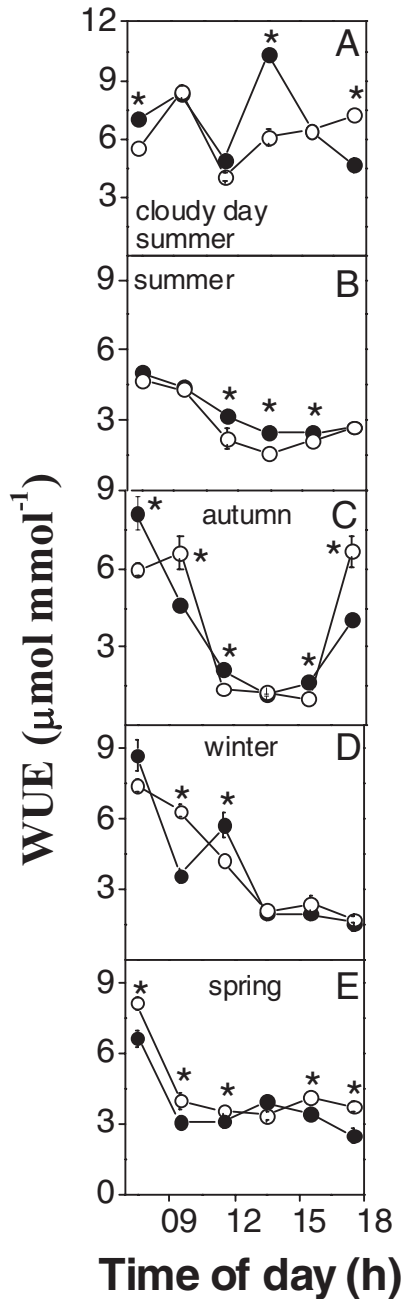


Figure 6. Diurnal courses water use efficiency (WUE) in leaves of non-grafted (●) and grafted (○) individuals of *Coffea arabica* on cloudy (March 2003, A) and sunny (March 2002, B) summer days, and during sunny days of autumn (May 2002, C), winter (August 2002, D) and spring (October 2002, E). Symbols represent average values, bars denote standard error and asterisks indicate significant differences ( $p < 0.05$ ) between non-grafted and grafted treatments at each time of the diurnal courses.

Table 1. Integrated net photosynthesis ( $IP_N$ ) and integrated potential net photosynthesis ( $IPP_N$ ) from 10:00 to 16:00 hours during sunny days of summer and spring, when average values of net photosynthesis ( $P_N$ ) were significantly different ( $p < 0.05$ ) between non-grafted (NG) and grafted (G) individuals of *Coffea arabica*.

	Summer		Spring	
	NG	G	NG	G
Average $P_N$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) $\pm$ s.d.	2.29 $\pm$ 0.47	3.58 $\pm$ 0.52	2.56 $\pm$ 0.66	3.97 $\pm$ 0.52
$IP_N$ ( $\text{mol m}^{-2}$ )	35	54	36	60
$IPP_N$ ( $\text{mol m}^{-2}$ )	209	180	151	108
$IP_N/IPP_N$	0.17	0.30	0.24	0.55

(Figures 5G and 5J), when  $IP_N$ ,  $IPP_N$  and the ratio  $IP_N/IPP_N$  around midday were higher in grafted than in non-grafted plants (Table 1).

$P_N$  showed linear decrease with VPD in both treatments, although the value of  $r^2$  was significant higher ( $p < 0.05$ ) in non-grafted than in grafted treatment (Figure 7). In contrast,  $g_s$  decreased exponentially with VPD in non-grafted plants and there was no significant relationship between  $g_s$  and VPD in grafted individuals (Figure 7). Therefore, VPD did not affect significantly the values of  $g_s$  in grafted plants considering the pool of data obtained during the year.

#### DISCUSSION

The favourable conditions observed on cloudy days in summer attenuated the midday depression of coffee plant photosynthesis. Indeed, Ronquim *et al.* (2006) observed that irradiance attenuation during cloudy days increased the diurnal carbon assimilation of *C. arabica* in relation to sunny days in wet periods. The values of  $E$ ,  $g_s$ ,  $\Psi_{\text{leaf}}$  and  $P_N$  agree with those found by Ronquim *et al.* (2006) in non-grafted plants of *C. arabica* (cv. Catuai) in similar cloudy conditions. The behaviour of leaf gas exchange and  $\Psi_{\text{leaf}}$  in grafted and non-grafted *C. arabica* plants on cloudy days could be associated with their origin in the understory of high altitude tropical forests in Ethiopia. Reduced PPFD, VPD and the number of hours of sunshine are common during the whole year in Ethiopian forest (Marin *et al.*, 2005), where the average annual air temperature and rainfall are 20 °C and 2000 mm, respectively (Carr, 2001). On the other hand, grafted plants showed higher  $P_N$  than non-grafted ones around midday during cloudy diurnal courses. Therefore, grafting is important to carbon uptake even in the wet season when water availability to roots and atmospheric conditions are favourable to  $P_N$  as happened in cloudy days in summer.

High PPFD and VPD around midday resulted in great differences between grafted and non-grafted plants in sunny diurnal courses. Grafted plants had lower midday depression than non-grafted ones under more severe water stress in atmosphere or in soil. Less susceptibility of grafted than non-grafted treatment to midday depression was revealed by lower values of  $\Psi_{\text{leaf}}$  associated with higher values of  $P_N$  and  $E$  in sunny days of summer and spring and by higher values of  $g_s$  around midday in autumn, winter and spring. The differences in  $E$ ,  $g_s$ ,  $P_N$  and  $\Psi_{\text{leaf}}$  between treatments were even

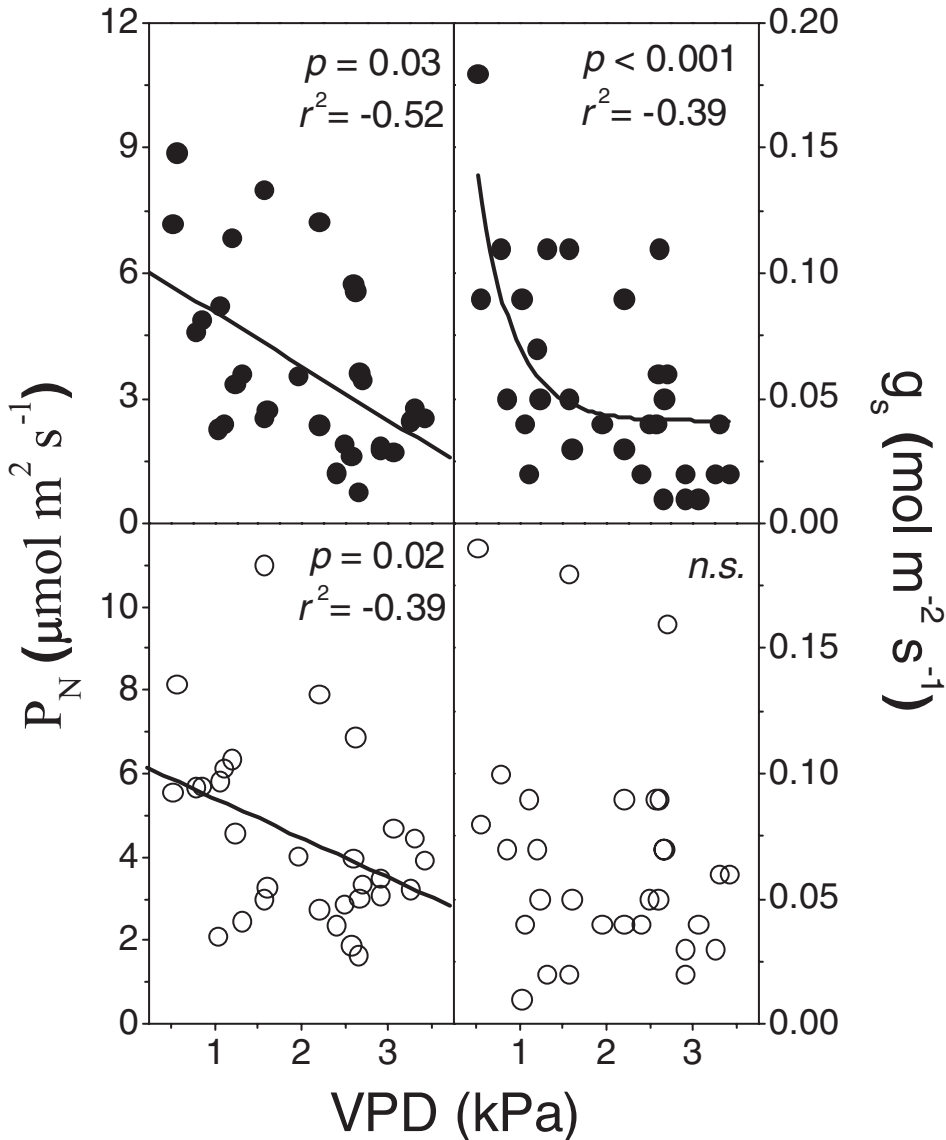


Figure 7. Average values of net photosynthesis ( $P_N$ ) and stomatal conductance ( $g_s$ ) as a function of vapour pressure deficit (VPD) on leaves of non-grafted (●) and grafted (○) individuals of *Coffea arabica*. Data were obtained during cloudy and sunny days in summer and during sunny days in autumn, winter, and spring under field conditions. *n.s.* = not significant.

higher in dry periods (winter and spring). Indeed, grafted plants showed WUE values significantly higher than non-grafted treatments at the peak of the dry season in spring. The better performance of grafted plants, principally in the dry period, could be linked to the bigger and deeper root system of *C. canephora*, which could acquire water at a rate that *C. arabica* could not (Fahl *et al.*, 2001). In fact, grafted plants showed great

capacity to restore leaf water status during the night in dry periods, when predawn leaf water potential in non-grafted and grafted plants was  $-1.25$  and  $-0.52$  MPa, respectively. Therefore, these physiological performances in grafted plants resulted in a better tolerance to soil and atmospheric water stresses, making them able to support higher rates of leaf gas exchange and greater tension in the xylem water column than non-grafted individuals.

The diurnal carbon assimilation in trees is usually influenced by midday depression of leaf gas exchange (Kikuzawa *et al.*, 2004). *Coffea* plants were able to hold  $IP_N/IPP_N$  near 1.0 in the absence of midday depression on a cloudy day (Ronquim *et al.*, 2006). Therefore, midday depression of leaf gas exchange was responsible for values of  $IP_N/IPP_N$  ratio lower than 0.55 in non-grafted and grafted plants. Midday depression in *C. arabica* growing in an open field in southeast of Brazil was also observed by Barros *et al.* (1997), who showed that  $g_s$  was high during the morning but decreased as air temperature and VPD increased after midday in summer. Minor susceptibility to midday depression of leaf gas exchange was confirmed by  $IP_N/IPP_N$  ratio two times higher in grafted than in non-grafted plants around midday in summer and spring. The values of  $E$ ,  $g_s$ ,  $\Psi_{\text{leaf}}$  and  $P_N$  of sunny diurnal courses agree with those found by Fahl *et al.* (2001) in grafted *C. arabica* over *C. canephora* and non-grafted plants (cv. Catuaí) between 08:00 and 12:00 hours during a sunny day in open fields. These authors also observed higher  $P_N$ ,  $g_s$  and  $E$  values in the grafted plants.

The behaviour of water relations and  $P_N$  during diurnal courses demonstrated that grafting on *C. canephora* is appropriate to increase the carbon balance of *C. arabica* and may result in higher vegetative and reproductive growth. Indeed, adult grafted *C. arabica* plants showed greater height, number of plagiotropic branches and grain production than non-grafted plants under field conditions (Fahl *et al.*, 2001). Lower susceptibility of grafted plants to midday depression as a result of better tolerance to soil and atmospheric water stress could contribute to better development and higher productivity of *Coffea arabica* during the year. The weaker connection between  $P_N$  and VPD and the independence of  $g_s$  in relation to VPD in grafted plants confirm the successes of grafting against water stress.

Conditions occurring on cloudy days are the opposite of the expected trend of climate in the future, i.e. projected simultaneous increase of air temperature and VPD in Brazil. The projected mean warming for Latin America for 2100 ranges from 1 to 6 °C according to different climate models (Bates *et al.*, 2008). In fact, low water availability in the rhizosphere and unfavourable temperatures during global climate change will be the major limitations to coffee production (DaMatta and Ramalho, 2006) in Brazil even under irrigation (Assad *et al.*, 2004). The increase of air temperature from 1.0 to 5.8 °C will induce an increase of 40–100% of unsuitable areas for *Coffea* plantations in the Brazilian states of Goiás, Minas Gerais and São Paulo, limiting appropriate areas to the south of Paraná state (Assad *et al.*, 2004). Grafted plants were less affected by drought since they showed greater tolerance to water stress in atmospheric and in soil and lower dependence of  $P_N$  and  $g_s$  to VPD during the year. Therefore, grafting *C. arabica* over *C. canephora* is highly recommended in a future

climate change, when air temperature and evaporative demand in atmosphere will increase simultaneously.

#### CONCLUSIONS

Higher values of  $P_N$  in grafted compared to non-grafted plants around midday showed that grafting was important even when environmental conditions were favourable in field conditions. Moreover, lower midday depression in grafted plants on sunny days confirmed that grafting is suitable to maintain the carbon uptake against environmental stresses. The differences in leaf gas exchange and leaf water potential in favour of grafted plants were higher in dry periods (winter and spring). Indeed, the  $IP_N/IPP_N$  ratio in grafted was double that in non-grafted individuals around midday in sunny periods in summer and spring. Low susceptibility of grafted plants to midday depression should contribute to better development and higher productivity during the year. In addition, low dependence of  $P_N$  and  $g_s$  on VPD in grafted *C. arabica* over *C. canephora* showed that grafting is strongly recommended under future climate scenarios in Latin America.

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