Tree Physiology 26, 517–525

Soluble sugars mediate sink feedback down-regulation of leaf photosynthesis in field-grown *Coffea arabica*

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Received April 27, 2005; accepted September 7, 2005; published online January 16, 2006

Summary Source–sink relationships of field-grown plants of *Coffea arabica* L. cultivar 'Caturra' were manipulated to analyze the contribution of soluble sugars to sink feedback down-regulation of maximal leaf net CO_2 assimilation rate (A_{max}). Total soluble sugar concentration (SSC_m) and A_{max} were measured in the morning and afternoon on mature leaves of girdled branches bearing either high or low fruit loads. Leaf A_{max} was negatively correlated to SSC_m, increased with fruit load and decreased during the day, indicating that limiting sink demand for carbohydrates caused SSC_m to accumulate in the leaf tissue which results in down-regulation of A_{max}. To further analyze source–sink feedback on A_{max}, we compared A_{max} of mature, non-sink-limited coffee leaves fed with water or sucrose for 5, 10 or 30 min with that of non-fed control leaves. Sucrose-feeding reduced A_{max} compared with the control and water-feeding treatments, indicating that down-regulation of A_{max} is related to phloem sucrose concentration in coffee source leaves, independent of SSC_m concentration in other leaf tissues. Although sucrose appeared to be more closely related to the mechanism underlying sink feedback down-regulation of A_{max} in coffee leaves than SSC_m, A_{max} was closely related to SSC_m by a non-linear equation that may be useful for integrating sink limitations in coffee leaf photosynthetic models.

Keywords: fruit load, photosynthesis, sink feedback, soluble sugars, source–sink relationships, sucrose.

Introduction

Increased understanding of source–sink relationships and their effect on photosynthesis (A) can be useful for predicting the effects of agronomical practices affecting fruit load (sinks) or leaf area (source), or both. In the case of coffee (*Coffea arabica* L.), cultivation in agroforestry systems is regaining popularity (Beer et al. 1997) and shade cast by associated trees strongly alters source–sink relationships through its effect on vegetative growth, leaf morphology, flower induction and resulting fruit set (Cannell 1971, Da Matta 2004, Campanha et al. 2005, Vaast et al. 2005*b*). In such systems, quantification of sink feedback down-regulation of A and a detailed understanding of the mechanism controlling this down-regulation are of crucial importance for modeling coffee primary production. Close coordination of source organ photosynthetic activity with carbon demand of sink organs has been clearly documented in many species, indicating a decrease in A when sink demand for carbohydrate is limited (Gucci et al. 1994, Myers et al. 1999, Iglesias et al. 2002, De Groot et al. 2003, Syvertsen et al. 2003, Quilot et al. 2004). For coffee, evidence of such coordination exists. Cannell (1971) observed that when coffee plants were completely deblossomed, A decreased by nearly 30%, and Vaast et al. (2005*a*) recently observed that A was 60% lower in girdled coffee branches with no fruit than in girdled branches with a high fruit load. A strong body of evidence indicates that this coordination between sink strength and source activity is the result of a feedback signal from sink to source organs mediated through the carbohydrate concentration in mature source leaves (Sharkey et al. 1986, Paul and Foyer 2001, Paul and Pellny 2003). Furthermore, genes involved in the photosynthetic pathway are inhibited by sugars (Sheen 1990, Lalonde et al. 1999, Pego et al. 2000, Vaughn et al. 2002, Rook and Bevan 2003) as confirmed by studies on in vitro cultures of cell suspensions of photoautotrophic tissue (Krapp et al. 1993, Jang and Sheen 1994). Additionally, studies of phloem loading in normal plants (Komor et al. 1996) and genetically modified plants (Von Schaewen et al. 1990) show that increasing sugar concentration in the phloem causes carbohydrates to accumulate in chlorenchymatic cells, which in turn decreases A (Von Schaewen et al. 1990, Vaughn et al. 2002). In nature, low sink demand causes an accumulation of sucrose in the source leaf phloem that inhibits phloem loading

resulting in carbohydrate accumulation in the surrounding mesophyll and a concomitant reduction in *A* (Chiou and Bush 1998, Vaughn et al. 2002). This sucrose-mediated mechanism has been proposed to control sink feedback down-regulation of *A* (Sheen 1990, Komor et al. 1996).

Soluble sugar accumulation in tissues of autotrophic leaves in response to decreased sink demand has been related to the down-regulation of *A* in several species, including tobacco (Paul and Driscoll 1997), loblolly pine (Myers et al. 1999), maize (Jeannette et al. 2000), *Poa alpina* L. (Baxter et al. 1995), citrus (Iglesias et al. 2002, Syvertsen et al. 2003), peach (Quilot et al. 2004) and mango (Urban et al. 2004). In some studies, the effect of leaf carbohydrate concentration on *A* was quantified through negative linear (Iglesias et al. 2002) or nonlinear (Baxter et al. 1995, Quilot et al. 2004) relationships. In other studies, down-regulation of *A* by sink feedback has been directly related to sink demand (Gucci et al. 1994, Ben Mimoun et al. 1996).

Various experimental techniques have been used to manipulate and study source–sink relationships in plants, including girdling (Krapp et al. 1993, Myers et al. 1999, Jeannette et al. 2000, Iglesias et al. 2002, Urban et al. 2004, Vaast et al. 2005*a*). Girdling, the removal of a whole ring of phloem around a vegetative axis, stops basipetal movement of assimilates through the phloem creating a closed-system environment for carbon metabolism and transport (Roper and Williams 1989, Di Vaio et al. 2001, Li et al. 2003). In such closed systems, contrasting source:sink ratios can easily be achieved by removing leaves or fruits or both (Myers et al. 1999, Iglesias et al. 2002, Urban et al. 2004, Vaast et al. 2005*a*). Another technique involves feeding sugars to a given plant organ (Fondy and Geiger 1977, Krapp et al. 1993, Komor et al. 1996, Chiou and Bush 1998, Iglesias et al. 2002) and thereby mimicking the effect of an increased carbohydrate concentration in the phloem as observed under limited sink demand (Chiou and Bush 1998, Voitsekhovskaja et al. 2000, Vaughn et al. 2002, Li et al. 2003). Sucrose represents the major transport form of photosynthetically assimilated carbon in plants (Lalonde et al. 1999) and is hence the major carbohydrate form found in the phloem of most plant species (Taiz and Zeiger 1991).

The goal of our study was to investigate the involvement of soluble sugars in source–sink relationships and to quantify their effect on maximal leaf net CO_2 assimilation rate (A_{max}) of producing coffee plants in the field. Specifically, we measured leaf soluble sugar concentration together with A_{max} on girdled coffee bearing branches with two contrasting fruit loads in the morning and afternoon of each day. The data set, which covered a broad spectrum of soluble sugar concentrations and A_{max}, was used: (1) to assess the effects of fruit load and time of day on the studied variables; (2) to determine how the concentration of leaf soluble sugars relates to A_{max}; and (3) to analyze the nature of this relationship. To investigate the feedback mechanism in detail, a sucrose-feeding trial was implemented based on the technique developed by Fondy and Geiger (1977). Assuming exogenous sucrose does not migrate into the symplast of the mesophyll parenchyma of source leaves (Fondy and Geiger 1977, Taiz and Zeiger 1991, Roberts et al. 1997), we tested the hypothesis that the phloem sucrose concentration of the source leaf acts as a feedback signal controlling down-regulation of A_{max} in mature coffee leaves.

Materials and methods

Experimental site and plant material

Measurements were performed on mature leaves of Arabica coffee plants (*Coffea arabica*) of the highly productive, dwarf cv. 'Caturra' in a homogeneous sun-exposed commercial orchard in the Orosi valley of Costa Rica (9.79 °N, 83.82 °W, 1108 m a.s.l.) planted in 1999 on an Inceptisol. The coffee plants were in their second (2003) and third (2004) production cycles, about 2 m high and spaced 1 m apart along the row. The rows were 2 m apart and oriented east–west. Fertilizers were applied and pests and diseases were controlled according to the locally recommended practices. Leaf analyses performed during the measurement periods showed that all nutrient concentrations were within the ranges recommended for coffee (data not shown). During the measurement periods, we registered a mean day time (dusk to dawn) photosynthetic photon flux (PPF) of 634 ± 451 µmol m^{-2} s^{-1} (with a midday maximum of 2038 µmol m^{-2} s^{-1}), air temperature of 23.3 ± 3.3 °C (15.6–32.0 °C), relative humidity of 79.5 ± 10.5 % (33.4–98.8 %) and water vapor pressure deficit (VPD) of 0.7 ± 0.4 kPa (0.37–2.95 kPa). Rainfall was concentrated in the afternoons (7.5 ± 7.9 mm) versus 0.1 ± 0.1 mm in the mornings and averaged 12.3 ± 11.0 mm day^{-1}.

Fruit load trial

Measurements were performed on pairs of opposite plagiotropic branches at mid-crown height (i.e., the crown region with high fruit load) on 12 randomly selected coffee plants. At the beginning of July 2003, each plant was decapitated 3 cm above the orthotropic stem node bearing the selected branch pair in order to obtain full sunlight exposure. Branches were then girdled at their base by bark removal in a 3-cm-wide band. The exposed tissues were protected with pruning seal to avoid drying and fungal infection. Contrasting fruit loads were set on each branch of a pair: a high fruit load treatment (HFL) consisted of removing leaves and some fruits to achieve a ratio of 10 fruits per leaf. (The normal full fruit load of similar branches outside the trial was 6.1 ± 2.8 fruits per leaf, n = 40, data not shown). A low fruit load treatment (LFL), applied on the opposite branch, involved removing fruits to achieve a ratio of 1 fruit per leaf. Selected branches carried a minimum of 10 mature leaves. To restrict sink demand for assimilates to fruits, immature leaves and the apical bud were removed at the onset of the treatment. Axillary branches that elongated thereafter were immediately removed. The rest of the tree was maintained unchanged at its initial full fruit load. We measured A_{max} and stomatal conductance to water vapor (g_s) three times a day (AM: 0600–0800 h; Noon: 1100–1300 h; and PM: 1600–1800 h) on one mature leaf of each of the four pairs of branches. The measurements were made during September 2003 when absolute fruit growth was highest (assessed by

monthly fruit dry mass increments, data not shown) to obtain the highest carbohydrate demand. The six days of measurement were completed between September 7 and 19, avoiding days with cloudy mornings and discarding data collected on days with rainy afternoons. On each measurement day, four plants (i.e., four branch pairs) were sampled among the 12 plants. Plant selection was such that each plant was sampled twice with no repeated combination of four plants per day. Leaf dimensions (blade length and width) were measured after each morning and afternoon measurement of A_{max}. The leaves were then detached and pooled according to fruit load and period of day to form composite four-leaf samples (i.e., four groups of four leaves, for each of the six days). Each time leaves were collected, fruits were removed to maintain the original fruit per leaf ratio. The composite leaf samples were placed in a cooler and taken to a freezer in the proximity of the experimental plot. Once the set of composite leaf samples was completed, the frozen samples were carried to the laboratory for further manipulation and analysis.

Sucrose feeding trial

In September 2004, six trees were selected for their high fruit load. Mature leaves with a high A_{max} (i.e., A_{max} > 10.5 µmol CO_2 m^{-2} s^{-1}, corresponding to the maximal A values obtained from A:PPF response curves, n = 16, unpublished data), were selected in the early morning (0530–0730 h) to limit the risk of sampling photoinhibited leaves. The A_{max} value was recorded as the initial A_{max} (A_{maxINI}). Directly thereafter, the branch segment bearing the monitored leaf was excised under water approximately 2 cm above and below its axillating node. With the branch segment immersed, the sample was taken to an open-sided shelter in the field, where PPF was 55.4 ± 17.2 µmol m^{-2} s^{-1}, and transferred to a water-filled tray (5 cm deep) set into a table. Maintaining the branch segment immersed, the leaf blade was fixed horizontally on the table by means of elastic bands with the adaxial side face upwards. The petiole was then cut under water and the branch segment removed. Three treatments were applied to the leaves: a control treatment in which leaves were left intact and two feeding treatments in which an area of ~2 cm^2 on the apical adaxial third of the leaf blade was gently rubbed with fine sandpaper to remove the cuticle. Water or a 0.7 M sucrose solution was then applied to the cuticle-free area with a fine paint brush, taking care to keep the whole surface wet throughout the 5-, 10- and 30-min treatments. A 0.7 M sucrose concentration was chosen because it is in the high range of normal leaf phloem sucrose concentrations (Lohaus and Moellers 2000, Knop et al. 2001) and osmotic potentials (Taiz and Zeiger 1991) reported for several species. At the end of the treatment, the wet area was dried with blotting paper and gas exchange was measured on the undisturbed leaf area to obtain the final A_{max} (A_{max5}; A_{max10}; A_{max30}). Six replicates were used for each combination of feeding × treatment duration, each of the replicates originating from a different plant among the six selected. Subsequently, the feeding treatments were repeated following the same procedure and leaf water potential (Ψ_l) was measured with a pressure chamber (Scholander et al. 1965).

Measurements: gas exchange

For both the fruit load and the sucrose feeding trials, A_{max} and g_s were measured on fully developed leaves (third to sixth pair of leaves from the branch tip) with a CO_2/H_2O infrared gas analyzer (LCPro, ADC BioScientific Ltd., Hoddesdon, U.K.) connected to a broadleaf chamber and with automatic control of leaf temperature, PPF and CO_2 concentration. We measured A_{max} and g_s at a saturating PPF of 1150 µmol m^{-2} s^{-1}, a leaf temperature of 25 °C and ambient CO_2 and H_2O vapor concentrations. The saturating PPF value was selected based on A:PPF response curves at a leaf temperature of 25 °C showing that A reaches a maximal value at a PPF of ~900 µmol m^{-2} s^{-1} (n = 16, unpublished data). Before each A_{max} measurement, leaves were preconditioned at a PPF of 750 µmol m^{-2} s^{-1} and a CO_2 concentration of 50 µmol mol^{-1} for 3 min to induce stomatal opening so as to avoid stomatal limitation of photosynthesis later on. To ensure that A_{max} values were not limited by g_s, a minimal threshold value for g_s (g_{smin}) was estimated as proposed by Farquhar and Sharkey (1982), on the basis of CO_2 supply and demand functions. First, the CO_2 concentration in the leaf mesophyll (C_i) necessary to achieve each measured A_{max} value was estimated from the demand function derived from A:C_i response curves performed at saturating PPF (n = 16, unpublished data). Then the g_{smin} value needed to achieve the calculated C_i (C_i^c) was estimated with the supply function through $g_{smin} = 1.37(A_{max}/[C_a - C_i^c])$ with C_a being the ambient atmosphere CO_2 concentration and 1.37 the ratio between conductance to water vapor and conductance to CO_2. Only measurements performed on leaves with g_s values higher than 1.1 times the calculated g_{smin} for a given A_{max} were retained for further analysis.

Measurements: specific leaf mass and soluble sugar concentration

Before the fruit load trial measurements, 60 leaves from branches on trees not included in the trial were collected and their leaf blade length and width recorded. The area of these leaves was measured with a scanner and image analysis software (WinRHIZO, Regent Instruments, Québec, Canada). A regression analysis was performed on the data set to fit an allometric function relating the product of leaf blade length and width to leaf area. The resulting linear function (r^2 = 0.99; data not shown) was then used to estimate leaf area from the product of leaf length and width. Frozen leaf samples of the fruit load trial were lyophilized and weighed and their mean leaf area ratio (SLM) determined by dividing the dry mass of the four-leaf samples by the sum of the four corresponding estimated leaf areas. Total soluble sugar concentration (SSC) in the lyophilized samples was estimated by the anthrone color reaction (Dische 1962) and concentrations calculated per unit leaf dry mass (SSC_m). Mass-based concentration was transformed to unit leaf area concentration (SSC_a) by multiplying by the estimated leaf area ratio.

Statistical analysis

One and two-way analyses of variance (ANOVA) were per-

formed for all variables obtained from the two trials. Means were separated by the Tukey's test ($\alpha = 0.05$). Pearson correlation analysis was performed to relate the carbohydrate concentration variables to A_{max}. All statistical analyses were performed with the Analyse-It software (Analyse-It Software, Leeds, Yorkshire, U.K.).

Results

Effects of fruit load and time of day on leaf soluble sugar concentration

Fruit load and time of day significantly affected SSC_m and SSC_a (Table 1) with higher values registered for LFL and PM. The relative increments from morning to afternoon were 40.2% for SSC_m and 59.8% for SSC_a, whereas the relative increments from HFL to LFL were 30.2% for SSC_m and 36.1% for SSC_a. Time of day had a significant effect on SLM with higher PM values, but there was no significant effect of fruit load on SLM (Table 1). The combination of fruit load and time of day had no significant effect on SLM (Figure 1c), but values of SSC_m and SSC_a were significantly higher for LFL-PM measurements than for HFL-AM measurements, with LFL-PM to HFL-AM ratios of 1.9 for SSC_m and 2.2 for SSC_a. For SSC_m, the HFL-PM and LFL-AM treatments did not differ significantly, but SSC_m differed significantly between the HFL-AM and LFL-PM treatments (Figure 1b). The absolute difference between AM and PM values was not significantly affected by fruit load treatment for any of the leaf variables ($F = 0.001$; $P = 0.9774$; $n = 6$, data not shown).

Effects of fruit load and time of day on photosynthesis

In the fruit load trial, all measured g_s values were higher than the calculated g_{smin} values, indicating that A_{max} was not limited by g_s (Figure 2a). As shown in Table 1, A_{max} was significantly affected by fruit load and time of day but the interaction of the two factors was not significant. In general, the effects of fruit load and time of day on A_{max} were opposite to the effects on SSC_m and SSC_a (Table 1 and Figures 1a and 1b). Fruit load resulted in an A_{max} 2.1 times higher at HFL than at LFL and

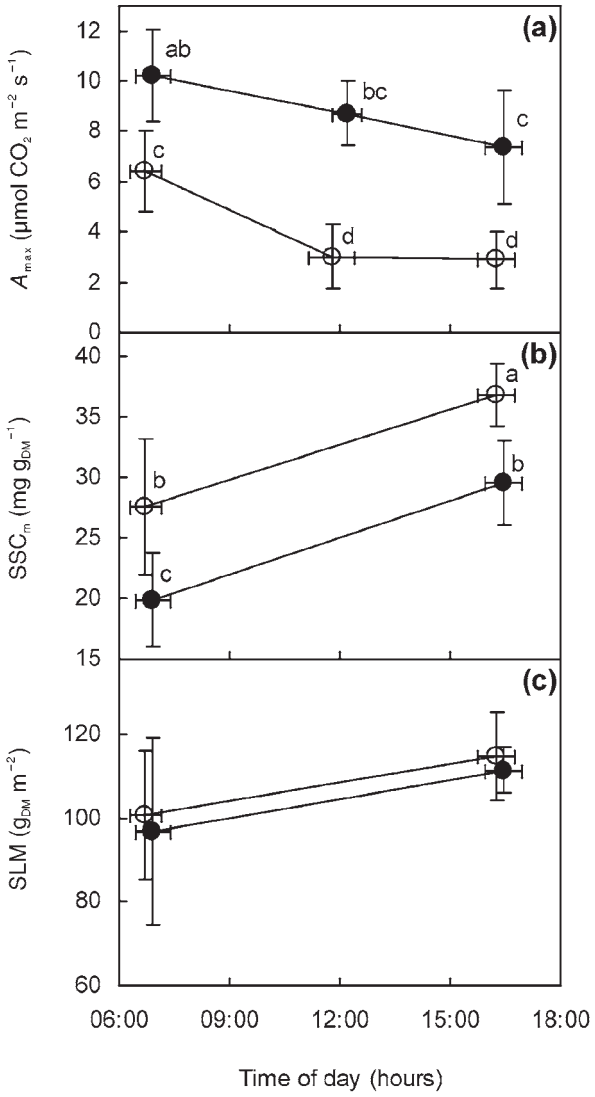

Figure 1. Changes in leaf variables during the day for high fruit load (●) and low fruit load (○) *Coffea arabica* leaves: (a) maximal CO_2 assimilation rate (A_{max}); (b) soluble sugar concentration per unit dry mass (SSC_m); and (c) leaf area ratio (SLM). Bars represent ± 1 SD; $n = 6$; within a panel, different letters indicate significant differences (Tukey's test; $\alpha < 0.05$).

Table 1. Summary of 2-way analysis of variance of the effects of fruit load and time of day on leaf variables and the correlation of these variables to maximal leaf CO_2 net assimilation rate (A_{max}). Variables: total soluble sugar concentration per unit dry mass (SSC_m) and unit leaf area (SSC_a); leaf area ratio (SLM); and A_{max}. Treatments: high fruit load (HFL); low fruit load (LFL); morning (AM); midday (Noon); and afternoon (PM). Means ($n = 6$) are given ± 1 standard deviation. Within a row, means followed by different letters are significantly different ($\alpha < 0.05$; lowercase letters for fruit load and uppercase letters for period of day). Correlation parameters: r = Pearson product-moment coefficient; and P = two-tailed probability; $n = 24$. Abbreviations: DM = dry mass; and ns = not significant.

Variable	Fruit load		Period of day			Correlation to A_{max}	
	HFL	LFL	AM	Noon	PM	r	P
SSC_m (mg_{DM} g_{DM}^{-1})	24.7 ± 6.2 b	32.2 ± 6.4 a	23.7 ± 6.1 B	–	33.2 ± 4.8 A	–0.87	< 0.001
SSC_a (g_{DM} m^{-2})	2.59 ± 0.82 b	3.53 ± 1.01 a	2.36 ± 0.82 B	–	3.76 ± 0.64 A	–0.75	< 0.001
SLM (g_{DM} m^{-2})	104.1 ± 17.3ns	107.8 ± 14.6ns	98.7 ± 18.3 B	–	113.2 ± 8.2 A	–0.14	0.504
A_{max} (µmol CO_2 m^{-2} s^{-1})	8.8 ± 2.1 a	4.1 ± 2.1 b	8.3 ± 2.6 A	5.9 ± 3.2 AB	5.1 ± 2.9 B	–	–

1.6 times higher in the morning than in the afternoon. Measurements made at noon had intermediate values that were not significantly different from either morning or afternoon measurements. The change in A_{max} during the day differed between the fruit load treatments (Figure 1a): A_{max} decreased linearly with time in the HFL treatment, whereas it reached a minimum at noon in the LFL treatment and remained around this low value in the afternoon. The mean morning to afternoon decrease in A_{max} was 3.52 ± 0.68 µmol CO_2 m^{-2} s^{-1} in the LFL treatment and 2.86 ± 0.73 µmol CO_2 m^{-2} s^{-1} in the HFL treatment and did not differ significantly between treatments (F = 2.63; P = 0.1358; n = 6, data not shown).

Relationship between soluble sugar concentration and photosynthesis

To analyze the correlation between the studied leaf variables and A_{max}, Pearson correlation analyses were performed on the whole fruit load trial data set (Table 1). Significant negative correlations were found for SSC_m and SSC_a, but not for SLM. The strongest Pearson product-moment correlation coefficient was for SSC_m (Table 1). To further analyze the relationship between SSC_m and A_{max}, nonlinear equations were fit on the whole data set with SSC_m as an independent variable and A_{max} as a dependent variable. The best fit was obtained with a power equation with three parameters (Figure 3).

Effects of sucrose feeding on photosynthesis

As in the case of the fruit load trial, all g_s values recorded in the sucrose feeding trial were higher than g_{smin} values indicating that A_{max} was not limited by g_s (Figure 2b). No effect of feeding treatment on Ψ_l was found (Figure 4). Values of A_{maxINI} in all feeding treatments averaged 11.78 ± 1.09 µmol CO_2 m^{-2} s^{-1} and showed no significant difference (Tukey's test; α = 0.05). Excluding A_{maxINI} values from the subsequent statistical analysis, all factors had a significant effect on A_{max} (feeding treatment: F = 80.83, P < 0.0001; treatment duration: F = 115.56, P < 0.0001; interaction term: F = 16.30, P < 0.0001; n = 18, data not shown). For the control treatment, A_{max} remained at a stable and high value, with no significant difference (Figure 5) or correlation to treatment duration (r = –0.10; $P_{(2\text{-tailed})}$ = 0.69; n = 18; data not shown). The water-feeding treatment had no significant effect on A_{max} for the first 10 min, but A_{max30} was significantly lower (36%) than control A_{max30} (Figure 5). Feeding sucrose to the leaves resulted in a significant decrease in A_{max} relative to the control and water-feeding treatments at all treatment times (Figure 5). The reductions in A_{max} relative to control values were 26% for A_{max5}, 62% for A_{max10} and 66% for A_{max30}, showing stabilization at a minimum value around 3 µmol CO_2 m^{-2} s^{-1} after 10 min (Figure 5).

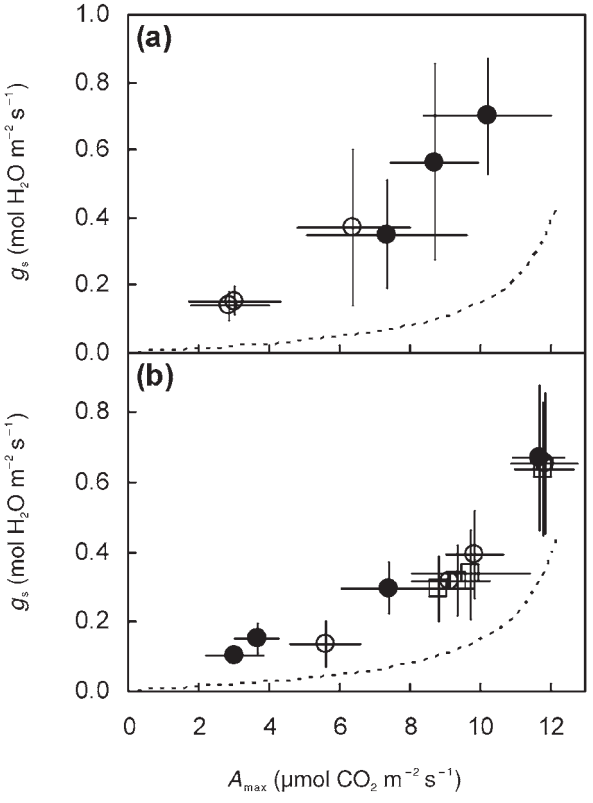

Figure 2. Relationships between maximal leaf net CO_2 assimilation rate (A_{max}) and leaf stomatal conductance to H_2O vapor (g_s) for *Coffea arabica* leaves in the fruit load (a) and sugar feeding (b) trials. Treatments: (a) ● high fruit load and ○ low fruit load; and (b) □ control, ○ water feeding and ● sucrose feeding. The dashed lines indicate the minimal threshold values for g_s. Bars represent ± 1 SD; n = 6.

Discussion

Effects of time of day and fruit load on leaf photosynthesis and soluble sugar concentration

Leaf A_{max} increased with sink demand (i.e., higher A_{max} in HFL than in LFL; Table 1, Figure 1a) which is consistent with the positive correlation between sink demand and A previously reported for apple (Gucci et al. 1994) and peach trees (Ben

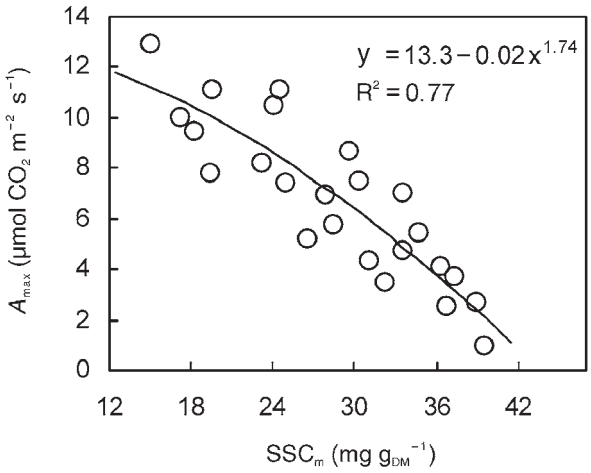

Figure 3. Maximal leaf net CO_2 assimilation rate (A_{max}) versus leaf total soluble sugar concentration per unit dry mass (SSC_m) in *Coffea arabica* leaves (n = 24).

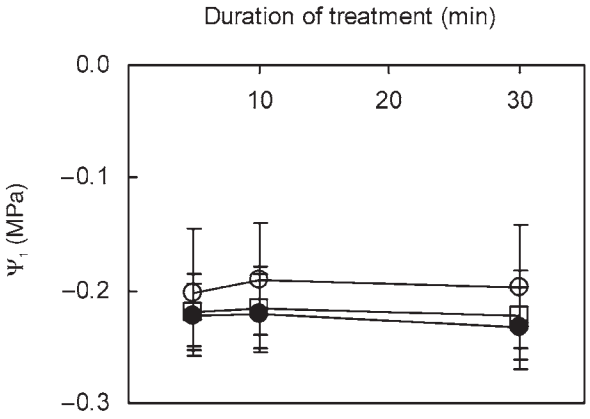

Figure 4. Leaf water potential (Ψ_l) in *Coffea arabica* leaves: control treatment (□), water-feeding treatment (○) and sucrose-feeding treatment (●), as a function of treatment duration. Bars represent ± 1 SD; n = 6; there were no significant differences between treatments (Tukey's test; $\alpha < 0.05$).

Mimoun et al. 1996). The AM measurements for the HFL treatment gave the lowest SSC_m (19.9 ± 3.9 mg g_{DM}^{-1}) and the highest A_{max} values (10.2 ± 1.8 µmol CO_2 m^{-2} s^{-1}), which were close to the A_{max} values obtained with A:PPF response curves (10.5 ± 0.5 µmol CO_2 m^{-2} s^{-1}, n = 16, unpublished data), suggesting that A_{max} was not (or only slightly) down-regulated by sink feedback (Figures 1a and 1b), presumably because, at high sink demand, carbohydrates accumulated in leaves during the previous day were almost completely consumed by the fruits during the night (Paul and Foyer 2001). Conversely, down-regulation of A_{max} in the early morning can be expected when sink demand is low (LFL), because assimilates accumulating in the leaves during the day are not completely consumed during the following night as shown by significantly

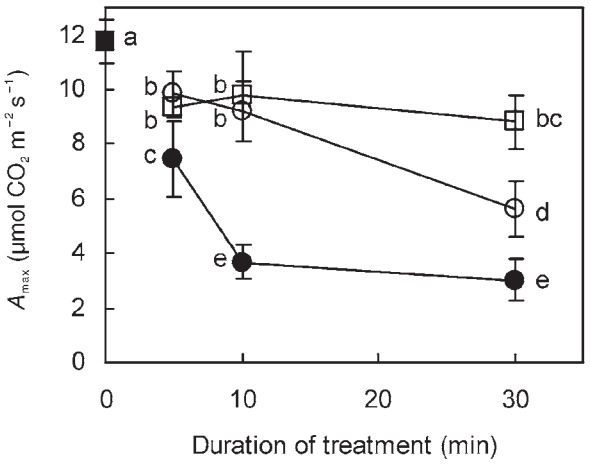

Figure 5. Maximal leaf net CO_2 assimilation rate (A_{max}): mean initial value for all treatments (■); control treatment (□), water-feeding treatment (○) and sucrose-feeding treatment (●), as a function of the treatment duration in *Coffea arabica* leaves. Bars represent ± 1 SD; n = 6; different letters indicate significant differences (Tukey's test; $\alpha < 0.05$).

higher leaf SSC_m in LFL-AM compared with HFL-AM (Figure 1b).

The magnitude of the decrease in A_{max} during the day was unexpectedly high in the HFL treatment (Figure 1a). Although similar responses have been reported previously in fully fruit-loaded coffee plants (Nutman 1937, Gutierrez et al. 1994) and fully fruit-loaded girdled coffee branches (Vaast et al. 2005*a*), they may have been due to photoinhibition (Ramalho et al. 1999, 2000) or g_s limitation during dry and hot midday conditions (Nunes 1988, Gutierrez et al. 1994, Vaast et al. 2005*b*). In our study, however, g_s did not limit A (Figure 2a) and the concomitant increase in SSC_m and decrease in A_{max} (Figures 1a and 1b) suggest that sink feedback down-regulation was the major factor depressing A in the afternoon, despite high sink demand (HFL), as also reported for apple trees by Gucci et al. (1994).

Thus, the accumulation of soluble sugars in leaves during the day when sink demand is high may be explained in two ways: (1) during the daytime, total fruit demand for assimilates in the HFL treatment was lower than the total amount produced by the leaves or (2) the export rate of assimilates from leaves to fruits was limited at some point(s) in the pathway linking these organs. As for the first hypothesis, Vaast et al. (2005*a*) observed a decreased fruit growth rate on heavily fruit-loaded girdled coffee branches, suggesting that fruit demand for assimilates was not fully met by assimilates produced in the leaves. For the LFL treatment, the stabilization of A_{max} at a low value from midday onward appeared to be caused by rapid accumulation of soluble sugars in the leaves and, hence, strong down-regulation of A_{max} as a result of low sink demand for assimilates (Figures 1a and 1b).

Relationship between leaf soluble sugar concentration and photosynthesis

Coffee leaves exhibited a strong negative correlation between SSC_m and A_{max}, supporting the prediction that soluble sugars are involved in sink feedback down-regulation of A (Table 1, Figure 3). A stronger correlation with A_{max} was obtained when soluble sugar concentration was expressed per unit leaf dry mass rather than per unit soluble sugar concentration in the leaf tissue (Table 1), perhaps reflecting the close relationship between leaf area and A_{max}. Down-regulation of A by SSC_m has also been reported for *Poa alpina* (Baxter et al. 1995). Consistent with the results reported by Baxter et al. (1995) and Quilot et al. (2004), there was a nonlinear relationship between leaf carbohydrate concentration and A_{max} (Figure 3). This relationship predicts that the down-regulation of A_{max} becomes steeper when SSC_m reaches high values (Figure 3). Conversely, A_{max} should tend to a maximum of 13.3 µmol CO_2 m^{-2} s^{-1} when SSC_m tends toward 0 mg g_{DM}^{-1} (Figure 3). According to our field observations, such high A_{max} values are unusual in 'Caturra' coffee leaves. This suggests that, under field conditions, SSC_m does not decrease beyond a physiological minimum, probably close to the lowest SSC_m value of 15.1 mg g_{DM}^{-1} measured in this study. In other species (Baxter et al. 1995, Quilot et al. 2004), A_{max} stabilizes at low values when leaf carbohy-

drate concentrations tend to high values. This feature was not observed in our study (Figure 3), possibly because high SSC_m values were not attained under our experimental conditions.

Effect of sucrose feeding on leaf photosynthesis

The A_{maxINI} value (measured when leaves were still attached to the plants) was significantly higher than the stable and relatively high A_{max} values of the control treatment (Figure 5), perhaps reflecting an effect of wounding on photosynthesis following petiole excision. Biochemical responses to wounding, which can occur within a few minutes (León et al. 2001), can induce down-regulation of *A* (Peña-Cortés et al. 1988, Jang and Sheen 1994). Nevertheless, because A_{max} of the control treatment remained at stable high values after leaf ablation, it may be considered a reliable reference for the other treatments. Similarly, wounding produced by gently rubbing the leaf surface for the feeding treatments could explain why A_{max30} decreased in water-fed leaves relative to the control value (Figure 5). Alternatively, the decreases in A_{max} might be caused by water stress effects in response to a hyperosmotic effect on *A* of the sucrose-feeding treatment and a hypoosmotic effect of the water-feeding treatment (Berkowitz and Gibbs 1982). However, Ψ_l did not differ significantly between treatments (Figure 4). Any water stress occurring in response to the treatments appears to have been mild (Hsiao 1973) and is unlikely, therefore, to account for the observed reductions in A_{max} in response to sucrose-feeding or water-feeding.

In sucrose-feeding studies, Fondy and Geiger (1977) showed that ^{14}C-sucrose fed to mature sugar beet leaves resulted in sucrose accumulation exclusively in the leaf phloem. This finding is consistent with the inability of the mesophyll of autotrophic leaves to absorb sucrose from the phloem (Taiz and Zeiger 1991, Roberts et al. 1997, Kühn et al. 1999, Voitsekhovskaja et al. 2000). Therefore, the observed decrease in A_{max} in response to sucrose feeding suggests that phloem sucrose concentration is involved in the feedback signaling of low sink demand. Chiou and Bush (1998) proposed that low sink demand results in an increased sucrose concentration in the source leaf phloem that down-regulates the activity of the sucrose symporter responsible for phloem loading. This would cause carbohydrates to build up in the surrounding mesophyll and result in a concomitant down-regulation of photosynthetic activity as observed in our LFL treatment (Figures 1a and 1b).

Sucrose regulation of sucrose symporter activity could be a key control that maintains tight coordination between *A* and sink utilization of assimilates (Vaughn et al. 2002). Some studies have shown that sucrose operates a direct (Grusak et al. 1990, Chiou and Bush 1998, Kehr et al. 1998) or coarse (Michin et al. 2002) control on phloem loading. Is the resulting accumulation of carbohydrates in the surrounding source leaf mesophyll the cause of the concomitant down-regulation of A_{max} observed in coffee leaves? In our sucrose-feeding treatment, down-regulation of A_{max} occurred in leaves that had a theoretically low mesophyll soluble sugar concentration, as indicated by the high A_{maxINI} values of all treatments and the stable high $A_{max(5–30)}$ of the control treatment (Figure 5). Our finding of down-regulation of *A*, apparently by phloem sucrose concentration, and its independence of carbohydrate accumulation in other leaf compartments are compatible with the results of Goldschmidt and Huber (1992) who reported that, in tobacco (a starch accumulator), starchless mutants showed down-regulation of *A* in response to low sink activity. Furthermore, Quereix et al. (2001) concluded that, in the case of grapevines, down-regulation of *A* was mediated by the carbohydrate concentration in the phloem of sink or source organs but not by the carbohydrate concentration in other source mesophyll tissues. Because we measured detached leaves, the signal could only arise from the sucrose concentration in the source phloem.

Sucrose concentration in the leaf phloem depends on the rate of sucrose loading in source organs and the rate of sucrose unloading in sink organs (Chiou and Bush 1998, Voitsekhovskaja et al. 2000, Vaughn et al. 2002, Li et al. 2003). This indicates that the sucrose concentration in phloem is more closely related to sink demand than the carbohydrate concentration in other leaf tissues, which is indirectly controlled by phloem sucrose concentration (Chiou and Bush 1998). This could explain why, in some species (Gucci et al. 1994, Ben Mimoun et al. 1996), down-regulation of *A* was better correlated to sink demand than to leaf carbohydrate content. Furthermore, sucrose is the principal soluble sugar found in coffee leaves (87.5 ± 0.06 % of the total soluble sugar concentration per unit dry mass; $n = 64$; unpublished data) indicating that part of the variation in SSC_m observed in the fruit load trial might be explained by variations in phloem sucrose concentration.

In conclusion, our field experiments on girdled coffee branches with high or low fruit load showed that source–sink down-regulation of A_{max} of autotrophic coffee leaves is highly correlated to their SSC_m. A single relationship between SSC_m and A_{max} for HFL and LFL was obtained (Figure 3) and revealed that the accumulation of soluble sugars in the leaves, as induced by LFL or with increasing time of the day, may account for a reduction in A_{max} of up to 92.5%. This shows that down-regulation of *A* by sink feedback can be a major factor limiting coffee photosynthesis, especially when plants are grown in agroforestry systems and carry low fruit loads (Cannell 1971, Da Matta 2004, Campanha et al. 2005, Vaast et al. 2005*b*). Additionally, we observed that feeding sucrose to leaves with a theoretically low SSC_m led to a similar down-regulation of A_{max}. Based on this result and the theory associated with source–sink relationships and sucrose transport (Chiou and Bush 1998, Voitsekhovskaja et al. 2000, Vaughn et al. 2002, Li et al. 2003), we conclude that down-regulation of A_{max} is mediated by sucrose concentration in the leaf phloem and is independent of SSC_m in other leaf compartments. The correlation between SSC_m and A_{max} would therefore imply that, under field conditions, sugar concentrations in the leaf phloem and the surrounding mesophyll are in equilibrium in coffee leaves. Therefore, the nonlinear equation relating A_{max} to SSC_m derived from our experiments may be sufficient for integrating sink limitation in coffee leaf CO_2 assimilation models.

Acknowledgments

The authors thank Mr. Ricardo Falla from "Finca las Chúcaras" for facilitating the use and maintenance of the experimental plot in Orosi, Costa Rica, CIRAD and the European Commission (ICA-4-CT-2001-10071) for their financial support of scientific equipment and field measurements performed within the framework of the Central American Coffee Agroforestry Project (www.casca-project.com) and Mrs. Alejandra Larraín for her valuable help in the collection of data.

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