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 CO2 assimilation rate (Amax). Total soluble sugar concentration (SSCm) and Amax were measured in the morning and afternoon on mature leaves of girdled branches bearing either high or low fruit loads. Leaf Amax was negatively correlated to SSCm increased with fruit load and decreased during the day, indicating that limiting sink demand for carbohydrates caused SSCm to accumulate in the leaf tissue which results in down-regulation of Amax. To further analyze source–sink feedback on Amax, we compared Amax 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 Amax compared with the control and water-feeding treatments, indicating that down-regulation of Amax is related to phloem sucrose concentration in coffee source leaves, independent of SSCm concentration in other leaf tissues. Although sucrose appeared to be more closely related to the mechanism underlying sink feedback down-regulation of Amax in coffee leaves than SSCm, Amax was closely related to SSCm 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. 2005a). 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. (2005a) 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. 2005a). 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. 2005a). 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 CO2 assimilation rate (A\textsubscript{\text{max}}) of producing coffee plants in the field. Specifically, we measured leaf soluble sugar concentration together with A\textsubscript{\text{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\textsubscript{\text{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\textsubscript{\text{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\textsubscript{\text{max}} in mature coffee leaves.

**Materials and methods**

**Experimental site and plant material**

Measurements were performed on mature leaves of Arabica coffee plants (*Coffee 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\textsuperscript{-2} s\textsuperscript{-1} (with a midday maximum of 2038 µmol m\textsuperscript{-2} s\textsuperscript{-1}), air temperature of 23.3 ± 3.3 °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\textsuperscript{-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\textsubscript{\text{max}} and stomatal conductance to water vapor (g\textsubscript{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 $A_{\text{max}}$. 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 $A_{\text{max}}$ (i.e., $A_{\text{max}} > 10.5$ $\mu$mol 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 $A_{\text{max}}$ value was recorded as the initial $A_{\text{max}}(A_{\text{maxini}})$. 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 $\mu$mol 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 $A_{\text{max}}(A_{\text{max10}}; A_{\text{max30}})$. 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 ($\Psi_t$) was measured with a pressure chamber (Scholander et al. 1965).

**Measurements: gas exchange**

For both the fruit load and the sucrose feeding trials, $A_{\text{max}}$ and $g_s$ were measured on fully developed leaves (third to sixth pair of leaves from the branch tip) with a CO$_2$/H$_2$O 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 $A_{\text{max}}$ and $g_s$ at a saturating PPF of 1150 $\mu$mol m$^{-2}$ s$^{-1}$, a leaf temperature of 25 °C and ambient CO$_2$ and H$_2$O 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 $\mu$mol m$^{-2}$ s$^{-1}$ ($n = 16$, unpublished data). Before each $A_{\text{max}}$ measurement, leaves were preconditioned at a PPF of 750 $\mu$mol m$^{-2}$ s$^{-1}$ and a CO$_2$ concentration of 50 $\mu$mol mol$^{-1}$ for 3 min to induce stomatal opening so as to avoid stomatal limitation of photosynthesis later on. To ensure that $A_{\text{max}}$ values were not limited by $g_s$, a minimal threshold value for $g_s$ ($g_{\text{min}}$) 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 $A_{\text{max}}$ value was estimated from the demand function derived from $A$:C$_i$ response curves performed at saturating PPF ($n = 16$, unpublished data). Then the $g_{\text{min}}$ value needed to achieve the calculated $C_i$ ($C_i^*$) was estimated with the supply function through $g_{\text{min}} = 1.37(A_{\text{max}}/C_i - C_i^*)$ with $C_a$ being the ambient atmosphere CO$_2$ concentration and 1.37 the ratiobetween 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 $g_{\text{min}}$ for a given $A_{\text{max}}$ 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 (α = 0.05). Pearson correlation analysis was performed to relate the carbohydrate concentration variables to $A_{\text{max}}$. 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 $g_{s\text{min}}$ values, indicating that $A_{\text{max}}$ was not limited by $g_{s}$ (Figure 2a). As shown in Table 1, $A_{\text{max}}$ 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 $A_{\text{max}}$ were opposite to the effects on SSC$_{m}$ and SSC$_{a}$ (Table 1 and Figures 1a and 1b). Fruit load resulted in an $A_{\text{max}}$ 2.1 times higher at HFL than at LFL and

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 ($A_{\text{max}}$). Variables: total soluble sugar concentration per unit dry mass (SSC$_{m}$) and unit leaf area (SSC$_{a}$); leaf area ratio (SLM); and $A_{\text{max}}$. 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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fruit load</th>
<th>Period of day</th>
<th>Correlation to $A_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HFL</td>
<td>LFL</td>
<td>AM</td>
</tr>
<tr>
<td>SSC$<em>{m}$ (mg DM$^{-1}$ g$</em>{DM}$$^{-1}$)</td>
<td>24.7 ± 6.2 b</td>
<td>32.2 ± 6.4 a</td>
<td>23.7 ± 6.1 B</td>
</tr>
<tr>
<td>SSC$_{a}$ (g DM$^{-1}$ m$^{-2}$)</td>
<td>2.59 ± 0.82 b</td>
<td>3.53 ± 1.01 a</td>
<td>2.36 ± 0.82 B</td>
</tr>
<tr>
<td>SLM (g DM$^{-1}$ m$^{-2}$)</td>
<td>104.1 ± 17.3ns</td>
<td>107.8 ± 14.6ns</td>
<td>98.7 ± 18.3 B</td>
</tr>
<tr>
<td>$A_{\text{max}}$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$)</td>
<td>8.8 ± 2.1 a</td>
<td>4.1 ± 2.1 b</td>
<td>8.3 ± 2.6 A</td>
</tr>
</tbody>
</table>
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 \( A_{\text{max}} \) during the day differed between the fruit load treatments (Figure 1a): \( A_{\text{max}} \) 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 \( A_{\text{max}} \) 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 \((F = 2.63; P = 0.1358; n = 6, \text{data not shown}).\)

Relationship between soluble sugar concentration and photosynthesis

To analyze the correlation between the studied leaf variables and \( A_{\text{max}} \), Pearson correlation analyses were performed on the whole fruit load trial data set (Table 1). Significant negative correlations were found for SSC\(_{\text{m}}\) and SSC\(_{\text{a}}\), but not for SLM. The strongest Pearson product-moment correlation coefficient was for SSC\(_{\text{m}}\) (Table 1). To further analyze the relationship between SSC\(_{\text{m}}\) and \( A_{\text{max}} \), nonlinear equations were fit on the whole data set with SSC\(_{\text{m}}\) as an independent variable and \( A_{\text{max}} \) 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 \( g_{\text{min}} \) values indicating that \( A_{\text{max}} \) was not limited by \( g_{s} \) (Figure 2b). No effect of feeding treatment on \( \Psi_{l} \) was found (Figure 4). Values of \( A_{\text{maxINI}} \) in all feeding treatments averaged 11.78 ± 1.09 µmol CO\(_2\) m\(^{-2}\) s\(^{-1}\) and showed no significant difference (Tukey’s test; \( \alpha = 0.05 \)). Excluding \( A_{\text{maxINI}} \) values from the subsequent statistical analysis, all factors had a significant effect on \( A_{\text{max}} \) (feeding treatment: \( F = 80.83, P < 0.0001; \) treatment duration: \( F = 115.56, P < 0.0001; \) interaction term: \( F = 16.30, P < 0.0001; n = 18, \text{data not shown} \)). For the control treatment, \( A_{\text{max}} \) remained at a stable and high value, with no significant difference (Figure 5) or correlation to treatment duration \((r = –0.10; P_{\text{2-tailed}} = 0.69; n = 18; \text{data not shown})\). The water-feeding treatment had no significant effect on \( A_{\text{max}} \) for the first 10 min, but \( A_{\text{max}30} \) was significantly lower (36%) than control \( A_{\text{max}30} \) (Figure 5). Feeding sucrose to the leaves resulted in a significant decrease in \( A_{\text{max}} \), relative to the control and water-feeding treatments at all treatment times (Figure 5). The reductions in \( A_{\text{max}} \) relative to control values were 26% for \( A_{\text{max}5} \), 62% for \( A_{\text{max}10} \) and 66% for \( A_{\text{max}30} \), showing stabilization at a minimum value around 3 µmol CO\(_2\) m\(^{-2}\) s\(^{-1}\) after 10 min (Figure 5).

Discussion

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

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

Figure 2. Relationships between maximal leaf net CO\(_2\) assimilation rate \( (A_{\text{max}}) \) and leaf stomatal conductance to H\(_2\)O 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; \( n = 6 \).

Figure 3. Maximal leaf net CO\(_2\) assimilation rate \( (A_{\text{max}}) \) versus leaf total soluble sugar concentration per unit dry mass (SSC\(_{\text{m}}\)) in Coffea arabica leaves \( (n = 24) \).
higher leaf SSC\textsubscript{m} in LFL-AM compared with HFL-AM (Figure 1b).

The magnitude of the decrease in \(A_{\text{max}}\) 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. 2005a), they may have been due to photo-inhibition (Ramalho et al. 1999, 2000) or \(g_s\) limitation during dry and hot midday conditions (Nunes 1988, Gutierrez et al. 1994, Vaast et al. 2005b). In our study, however, \(g_s\) did not limit \(A\) (Figure 2a) and the concomitant increase in SSC\textsubscript{m} and decrease in \(A_{\text{max}}\) (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. (2005a) 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 \(A_{\text{max}}\) 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 \(A_{\text{max}}\) 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\textsubscript{m} and \(A_{\text{max}}\), supporting the prediction that soluble sugars are involved in sink feedback down-regulation of \(A\) (Table 1, Figure 3). A stronger correlation with \(A_{\text{max}}\) 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 \(A_{\text{max}}\). Down-regulation of \(A\) by SSC\textsubscript{m} has also been reported for \textit{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 \(A_{\text{max}}\) (Figure 3). This relationship predicts that the down-regulation of \(A_{\text{max}}\) becomes steeper when SSC\textsubscript{m} reaches high values (Figure 3). Conversely, \(A_{\text{max}}\) should tend to a maximum of 13.3 \(\mu\text{mol CO}_2\text{ m}^{-2}\text{s}^{-1}\) when SSC\textsubscript{m} tends toward 0 mg g\textsubscript{DM}^{-1} (Figure 3). According to our field observations, such high \(A_{\text{max}}\) values are unusual in ‘Catuorra’ coffee leaves. This suggests that, under field conditions, SSC\textsubscript{m} does not decrease beyond a physiological minimum, probably close to the lowest SSC\textsubscript{m} value of 15.1 mg g\textsubscript{DM}^{-1} measured in this study. In other species (Baxter et al. 1995, Quilot et al. 2004), \(A_{\text{max}}\) 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 \( A_{\text{max}30} \) value (measured when leaves were still attached to the plants) was significantly higher than the stable and relatively high \( A_{\text{max}} \) 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 \( A_{\text{max}} \) 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 \( A_{\text{max}30} \) decreased in water-fed leaves relative to the control value (Figure 5). Alternatively, the decreases in \( A_{\text{max}} \) 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, \( \Psi_t \) 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 \( A_{\text{max}} \) in response to sucrose-feeding or water-feeding.

In sucrose-feeding studies, Fondy and Geiger (1977) showed that \( ^{14}\text{C}\)-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 \( A_{\text{max}} \) in response to sucrose feeding suggests that phloem sucrose concentration is involved in the feedback signaling of low sink demand. Chiu 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).

Sugarcane 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, Chiu and Bush 1998, Kehr et al. 1998) or coarse (Michon 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 \( A_{\text{max}} \) observed in coffee leaves? In our sucrose-feeding treatment, down-regulation of \( A_{\text{max}} \) occurred in leaves that had a theoretically low mesophyll soluble sugar concentration, as indicated by the high \( A_{\text{max}30} \) values of all treatments and the stable high \( A_{\text{max}5-30} \) 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 is 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 (Chiu 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 (Chiu 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 \( A_{\text{max}} \) of autotrophic coffee leaves is highly correlated to their SSC_m. A single relationship between SSC_m and \( A_{\text{max}} \) 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 \( A_{\text{max}} \) 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. 2005b). Additionally, we observed that feeding sucrose to leaves with a theoretically low SSC_m led to a similar down-regulation of \( A_{\text{max}} \). Based on this result and the theory associated with source–sink relationships and sucrose transport (Chiu and Bush 1998, Voitsekhovskaja et al. 2000, Vaughn et al. 2002, Li et al. 2003), we conclude that down-regulation of \( A_{\text{max}} \) 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 \( A_{\text{max}} \) 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 \( A_{\text{max}} \) 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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