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# EFFECT OF ATMOSPHERIC CO<sub>2</sub> ENRICHMENT ON THE ESTABLISHMENT OF SEEDLINGS OF JATOBÁ, *HYMENAEA COURBARIL* L. (LEGUMINOSAE, CAESALPINIOIDEAE)

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## Abstract

Plants grown in elevated CO<sub>2</sub> environments may exhibit photosynthetic acclimation or down regulation, which is characterised by reduced rates of photosynthesis. In most cases of CO<sub>2</sub>-induced photosynthetic acclimation, the reduced rates of photosynthesis were still higher than those detected in plants growing at ambient CO<sub>2</sub> concentrations. In this work we present a study on the behaviour of seedlings of *Hymenaea courbaril*, a late secondary/climax species that is one of the most important trees in mature tropical forests of the Americas. After germination, the seedling of *H. courbaril* increases its rate of growth due to the mobilisation of massive amounts of a storage cell wall polysaccharide (xyloglucan) from its cotyledons. In our experiments, germinated seeds were incubated in open top chambers with increased concentration of atmospheric CO<sub>2</sub> (720 ppm) (control at 360 ppm). To test the effects of the presence of the storage compound on the responses of growing seedlings, cotyledons were detached before the start of polysaccharide mobilisation and parameters such as dry mass, leaf area, CO<sub>2</sub> assimilation rates and chlorophyll *a* fluorescence were measured during 98 days. A comparison between 360 and 720ppm growing seedlings showed a significant increase in leaf area only in metaphylls of seedlings growing under higher CO<sub>2</sub>. However, a marked and persistent increase (2 fold) in photosynthesis (CO<sub>2</sub> assimilation) was observed in all cases (with or without cotyledons). Changes in the levels of sucrose have been suggested to act as a signalling mechanism that switches on/off the storage or development mode in plant tissues. Thus, the explanation for our general observation that the differential response in terms of growth of seedlings ceases to exist when storage mobilisation is functioning, might be related to the fact that higher levels of sucrose are produced as a result of carbon storage compounds degradation. By the results obtained, it appears that plants grown under enriched CO<sub>2</sub> did not acclimate and therefore under the climatic conditions forecasted on the basis of the present carbon dioxide emissions, *Hymenaea courbaril* should establish faster in its natural environment and might also serve as an efficient mechanism of carbon sequestration within the forest.

**Key Words:** Photosynthesis; CO<sub>2</sub> enrichment; *Hymenaea courbaril*; storage mobilisation; root:shoot ratio; seedling growth; cotyledons; open top chamber; xyloglucan; biodiversity

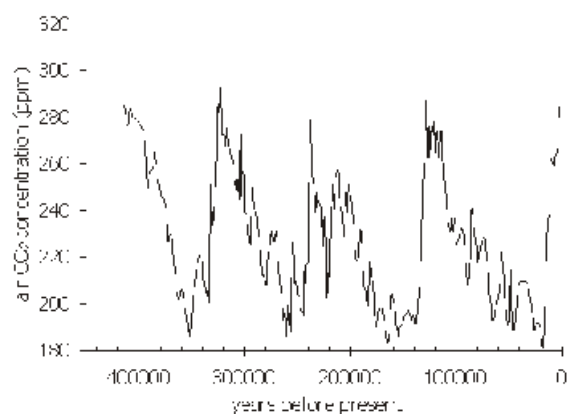
## Resumo

Plântulas de jatobá crescidas em ambiente com concentrações elevadas de CO<sub>2</sub> podem exibir aclimação fotossintética ou retro-inibição, a qual é caracterizada pela redução das taxas fotossintéticas. Em muitos casos de aclimação induzida por alto CO<sub>2</sub>, taxas reduzidas de fotossíntese são observadas, mas ainda são mais altas que aquelas detectadas em plantas crescendo em concentrações atuais de CO<sub>2</sub> atmosférico (360ppm). No presente trabalho, realizamos um estudo do comportamento de plântulas de *Hymenaea courbaril*, uma espécie secundária tardia/clímax de grande importância em florestas tropicais maduras nas Américas. Em nossos experimentos, sementes recém germinadas foram cultivadas em câmaras de topo aberto com concentrações alteradas de CO<sub>2</sub> (720 ppm e controle a 340 ppm). Para testar os efeitos da presença de compostos de reserva sobre as respostas de plântulas em crescimento, cotilédones foram destacados antes do início da mobilização do polissacarídeo de reserva (xiloglucano) e parâmetros tais como massa seca, área foliar, assimilação de CO<sub>2</sub> e fluorescência da clorofila *a*, foram medidos durante 98 dias. A comparação entre plântulas crescendo em atmosfera de CO<sub>2</sub> a 360 e 720 ppm, mostraram aumento significativo em área foliar apenas nos metáfilos de plântulas crescendo nas concentrações mais altas. No entanto, um efeito marcado e consistente de aumento na fotossíntese (assimilação de CO<sub>2</sub>) foi observado em todos os casos (com e sem cotilédones). Acredita-se que as variações nos níveis de sacarose podem funcionar como um mecanismo sinalizador de alterações nos estados de armazenamento ou desenvolvimento de tecidos vegetais. Portanto, uma possível explicação para nossas observações gerais de que uma resposta diferencial em termos de crescimento de plântulas deixa de existir quando a mobilização de reservas está em curso, pode estar relacionada com o fato de que altos níveis de sacarose são produzidos quando há degradação de compostos de reservas. De acordo com nossos resultados, parece que as plantas crescidas sob concentrações enriquecidas de CO<sub>2</sub> não aclimataram e portanto, sob as condições climáticas previstas com base nos níveis atmosféricos atuais, plântulas de *Hymenaea courbaril* deverão estabelecer mais rapidamente em seu ambiente natural e podem também servir como um mecanismo eficiente de seqüestro de carbono pela floresta. *schottiana* Mart.. Phytoseiidae foi a segunda família mais numerosa (11,2% dos ácaros coletados), sendo a mais abundante em folíolos.

**Palavras-chave:** Fotossíntese; enriquecimento CO<sub>2</sub> atmosférico; *Hymenaea courbaril*; mobilização de reservas; razão raiz:parte aérea; crescimento e estabelecimento de plântula; cotilédones; câmara de topo aberto; xiloglucano; biodiversidade

## INTRODUCTION

When plants conquered the terrestrial environment, about 400 million years ago, atmospheric CO<sub>2</sub> concentration was up to 16 times higher than today (Berner, 1991). Since then, it has varied considerably and the Vostok ice cores indicate that in the last four hundred thousand years it has oscillated between 300 and 180 mmol mol<sup>-1</sup> (ppm) (Figure 1). Since the last glacial maximum (ca. 18,000 years ago), atmospheric CO<sub>2</sub> concentration has raised to 360 ppm, but it is still relatively low, and it is not sufficient to saturate C<sub>3</sub> photosynthesis, the CO<sub>2</sub> assimilation mechanism of some 95% of terrestrial species.



**Figure 1** Vostok ice core air CO<sub>2</sub> concentration for the last four hundred thousand years. Data from Petit et al. (1999).

Photosynthesis is a physiological process by which plants sense directly the rising atmospheric CO<sub>2</sub>. As photosynthesis is a process that involves responses of the whole plant, any prediction on how a given plant will respond to increasing CO<sub>2</sub> concentration will have to take photosynthetic responses into consideration (Long, 1999; Martinez, 2002).

The rise in atmospheric CO<sub>2</sub> due to the burning of fossil fuels is expected to reach ca. 700 ppm by the year 2075 (Houghton et al., 1996). The higher CO<sub>2</sub> concentration has the potential to increase photosynthesis and to decrease photorespiration by reduction of the oxygenase activity of RUBISCO (Ribulose 1,5 bisphosphate carboxylase/oxygenase). Other expected effects of the rise in atmospheric CO<sub>2</sub> will be the reduction of stomatal conductance and enhance of water use efficiency, increase in C/N ratios and lowering of dark respiration. Altogether, these effects should result in an increase in growth, but on the other hand, such an "efficiency effect" may not be reached in all cases because of acclimation of photosynthesis (Bowes 1996).

Plants grown in elevated CO<sub>2</sub> environments may exhibit photosynthetic acclimation or down regulation, which is characterised by reduced rates of photosynthesis resulting from decreased activity and/or amount of RUBISCO (Fernandez et al., 1998; Garcia et al., 1998; Ludewig et al., 1998). CO<sub>2</sub>-induced photosynthetic

acclimation often results from insufficient plant sink strength, which can lead to carbohydrate accumulation in source leaves and the triggering of photosynthetic end product feedback inhibition (Gesch et al., 1998; Reekie et al., 1998). This phenomenon results in the redistribution of limiting resources within a plant and appears to be closely related to the source:sink unbalance. In most of the reported cases of CO<sub>2</sub>-induced photosynthetic acclimation, the reduced rates of photosynthesis were still significantly higher than those detected in plants growing at ambient CO<sub>2</sub> concentrations (Centritto and Jarvis, 1999; Egli et al., 1998; Pan et al., 1998; Rey and Jarvis, 1998; Tjoelker et al., 1998; Turnbull et al., 1998).

Forests exchange large amounts of CO<sub>2</sub> with the atmosphere and can influence and be influenced by the concentration of this gas. Recently, there has been a proliferation of literature on the effects of atmospheric CO<sub>2</sub> on forest trees that indicates a potential for a persistent enhancement of tree growth for several years. However, the only relevant long-term data sets currently available are for juvenile trees (Saxe et al., 2001)

Tropical forest dynamics include tree species specialised to occupy different microenvironmental conditions defined mainly by the gradient of light that occurs between a gap and mature forest. In the latter, one of the tree species of widest geographical and physiognomic distribution in neotropical forests is *Hymenaea courbaril* L. (common name jatobá).

*H. courbaril* is considered a late secondary species (Gandolfi et al. 1995, Aidar et al., 2001) and is one of the most important trees in mature forests in southeastern Brazil, occurring in more than 30% of 43 inventories made in the extra-Amazonian riparian forests (Rodrigues & Nave 2000). Oliveira Filho & Fontes (1999) suggest that the jatobá is a "super tramp" species regarding its distribution among the major groups of Atlantic Forest formations. The genus *Hymenaea* has originated from Africa (Langenheim, 1981), but the oldest register for Tropical America is around at least 15 million years ago, as described by the fossil amber found in the Caribbean region (Iturralde-Vinent & MacPhee, 1996). *H. courbaril* is a 20-30 m high tree with a diameter up to 200 cm, with compound leaves with 2 sessile leaflets that are shiny green. The indehiscent woody pods are 10 to 15 cm long and contain 3 to 4 hard reddish-brown seeds, embedded in a sweet and odorous pulp. The species shows wide distribution in neotropics, from the Caribbean isles, Mexico and Peru to Southeastern Brazil (Allen & Allen, 1981). In this latter country it occurs in Seasonal and Ombrophylous Dense Forests (IBGE 1992) in areas with precipitation between 600 and 4200mm year<sup>-1</sup>, average temperature between 22 and 28°C, soils with pH between 4 and 7.5. Bats pollinate the flowers. Fruits are composed by 5.9% of protein, 74.3% total carbohydrate (including high starch content) and 13.4% fiber (Langenheim 1981). Seeds are composed of ca. 40% of a cellulose like polysaccharide (xyloglucan), 20% protein and 2-4% raffinose/sucrose (Buckeridge & Dietrich, 1990, Tiné et al. 2000).

The germination of seeds from *H. courbaril* occurs inside the shaded forest and seedlings whose cotyledons have large amounts of carbohydrate reserves, form a seedling bank characteristic of late secondary and/or climax species (Kitajima 1996). The mobilisation of the storage polysaccharide during seedling development of *H. courbaril* has been studied by Tiné et al. 2000. At the beginning of imbibition, the seed starts to mobilise soluble sugars (sucrose and raffinose) and proteins, which are reserves thought to be related mainly to germination. Around 30 days after imbibition, the embryo starts to grow and at the same time as the storage polysaccharide (xyloglucan) begin to be mobilised from the cotyledons to the developing seedling. This reserve has been demonstrated to contribute to leaf development (eophyll and first metaphyll) and establishment of photosynthesis in the new autotrophic organism (Tiné et al. 2000; Santos, 2002).

In this study, we investigated the effects of the rise in atmospheric CO<sub>2</sub> on photosynthesis performance and growth of developing seedlings of *Hymenaea courbaril*, with and without cotyledons. To characterise the potential effect of CO<sub>2</sub> enriched atmosphere over the seedling establishment, our experiment was designed to evaluate the response of seedlings to two atmospheric CO<sub>2</sub> concentrations, 360 and 720 ppm and to understand whether the presence of storage compounds would interfere with the responses of growth parameters and photosynthesis. In summary, we found that photosynthetic CO<sub>2</sub> assimilation doubled when seedlings were grown under doubled CO<sub>2</sub> concentration.

## MATERIAL AND METHODS

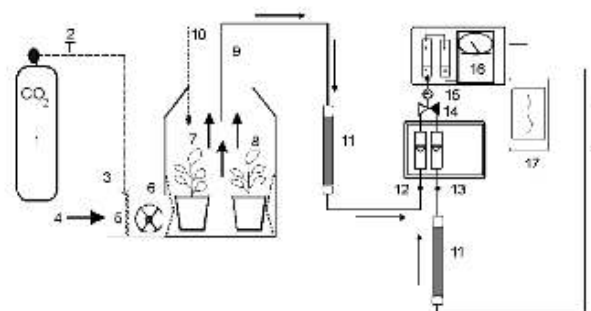
### Plant material

Seeds of *Hymenaea courbaril* were incubated at 25°C for 28 days in plastic trays covered with wet filter paper. After this period, seedlings were transferred to pots (3.8 litres; diameter 16 cm x height 15 cm) with substrate composed of fine sand:vermiculite (2:1 w/w) and watered weekly with Hoagland nutritive solution.

### Open-top chambers

Open-top chambers (OTCs) were constructed with aluminium and plastic as illustrated in Figure 2. Air sampling and automated measurement were performed at 5-min. intervals in continuous fashion throughout experimental period. Air samples were dried out and filtered before injection through a selective pump (ADC model WA-161-Mk2/3 Analytical Development Company Ltd, Hoddesdon, UK) in an ADC infra-red gas analyser model 225 MK3 (Analytical Development Company Ltd, Hoddesdon, UK). Data registration was performed with OMEGA 2020/2030 recorder (Intralab S.A., Brasil). Microenvironment was monitored with an automated station (LI-1200-Data Set Recorder; Li-Cor, Lincoln, Nebraska, USA) with sensors of temperature (LI-1000-15, Li-Cor, Lincoln, Nebraska, USA) and solar irradiation (LI-

200AS, Li-Cor, Lincoln, Nebraska, USA).



**Figure 2** Enriched CO<sub>2</sub> atmosphere system: 1 CO<sub>2</sub> cylinder; 2/3 CO<sub>2</sub> valve and injection; 4 atmospheric air entrance; 5 homogenisation chamber; 6 fan; 7/8 open-top chamber (360 and 720 ppm); 9 air sampling inside chambers; 10 temperature sensor; 11 humidity filter; 12 air flux meter from chambers; 13 air flux meter from atmosphere; 14 sampling selector valve; 15 pump; 16 Infra Red Gas Analyser (IRGA); 17 plotter.

Two OTCs were kept with 720 ppm of atmospheric CO<sub>2</sub> and other two in normal ambient CO<sub>2</sub> atmospheric concentration (360ppm) in the glasshouse of the Plant Ecophysiology and Crop Productivity Lab, Federal University of Viçosa, MG. CO<sub>2</sub> source was pressurised cylinders containing 99.8% CO<sub>2</sub>, 58.3 KgF cm<sup>-2</sup> (White Martins Ltda). Following recommendations of Langhans and Tibbitts (1997) air homogenisation and injection were done through forced ventilation in the chamber base, which renovated all volume in ca. 40 seconds.

### Experimental design

The pots were held in OTCs for 70 days in two different atmospheric CO<sub>2</sub> concentrations: 360 and 720 ppm. Each CO<sub>2</sub> treatment was performed in duplicate chambers. Ten pots with 4 seedlings each were held in each OTC. Half of the seedlings in each OTC had their cotyledons removed just after transplanting. Chambers were held inside the glasshouse and covered with shade cloth to allow maximum irradiation of 300 mmol m<sup>-2</sup> s<sup>-1</sup>. Temperature inside the glasshouse was monitored as described above.

Parameters were evaluated as described in Table 1. Photosynthesis was measured with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, Nebraska, USA). Photosynthetic evaluations were performed as curves of CO<sub>2</sub> assimilation (A) x Photosynthetic Active Radiation (PAR) and actual gas exchange (light intensity 400 mmol m<sup>-2</sup> s<sup>-1</sup> PAR). For plants growing in atmospheric CO<sub>2</sub> the concentration of 360 ppm was used as a reference concentration. For plants growing in doubled CO<sub>2</sub>, 720 ppm was used as reference concentration. Chlorophyll *a* fluorescence was measured with Modulated Chlorophyll Fluorometer (Opti-sciences, model OS5-FL). Dry mass was obtained after oven drying

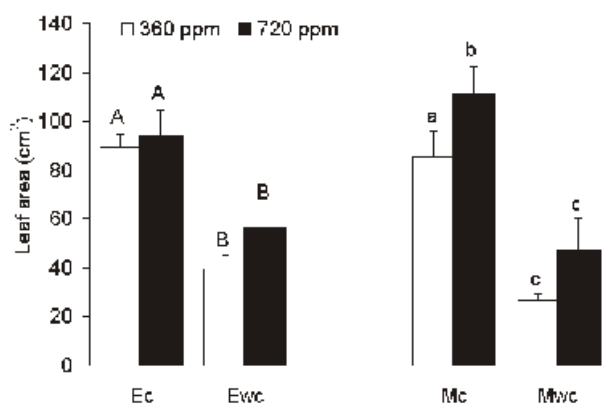
DATA	days after initiation	days of treatment	parameters		
			A	F	DW/LA
18/05/01	0	0			
15/06/01	28	0			
29/06/01	42	14	X	X	X
06/07/01	49	21	X	X	
16/07/01	59	28	X	X	
20/07/01	63	35	X	X	
27/07/01	70	42	X	X	X
06/08/01	80	49	X	X	
24/08/01	98	70	X	X	X

**Table 1** Schedule of parameters evaluation: A (net photosynthetic rate); F (chlorophyll a fluorescence); DW/LA (dry weight/leaf area ratio). Days of initiation refer to initiation of imbibition. Days of treatment refers days of CO<sub>2</sub> treatments.

plant material at 80°C. Leaf Area was measured through Portable Area meter (LI-3000A, Li-Cor, Lincoln, Nebraska, USA). The measurements were performed in the morning (between 9 - 11am) in the one of the eophylls and in the first metaphyll of each plant under treatment.

The experimental period in which seedlings of *Hymenaea courbaril* were grown under atmospheric (360ppm) and elevated (720ppm) CO<sub>2</sub> concentrations was between June and the end of August of the year 2001. Air temperature during this period varied between 15 °C and 23 °C with average of 17.9 °C.

## RESULTS & DISCUSSION

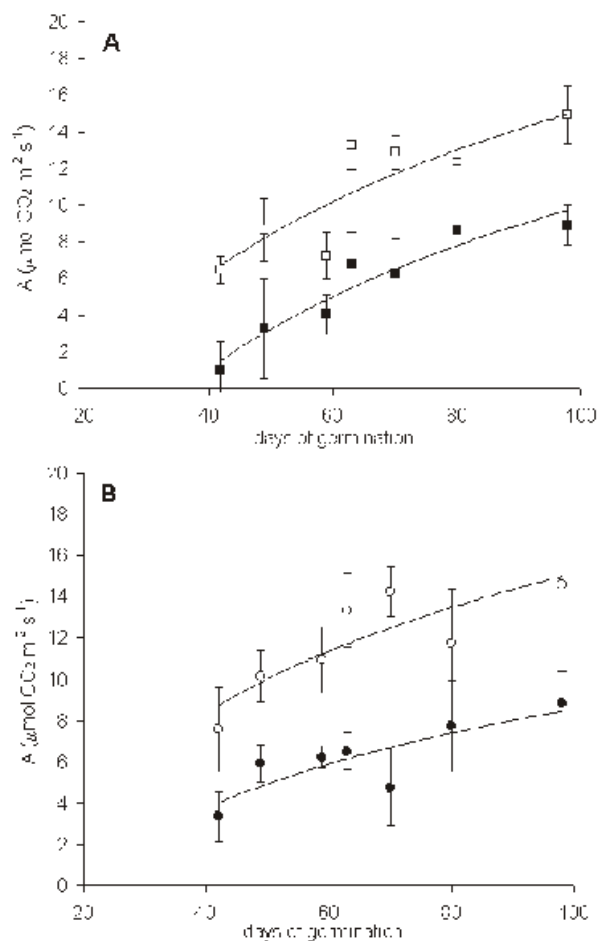


**Figure 3** Average leaf area of 98 days old seedlings of *Hymenaea courbaril* submitted to CO<sub>2</sub> atmospheric concentrations of 360 and 720 ppm. Ec eophyll, seedling with cotyledons; Ewc eophyll, seedling without cotyledons; Mc first metaphyll, seedling with cotyledons; Mwc first metaphyll, seedling without cotyledons. Statistically significant differences ( $P < 0.05$ ) between groups are indicated by different letters (ANOVA, LSD post-hoc test or *t* test); capitals = eophylls; small caps = metaphylls;  $n = 5$ .

### Effect of the presence of seed storage compounds and atmospheric enriched CO<sub>2</sub> on the leaf area

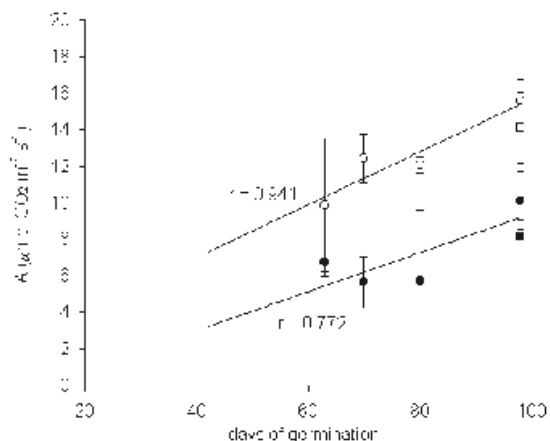
Figure 3 shows both effects on the area of the eophyll (the first pair of leaves that develop at the expenses of the carbon stored in the cotyledons), and the first metaphyll (the second leaf produced, initially with the use of some of the cotyledon storage carbon reserve and also on the basis of the photosynthesis) (Santos, 2002). As a consequence of the partial dependence of leaf development on the mobilisation of xyloglucan (the storage polysaccharide in *Hymenaea courbaril* cotyledons), an average of 50% less of leaf area was observed in both eophylls and metaphyll when cotyledons were removed. On the other hand, no effect of the rise in CO<sub>2</sub> concentration was observed on the eophylls of seedlings with cotyledons, but in this case the metaphyll presented a significant increase ( $p < 0.05$ ) in leaf area under 720ppm of CO<sub>2</sub>.

### Effect of the presence of seed storage compounds and atmospheric enriched CO<sub>2</sub> on photosynthesis



**Figure 4** Net photosynthetic rate (A) of Eophyll of *Hymenaea courbaril* seedlings by effect of two atmospheric CO<sub>2</sub> concentration: 360 ppm (filled symbols) and 720 ppm (open symbols) without (A) and with (B) cotyledons.

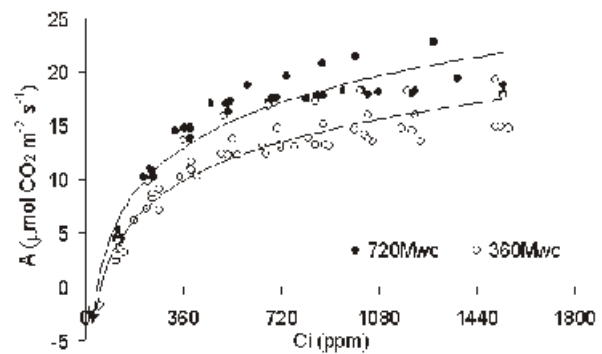




**Figure 5** Net photosynthetic rate ( $A$ ) of metaphylls of *Hymenaea courbaril* seedlings by effect of two Atmospheric  $\text{CO}_2$  concentration: 360 ppm (filled symbols) and 720 ppm (open symbols) without (squares) and with (circles) cotyledons. Each value is the mean of five individual replicates (SD). For seedlings wit cotyledons: 720 ppm,  $r = 0.941$ ; 360 ppm,  $r = 0.772$

Figures 4 and 5 show the effects of the presence or absence as well as the two  $\text{CO}_2$  concentrations on the levels of photosynthesis of eophylls during initial plant development. The presence of the storage compounds showed no effect on the maximum level reached at the end of the period. However, the slopes of the curves are higher for eophylls of seedlings growing without cotyledons (minimum level at ca. 6 and 1  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for 720 and 360 ppm respectively; Figure 4) whereas for seedlings growing with cotyledons the minimum was higher (ca. 8 and 4  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for 720 and 360 ppm respectively; Figure 5). This can be explained by the fact that leaves of seedlings growing with cotyledons have their photosynthetic system established earlier in relation to seedlings growing without cotyledons. Figure 5 shows the photosynthetic assimilation of the metaphyll, where the maximum rates were similar for seedlings with and without cotyledons when growing in the same  $\text{CO}_2$  concentration, but with consistent higher values (ca. 2 fold) for seedlings growing in the 720ppm treatment.

The  $A \times C_i$  curves indicate that photosynthetic saturation ( $A_{\text{sat}} = 0.9A_{\text{max}}$ ) and maximum photosynthesis rate ( $A_{\text{max}}$ ) occurred in a similar levels under both  $\text{CO}_2$  concentrations in eophylls of seedlings growing with and without cotyledons and in metaphylls of seedlings with cotyledons ( $A_{\text{sat}} = 15\text{--}16 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ;  $A_{\text{max}} = 17\text{--}18 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). However, in metaphylls of seedlings without cotyledons these parameters showed relatively different values: metaphylls of seedlings under 360 ppm showed lower values ( $A_{\text{sat}} = 14 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ;  $A_{\text{max}} = 16 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and under 720 ppm of  $\text{CO}_2$ , the values were higher ( $A_{\text{sat}} = 17 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ;  $A_{\text{max}} = 19 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) (Figure 6). These results support the hypothesis that the process of storage mobilisation interferes with photosynthetic maturation of metaphylls under higher  $\text{CO}_2$  concentrations.



**Figure 6** Net Photosynthetic saturation ( $A_{\text{sat}} = 0.9A_{\text{max}}$ ) and maximum net photosynthesis rate ( $A_{\text{max}}$ ) for metaphyll of seedlings of *Hymenaea courbaril* without (Mwc) cotyledons submitted to 360 ( $n = 5$ ) and 720 ppm  $\text{CO}_2$  ( $n = 4$ ). Measurements were performed in 98 days old seedlings.

The observations regarding slopes of photosynthetic assimilation are corroborated by the data on chlorophyll *a* fluorescence. The  $F_v/F_m$  ratios accounts for an estimate of the photochemical efficiency of PSII. Santos (2002) has previously determined that metaphylls of seedling of *H. courbaril* reach maturity when the ration  $F_v/F_m$  attain 0.77 (Figure 7A). Taking this parameter into consideration, the observations presented here showed that seedlings growing without cotyledons under 360ppm (Figure 7D), both the eophyll and metaphyll took about 80 days to reach maturity, whereas seedlings growing without cotyledons, but in atmosphere enriched with  $\text{CO}_2$  (720ppm, Figure 7E) reached maturity at ca. 59 days. However, when plantlets were grown with their cotyledons these differences were not observed and the ones growing under 360ppm of  $\text{CO}_2$  reached maturity at 59 days.

These results suggest that the rising in  $\text{CO}_2$  concentration partially compensates the effect of the lack of the reserves on the maturation of photosynthetic apparatus.

#### Effect of the presence of seed storage compounds and atmospheric enriched $\text{CO}_2$ on the shoot:root ratio

The seedlings of *H. courbaril* showed a marked difference in respect to shoot:root ratios when submitted to the different treatments (Figure 8). Independently of the concentration of atmospheric  $\text{CO}_2$ , seedlings growing without cotyledons maintained a constant shoot:root ratio during all the experimental period (approximately 0.2). On the other hand, the presence of the cotyledons (and reserves) strongly affected the carbon partitioning in the growing seedlings. In seedlings growing under 360ppm of  $\text{CO}_2$ , the investment of carbon was relatively higher in the shoot while storage mobilisation occurred (46days) and the "average" ratio (0.2) was attained at 63 days. When seedlings were growing under 720ppm of  $\text{CO}_2$ , the average ratio was reached only after 90 days, indicating that investment in the shoot continued for a longer period as a consequence of the change in the carbon dioxide atmosphere.

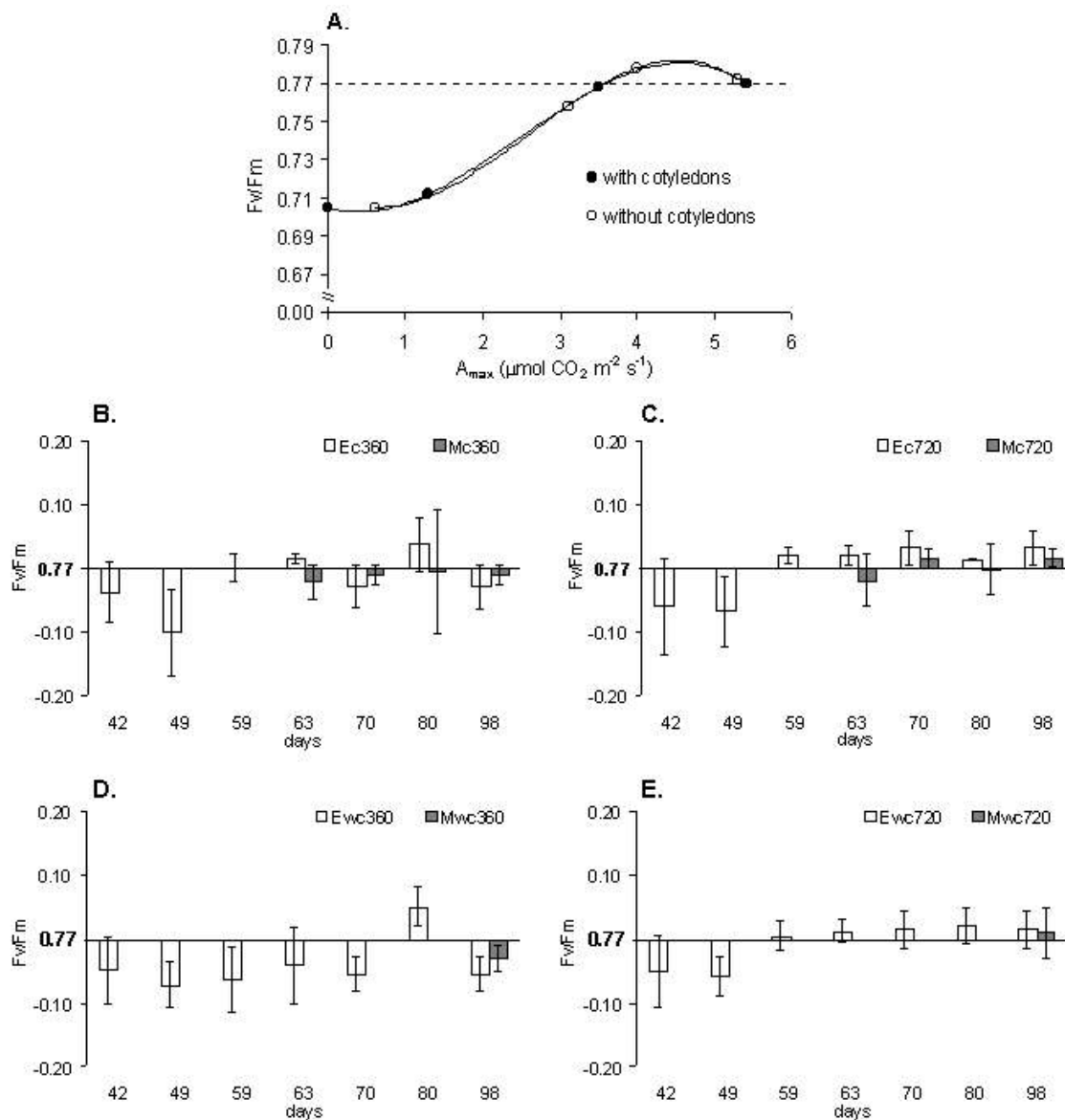


Figure 7 A.) relationship between  $F_v/F_m$  ratio and maximum photosynthetic assimilation ( $A_{max}$ ) during the period of leaf expansion (32-54 days of growth) in seedlings growing with and without cotyledons, indicating the leaf photosynthetic mature state  $F_v/F_m = 0.77$  (adapted from Santos, 2002). B-E) Photochemical efficiency of PSII ( $F_v/F_m$ ) in seedlings of *Hymenaea courbaril* subjected to the different treatments: B.) eophyll (E) and metaphyll (M) from seedling with cotyledons (c) subjected to 360 ppm of  $\text{CO}_2$ ; C.) eophyll and metaphyll from seedling with cotyledons subjected to 720 ppm of  $\text{CO}_2$ ; D.) eophyll and metaphyll from seedling without cotyledons (wc) subjected to 360 ppm of  $\text{CO}_2$ ; E.) eophyll and metaphyll from seedling without cotyledons subjected to 720 ppm of  $\text{CO}_2$ ; v

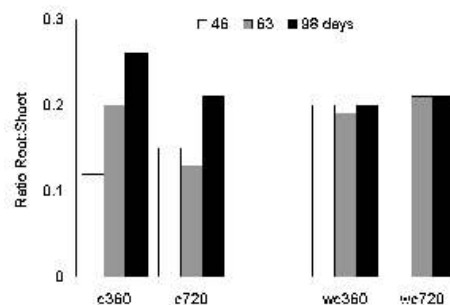
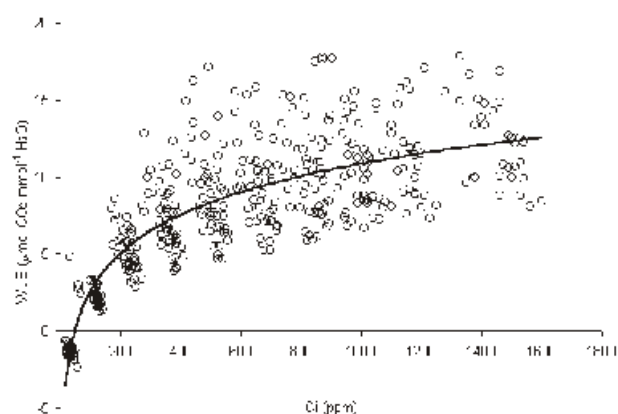


Figure 8 Ratio root:shoot in seedlings of *Hymenaea courbaril* subjected to two atmospheric concentration: 360 and 720 ppm. c seedling with cotyledons; wc seedling without cotyledons.

### Water use efficiency

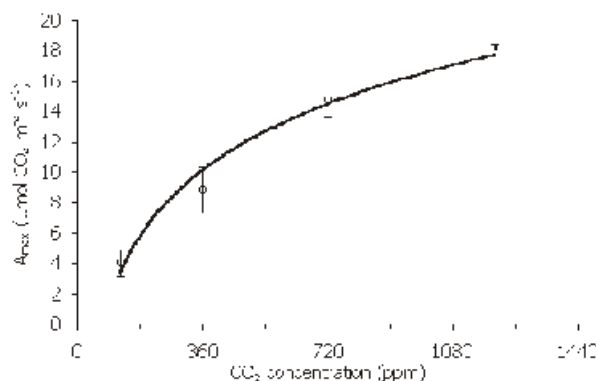
Using the parameters net photosynthetic rate ( $A$ ) and transpiration rate ( $E$ ) we calculated the instantaneous water use efficiency ( $WUE = \mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ ) of eophylls and metaphylls of *H. courbaril* under increasing  $\text{CO}_2$  concentrations. A curve with a parabolic shape was obtained, indicating an increase in the WUE with increase of  $C_i$  (internal  $\text{CO}_2$  concentration) (Figure 9). The curve indicates an average increase of 34.9% in the WUE from 360 to 720 ppm. This suggests that as atmospheric  $\text{CO}_2$  concentration rise, seedlings of *H. courbaril* will have lower transpiration rates as consequence of lower stomatal conductance, therefore improving their water use efficiency. As a result, this species might be able to tolerate dryer and more open environments, which should allow them to better cope with drought stress or a more seasonal climate.



**Figure 9** Relationship between instantaneous water use efficiency ( $WUE$ ,  $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ ) and internal  $\text{CO}_2$  concentration ( $C_i$ , ppm) for eophylls and metaphylls of *Hymenaea courbaril* seedlings submitted to two  $\text{CO}_2$  concentrations;  $r^2 = 0.715$ . Figure 9 Relationship between instantaneous water use efficiency ( $WUE$ ,  $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ ) and internal  $\text{CO}_2$  concentration ( $C_i$ , ppm) for eophylls and metaphylls of *Hymenaea courbaril* seedlings submitted to two  $\text{CO}_2$  concentrations;  $r^2 = 0.715$ .

### Photosynthesis under different atmospheric $\text{CO}_2$ concentrations

A calibration of the photosynthetic responses based on the results obtained from eophylls of seedlings that had actually grown under 360 and 720 ppm with the responses of  $A_{\text{max}}$  obtained using the Infra Red Gas Analyser at 120 and 1200 ppm  $\text{CO}_2$  concentration showed an steady increase in the maximal assimilation rates compatible with a proportional correlation between atmospheric  $\text{CO}_2$  concentration, assimilation and growth (Figure 10).



**Figure 10** Responses of the light saturated net photosynthesis ( $A_{\text{max}}$ ) for eophylls from *Hymenaea courbaril* seedlings with cotyledons to  $\text{CO}_2$  concentrations ( $R^2 = 0.979$ ). Values for 360 and 720 ppm  $\text{CO}_2$  concentrations were measured in the open top chambers; values for  $\text{CO}_2$  concentration of 120 and 1200 ppm were obtained through the  $A \times C_i$  curves simulated by IRGA (Li-Cor 6400).

### A raise in $\text{CO}_2$ concentration increases photosynthesis and growth that probably increases carbon sequestration capacity of seedlings of *H. courbaril*

According to Chambers et al. (1998), increased rates of photosynthesis and biomass will probably allow long-living perennial species from forest ecosystems to sequester large amounts of carbon within their wood. This is likely to help in mitigation of the  $\text{CO}_2$  emissions, since on the basis of several different types of empirical data, it has been concluded that current rates of carbon sequestration are robust and that future rates will increase with increasing atmospheric  $\text{CO}_2$  concentrations. Fan et al. (1998) calculated that the broad-leaved forested region of North America possess a current carbon sink that can annually remove all the  $\text{CO}_2$  emitted from fossil fuel combustion in both Canada and the United States. On another study, it has been shown that average forest biomass in the tropics has increased substantially over the last 40 years and that growth in the Neotropics alone can account for 40% of the missing carbon in the planet (Phillips et al. 1998).

In a review, Idso (1999) reported that the average growth enhancement of three tree species exposed to twice-ambient level of atmospheric  $\text{CO}_2$  was 90% after five years, which is consistent with data reported for mature trees growing near  $\text{CO}_2$ -emitting springs and vents. Lovelock et al. (1998) working with ten tropical tree species grown in open-top chambers in a tropical forest in Panama showed that leaf area ratio was much lower in trees grown in double  $\text{CO}_2$  concentration than that of trees grown in ambient  $\text{CO}_2$ , indicating that even with less resources invested in their leaves, trees grown in elevated  $\text{CO}_2$  still synthesised enough carbohydrate to support biomass production equivalent to that of ambiently-grown trees, suggesting an increase in photosynthetic efficiency in the doubled  $\text{CO}_2$  environment.

Our results confirm the general view that the doubling in ambient CO<sub>2</sub> concentration will provide a proportional increase in the capacity of *Hymenaea courbaril* to sequester carbon during the establishment of seedlings. The fact that our investigation took into consideration the presence of an important source of carbon in the form of reserves of carbohydrate, allowed us to probe the importance of the status of the carbohydrate reserves in a situation where the CO<sub>2</sub> concentration is higher. The presence of storage compounds had a significant effect on *H. courbaril* metaphyll development, since its eophylls, which are relatively more dependent on the reserves of the cotyledons, had their development apparently buffered by the storage compounds. This was reflected in the status of the photosynthetic apparatus of eophylls and metaphylls, denoting a close correlation between the two phenomena.

One has also to take into consideration the fact that the observed raise in growth and photosynthesis might not persist if *H. courbaril* has the capacity to acclimate. Although it did not happen during our experiments, the fact that the presence of high concentrations of sugars (as a consequence of storage polysaccharide mobilisation - Tiné et. al. 2000, Santos, 2002) buffered the effect of the higher CO<sub>2</sub> concentration might mean that *H. courbaril* could reach acclimation in older stages of its life cycle. Thus, although our experiments point out that *H. courbaril* might be an important species in carbon sequestration in tropical forests in the future, more experiments must be done with the tree in order to confirm this hypothesis.

### Relevance for effects on biodiversity

Seeds and seedlings are implicated in various ecological phenomena, extending beyond population process to influence the distribution, dynamics and diversity. With increasing evidence of the impact of climate change upon vegetation, there is a need to model and predict regenerative processes at these scales, which is a critical feature of plant community dynamics. Regarding the relationship between the regenerative strategies and vegetation dynamics, the most important contribution of seedling regeneration is likely to be the establishment of founder populations during the initial open stages of succession (pioneer).

In a meta-analysis of the growth response of wood shade and light tolerant species to elevated CO<sub>2</sub>, Kerstiens (2001) found that the shade-tolerant ones respond with a bigger increase in biomass (ca. 51%) in relation to shade-intolerant species (ca.18%). Our results seem to corroborate Kerstiens hypothesis, since we found that leaves of growing seedlings of *H. courbaril*, respond to high CO<sub>2</sub> (720ppm) by doubling CO<sub>2</sub> assimilation, with higher leaf area and dry mass. The increment in biomass is probably correlated with the decrease of photorespiration, which occurs in high CO<sub>2</sub> concentrations as a result of lower oxygenation activity of the RUBISCO. Ammonia recycling is lowered concomitantly with the decrease in

photorespiration, lowering amino acid biosynthesis, slowing down the development and increasing biomass. Another explanation for this behaviour is that their leaves present saturation of assimilation rate only to atmospheric CO<sub>2</sub> at concentrations as high as 1000ppm, independently of the presence or absence cotyledons (data not shown).

It is becoming increasingly clear that the future behaviour of plants to face the raise in atmospheric CO<sub>2</sub> can be evaluated through the performance of their photosynthetic apparatus. The results presented in Figure 10 suggest that the performance of leaves of *H. courbaril* to increasing concentrations of CO<sub>2</sub> may be an interesting approach to evaluate the capacity of plants to grow differentially according to the atmospheric concentration of CO<sub>2</sub>. We have measured the saturation level of some other tropical trees from the rain forest (*Caesalpinia echinata*, *Piptadenia gonoacantha*, *Tibouchina granulosa*, *T. pulchra*) and all of them saturate at relatively high CO<sub>2</sub> concentrations (ca. 800ppm). On the other hand, the bromeliad *Alcantaria imperialis* for example, saturated at about 500ppm. It is not yet known whether species like these will have a growth rate proportional to their response to CO<sub>2</sub>, as measured in the atmospheric CO<sub>2</sub> facilities using the IRGA, but in case this pattern is confirmed for other species besides *H. courbaril* this will be a valuable tool to predict the impact on plant communities and therefore possible changes in plant biodiversity patterns.

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