

## Effects of Elevated CO<sub>2</sub> and Defoliation on Compensatory Growth and Photosynthesis of Seedlings in a Tropical Tree, *Copaifera aromatica*<sup>1</sup>

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### ABSTRACT

After defoliation by herbivores, some plants exhibit enhanced rates of photosynthesis and growth that enable them to compensate for lost tissue, thus maintaining their fitness relative to competing, undefoliated plants. Our aim was to determine whether compensatory photosynthesis and growth would be altered by increasing concentrations of atmospheric CO<sub>2</sub>. Defoliation of developing leaflets on seedlings of a tropical tree, *Copaifera aromatica*, caused increases in photosynthesis under ambient CO<sub>2</sub>, but not under elevated CO<sub>2</sub>. An enhancement in the development of buds in the leaf axils followed defoliation at ambient levels of CO<sub>2</sub>. In contrast, under elevated CO<sub>2</sub>, enhanced development of buds occurred in undefoliated plants with no further enhancement in bud development due to exposure to elevated CO<sub>2</sub>. Growth of leaf area after defoliation was increased, particularly under elevated CO<sub>2</sub>. Despite this increase, defoliated plants grown under elevated CO<sub>2</sub> were further from compensating for tissue lost during defoliation after 5½ weeks than those grown under ambient CO<sub>2</sub> concentrations.

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### RESUMEN

Luego de ser defoliadas por herbívoros, algunas plantas muestran aumentos en las tasas de fotosíntesis y crecimiento que les permiten compensar por el tejido perdido y así mantener su aptitud (*fitness*), relativo a plantas no defoliadas que están compitiendo con ellas. Nuestro propósito era determinar si la fotosíntesis compensatoria y el crecimiento eran alterados, o no, por incrementos en la concentración de CO<sub>2</sub> atmosférico. La defoliación de folíolos en desarrollo, de plántulas del árbol tropical *Copaifera aromatica*, causó incrementos en fotosíntesis bajo CO<sub>2</sub> ambiente, pero no bajo CO<sub>2</sub> elevado. Una mejora en el desarrollo de yemas localizadas en el eje de las hojas ocurrió, luego de que las plantas en niveles de CO<sub>2</sub> ambiente fueran defoliadas. En cambio, una mejora en el desarrollo de yemas ocurrió en plantas control no defoliadas creciendo bajo CO<sub>2</sub> elevado, sin que se observaran mejoras adicionales en la producción de yemas en plantas defoliadas. El crecimiento del área foliar después de la defoliación fue incrementado, especialmente bajo CO<sub>2</sub> elevado. Pero, a pesar de este aumento, después de 5½ semanas las plantas defoliadas que crecieron bajo CO<sub>2</sub> elevado estaban más lejos de haber compensado por el tejido perdido durante la defoliación, que aquellas que crecieron bajo concentraciones de CO<sub>2</sub> ambiente.

*Key words:* compensatory growth; *Copaifera aromatica*; elevated CO<sub>2</sub>; Panama; photosynthesis; tropical forest.

DEFOLIATION DUE TO HERBIVORY may reduce plant fitness (Marquis 1984, Doak 1992, Maurico *et al.* 1993). One strategy that can mitigate the long-term negative impact of herbivore attack is the mobilization of stored reserves and altering of carbon allocation patterns to accelerate the replacement of damaged tissue (reviewed by Trumble *et al.* 1993, Rosenthal & Kotanen 1994). The extent of mo-

bilization of stored reserves and changes in carbon allocation to support growth of new leaves depends on how herbivory influences the source-sink relationships within the plant (Trumble *et al.* 1993, Honkanen *et al.* 1994). For example, removal of mature leaves will reduce the size of the photosynthetic area and hence the carbon source, potentially leading to a reduction in growth. In contrast, removal of young, immature leaves or buds could decrease the strength of carbon sinks and may stimulate compensatory new leaf growth.

Source-sink relationships also are altered in plants when they are grown under elevated CO<sub>2</sub> (Arp 1991). Elevated concentrations of CO<sub>2</sub> have generally been observed to increase levels of non-structural carbohydrates (source) due to enhanced rates of photosynthesis (Körner & Miglietta 1994,

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Xu *et al.* 1994). Because both elevated CO<sub>2</sub> and defoliation caused by herbivory alter strengths of sources and sinks of carbohydrates, it is likely that elevated CO<sub>2</sub> will modify plant responses to defoliation. We expected that increased rates of photosynthesis and carbohydrate accumulation under elevated CO<sub>2</sub> would enhance rates of compensatory growth after the defoliation of young, developing leaves. Enhanced compensatory growth under elevated CO<sub>2</sub> could decrease the effect of herbivore defoliation on plant fitness. To test the hypothesis that elevated CO<sub>2</sub> increases the extent of compensatory photosynthesis and growth, we simulated herbivory on seedlings of the tropical tree species, *Copaifera aromatica*. This species belongs to the family, Leguminosae, which has the greatest number of species for a single family in Neotropical forests (Gentry 1982). Young leaves are the primary site of folivore damage in terrestrial tropical forests (Coley & Kursar 1996; cf. Feller & McKee 1998 for defoliation caused by wood-boring insects in tropical mangrove forests). Although no specific information on herbivore damage for *C. aromatica* is available, tropical trees within the Leguminosae can have up to 20 percent of their leaf area damaged during leaf expansion (Coley & Kursar 1996). A congener of *C. aromatica* (*C. langsdorffii*) which occurs in Brazil has been observed to experience high levels of leaf damage from microlepidoptera (leaf-tiers from the families Oecophoridae and Gelechiidae; Macedo & Langenheim 1989). The spectacularly destructive gelechiids, reported to defoliate 50 percent of the studied trees in one season (Macedo & Langenheim 1989), preferred to utilize young, not fully expanded leaves. In this study of *C. aromatica*, we removed the developing leaflets on the youngest leaf of seedlings growing under elevated and ambient levels of CO<sub>2</sub>. After the simulated herbivory we monitored photosynthesis and regrowth.

## MATERIALS AND METHODS

**PLANT MATERIAL AND GROWTH CONDITIONS.**—We used seedlings of the tropical tree, *Copaifera aromatica* Dwyer (Leguminosae, Caesalpinoideae). This species occurs in young, seasonally dry forest, below 1000 m in altitude. Its leaves are pinnately compound, with 8–12 leaflets/leaf in mature trees and 4–8 leaflets/leaf in seedlings. Seeds were collected in March 1994 on the bank of the Curundu River, Parque Natural Metropolitano, Panamá. Seeds were stored for nine months after which they were germinated in trays filled with a soil-sand mix (1:1) at low light intensities (20–50  $\mu\text{mol}/\text{m}^2/\text{sec}$ ). Ger-

mination occurred between 1 February and 28 March 1995. In April, plants were gradually transferred from low light intensities to those light intensities used in the experimental growth chambers (see below). On 28 April 1995, seedlings were transferred to 40-cm high, 15-liter pots. The bottoms of the pots were filled with gravel 1–2 cm in diameter to facilitate drainage. Soil was collected locally and mixed with sand (2:1 mix) to improve drainage.

At the beginning of the experiment, seedlings were randomly assigned to four open-top chambers on Barro Colorado Island (Panamá) in an artificial forest clearing whose west, north, and south sides were surrounded by vegetation *ca* 8 m tall. The east side was open, allowing direct sunlight to reach the chambers from 0700–1430 h. The open-top chambers consisted of an aluminum frame (1.8 m in diameter and 2 m in height) covered with clear polyethylene film. The chambers were covered with shade cloth which reduced the incident solar radiation by *ca* 80 percent. Photon flux density was measured in the open-top chambers with gallium-arsenide photodiodes (Pontallier 1990) and outside the chambers with a quantum sensor (Li-189, Li-Cor, Lincoln, Nebraska, U.S.A.) over three days. All five sensors were connected to an electronic datalogger (Li-1000 Datalogger, Li-Cor, Lincoln, Nebraska, U.S.A.). Average ambient photon flux density was  $21.1 \pm 4.4 \text{ mol}/\text{m}^2/\text{d}$  ( $\bar{x} \pm \text{SE}$ ) while inside the chambers it was  $4.4 \pm 0.5 \text{ mol}/\text{m}^2/\text{d}$ . For each pair of chambers, ventilation was provided by blowers in aluminum housings connected to the chambers by 10-cm diameter PVC tubing. In order to raise the CO<sub>2</sub> concentration in one of each pair of chambers, pure CO<sub>2</sub> was injected continuously at a rate of 2 liters/min into the ventilation stream. CO<sub>2</sub> concentration was measured between 0900 and 1600 h over five days. The mean elevated CO<sub>2</sub> concentration was  $861 \pm 34 \mu\text{l}/\text{liter}$  (range 600–1000  $\mu\text{l}/\text{liter}$ ) and the mean ambient CO<sub>2</sub> concentration was  $386 \pm 8 \mu\text{l}/\text{liter}$  (range 350–500  $\mu\text{l}/\text{liter}$ ). Plants were watered daily. At the beginning of the experiment, an average of  $5.42 \text{ g} \pm 0.05$  of OSMOCOTE (14:14:14 N:P:K, Hyponex Corporation, Atlanta, Georgia, U.S.A.) was added to the soil surface of each pot. Directly before the experiment and on days 4, 14, and 37, plants were sprayed with a commercially available garden fungicide (Chevron-ORTHOCIDE, Captan 48.9% of weight, Monsanto, San Ramon, California, U.S.A.) at a concentration of 1 g/L of water. Within each chamber, pots were moved to a new position each week in order to reduce any effects of variations in growth conditions.

**EXPERIMENTAL PROCEDURE.**—Two treatments were imposed on the seedlings. Seedlings were grown either at ambient or elevated CO<sub>2</sub> concentrations. After 14 days under these conditions, they then either were exposed to simulated herbivory or left intact. Herbivory was simulated by removing the leaflets, but not the central midrib, of the youngest leaf on each plant using scissors. Scissors were sterilized before removing the leaflets from each plant. After simulated herbivory, plants had 1–2 remaining leaves (6–12 remaining leaflets). Mean leaf area remaining on defoliated plants was  $59.1 \pm 0.2\%$  of their original leaf area. Plants were grown under elevated or ambient CO<sub>2</sub> concentrations for a period of 53 days, over which growth and photosynthesis were measured periodically.

Leaf area was measured non-destructively on every plant on days 1, 14, 15, 22, 29, 37, and 49. Leaves were held between two rectangles of transparent plastic and leaf shapes were traced onto a transparency placed on top of the plastic. The transparencies were photocopied, the leaf shapes cut out with scissors, and their area measured with a leaf area meter (Li-3000A, attached to a transparent belt conveyer Li-3050A, Li-Cor, Lincoln, Nebraska, U.S.A.). Relative growth rate of leaf area was calculated for each time interval as  $[\ln(\text{leaf area}_{T2}) - \ln(\text{leaf area}_{T1})] / (T2 - T1)$ , where T is time in days. The number of axillary leaf buds released from dormancy on each plant was counted on days 25, 29, 34, 39, 44, and 51 of the experiment. On days 9, 11, 23, 27, and 47, photosynthetic rates of mature leaves were measured *in situ* using a portable photosynthesis measuring system (CI-301PS, CID Inc., Vancouver, Washington, U.S.A.) in the closed mode with a 0.5 liter chamber. Measurements were made on one randomly chosen mature leaf per plant between 0900–1500 h when natural light levels were relatively uniform within the open-top chambers. On each day of measurement, the same leaf on each plant was utilized. Mean light intensity within the chambers during the measurements was  $208 \pm 5 \mu\text{mol}/\text{m}^2/\text{sec}$  (range 12–758  $\mu\text{mol}/\text{m}^2/\text{sec}$ ,  $N = 584$ ). Mean air temperature was  $33 \pm 0.3^\circ\text{C}$  (range 26–39,  $N = 735$ ) and mean relative humidity was  $63 \pm 0.3\%$  (range 51–83%,  $N = 735$ ). Approximately 6–15 cm<sup>2</sup> of leaf area was placed in the gas-exchange chamber. Photosynthesis was measured with a flow rate of 0.5 liter/min for 30 sec after the CO<sub>2</sub> concentration within the chamber had stabilized.

The daily courses of photosynthesis in mature leaves also were measured under controlled condi-

tions in growth cabinets 20–21 days after simulated herbivory (days 34 and 35). Five plants per treatment were randomly chosen and placed in the growth cabinets at ca 0800 h. Photon flux density within the growth cabinets was between 440–460  $\mu\text{mol}/\text{m}^2/\text{sec}$ , air temperature was maintained at 30°C, and relative humidity at 80 percent. The concentration of CO<sub>2</sub> was 775  $\mu\text{l}/\text{liter}$  (range 760–790) for elevated CO<sub>2</sub>-grown plants and 390  $\mu\text{l}/\text{liter}$  (range 380–400) for ambient CO<sub>2</sub>-grown plants. Measurements of photosynthetic rates were made *ca* every two hours until 0630 h in the same manner as described above.

At the beginning of the experiment, ten seedlings were harvested to obtain initial dry weights and determined biomass allocation. At the completion of the experiment, all plants were harvested, dried, and weighed. Average relative growth rate (g/g/wk) was calculated as the  $[\ln(\text{final weight}) - \ln(\text{average initial weight})] / 7.5 \text{ wk}$ . Leaf area ratio (m<sup>2</sup>/kg) was calculated as the ratio of the final leaf area to the final plant weight. Specific leaf area (m<sup>2</sup>/kg) was calculated as the ratio of total leaf area to total leaf dry weight. Nitrogen contents of mature leaves used for photosynthesis measurements were determined at the University of Würzburg (Germany) using a CHN Elemental Analyzer (Heraeus, Hanau, Germany).

**DATA ANALYSIS.**—Biomass accumulation and leaf nitrogen contents were analyzed using analysis of variance (ANOVA). CO<sub>2</sub> concentration and simulated herbivory were considered as fixed effects and pairs of chambers as random blocks. For analysis of relative growth rate of leaf area and buds per plant, multivariate analysis of variance was used (MANOVA), with relative growth rates or number of buds for each time period as independent variables. Photosynthetic rates under controlled conditions within growth cabinets were analyzed using ANOVA, with CO<sub>2</sub> concentration and defoliation as fixed effects and time of day as a continuous, random variable. Adequacy of the models was assessed by the distribution of residuals. All analyses were done using the statistical package, Data Desk 4.0 (Data Description Inc., Ithaca, New York, U.S.A.).

## RESULTS

Growth in leaf area of undefoliated plants was enhanced under elevated CO<sub>2</sub> compared to those growing under ambient CO<sub>2</sub> concentrations (Figs. 1a, b). After defoliation, rates for leaf area growth

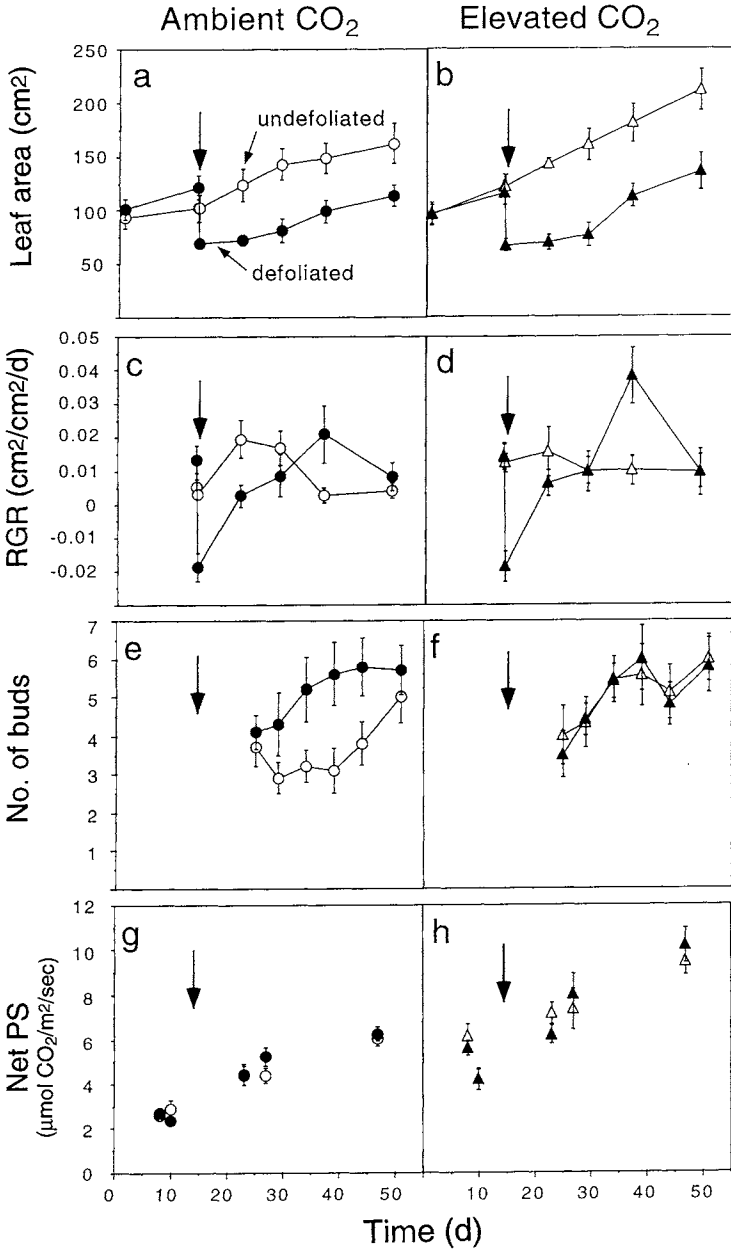


FIGURE 1. Mean leaf area (a and b), relative growth rate of leaf area (c and d), number of buds per plant (e and f), and *in situ* net photosynthetic rate (g and h) of plants grown under ambient (circles) and elevated (triangles) CO<sub>2</sub> concentrations. Plants were either subjected to simulated herbivory by defoliation on day 14 of the experiment (filled symbols) or left intact (undefoliated; open symbols). Arrows indicate the time of defoliation. Bars are the standard errors of the means of 9–10 plants.

in defoliated plants were lower compared to the undefoliated plants for ca 15 days, after which relative growth rate of leaf area increased in both elevated and ambient CO<sub>2</sub> (Figs. 1c, d). Sixteen to

23 days after defoliation (days 30–37), leaf growth was greater in defoliated plants compared to undefoliated plants (main effect of defoliation:  $F_{1,1} = 228.7, P = 0.042$ ). The increase in leaf area growth

of defoliated plants during this time tended to increase faster in plants growing under elevated CO<sub>2</sub> compared to those growing in ambient CO<sub>2</sub> concentrations, although this trend was not significant (CO<sub>2</sub> x defoliation interaction:  $F_{1,1} = 11.96$ ,  $P = 0.179$ ). Similar rates for leaf area growth of defoliated plants in elevated and ambient CO<sub>2</sub> concentrations were resumed between days 38 and 48. Despite the enhancement of relative growth of leaf area in defoliated plants, especially under elevated CO<sub>2</sub>, this response was not sufficient to compensate for the area lost in simulated herbivory by the completion of the experiment (Figs. 1a, b).

The release of leaf buds from dormancy and their development was influenced by both growth under elevated CO<sub>2</sub> and defoliation (Figs. 1e, f). Under ambient CO<sub>2</sub>, defoliation resulted in an increase in active buds *ca* ten days after defoliation. The number of active buds per plant on defoliated plants was more than double that of the undefoliated plants 15–29 days after defoliation (Scheffe posthoc test for differences between defoliated and undefoliated plants at ambient CO<sub>2</sub>: day 29,  $P = 0.020$ ; day 36,  $P = 0.002$ ; day 43,  $P = 0.081$ ). Under ambient CO<sub>2</sub>, higher numbers of active buds in defoliated plants were coincident with increasing relative growth rate of leaf area (Figs. 1a, c). In contrast, under elevated CO<sub>2</sub>, the number of active buds per plant increased in both defoliated and undefoliated plants, and was similar to defoliated plants growing under ambient CO<sub>2</sub>. In plants grown under elevated CO<sub>2</sub>, increased numbers of active buds per plant in undefoliated plants were not associated with increased relative growth rate of leaf area as was observed in defoliated plants.

Plants growing under elevated CO<sub>2</sub> had generally higher rates of photosynthesis compared to those growing under ambient CO<sub>2</sub> conditions (Figs. 1g, h). There were no detectable differences in net photosynthesis between undefoliated and defoliated plants. Net photosynthesis measured during the course of the experiment increased over time. Light conditions were highly variable over the experiment, and increases in photosynthesis over time may have been due to variations in light levels over measurement days. However, slow maturation of leaves and concurrent increases in photosynthetic rates of leaves also could have been responsible. Measurements of net photosynthesis for mature leaflets under controlled conditions at the end of the experiment showed a strong decrease in net photosynthesis throughout the day (Fig. 2). There was no significant influence of defoliation on net photosynthesis at elevated CO<sub>2</sub>, but there was a

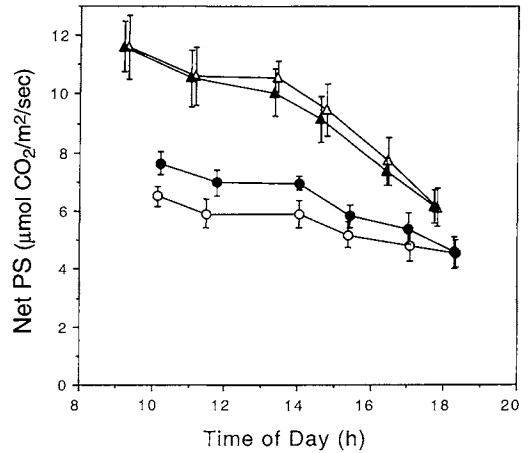


FIGURE 2. Mean net photosynthetic rate measured under controlled conditions over days 34 and 35 of the experiment. Plants were grown under ambient (circles) and elevated (triangles) CO<sub>2</sub> concentrations, and either were subjected to simulated herbivory by defoliation on day 14 of the experiment (filled symbols), or left intact (undefoliated; open symbols). Photon flux densities under controlled conditions were between 440–460 µmol/m<sup>2</sup>/sec. Values are the means of five plants and bars are standard errors.

significant, ten percent increase in net photosynthesis in defoliated plants grown at ambient CO<sub>2</sub> (CO<sub>2</sub> X defoliation interaction:  $F_{1,114} = 4.095$ ,  $P = 0.045$ ). Analysis of the nitrogen content in mature leaves showed a small increase (*ca* 10%) in the nitrogen concentration in leaves of defoliated plants at ambient CO<sub>2</sub> compared to controls (CO<sub>2</sub> X defoliation interaction:  $F_{1,1} = 382.5$ ,  $P = 0.033$ ). In contrast, under elevated CO<sub>2</sub> leaves of defoliated plants tended to have similar nitrogen concentrations as undefoliated plants (Table 1).

At harvest, plant biomass and average relative growth rate for biomass of undefoliated plants were enhanced in elevated CO<sub>2</sub>-grown plants compared to those grown under ambient CO<sub>2</sub> (CO<sub>2</sub> x defoliation interaction for biomass:  $F_{1,1} = 610.9$ ,  $P = 0.026$ ; and relative growth rate,  $F_{1,1} = 61.56$ ,  $P = 0.081$ ; Table 1). Defoliation resulted in lower biomass accumulation and relative growth rate under both elevated and ambient CO<sub>2</sub> conditions. However, the difference between defoliated and undefoliated plants was greater under elevated CO<sub>2</sub>. The proportion of defoliated to control biomass was 77 percent in ambient CO<sub>2</sub>-grown plants and 67 percent in elevated CO<sub>2</sub>-grown plants. Average relative growth rate of defoliated plants as a proportion of the undefoliated plants was 72 percent under ambient CO<sub>2</sub> and 53 percent under elevated

TABLE 1. Characteristics of seedlings of *Copaifera aromatica* at harvest after growth under ambient or elevated CO<sub>2</sub> concentrations for 50 days. Plants either were subjected to simulated herbivory by defoliation of developing leaflets on day 14 of the experiment (Defoliated), or left intact (Undefoliated). Values are the means of 9–10 plants with standard errors of means in parenthesis.

	Ambient CO <sub>2</sub>		Elevated CO <sub>2</sub>	
	Undefoliated	Defoliated	Undefoliated	Defoliated
Final dry weight (g)	2.16 (0.26)	1.66 (0.16)	2.86 (0.24)	1.91 (0.14)
Relative growth rate (g/g/wk)	0.091 (0.016)	0.065 (0.012)	0.140 (0.012)	0.075 (0.012)
Leaf area ratio (m <sup>2</sup> /kg)	8.18 (0.42)	7.26 (0.40)	7.93 (0.46)	6.27 (0.40)
Specific leaf area (m <sup>2</sup> /kg)	20.3 (0.3)	19.6 (0.4)	19.4 (0.7)	18.7 (0.4)
Root:shoot ratio (g/g)	0.37 (0.02)	0.43 (0.02)	0.38 (0.02)	0.47 (0.03)
Leaf nitrogen (g N/m <sup>2</sup> )	1.18 (0.05)	1.27 (0.03)	1.25 (0.06)	1.19 (0.03)
Leaf carbon:nitrogen ratio (g/g)	24.9 (0.7)	24.0 (0.4)	25.0 (1.2)	25.9 (0.6)

CO<sub>2</sub>. Thus, although biomass and average relative growth rate were enhanced under elevated CO<sub>2</sub>, at the end of the experiment defoliated plants under elevated CO<sub>2</sub> were further from compensating for tissue lost during simulated herbivory than plants growing under ambient CO<sub>2</sub>.

The ratio of leaf area to whole plant biomass (leaf area ratio) was similar in undefoliated plants under elevated and ambient CO<sub>2</sub> (Table 1). The leaf area ratio tended to be lower in defoliated plants (main effect of defoliation:  $F_{1,1} = 39.03$ ,  $P = 0.087$ ). Specific leaf area tended to be lower in defoliated plants (main effect of defoliation:  $F_{1,1} = 36.94$ ,  $P = 0.104$ ) and under elevated CO<sub>2</sub> (main effect of CO<sub>2</sub>:  $F_{1,1} = 21.13$ ,  $P = 0.136$ ).

## DISCUSSION

Under elevated CO<sub>2</sub>, we found that net photosynthetic rates were *ca* 50–100 percent higher than those under ambient CO<sub>2</sub> (Figs. 1 & 2). This increase is comparable to what has been reported in both temperate and tropical species grown under elevated CO<sub>2</sub> (Ziska *et al.* 1991, Drake *et al.* 1997). But compensatory increases in photosynthesis after defoliation were only evident in plants growing under ambient CO<sub>2</sub> (Fig. 2). Increased nitrogen concentrations in leaves corresponded with enhanced photosynthetic rates in defoliated plants under ambient CO<sub>2</sub> (Table 1), suggesting that defoliation of young leaf tissue may result in allocation of additional nitrogen to photosynthetic enzymes of mature tissue. This has been observed in other species (*e.g.*, von Caemmerer & Farquhar 1984, Senock *et al.* 1991, Morrison & Reekie 1995), and appears to be a characteristic response when tissue that provides a sink for carbohydrates is removed (Trumble *et al.* 1993).

Under ambient CO<sub>2</sub>, however, increases in photosynthesis and leaf nitrogen after defoliation were only on the order of 10 percent. Other studies have observed up to a 400 percent increase in photosynthesis after defoliation relative to undefoliated controls (Morrison & Reekie 1995). This relatively small effect of defoliation on photosynthesis in *C. aromatica* could be because most of the resources needed for leaf development were already accumulated within the developing leaflets. Thus, the removed leaflets may not have been strong sinks for resources. However, a 100 percent increase in photosynthesis was observed in herbaceous plants that had been defoliated to a similar extent as *C. aromatica* (*ca* 40% of leaf area removed; Morrison & Reekie 1995). Another possible explanation for the small effect of defoliation on photosynthesis is that removing leaflets, rather than severing the midrib and removing of whole leaves, may cause a less severe compensatory response. Diffuse leaf damage or wounding has been observed in other species to cause less pronounced effects on growth and photosynthesis compared to defoliation of whole leaves (Marquis 1992, Morrison & Reekie 1995). Levels of nutrient resources also are likely to be important in determining plant responses to defoliation. For example, low nutrient levels, or low levels of nutrients that are not readily retranslocatable (*e.g.*, calcium), may restrict the potential for compensatory photosynthesis and growth after defoliation. In the experiment described here, seedlings of *C. aromatica* were fertilized and roots were nodulated. Despite this, mature leaf nitrogen concentrations fell in the low range of published values for tropical tree species (Reich *et al.* 1995). Thus, there may have been insufficient time during the experiment for plants to respond to fertilization, which may have limited the response of plants to defoliation.

Responses to defoliation also are likely to vary among species, being highly dependent on factors such as the levels of chemical and physical defenses against herbivory (Rosenthal & Kotanen 1994, Coley & Kursar 1996). The relatively small response of *C. aromatica* to defoliation possibly could be due to its reliance on alternative strategies to minimize the effects of herbivory, for example, having high levels of chemical or physical defenses, or synchronous leaf production (Coley & Kursar 1996). In a Brazilian congener, levels of chemical defenses and leaf toughness were low in young leaves but increased *ca* four-fold during maturation (Macedo & Langenheim 1989). The synchronous production of leaves could be important in this genus for reducing the impacts of herbivory.

In plants grown under elevated CO<sub>2</sub>, there was no increase in rates of photosynthesis or in leaf nitrogen concentrations after defoliation (Figs. 1, 2; Table 1). This may be due to the acclimation of plants to elevated CO<sub>2</sub>. Under elevated CO<sub>2</sub>, plants have typically 15–20 percent lower concentrations of tissue nitrogen (Drake *et al.* 1997). Therefore, any increases in tissue nitrogen due to defoliation may have been counterbalanced by decreases in tissue nitrogen due to acclimation to elevated CO<sub>2</sub>. The implications of this change in nitrogen allocation under elevated CO<sub>2</sub> may be an increase in the pool size of available nitrogen that could be utilized elsewhere and an enhancement of whole plant nitrogen use efficiency.

Under both elevated and ambient CO<sub>2</sub> concentrations, defoliation of young leaf tissue resulted in increased relative growth rate of leaf area between days 30 and 37 (Figs. 1c, d). This effect was particularly pronounced under elevated CO<sub>2</sub>. Increased leaf growth rates after defoliation have been observed in a range of taxa (*e.g.*, Oosterheld & McNaughton 1991, Wandera *et al.* 1992, Edinius *et al.* 1993, Honkanen *et al.* 1994). Despite the pronounced increase in the rate of leaf area growth in defoliated plants, especially in plants growing under elevated CO<sub>2</sub> (Fig. 1d), the compensatory response failed to lead to the replacement of lost tissue within the 5<sup>1</sup>/<sub>2</sub> weeks of the experiment. Birch saplings also have been observed as unable to replace tissue lost to herbivory (Ovaska *et al.* 1993). In contrast, a South American grass species was able to replace lost leaf area in *ca* three weeks after simulated herbivory (Oosterheld & McNaughton 1991).

The final plant weight of defoliated plants as a proportion of the undefoliated controls was lower in plants growing under elevated CO<sub>2</sub> compared

to those under ambient CO<sub>2</sub> (Table 1). Defoliated, elevated CO<sub>2</sub>-grown plants also had lower average growth rates when expressed as a proportion of controls than their defoliated, ambient CO<sub>2</sub>-grown counterparts (Table 1). Thus, under elevated CO<sub>2</sub>, the compensatory growth response appears to be less effective than under ambient CO<sub>2</sub>. In contrast, a study of defoliation in *Plantago lanceolata* found that plants growing under elevated CO<sub>2</sub> were able to compensate fully for leaf area removed by defoliation after 13 weeks of growth (Fajer *et al.* 1991). *Plantago lanceolata* appeared to achieve this compensation for leaf area by reallocating carbon from roots to shoots. In *C. aromatica* the root: shoot ratio did not differ between defoliated plants grown under elevated and ambient CO<sub>2</sub>, indicating that detectable levels of carbon reallocation from roots to shoots did not occur within 6 weeks of simulated herbivory in this experiment.

The development of axillary leaf buds after defoliation also showed modifications in response to elevated CO<sub>2</sub>. Under ambient CO<sub>2</sub>, defoliation caused a steep rise in the number of active leaf buds in comparison to undefoliated plants (Fig. 1e). Increased carbohydrate concentrations, due to the reduction in sink strength when the young leaves were removed, or increased auxin concentrations, may be responsible (Honkanen *et al.* 1994). In contrast, under elevated CO<sub>2</sub>, high rates of bud initiation occurred regardless whether the plants were defoliated or not (Fig. 1f). Therefore, under elevated CO<sub>2</sub>, defoliation ceased to stimulate the initiation of more buds above that initiated by undefoliated plants. High levels of carbohydrates in other trees species growing under elevated CO<sub>2</sub> have also been correlated with the stimulation of bud formation, leading to increased plant "bushiness" (Downton *et al.* 1990). However, enhanced levels of bud initiation under elevated CO<sub>2</sub> in undefoliated plants were not associated with enhanced leaf area growth between days 30 to 37, as was evident in defoliated plants (compare leaf bud initiation and leaf area growth at elevated CO<sub>2</sub>; Figs. 1b, d). Although high CO<sub>2</sub> resulted in bud initiation, defoliation appears necessary to initiate high rates of new leaf growth.

Reduction in the effectiveness of short-term responses compensating for defoliation under elevated CO<sub>2</sub> in *C. aromatica* raises interesting questions about the effect of rising CO<sub>2</sub> concentrations on the fitness of species that rely on compensatory responses to herbivory. The results of this study suggest that if plants do achieve enhanced growth rates under elevated CO<sub>2</sub>, which in tropical ecosystems

is not yet certain (Reekie & Bazzaz 1989, Ziska *et al.* 1991, Körner & Arnone 1992, Lovelock *et al.* 1998, Winter & Lovelock in press), plants that undergo herbivory may be at an even greater competitive disadvantage compared to their undefoliated neighbors than they are under the current ambient CO<sub>2</sub> concentrations.

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## LITERATURE CITED

- ARP, W. J. 1991. Effects of source-sink relations on photosynthetic acclimation to elevated CO<sub>2</sub>. *Plant Cell Environ.* 14: 869–875.
- COLEY, P. D., AND T. KURSAR. 1996. Anti-herbivore defenses of young tropical leaves: Physiological constraints and ecological tradeoffs. In S. S. Mulkey, R. L. Chazdon, and A. P. Smith (Eds.). *Tropical forest plant ecophysiology*, pp. 305–336. Chapman and Hall, New York, New York.
- DOAK, D. F. 1992. Lifetime impacts of herbivory for a perennial plant. *Ecology* 73: 2086–2099.
- DOWNTON, W. J. S., W. J. R. GRANT, AND E. K. CHACKO. 1990. Effect of elevated carbon dioxide on the photosynthesis and early growth of mangosteen (*Garcinia mangostana* L.). *Scientia Hort.* 44: 215–225.
- DRAKE, B. G., M. A. GONZALEZ-MELER, AND S. P. LONG. 1997. More efficient plants: A consequence of rising atmospheric. *Annu. Rev. Plant Physiol. Mol. Biol.* 48: 607–637.
- EDINIUS, L., K. DANELL, AND R. BERGSTROM. 1993. Impact of herbivory and competition on compensatory growth in woody plants: winter browsing by moose on Scots pine. *Oikos* 66: 286–292.
- FAJER, E. D., M. D. BOWERS, AND F. A. BAZZAZ. 1991. Performance and allocation patterns of the perennial herb, *Plantago lanceolata*, in response to simulated herbivory and elevated CO<sub>2</sub> environments. *Oecologia (Berl.)* 87: 37–42.
- FELLER, I. C., AND K. L. MCKEE. 1998. Creation of light gaps by wood-boring insects in mangrove forests in Belize. *Biotropica*. In press.
- GENTRY, A. H. 1982. Patterns of Neotropical plant species diversity. *Evol. Biol.* 15: 1–84.
- HONKANEN, T., E. HAUKIOJA, AND J. SUOMELA. 1994. Effects of simulated defoliation and debudding on needle and shoot growth in Scots pine (*Pinus sylvestris*): implications of plant source/sink relationships for plant-herbivore studies. *Funct. Ecol.* 8: 631–639.
- KÖRNER, C., AND J. A. ARNONE. 1992. Responses to elevated carbon dioxide in artificial tropical ecosystems. *Science (Wash. DC)* 257: 1672–1675.
- , AND F. MIGLIETTA. 1994. Long term effects of naturally elevated CO<sub>2</sub> on Mediterranean grassland and forest trees. *Oecologia (Berl.)* 99: 343–351.
- LOVELOCK, C. E., K. WINTER, R. MERSITS, AND M. POPP. 1998. Responses of communities of tropical tree species to elevated CO<sub>2</sub> in a forest clearing. *Oecologia (Berl.)* 116: 207–218.
- MACEDO, C. A., AND J. H. LANGENHEIM. 1989. A further investigation of leaf sesquiterpene variation in relation to herbivory in two Brazilian populations of *Copaifera langsdorffii*. *Biochem. Syst. Ecol.* 17: 207–218.
- MARQUIS, R. J. 1984. Leaf herbivores decrease fitness of a tropical plant. *Science (Wash. DC)* 226: 537–539.
- . 1992. A bite is a bite? Constraints on responses to folivory in *Piper arizianum* (Piperaceae). *Ecology* 73: 143–152.
- MAURICO, R., M. D. BOWERS, AND F. A. BAZZAZ. 1993. Pattern of leaf damage affects fitness of the annual plant *Raphanus sativus* (Brassicaceae). *Ecology* 74: 2066–2071.
- MORRISON, K. D., AND E. G. REEKIE. 1995. Pattern of defoliation and its effect on photosynthetic capacity in *Oenothera biennis*. *J. Ecol.* 83: 759–767.
- OESTERHELD, M., AND S. J. MCNAUGHTON. 1991. Effect of stress and time for recovery on the amount of compensatory growth after grazing. *Oecologia (Berl.)* 85: 305–313.
- OVASKA, J., M. WALLS, AND E. VAPAAVUORI. 1993. Combined effects of partial defoliation and nutrient availability on cloned *Betula pendula* saplings. *J. Exp. Bot.* 44: 1385–1393.
- PONTAILLER, J. Y. 1990. A cheap quantum sensor using a gallium arsenide photodiode. *Funct. Ecol.* 4: 591–596.
- REEKIE, E. G., AND F. A. BAZZAZ. 1989. Competition and patterns of resource use among seedlings of five tropical trees grown at ambient and elevated CO<sub>2</sub>. *Oecologia (Berl.)* 79: 212–222.
- REICH, P. B., D. S. ELLESWORTH, AND C. UHL. 1995. Leaf carbon and nutrient assimilation and conservation in species of differing successional status in an oligotrophic Amazonian forest. *Funct. Ecol.* 9: 65–76.
- ROSENTHAL, J. P., AND P. M. KOTANEN. 1994. Terrestrial plant tolerance to herbivory. *Trends Ecol. Evol.* 9: 145–148.
- SENOK, R. S., W. B. SISSON, AND G. B. DONART. 1991. Compensatory photosynthesis of *Sporobolus flexuosus* (Thurb.) Rydb. following simulated herbivory in the northern Chihuahuan desert. *Bot. Gaz.* 152: 275–281.
- TRUMBLE, J. T., D. M. KOLODNY-HIRSCH, AND I. P. TING. 1993. Plant compensation for arthropod herbivory. *Annu. Rev. Entomol.* 38: 93–119.
- VON CAEMMERER, S., AND G. D. FARQUHAR. 1984. Effects of partial defoliation, changes of irradiance during growth,



- short term water stress and growth at enhanced p(CO<sub>2</sub>) on the photosynthetic capacity of leaves of *Phaseolus vulgaris* L. *Planta* 160: 320–329.
- WANDERA, J. L., J. H. RICHARDS, AND R. J. MUELLER. 1992. The relationship between relative growth rate, meristematic potential and compensatory growth of semiarid-land shrubs. *Oecologia* 90: 391–398.
- WINTER, K., AND C. E. LOVELOCK. 1999. Growth responses of seedlings of early and late successional tropical forest trees to elevated atmospheric CO<sub>2</sub>. *Flora* (in press).
- XU, D. Q., R. M. GIFFORD, AND W. S. CHOW. 1994. Photosynthetic acclimation in pea and soybean to high atmospheric CO<sub>2</sub> partial pressure. *Plant Physiol.* 106: 661–671.
- ZISKA, L. H., K. P. HOGAN, A. P. SMITH, AND B. G. DRAKE. 1991. Growth and photosynthetic response of nine tropical species with long term exposure to elevated carbon dioxide. *Oecologia (Berl.)* 86: 383–389.
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