

Effect of CO₂-enrichment on seedling physiology and growth of two tropical tree species

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Seedlings of two tree species from the Atlantic lowlands of Costa Rica, *Ochroma lagopus* Swartz, a fast-growing pioneer species, and *Pentaclethra macroloba* (Willd.) Kuntze, a slower-growing climax species, were grown under enriched atmospheric CO₂ in controlled environment chambers. Carbon dioxide concentrations were maintained at 350 and 675 $\mu\text{l l}^{-1}$ under photosynthetic photon flux densities of 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and temperatures of 26°C day and 20°C night. Total biomass of both species increased significantly in the elevated CO₂ treatment; the increase in biomass was greatest for the pioneer species, *O. lagopus*. Both species had greater leaf areas and specific leaf weights with increased atmospheric CO₂. However, the ratio of non-photosynthetic tissue to leaf area also increased in both species leading to decreased leaf area ratios. Plants of both species grown at 675 $\mu\text{l l}^{-1}$ CO₂ had lower chlorophyll contents and photosynthesis on a leaf area basis than those grown at 350 $\mu\text{l l}^{-1}$. Reductions in net photosynthesis occurred despite increased internal CO₂ concentrations in the CO₂-enriched treatment. Stomatal conductances of both species decreased with CO₂-enrichment resulting in significant increases in water use efficiency.

Additional key words – CO₂ concentration, *Ochroma lagopus*, *Pentaclethra macroloba*, photosynthesis, stomatal conductance, water use efficiency.

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Introduction

As a result of human activities, the concentration of atmospheric CO₂ is increasing. One of the largest sinks for atmospheric carbon dioxide is photosynthesis by woody plants; tropical forests alone store an estimated 46% of the world's living terrestrial carbon pool (Brown and Lugo 1982). Nevertheless, very little information is available on the response of woody plants, particularly from the tropics, to enriched atmospheric CO₂ concentrations (Sionit and Kramer 1985).

Numerous studies of herbaceous plants indicate that the response to CO₂-enrichment is species dependent; differences have been found in terms of growth, morphology and physiology (Gates et al. 1983, Kimball 1983). It has been suggested that these differences in response to elevated CO₂ concentrations will change com-

petitive balances within ecosystems, and hence ecosystem production and composition (Carter and Peterson 1983, Gates et al. 1983, Strain and Bazzaz 1983). In order to predict the impact of elevated CO₂ on tropical as well as temperate forests, studies of the component species of these communities are needed.

The objective of this study was to test the effect of enriched atmospheric CO₂ levels on the growth and physiology of two ecologically different tree species from the Atlantic lowland rain forest of Costa Rica. Such information is of particular interest in view of the rapid deforestation in the tropics and the resulting shifts toward early successional species (National Research Council 1980).

The two tree species examined in this study, *Ochroma lagopus* Swartz (Balsa) and *Pentaclethra macroloba* (Willd.) Kuntze, are on opposite ends of the suc-

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cessional spectrum. *Ochroma lagopus* (Bombacaceae) is a widespread pioneer of large clearings and is characterized by small seeds, rapid growth and low density wood. *Pentaclethra macroloba* (Mimosoideae), an abundant climax species in the wet forest of the Atlantic lowlands of Costa Rica, has larger seeds, slower growth and higher density wood than *O. lagopus*.

Abbreviations – LAR, leaf area ratio; PPF, photosynthetic photon flux density; SLA, specific leaf area; WUE, water use efficiency.

Materials and methods

Seeds from field populations were collected at the Organization for Tropical Studies La Selva Biological Station, Costa Rica, (84°02', 10°26'N). La Selva is classified as premontane wet forest in the Holdridge life zone system (Holdridge et al. 1971).

Seeds were germinated in a 1:1 mixture of gravel and vermiculite in controlled-environment chambers of the Duke University Phytotron. The larger seeded *P. macroloba* (seed fresh weight of approximately 6 g) were grown in 18 cm diameter pots while *O. lagopus* (seed fresh weight of 0.008 g) were grown in 11 cm pots. Photon flux density in the wavelength range of 400–700 nm was $500 \pm 25 \mu\text{mol m}^{-2} \text{s}^{-1}$ at plant level. Illumination was provided by a combination of 215 W fluorescent (FR96T12/cw/1500) and 105 W incandescent (105A-23/12 clear) lamps manufactured by General Electric Co., Bridgeport, CT, USA, on a 70/30% wattage ratio. The photoperiod was 12 h and light/dark temperatures were 26/20°C. (Mean maximum and minimum temperatures in the canopy at La Selva are approximately 27.0 and 21.0°C, Fetcher et al. 1985). Vapour pressure deficits were maintained near 1.05 kPa (70% relative humidity) during the day and near zero at night. Plants were watered to the dripping point twice daily with demineralized water in the morning and modified 1/2 strength Hoagland's solution (Downs and Hellmers 1975) in the afternoon. Carbon dioxide concentrations were maintained by a computer-controlled injection system (Hellmers and Giles 1979) at 350 ± 10 (low) and $675 \pm 20 \mu\text{l l}^{-1}$ (high).

All plants were harvested after growth periods of 60 days for *O. lagopus* and 123 days for *P. macroloba*. Leaf area was determined with an area meter (Li-3100, LiCor Inc, Lincoln, Nebraska). Plants were separated into leaves, stems and roots and dried at 70°C for 48 h, and then weighed.

Light-saturated photosynthesis and stomatal conductance of plants from both CO₂ treatments were measured at 350 and 675 $\mu\text{l l}^{-1}$ CO₂. Measurements of photosynthesis of *P. macroloba* were made using an Anarad Ar-500R infrared gas analyzer with two dewpoint hygrometers (General Eastern System 1000AP). Flow rates were controlled and measured by Brooks Instruments model 5815 mass flow valves. Photosynthesis values

were calculated according to Šesták et al. (1971). Rates are expressed on the basis of one leaf surface only. Measurements of photosynthesis of *O. lagopus* were made with the Lawrence Livermore cuvette system with a Horiba TIR-2000 infrared gas analyzer (Bingham et al. 1980). Flow rates were determined using Foxboro Model 1710 pressure transducers and calibrated capillary tubes. Stomatal conductance was calculated according to Bingham et al. (1980) from humidity measurements made with a Vaisala Humicap 6061 sensor. Internal CO₂ concentrations were calculated using equations from Nobel et al. (1975). Measurements were made at photon flux densities of 1 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for *O. lagopus* and 1 000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for *P. macroloba* the week before harvesting. However, because of time constraints, the measurements of *O. lagopus* at 675 $\mu\text{l l}^{-1}$ were made six days later than those at 350 $\mu\text{l l}^{-1}$.

Chlorophyll was extracted from leaf samples of known area with 80% acetone. Chlorophyll was measured photometrically and concentrations were calculated with equations from MacKinney (1941). The thickness of fresh leaf sections was measured using a calibrated ocular micrometer at 400×. Stomatal densities of *P. macroloba* and *O. lagopus* were determined with a light microscope at 400×.

Results

Growth of both *O. lagopus* and *P. macroloba* increased significantly with CO₂-enrichment of the atmosphere (Tab. 1). The early successional species, *O. lagopus*, responded more to CO₂-enrichment than did the late successional species, *P. macroloba*. Total plant weights of *O. lagopus* increased 79% compared to a 30% increase for *P. macroloba* in response to CO₂-enrichment. When

Tab. 1. Mean growth characteristics of two species of tropical trees, *Ochroma lagopus* and *Pentaclethra macroloba*, grown at two atmospheric carbon dioxide concentrations, 350 and 675 $\mu\text{l l}^{-1}$. Means with different letters are significantly different at the 0.05 level.

Character	<i>Ochroma lagopus</i> at 60 days (n=5)		<i>Pentaclethra macroloba</i> at 123 days (n=8)	
	350 $\mu\text{l l}^{-1}$ CO ₂	675 $\mu\text{l l}^{-1}$ CO ₂	350 $\mu\text{l l}^{-1}$ CO ₂	675 $\mu\text{l l}^{-1}$ CO ₂
Total weight (g)	4.80b	8.61a	13.24b	17.15a
Leaf area ratio (cm ² g ⁻¹)	116a	90b	57a	47b
Leaf area (cm ²)	555b	770a	759a	793a
Leaf weight (g)	2.88b	5.58a	4.64a	5.42a
Root shoot ratio	0.34a	0.30a	0.73a	0.66a
Height (cm)	14.3a	16.4a	30.8a	36.7a

the effect of the difference in seed size between the two species was excluded by subtracting the average seed dry weight from total weights at harvest, the increase in biomass for *P. macroloba* at high CO₂ (38%) was still less than half that of *O. lagopus*. Total weights of *O. lagopus* were lower overall than those of *P. macroloba* as a result of smaller seed weights for *O. lagopus* (<0.2% of *P. macroloba*) and a shorter growth period.

The ratios of photosynthetic leaf area to the total biomass (leaf area ratio, LAR) decreased approximately 21–22% for both species in the higher CO₂ treatment (Tab. 1). This reduction occurred even though mean leaf areas and leaf weights increased. Mean leaf weights were 48 and 14% greater under high CO₂ for *O. lagopus* and *P. macroloba*, respectively. Root to shoot ratios decreased and plant height increased for both species in the high CO₂ treatment, although differences were not statistically significant (Tab. 1).

Plants of both species grown at 350 μl l⁻¹ had higher photosynthesis, stomatal conductances, chlorophyll

contents and specific leaf areas (SLA) than plants grown at 675 μl l⁻¹ (Tab. 2). The difference between the two treatments was greatest for the pioneer species, *O. lagopus*. Higher internal CO₂ concentrations calculated for leaves in the elevated CO₂ treatment indicate that lower photosynthesis was not due to internal CO₂ limitation by stomatal closure in either species (Tab. 2).

Water use efficiencies (WUE, μmol CO₂ mol⁻¹ H₂O) at low CO₂ were highest for *O. lagopus*, which had photosynthetic rates five times as high as those of *P. macroloba* (Tab. 2). At 675 μl l⁻¹, WUE for both species was similar and substantially higher than those at low CO₂.

Leaves of *Ochroma lagopus* grown at high CO₂ had lower photosynthetic rates and leaf conductances than leaves grown at low CO₂ when measured at both 350 and 675 μl l⁻¹ (Tab. 3). However, photosynthesis measured at 675 μl l⁻¹ was slightly lower than at 350 μl l⁻¹ for leaves grown at both CO₂ levels, perhaps because of the greater age of leaves when measured at 675 μl l⁻¹. Internal CO₂ limitation was not the cause for reductions of photosynthesis at either CO₂ concentration. The water use efficiency of *O. lagopus* was lowest for plants grown at 675 μl l⁻¹ and measured at 350 μl l⁻¹.

Leaves of *P. macroloba* grown at both CO₂ levels had lower conductance but higher photosynthesis and WUE when measured at 675 μl l⁻¹ than when measured at 350 μl l⁻¹ (Tab. 3). Leaves grown at high CO₂ but measured at low CO₂ had lower photosynthetic rates and WUE than leaves grown and measured at low CO₂. Internal CO₂ limitation was not the cause of the reduction in photosynthesis for plants grown under high CO₂ measured at 350 μl l⁻¹, but at 675 μl l⁻¹ some internal CO₂ limitation cannot be ruled out.

Elevated CO₂ concentrations during growth produced only small differences in stomatal density. Stomatal densities were not significantly affected on either surface of *Ochroma lagopus* leaves. *Pentaclethra macroloba* had significantly lower stomatal densities on the abaxial surface when grown at 675 μl l⁻¹ than at 350

Tab. 2. Mean leaf characteristics of two species of tropical trees, *Ochroma lagopus* and *Pentaclethra macroloba*, grown at two atmospheric carbon dioxide concentrations, 350 and 675 μl l⁻¹. Photosynthesis (J_{CO₂}), stomatal conductance to water vapour (g_s), CO₂ concentration of internal air spaces (C_i), and water use efficiency (WUE) are values at light saturation. Total chlorophyll (Chl) and specific leaf area (SLA) are values one week before harvest. Significances as in Tab. 1 (n=5 or 6).

[CO ₂] μl l ⁻¹	J _{CO₂} μmol m ⁻² s ⁻¹	g _s mm s ⁻¹	Chl g m ⁻²	SLA m ² g ⁻¹	C _i μl l ⁻¹	WUE μmol CO ₂ (mol H ₂ O) ⁻¹
<i>Ochroma lagopus</i>						
350	27.5a	7.3a	0.544a	253a	199b	7850b
675	18.4b	3.1b	0.429b	157b	429a	12460a
<i>Pentaclethra macroloba</i>						
350	5.4a	3.9a	0.431a	214a	286b	5751b
675	4.7a	1.1b	0.295b	171b	482a	11226a

Tab. 3. Mean leaf characteristics of two species of tropical trees, *Ochroma lagopus* and *Pentaclethra macroloba*, measured and grown at two different atmospheric carbon dioxide concentrations, 350 and 675 μl l⁻¹. Photosynthesis (J_{CO₂}), stomatal conductance to water vapour (g_s), CO₂ concentration of internal air spaces (C_i), and water use efficiency (WUE) are values at light saturation. Significances as in Tab. 1 (n=5 or 6).

Growth [CO ₂] μl l ⁻¹	Measurement [CO ₂]							
	350				675			
	JCO ₂ μmol m ² s ⁻¹	g _s mm s ⁻¹	C _i μl l ⁻¹	WUE μmol CO ₂ mol H ₂ O ⁻¹	JCO ₂ μmol m ² s ⁻¹	g _s mm s ⁻¹	C _i μl l ⁻¹	WUE μmol CO ₂ mol H ₂ O ⁻¹
<i>Ochroma lagopus</i>								
350	27.5a	7.3a	199b	7850a	24.6a	3.5a	391a	14607a
675	20.6b	6.1b	217a	6694b	18.4b	3.1a	429a	12460a
<i>Pentaclethra macroloba</i>								
350	5.4a	3.9a	286a	5751a	6.0a	2.7a	578a	6355b
675	3.7b	2.6a	277a	4042a	4.7a	1.1b	482b	11226a

$\mu\text{l l}^{-1}$ (308/ mm^2 vs. 332/ mm^2). Mean leaf thickness of both species increased slightly, but not significantly, with CO_2 -enrichment.

Discussion

A doubling of CO_2 concentration stimulated growth of the early successional species, *Ochroma lagopus*, twice as much as that of the late-successional species, *Pentaclethra macroloba*. This difference can be attributed in part to the greater allocation of biomass to leaf tissue and higher photosynthetic rates in *O. lagopus*. Similar results have been noted for some temperate species (Carlson and Bazzaz 1980, Tolley and Strain 1984). Tolley and Strain (1984) found a larger growth response to CO_2 -enrichment in the faster growing of two temperate early-successional trees, *Liquidambar styraciflua* and *Pinus taeda*.

Both species in this study had larger growth in the high CO_2 treatment despite having lowered photosynthetic rates immediately before harvesting. Lowered stomatal conductances at the same time resulted in a large increase in the water use efficiency, but the reduction in conductance was not sufficient to account for lowered photosynthetic rates. Previous studies have also found enhanced growth combined with lowered photosynthesis, determined by gas analysis, or lowered net assimilation rates, determined by mathematical growth analysis, in response to long term exposure to elevated CO_2 concentrations (Raper and Peedin 1978, Sionit et al. 1981, Wulff and Strain 1982, Tolley and Strain 1984). Tolley and Strain (1984) found that net assimilation rates of sweetgum seedlings under CO_2 -enrichment were initially higher than those of controls, but declined with time. After 84 days, net assimilation rates under elevated CO_2 were lower than controls. Nevertheless, overall growth was largest for the high CO_2 treatment plants because of higher net assimilation rates early in their development. Both *P. macroloba* and *O. lagopus* in this study showed similar patterns of larger overall growth despite lower photosynthesis after long periods under elevated CO_2 concentrations. Reductions in photosynthesis or net assimilation rates have been accompanied by chlorosis of leaves and abnormal starch accumulation in chloroplasts (Madsen 1976, Patterson and Flint 1980, Cave et al. 1981, Wulff and Strain 1982, DeLucia et al. 1985). Damage caused by the accumulation of excess starch in chloroplasts has been suggested as a causative factor in this reduction in photosynthesis (Cave et al. 1981, Wulff and Strain 1982). Reductions in chlorophyll concentration have been associated with this excess starch (Cave et al. 1981, DeLucia et al. 1985). Although starch contents were not measured in this study, SLA, which is often negatively correlated with starch content, significantly decreased with CO_2 -enrichment. Slight increases in leaf thickness may also be involved in the decrease in SLA as has been

previously reported for some species (Hurd 1968, Rogers et al. 1983). Chlorophyll contents of both species examined in this study were significantly reduced with CO_2 -enrichment as has been reported in studies that have found abnormal starch accumulation in chloroplasts (Cave et al. 1981, Wulff and Strain 1982).

The current trend in tropical regions is toward large-scale deforestation with replacement of late successional trees by early-successional herbs, shrubs and trees (National Research Council 1980). If the growth of early successional species is disproportionately favored by increased atmospheric CO_2 , then we may expect that longer regeneration times will be required for tropical forests because of suppression of late-successional species.

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