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Nutrient and genotypic effects on CO_2-responsiveness: photosynthetic regulation in *Leucadendron* species of a nutrient-poor environment

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Abstract

Four South African *Leucadendron* congenerics with divergent soil N and P preferences were grown as juveniles at contrasting nutrient concentrations at ambient (350 μmol mol^{-1}) and elevated (700 μmol mol^{-1}) atmospheric CO_2 levels. Photosynthetic parameters were related to leaf nutrient and carbohydrate status to reveal controls of carbon uptake rate. In all species, elevated CO_2 depressed both the maximum Rubisco catalytic activity ($V_{c,max}$, by 19–44%) and maximum electron transport rate (J_{max}, by 13–39%), indicating significant photosynthetic acclimation of both measures. Even so, all species had increased maximum light-saturated rate of net CO_2 uptake (A_{max}) at the elevated growth CO_2 level, due to higher intercellular CO_2 concentration (c_i). Leaf nitrogen concentration was central to photosynthetic performance, correlating with A_{max}, $V_{c,max}$ and J_{max}. $V_{c,max}$ and J_{max} were linearly co-correlated, revealing a relatively invariable J_{max}:$V_{c,max}$ ratio, probably due to N resource optimization between light harvesting (RuBP regeneration) and carboxylation. Leaf total non-structural carbohydrate concentration (primarily starch) increased in high CO_2, and was correlated with the reduction in $V_{c,max}$ and J_{max}. Apparent feedback control of $V_{c,max}$ and J_{max} was thus surprisingly consistent across all species, and may regulate carbon exchange in response to end-product fluctuation. If so, elevated CO_2 may have emulated an excess end-product condition, triggering both $V_{c,max}$ and J_{max} down-regulation. In *Leucadendron*, a general physiological mechanism seems to control excess carbohydrate formation, and photosynthetic responsiveness to elevated CO_2, independently of genotype and nutrient concentration. This mechanism may underlie photosynthetic acclimation to source:sink imbalances resulting from such diverse conditions as elevated CO_2, low sink strength, low carbohydrate export, and nutrient limitation.

Key words: Carbohydrate, elevated CO_2, nitrogen, photosynthesis, Proteaceae.

Introduction

The photosynthetic and growth responses of C_3 plants to elevated CO_2 show a bewildering diversity, ranging from highly positive to neutral and, in rare cases, even negative (Poorter, 1993; Gunderson and Wullschleger, 1994). This greatly complicates the accurate prediction of ecosystem changes as CO_2 continues to accumulate in the earth's atmosphere. Responses of C_3 plants to rising atmospheric CO_2 levels are clearly modified by growing conditions (Idso and Idso, 1994), and appear strongly species- (Poorter, 1993) and even ecotype- (Norton *et al.*, 1995) and genotype-specific (Curtis *et al.*, 1994; Zhang and Lechowicz, 1995). Because plant growth requires a nutritional balance, that is a balance between carbon uptake above-ground and nutrient uptake below-ground, it is has been suggested that nutrient limitation should con-

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Abbreviations: A_{max}, maximum light-saturated rate of net CO_2 uptake; $V_{c,max}$, maximum catalytic activity of the enzyme Rubisco; J_{max}, maximum electron transport rate; c_i, intercellular CO_2 concentration; g_s, stomatal conductance; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose 1,5-bisphosphate; PFD, photon flux density; PNUE, photosynthetic nitrogen use efficiency.

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strain plant CO_2-responsiveness (Rastetter *et al.*, 1997). However, data suggest that proportional responses to elevated CO_2 may be greater in some species under low than high nutrient conditions (Lloyd and Farquhar, 1996).

Plant evolutionary history also appears to influence CO_2-responsiveness, as demonstrated by the relationship between 'life history strategy' (*sensu* Grime, 1977) and CO_2-responsiveness (Hunt *et al.*, 1993). The latter view is supported by the finding that biome affinities are more important than photosynthetic type in predicting CO_2-responsiveness (Wilsey, 1996). It is vital to understand the relative roles of these extrinsic (e.g. nutrient limitation) and intrinsic (adaptive) limitations to CO_2-responsiveness, as global change involves changes to resource availability (Vitousek, 1994), but many ecosystems have evolved under resource-limited conditions. Are the mechanisms which control CO_2-responsiveness chiefly intrinsic, species-specific and a function of evolutionary history, or are they externally determined and a function of resource concentration?

Photosynthetic acclimation (also termed down-regulation) is a crucial component of plant productivity responses to elevated CO_2 (Long *et al.*, 1993), and many species show different degrees of photosynthetic acclimation in elevated CO_2 (Harley, 1995). Acclimation appears proximately due to either, or both, a reduction in the concentration or activation state of Rubisco (Sage *et al.*, 1989; Jacob *et al.*, 1995). This response may be due to repartitioning of nitrogen resources within photosynthetic cells (Bowes, 1991), feedback regulation by carbohydrate status (Stitt, 1991), or inorganic phosphate limitation (van Oosten *et al.*, 1992). Altered carbohydrate status itself may be due to a combination of factors which lead to an altered source:sink balance in elevated CO_2, such as low carbohydrate export rate (Körner *et al.*, 1995), low sink demand (Arp, 1991) or nutrient supply limitation (Paul and Driscoll, 1997). This is a complex chain of linked events, and the process of acclimation is poorly understood (Bowes *et al.*, 1996).

There is relatively little information about photosynthetic acclimation in species of nutrient-limited mediterranean-type ecosystems (MTEs). Sclerophyll-dominated South African MTEs are among the most nutrient-limited in the world (Kruger *et al.*, 1983). Harley (1995) has proposed that CO_2-responsiveness in mediterranean schlerophylls will depend on whether new sinks can be developed to capitalize on increased carbohydrate formation in elevated CO_2. Stock and Midgley (1995) concluded, from the limited available information, that mediterranean-type species with low growth rates might show muted photosynthetic responses to elevated CO_2. Empirical studies of CO_2-mediated photosynthetic responses of MTE perennials (Larigauderie *et al.*, 1988; Jenkins, 1993, in Oechel *et al.*, 1995; Miglietta *et al.*, 1995; Bettarini *et al.*, 1995) have produced a mixed bag of results including both down- and up-regulation.

At the leaf level, both carbohydrate status and leaf nitrogen content have been clearly shown to influence photosynthetic activity, and both are potentially altered in elevated CO_2. Plants must have mechanisms to sense carbohydrate status (van Oosten and Besford, 1996); the proven diurnal regulation of carbohydrate production (Geiger and Servaites, 1994) demonstrates that these exist, and may serve to regulate photosynthesis in the medium term. Leaf N content also plays a central role in photosynthesis, and is an important trait that covaries widely with photosynthetic capacity throughout the plant kingdom (Field and Mooney, 1986). Does elevated CO_2 affect these measures consistently across species and nutrient concentration conditions?

This study attempts to discern direct effects of nutrient concentration on the photosynthetic response to elevated CO_2, as distinct from species-specific effects, and to tease apart the relative roles of leaf N and carbohydrate status in modifying photosynthetic rate under elevated CO_2 conditions.

Materials and methods

Species selection

Four closely related *Leucadendron* (Proteaceae) species of congruent growth form, but with inherently different nutrient dependencies, were selected. *Leucadendron xanthoconus* (Kuntze) K. Schum. and *L. laureolum* (Lam.) Fourc. (dystrophic species) are associated with acidic sands of low N and P status, *L. coniferum* (L.) Meisn. (mesotrophic) is associated with neutral sands of intermediate N and higher P availability, and *L. meridianum* I. Williams (mesotrophic) is associated with basic sands of higher N and intermediate P status (Richards, 1997*a*, *b*; Midgley *et al.*, 1995). Members of the genus *Leucadendron* do not possess mycorrhizae so common in many fynbos genera (Allsopp and Stock, 1993), thus allowing nutrient concentration to be manipulated hydroponically in a sterile sand/perlite culture medium.

Plant material

Seeds were collected from at least five plants near Cape Town (*L. xanthoconus* and *L. laureolum*) and near Cape Agulhas (*L. meridianum* and *L. coniferum*) and stored in sealed containers at room temperature. Seeds were germinated in sterile sand, and 6–8-week-old seedlings transferred to 0.5 m deep pots (3.3 dm^3 volume) containing a sterile sand/perlite mix, and allowed to establish for a further 3–4 months. Plants were fed monthly with 100 ml of a complete Long Ashton solution diluted to 10% (containing 0.1 mM N as nitrate and ammonium), until cotyledonary reserves were exhausted. Plants were then transferred to open-top chambers, and appropriate CO_2 and nutrient treatments initiated.

Pots were watered daily, receiving approximately 0.5 l d^{-1} each during cool months, and 1.0 l d^{-1} during warm months. Plants were harvested 6 months later, aged between 11 and 12 months (*L. xanthoconus* and *L. laureolum*) and 12 and 13 months (*L. coniferum* and *L. meridianum*). Harvesting was conducted during the early morning, and plants were subdivided

into leaves, stems and roots. Bulked samples per plant were oven-dried to constant mass at 65 °C and milled.

Open-top chambers

Chambers were hexagonal with 0.38 m long sides and 0.5 m tall, constructed of polycarbonate (1.8 mm thickness) and topped by a removable frustum. They were placed on tables in a polycarbonate-clad greenhouse. Each chamber was ventilated individually by a 12 V DC brushless fan (model FP-108, Commonwealth, Taiwan) which drew air from outside and circulated it in a plenum surrounding the base of the chamber before entering through perforations in the inner plenum wall. For elevated CO_2 chambers, pure CO_2 was bled into the intake pipes at rates controlled by float metering flowmeters (model DK800, Krohne, Germany). Elevated CO_2 chambers were individually calibrated to 700 μmol mol^{-1} using an infra-red gas analyser (LI-6200, Li-Cor, Lincoln, NE, USA), and were generally within 80 μmol mol^{-1} of the target concentration during the first experiment (*L. xanthoconus* and *L. laureolum*), and within 50 μmol mol^{-1} for the second experiment (*L. coniferum* and *L. meridianum*). CO_2 concentrations in the ambient chambers were approximately 350 μmol mol^{-1}. The ventilation rate of the open-top chambers was controlled at somewhat more than four air changes per min.

Pots were suspended through holes in the table tops, thus preventing pot heating and allowing CO_2 fumigation of the soil surface and above-ground plant parts only.

Application of nutrient treatments

Nutrient treatments comprised a complete Long Ashton solution which was diluted to 20% for the high nutrient treatment (containing 0.20 mM nitrogen as ammonium and nitrate), and diluted a further four times for the low nutrient treatment (5% Long Ashton, containing 0.05 mM nitrogen as ammonium and nitrate). Plants were fed 100 ml each once weekly.

Non-structural carbohydrate and nitrogen concentrations

Foliar sugar and starch concentrations were analysed using a modified phenol-sulphuric acid method (Buysse and Merckx, 1993). A 50 mg dry sample was extracted overnight in 10 ml 80% ethanol (v/v) and the supernatant analysed for total sugars. The residue was boiled for 3 h in 5 ml 2% HCl (v/v) and the supernatant analysed for starch. Absorbance at 490 nm was measured using a Beckman DU640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA).

Total leaf nitrogen concentration was determined using micro-Kjeldahl digestion.

Gas exchange

Plant gas exchange characteristics were sampled after a minimum of 3 months using leaves which had developed after treatment initiation. All determinations were carried out using an LI-6200 portable photosynthesis system (Li-Cor, Lincoln, Nebraska, USA), configured as a closed system. For *L. laureolum* and *L. xanthoconus*, recently mature, fully expanded leaves were positioned singly in a 0.25 dm^3 cuvette, and for *L. coniferum* and *L. meridianum* the terminal portion of the shoot was enclosed in a 1 dm^3 cuvette, because leaves were too short to sample singly.

Plants were removed from the open-top chambers and measured in the laboratory on the same day. For the duration of the light-response measurements, the enclosed plant parts were maintained at their respective ambient growing CO_2 concentration by periodically injecting pure CO_2 to replace CO_2 removed by photosynthesis. Using a bank of 50 W tungsten-halogen incandescent lamps (Decostar 51, Osram, Germany), plants were gradually brought to light-saturation point, over a period of 20–30 min. This was determined empirically for each treatment (species × CO_2 × nutrient) and was always above 1000 μmol m^{-2} s^{-1} PFD. At this point, A_{max} and the reported g_s were recorded. Light levels were then reduced in steps of about 300 μmol m^{-2} s^{-1} above 200 μmol m^{-2} s^{-1} PFD and about 50 μmol m^{-2} s^{-1} below 200 μmol m^{-2} s^{-1} PFD, and net CO_2 exchange rates measured at each level after sufficient acclimation. To determine dark respiration rate, the cuvette was covered with a black cloth for 5 min.

Following the light-response measurements, plants were again exposed to saturating PFD until photosynthetic rate was within 5% of A_{max}. Thereafter, the CO_2 level was decreased in steps of about 150 μmol mol^{-1}, allowed to stabilize, and measurements of photosynthetic rate taken to construct an A:c_i curve. After the CO_2 compensation point had been closely approached or exceeded (a process which took about 40–60 min), the CO_2 concentration was increased to above 1400 μmol mol^{-1}. At this point, stomatal conductance had generally increased due to the depleted CO_2 concentration in the cuvette, allowing a rapid and substantial increase in c_i, and an accurate estimate of the light and CO_2-saturated photosynthetic rate, J_{max}. The CO_2 concentration was maintained at this level for at least 15 min for full stabilization, and then decreased in steps of about 200 μmol mol^{-1} until the ambient growing CO_2 concentration was reached. The photosynthetic rate at this point was again checked to be within 5% of A_{max}. For gas exchange measurements above 50 PFD, cuvette air temperature was typically maintained at 29 ± 1 °C and air vapour pressure at 20 ± 2 mb.

For gas exchange analysis, three individuals of each species were sampled in each treatment (CO_2 × nutrient combination). Response curves were fitted individually to light- and CO_2-response data for every leaf or shoot sampled, using iterative non-linear regression (Unistat 4.51 for Windows, Unistat Ltd., London, UK). A monomolecular hyperbola (Causton and Dale, 1990) was fitted to light-response data. The function is

$$y = a(1 - e^{b-cx}) \quad (1)$$

where y is the rate of CO_2 exchange and x is the independent variable (PFD). The coefficient a gives the light-saturated rate of CO_2 exchange (A_{max}) and apparent quantum yield (α, the slope, or derivative of the curve at $x = 0$) is given by ace^b. These parameters were derived individually for every shoot and leaf sampled, and used in statistical analysis.

Carbon dioxide response curves were analysed by fitting the model of Farquhar *et al.* (1980) to the data for each leaf or shoot sampled, using methods described by Hilbert *et al.* (1991). Photosynthesis was assumed to be either (a) RuBP saturated, or (b) limited by the light-dependent regeneration of RuBP. In the case of (a) the following holds:

$$A = V_{c,max}(C - \Gamma)/(C + k) - R_d \quad (2)$$

where $V_{c,max}$ is the maximum RuBPcase activity, C is the intercellullar partial pressure of CO_2, R_d is dark respiration rate, Γ is the CO_2 compensation point, and

$$k = k_C(1 + O/k_O) \quad (3)$$

where k_C and k_O are the Michaelis-Menten constants for CO_2 and O_2, and O is the partial pressure of O_2 at the site of carboxylation (Farquhar *et al.*, 1980).
In the case of (b) the following holds:

$$A = J(C - \Gamma)/(4.5C + 10.5\Gamma) - R_d \quad (4)$$

where

$$J = J_{max} I/(I + 2.1 J_{max}) \quad (5)$$

and I is the instantaneous photosynthetic photon flux density.

Iterative non-linear regression analysis was used first to derive $V_{c,max}$ from each A:c_i data set for each leaf or shoot. The conditions for (a) were assumed to be met with a c_i of less than 200 μmol mol^{-1}. R_d was derived from the light-response curve and substituted into equation 2 (R_d ranged between 0.45 μmol m^{-2} s^{-1} and 1.23 μmol m^{-2} s^{-1} with a mean of 0.74 μmol m^{-2} s^{-1}); thus only $V_{c,max}$ and Γ were unknowns. Values for J_{max} were derived by fitting equation 4 to $A : c_i$ data where c_i exceeded approximately 300 μmol mol^{-1} (substituting for J according to equation 5).

Statistical design

There were eight open-top chambers in the experimental array, each of which held four individuals of each of two species (eight plants in each chamber, 64 plants in total). Thus elevated CO_2 treatments were replicated four times. Within each open-top chamber, high and low nutrient treatments were replicated twice for each species. The experiments used a split-plot design, giving six degrees of freedom to test for elevated CO_2 effects, and 22 degrees of freedom for nutrient effects. The allocation of species and treatments within each open-top chamber was formally randomized *a priori*. Results for each species were analysed separately using split-plot ANOVA. Not all plants could be sampled for gas exchange characteristics due to time constraints, and a subset of three plants per treatment was sampled at random from the open-top chambers. Gas exchange data were tested statistically using standard analysis of variance. Correlations between variables were identified by linear-regression, and significant differences in regression slopes and intercepts due to elevated CO_2 tested using analysis of covariance. All statistical procedures were carried out using Unistat 4.51 for Windows.

Results

Main CO_2 effects and interactions

Elevated CO_2 had significant negative effects on $V_{c,max}$ and J_{max} (Tables 1, 2, 3). Elevated CO_2 reduced $V_{c,max}$ in all species under both nutrient treatments, and reduced J_{max} in all species at both nutrient concentrations, with two exceptions at the low nutrient concentration (*L. xanthoconus* and *L. coniferum*, Fig. 1). Elevated CO_2 had positive effects on A_{max}, PNUE and starch concentration, but did not affect sugar concentration, leaf N concentration or g_s (Tables 1, 2, 3). There was no significant $CO_2 \times$ nutrient interaction for any measured response (Table 3) and thus the ΔCO_2 values (relative effect of elevated CO_2) presented do not differentiate between nutrient concentrations. There was significant $CO_2 \times$ species interaction only for g_s, as two of the four species showed no g_s response to elevated CO_2 but *L. meridianum* decreased g_s and *L. coniferum* increased g_s in elevated CO_2 (Tables 1, 2, 3; Fig. 1). Nutrient × species interaction was significant for leaf N and sugar concentrations (Table 3).

Nutrient and species effects

The higher nutrient concentration significantly increased all measures except starch content, which it significantly decreased (Tables 1, 2, 3). Species differed significantly in all variables measured (Table 3). Dystrophic species displayed higher A_{max}, $V_{c,max}$, J_{max}, and leaf [N] (Table 1), and mesotrophic species higher g_s, sugar and

Table 1. *CO_2-responsiveness of foliar carbohydrate and nitrogen levels, and gas exchange measures of dystrophic fynbos* Leucadendrons *at the juvenile life stage (ΔCO_2 is the ratio of the means of data measured at elevated relative to ambient CO_2, all means given with standard errors below)*

Gas exchange measurements were carried out above light saturation level (PFD > 1000 μmol m^{-2} s^{-1}), with cuvette air temperature 29 ± 1 °C, cuvette air vapour pressure 20 ± 2 mb.

Species	*L. xanthoconus*				**ΔCO_2**	*L. laureolum*				**ΔCO_2**
Nutrient level	Low		High			Low		High		
CO_2 level (μmol mol^{-1})	350	700	350	700		350	700	350	700	
[Starch]	155.8	195.0	133.7	165.0	**1.24**	171.5	195.0	117.8	161.2	**1.23**
(mg g^{-1} dry mass)	14.8	13.7	9.4	8.3	**0.01**	17.4	14.6	6.1	13.8	**0.01**
[Sugar]	22.2	22.2	17.8	23.0	**1.13**	20.9	28.6	22.7	24.1	**1.21**
(mg g^{-1} dry mass)	1.4	1.9	2.6	2.2	**0.01**	1.9	3.9	1.8	1.5	**0.01**
[N]	61.2	60.5	85.2	79.3	**0.95**	54.4	52.6	69.8	63.0	**0.93**
(mmol m^{-2})	2.3	3.6	4.0	2.9	**0.01**	3.2	3.2	4.8	3.3	**0.00**
g_s	73.3	75.7	98.7	103.7	**1.04**	115.0	97.0	129.0	158.3	**1.05**
(mmol m^{-2} s^{-1})	14.9	17.9	8.4	22.4	**0.04**	26.9	11.6	17.4	5.7	**0.03**
A_{max}	7.4	13.7	10.2	15.2	**1.65**	6.2	8.8	11.1	13.8	**1.30**
(μmol m^{-2} s^{-1})	1.2	1.3	0.9	0.9	**0.04**	0.6	1.0	1.2	0.6	**0.06**
$V_{c,max}$	46.0	38.0	57.9	45.8	**0.81**	37.2	16.5	56.4	35.8	**0.56**
(μmol m^{-2} s^{-1})	6.5	1.9	3.0	8.8	**0.10**	3.7	2.1	3.4	3.1	**0.12**
J_{max}	100.1	96.6	134.1	107.4	**0.87**	78.2	40.0	124.6	83.5	**0.61**
(μmol m^{-2} s^{-1})	10.2	6.6	10.3	10.1	**0.11**	9.5	7.5	0.3	8.7	**0.13**
PNUE	121.1	229.1	120.1	192.4	**1.75**	114.1	168.6	161.0	219.6	**1.41**
(mmol CO_2 mol^{-1} N)	19.5	21.3	11.0	11.4	**0.18**	10.8	18.4	16.7	10.4	**0.18**

Table 2. *CO_2-responsiveness of foliar carbohydrate and nitrogen levels, and gas exchange measures of mesotrophic fynbos* Leucadendrons *at the juvenile life stage (ΔCO_2 is the ratio of the means of data measured at elevated relative to ambient CO_2, all means given with standard errors below)*

Gas exchange measurements were carried out above light saturation level (PFD > 1000 μmol $m^{-2}s^{-1}$), with cuvette air temperature 29 ± 1 °C, cuvette air vapour pressure 20 ± 2 mb.

Species	*L. meridianum*				ΔCO_2	*L. coniferum*				ΔCO_2
Nutrient level	Low		High			Low		High		
CO_2 level (μmol mol^{-1})	350	700	350	700		350	700	350	700	
[Starch]	208.6	248.0	176.7	221.4	**1.22**	164.4	231.6	177.5	180.1	**1.19**
(mg g^{-1} dry mass)	9.7	18.5	4.4	13.5	**0.01**	16.1	25.0	16.7	15.9	**0.01**
[Sugar	28.3	28.6	35.8	48.2	**1.20**	26.9	24.9	37.5	38.5	**1.00**
(mg g^{-1} dry mass)	1.6	4.2	3.3	3.7	**0.02**	1.7	2.2	3.5	6.6	**0.02**
[N]	45.2	41.9	46.4	53.1	**1.05**	40.9	34.0	44.3	40.4	**0.87**
(mmol m^{-2})	2.1	2.2	3.2	2.4	**0.00**	2.3	4.2	1.5	2.0	**0.00**
g_s	152.7	99.3	166.0	157.7	**0.81**	162.0	282.3	244.4	294.7	**1.42**
(mmol m^{-2} s^{-1})	13.0	22.3	17.6	23.3	**0.02**	19.7	41.2	18.7	43.1	**0.04**
A_{max}	5.0	5.2	6.7	9.3	**1.24**	5.3	8.9	7.9	9.6	**1.41**
(mol m^{-2} s^{-1})	0.3	0.6	0.8	0.5	**0.04**	0.5	0.1	0.8	1.2	**0.03**
$V_{c,max}$	22.7	15.3	36.1	23.0	**0.65**	26.6	23.9	40.9	27.3	**0.76**
(mol m^{-2} s^{-1})	2.0	2.1	4.5	0.2	**0.18**	2.4	0.7	3.8	2.5	**0.14**
J_{max}	42.3	30.5	58.1	49.8	**0.80**	53.1	54.5	78.3	47.9	**0.78**
(mol m^{-2} s^{-1})	1.7	4.8	2.1	0.1	**0.12**	6.4	1.5	6.8	14.3	**0.14**
PNUE	112.1	127.4	143.7	173.4	**1.18**	128.7	258.7	178.8	239.9	**1.62**
(mmol CO_2 mol^{-1} N)	7.5	14.4	18.1	8.9	**0.14**	13.1	1.9	18.6	30.5	**0.19**

Table 3. F-*values for treatment and interactive effects on leaf gas exchange and foliar carbohydrate and nutrient levels of four fynbos* Leucadendron *species at the juvenile life stage*

Results are for three-way ANOVA of the combined data sets given in Tables 1 and 2.

	CO_2 effect	Nutrient effect	Species effect	CO_2 × nutrient	Nutrient × species	CO_2 × species
[Starch] (dry mass)	24.55*	19.04**	12.93**	0.18	0.45	0.08
[Sugar] (dry mass)	6.98	20.34**	11.87**	0.83	7.77**	1.23
[N] (area)	2.91	53.39**	81.49**	0.03	5.94**	1.11
g_s	1.16	11.97*	40.31**	0.09	0.16	5.36*
A_{max}	90.25**	44.39**	25.73**	0.04	2.70	4.26
$V_{c,max}$	38.38**	37.32**	22.41**	1.63	1.55	2.14
J_{max}	24.98**	33.87**	44.43**	3.32	3.64	2.67
PNUE	69.19**	7.06	10.35**	1.84	3.58	4.53

**$P < 0.001$; *$P < 0.01$.

starch levels (Table 2). Leaf nitrogen levels of dystrophic species were more responsive to increased nutrient concentration than those of mesotrophic species, while sugar levels of mesotrophic species were more responsive to nutrient concentration than those of dystrophic species (resulting in significant nutrient × species interaction).

Correlations

A_{max} was positively correlated ($P < 0.02$) with leaf [N] for both ambient and elevated CO_2-grown plants (Fig. 2). The slope of these regressions did not differ significantly, but the intercept was significantly increased in elevated CO_2 ($P < 0.002$), and the regressions differed significantly ($P < 0.005$). Both J_{max} and $V_{c,max}$ were significantly-positively correlated with leaf [N] (Fig. 3), but regressions differed between CO_2 treatments only for $V_{c,max}$ ($P < 0.05$), due to a significantly increased intercept in elevated CO_2 ($P < 0.01$). $V_{c,max}$ and J_{max} were significantly co-correlated in both ambient and elevated CO_2-grown plants (Fig. 4), but these regressions barely differed significantly ($P = 0.047$), due to increased intercept in elevated CO_2 ($P < 0.02$).

Both J_{max} and $V_{c,max}$ were significantly negatively correlated with leaf starch concentrations, regardless of CO_2 treatment (Fig. 5). The coefficient of variation of the $V_{c,max}$:starch correlation was increased by expressing leaf carbohydrate status on a leaf dry mass basis (Fig. 5 insert). Both $V_{c,max}$ and J_{max} were poorly correlated with leaf sugar concentration (data not shown).

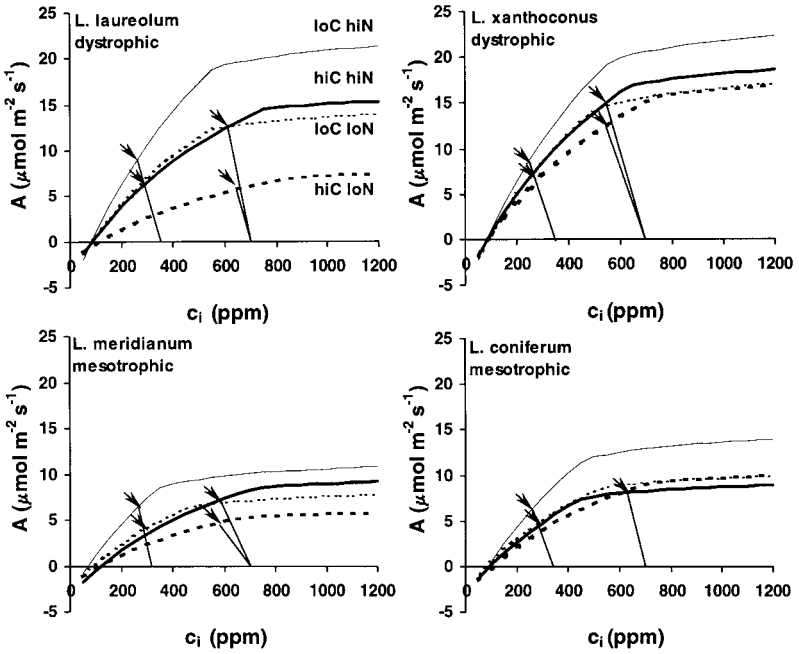

Fig. 1. CO_2 response curves (A:c_i curves) of four fynbos *Leucadendron* species, adapted to soils of different nutrient status, grown under two contrasting soil nutrient regimes and two atmospheric CO_2 levels. Gas exchange measurements were carried out above the light-saturation level (PFD >1000 μmol m^{-2} s^{-1}), with cuvette air temperature 29 ± 1 °C and cuvette air vapour pressure 20 ± 2 mb. Curves were plotted using mean parameter values, derived from three replicate plants, substituted into the model of Farquhar *et al.* (1980). Line representations given in the top left panel apply to all panels, thin lines refer to ambient CO_2 level (loC), and heavy lines to elevated CO_2 level (hiC), dashed lines to low nutrient concentration (loN) and continuous lines to high nutrient concentrations (hiN). Arrows indicate operational c_i at the growing CO_2 level, and lines connect these points to the growing CO_2 level on the x-axis (supply function [Sharkey, 1985], indicating stomatal limitation).

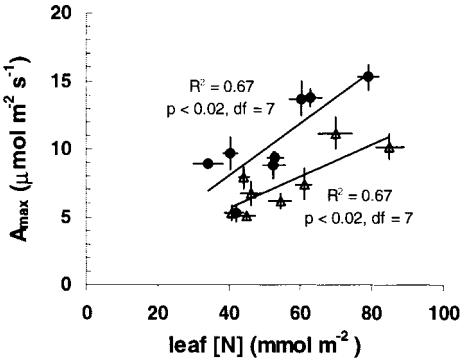

Fig. 2. Relationship between leaf [N] and A_{max} in juvenile individuals of four *Leucadendron* species adapted to soils of different nutrient status, grown under two contrasting soil nutrient regimes and two atmospheric CO_2 levels. Each symbol is a two way mean of eight nutrient values and three A_{max} values, with bars representing standard errors. Solid circles represent means for plants grown at 700 μmol mol^{-1} CO_2, and triangles those grown at 350 μmol mol^{-1} CO_2.

Discussion

Nutrient-induced and species differences in assimilation rate and CO_2-responsiveness

As expected, higher nutrient concentration led to generally increased leaf nutrient status and associated higher photosynthetic rates and stomatal conductance, patterns often reported in the literature (Field and Mooney, 1986; Evans, 1989). The significant species differences for all leaf nutrient and photosynthetic measures were also expected. Unexpectedly, however, dystrophic species had higher foliar N concentrations under both nutrient regimes than did mesotrophic species, and higher total plant N (data not shown). This suggests that the dystrophic species had an inherently higher nutrient uptake capacity than did mesotrophic species. Soil factors, rather than competitive interactions seem to explain species/soil specificity in nature among these and other Proteaceous species (Richards *et al.*, 1997*a*). It is possible that differences in nutrient uptake capacity may determine these patterns.

Even though species' photosynthetic characteristics differed significantly, the photosynthetic response of all four species to both increased nutrient concentration and elevated CO_2 was similar, as there were no species $\times$ CO_2 and species $\times$ nutrient interactions. Also, the lack of $CO_2 \times$ nutrient interaction suggests that nutrient concentration did not alter photosynthetic CO_2-responsiveness. If either species-specific characteristics or nutrient concentration were important determinants of CO_2-responsiveness, then significant interaction of these factors with CO_2 level would be expected.

The mechanisms which control photosynthetic CO_2-responsiveness in these species, therefore, do not seem to be primarily a function of nutrient concentration (i.e. not

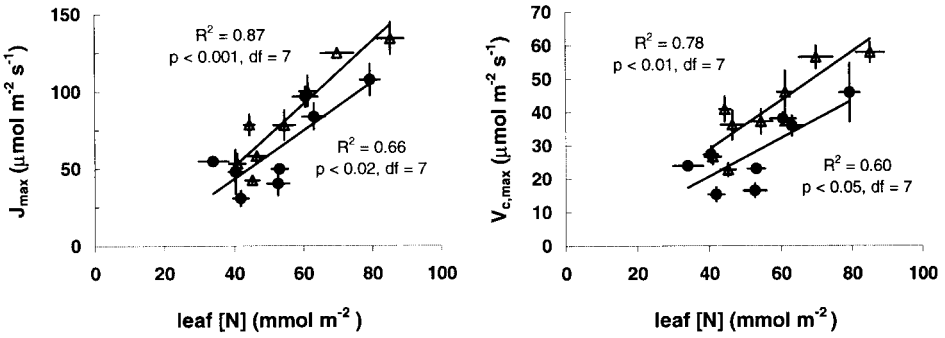

Fig. 3. Relationship between leaf [N] and J_{max} and between leaf [N] and $V_{c,max}$ in juvenile individuals of four *Leucadendron* species adapted to soils of different nutrient status, grown under two contrasting soil nutrient regimes and two atmospheric CO_2 levels. Each symbol is a two-way mean of eight nutrient values and three J_{max} or $V_{c,max}$ values, with bars representing standard errors. Solid circles represent means for plants grown at 700 μmol mol^{-1} CO_2, and triangles those grown at 350 μmol mol^{-1} CO_2.

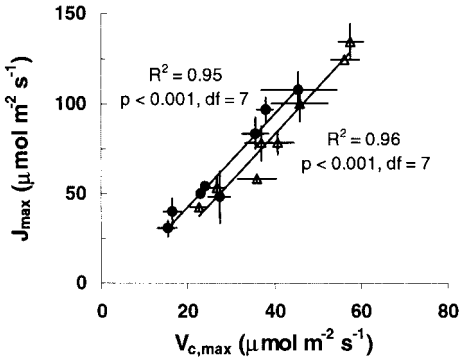

Fig. 4. Relationship between $V_{c,max}$ and J_{max} in juvenile individuals of four *Leucadendron* species adapted to soils of different nutrient status, grown under two contrasting soil nutrient regimes and two atmospheric CO_2 levels. Each symbol is a two-way mean of three J_{max} and $V_{c,max}$ values, with bars representing standard errors. Solid circles represent means for plants grown at 700 μmol mol^{-1} CO_2, and triangles those grown at 350 μmol mol^{-1} CO_2.

chiefly extrinsic). These mechanisms do not appear to be species-specific either, and so are independent of the recent evolutionary divergence that accompanied the development of their association with distinct soil types.

Leaf nitrogen content and photosynthetic capacity

Nitrogen is a central determinant of leaf photosynthetic capacity (A_{max}), and A_{max} is correlated with leaf [N] across the plant kingdom (Field and Mooney, 1986; Evans, 1989; Woodward and Smith, 1994). This study is consistent with that pattern, but reveals a significant increase in the efficiency of nitrogen use in elevated CO_2 represented by the increased intercept of the linear leaf A_{max}:[N] relationship (Fig. 2). This study also shows a related and clearly demonstrable increase in PNUE of high CO_2-grown plants (Table 2), and further that the principal determinants of A_{max}, namely carboxylation efficiency (i.e. $V_{c,max}$) and RuBP regeneration capacity (J_{max}), were also significantly correlated with leaf [N] (Fig. 3). Furthermore, $V_{c,max}$ and J_{max} were consistently co-correlated in the four selected species (Fig. 4). This pattern is virtually identical to that identified for a broad range of species (Wullschleger, 1993; Leuning, 1997), and is thought to represent optimal distribution of nitrogen between light-harvesting and carboxylation functions (Lloyd and Farquhar, 1996). Thus the dependence of A_{max} on leaf [N] seems to be largely due to a consistent partitioning ratio of N resources that may be more or less conserved across the plant kingdom, and the ubiquitous observation of increased PNUE in elevated CO_2 (Drake *et al.*, 1997) is the manifest result.

It is perhaps surprising that there was no apparent N repartitioning between carboxylation and RuBP regeneration in elevated CO_2, given that this would boost plant nitrogen-use efficiency even more than occurs by the reduction in photorespiration alone (Bowes, 1991; Sage, 1994). Nitrogen repartitioning in elevated CO_2 has been reported in only very few studies, such as for loblolly pine (Tissue *et al.*, 1993). Nitrogen repartitioning of this type often accompanies the process of shade adaptation (Woodward, 1990), but is probably triggered by a change in light quality, and not carbohydrate availability. Nitrogen allocation at canopy level approximates optimality with respect to carbon assimilation (Field, 1983; Pons *et al.*, 1993), but a lack of repartitioning at leaf level in elevated CO_2 suggests that current allocation patterns may not be optimal at future higher CO_2 levels (Lloyd and Farquhar, 1996). In fact, optimal partitioning of N between carboxylation and light-harvesting functions may be tuned to lower than current ambient atmospheric CO_2 levels, found prior to the industrial revolution ($\sim$270 μmol mol^{-1} CO_2), as appears from a model for Amazonian rainforest (Lloyd *et al.*, 1995). It is possible that species with greater plasticity of nitrogen partitioning in response to carbohydrate availability (such as loblolly pine, Tissue *et al.*, 1993), or genotypes with variable partitioning ratios, might be favoured as CO_2 continues to rise. Certainly, CO_2 responsiveness has been shown to differ between ecotypes (Norton *et al.*, 1995) and may vary, heritably, between genotypes (Curtis *et al.*, 1994).

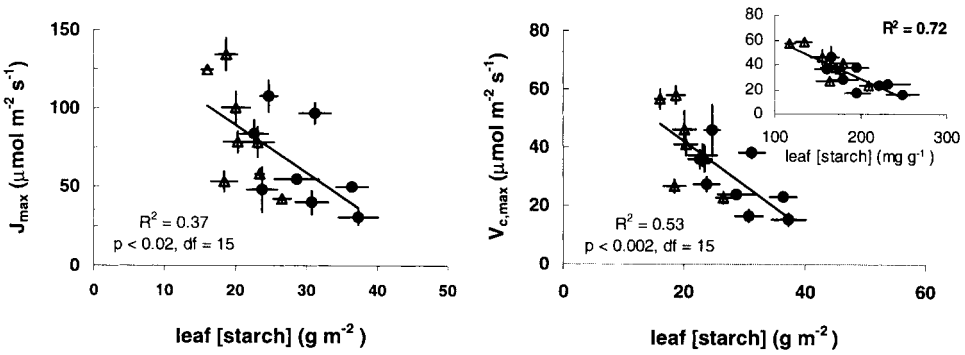

Fig. 5. Relationship between leaf starch content (leaf area basis) and J_{max} and $V_{c,max}$, (Fig. insert: starch expressed on a dry mass basis) in juvenile individuals of four *Leucadendron* species adapted to soils of different nutrient status, grown under two contrasting soil nutrient regimes and two atmospheric CO_2 levels. Each symbol is a two-way mean of eight carbohydrate values and three J_{max} or $V_{c,max}$ values, with bars representing standard errors. Filled symbols represent means for plants grown at 700 μmol mol^{-1} CO_2, and empty symbols those grown at 350 μmol mol^{-1} CO_2.

Photosynthetic acclimation in elevated CO_2

An understanding of photosynthetic acclimation processes in elevated CO_2 remains elusive (van Oosten and Besford, 1996). Although responses at leaf level are diverse, some generalizations can be made for woody species (Gunderson and Wullschleger, 1994). Net carbon uptake rate measured at growth [CO_2] is stimulated under elevated CO_2 by roughly 45%, even though the net CO_2 uptake rate of elevated CO_2-grown plants is 21% lower than that of ambient CO_2-grown plants when measured at elevated CO_2 concentration (Gunderson and Wullschleger, 1994). The results of this study are consistent with these generalizations.

The mechanism most commonly implicated in acclimation is feedback regulation of carboxylation by carbohydrate accumulation (Stitt, 1991; van Oosten *et al.*, 1994; Jacob *et al.*, 1995). Sugar repression of photosynthesis has been identified as a general trigger for the regulation of photosynthesis in response to changes in sink demand (van Oosten and Besford, 1996). Although relationships have been shown between carbohydrate status and photosynthetic measures in high CO_2-grown plants, results from elevated CO_2 studies are contradictory (Paul and Driscoll, 1997).

Photosynthetic acclimation in this study comprised apparently synchronized reductions in both carboxylation and RuBP regeneration capacity, and not only the often-cited reduction in carboxylation capacity. Feedback regulation of RuBP regeneration capacity has not received the same emphasis as short-term photosynthetic acclimation in response to tissue carbohydrate status (van Oosten and Besford, 1996) involving regulation of carboxylation capacity.

The consistent negative relationship found in the current study between starch accumulation and both $V_{c,max}$ and J_{max} (Fig. 5) identifies the central role of carbohydrate accumulation (which responded to both elevated CO_2 and nutrient concentration) in photosynthetic regulation. This pattern suggests a general mechanism of photosynthetic regulation in response to both nutrient and carbohydrate concentration, supporting the contention that photosynthetic responses to nutrient deficiency are almost identical to those to elevated CO_2 (Paul and Driscoll, 1997). This suggests that elevated CO_2 emulates an excess end-product condition, triggering photosynthetic downregulation in a response which may have evolved under conditions of source:sink imbalance, such as periodic nutrient limitation. This response is likely to be particularly well-developed in species subject to periodic episodes of nutrient stress and, therefore, may have an important genetic component (Sage *et al.*, 1989).

Plant species differ in their propensities for accumulating starch relative to sugars, and there is an identified need for a better understanding of how starch- versus sugar- accumulating species respond to elevated CO_2 (Bowes *et al.*, 1996). Species studied here showed roughly 5-fold greater starch than sugar concentrations, and starch status seemed more important in photosynthetic feedback regulation than in many other studies. Carbohydrate relations in source leaves are regulated both on a short-term (instantaneous and diurnal) basis (Fondy *et al.*, 1989; Geiger and Servaites, 1994) and on a longer term basis reflected by the baseline (morning) total non-structural carbohydrate (TNC) concentration. Jacob *et al.* (1995) reported increased sugar concentrations in photosynthetically-acclimated high CO_2-grown *Scirpus olneyi* only at midday, whereas both baseline and midday starch concentrations were higher. This would support the suggestion that baseline TNC status, rather than the more ephemeral diurnal sugar fluctuation, is the cue for *in vivo* photosynthetic downregulation in elevated CO_2 in the medium- to long-term.

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