

Control of PAR-saturated CO₂ exchange rate in some C₃ and CAM plants

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Abstract

We measured PAR-saturated CO₂ exchange rate (CER), and leaf N, P and chlorophyll (Chl) concentrations in 21 plant species, selected to encompass as broad a range in specific leaf area (SLA) as possible, and encompassing non-succulent C₃ as well as succulent CAM plants. We worked with plants growing under uniform conditions in the facilities of a biological research station to ensure that any correlations found were due to inherent, genetically controlled, relationships between the measured parameters and not due to variations in resource availability in different habitats. We found CER to be strongly correlated to SLA, leaf N concentration and Chl concentration. CER increased much faster with increasing leaf N concentration ($CER \approx N^{3.1}$) than with increasing SLA ($CER \approx SLA^{1.2}$). CER also increased much faster with leaf N concentration than with increasing Chl concentration ($CER \approx Chl^{1.3}$), indicating the photosynthetic N-use efficiency (NUE) to be higher for plants with high N concentration than for plants with low N concentration ($NUE \approx N^{2.1}$). Analysis of covariance showed that these relationships exist even when comparing plants of widely different growth forms - succulent or non-succulent, and of different photosynthetic pathways, as the C₃ and CAM plants compared here. Testing against scaling coefficients calculated using dimensional analysis, showed that the scaling of N, Chl and CER against SLA was not merely a result of diluting N and Chl with

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Abbreviations: ANCOVA - analysis of covariance, CAM - crassulacean acid metabolism, CER - CO₂ exchange rate, Chl - chlorophyll, IRGA - infrared gas analyzer, NUE - photosynthetic nitrogen use efficiency, PAR - photosynthetically active radiation, SLA - specific leaf area, SLM - specific leaf mass.

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carbon in thicker leaves but that SLA, probably through influencing light absorption and/or CO₂ diffusion pathway, played an independent role in controlling CER.

Additional key words: CER, leaf thickness, photosynthesis, photosynthetic N-use efficiency, succulence.

Introduction

PAR-saturated CO₂ exchange rate, CER, has been shown to be closely related to leaf N concentration (*e.g.* Evans 1983, 1989, Field and Mooney 1986, Chapin *et al.* 1987, Körner 1989, Reich *et al.* 1992, Connor *et al.* 1993, Terashima *et al.* 1993). Interestingly, although leaf N concentration and Chl concentration appear to be coupled, at least when comparing leaves of differing N status within a single species (Evans 1983, 1989), Chl concentration has been found to be a minor component of the variation in CER when comparing across a broad range of species (about 12 % of the variation, Nielsen and Sand-Jensen 1989 - calculated from Björkman 1981). Another factor that has been shown to be closely coupled to leaf N concentration and/or to CER is leaf longevity (*e.g.* Mooney and Dunn 1970, Williams *et al.* 1989, Körner 1989, Diemer *et al.* 1992, Reich *et al.* 1992). Diemer *et al.* (1992) found leaf longevity to be inversely related to leaf N concentration, but not to CER and concluded that leaf longevity must be associated with tissue qualities not related to assimilatory potential. In contrast, Mooney and Dunn (1970), Williams *et al.* (1989) and Reich *et al.* (1992) found a negative relationship between leaf longevity and CER, thus linking leaf longevity and payback period - the time needed for a leaf to produce photosynthate equalling the investment for production of the leaf. Leaf life span is negatively correlated to SLA, indicating that thick leaves tend to have longer life spans (Reich *et al.* 1991, Reich 1993). CER has also been shown to be correlated to the SLA (Reich *et al.* 1992). However, there is also a strong correlation between leaf N concentration and SLA (or the inverse - specific leaf mass, SLM) (Körner 1989, Reich *et al.* 1992), and it thus remains unclear whether the apparent coupling between SLA and CER is direct, for instance due to reduced light absorption per unit biomass in thick leaves (Agusti *et al.* 1994) or the increasing diffusion pathway of CO₂ with increasing thickness (Parkhurst 1986), or whether it is indirect and caused by thick leaves having inherently low N concentrations.

Experimental tests of the relationships between growth rate, CER, and leaf properties (*e.g.* SLA, thickness, and chemical composition), have been conducted within relatively narrow ranges of plant types. These are often varieties of individual species (*e.g.* Evans 1983) or plants with similar growth forms (*e.g.* Körner 1989, Poorter *et al.* 1990, Poorter and Remkes 1990, Diemer *et al.* 1992, Lambers and Poorter 1992, Kitajima 1992). Conclusions derived from extension of narrow plant ranges may lead to unsubstantiated disagreement when discussing general patterns (Reich 1993). Consequently, broader ranges of plants have been compared by compiling data from different sources, (*e.g.* Field and Mooney 1986, Chapin *et al.* 1987), where plants grew under widely different conditions. In nature, however, there is a clear tendency for thick-leaved plants with low SLA to be found in resource-poor

environments (Mooney *et al.* 1981), whether the low SLA reflects adaptations to increase water storage capacity, as in succulent plants, or is associated to adaptations to increase leaf longevity and thus reduce nutrient investment per unit time (Mooney and Dunn 1970, Chabot and Hicks 1982, Reich *et al.* 1992, Reich 1993). Plants with high SLA, on the other hand, are often found in nutrient rich, fertile, environments. Hence, the observed association between SLA, N concentration, and CER in comparisons of diverse plant types could be confounded by the tendency of thick-leaved plants to be confined to nutrient-poor habitats. There is, therefore, a need to test the observed association between leaf thickness, N concentration and CER across a broad range of plants growing under homogeneous resource availability. This approach avoids the confounding covariation of resource availability and leaf characteristics and focuses on relationships between CER and intrinsic properties of the leaves.

We examine here the relationships between CER and SLA, nutrient and Chl concentrations of a range of plants grown under similar conditions (soil properties and meteorological conditions). As the thickness - or SLA - of the photosynthetic tissue has been shown to be an important determinant of CER in a wide range of plant forms (Enriquez *et al.* 1996), we selected the plants as to encompass a broad range of SLA to test if such patterns could also be found in these terrestrial plants. We deliberately included plants with different photosynthetic pathways (C₃ and CAM), and, therefore, with succulent and non-succulent leaves in order to test whether the relationships that has been observed for narrow ranges of C₃ plants (Körner 1989, Poorter *et al.* 1990, Poorter and Remkes 1990, Diemer *et al.* 1992, Lambers and Poorter 1992) and for phototrophic organisms in general (Enriquez *et al.* 1996) can be demonstrated regardless of photosynthetic pathway.

In the literature, CER is often expressed on an area basis and nutrient and Chl concentrations on a mass basis. However, as stated by Field and Mooney (1986), this is confusing and can make interpretation of results difficult because SLM is subject to changes in response to nutrient availability. As our purpose is to examine the relationships between SLA, nutrient concentration, Chl concentration, and CER, we chose to express all parameters on a mass basis. As it has been shown that the correlation between nutrient content and CER is weak or non-existent when both parameters are expressed on an area basis (Field and Mooney 1986, Reich *et al.* 1992), there is a possibility that any relationship found between mass based CER and nutrient and Chl is caused by a "dilution effect" due to dilution of a constant amount of nutrients and Chl under a given leaf surface with carbon when increasing leaf thickness (decreasing SLA). To examine this possibility, we compare the scaling coefficients we find with those expected from a dilution effect, calculated using dimensional analysis (Niklas 1994).

Materials and methods

The plants used in this study grew under uniform soil and meteorological conditions within a restricted area (about 2000 m²) of the facilities of the International

Mediterranean Biological Station at Blanes, North-East Spain (41° 40.4' N, 2° 48.16' E). The plants were growing in a sandy soil (89 % sand), containing 17.5 mg kg⁻¹ inorganic N and 3 mg kg⁻¹ available phosphorous (Pedrola 1983). During the study period (second half of July) the mean maximum temperature was 29 °C and the mean minimum temperature was 17 °C. There was no precipitation during the study period, but the plants were watered regularly. We selected 21 plant species encompassing the broadest possible range in SLA, and including plants with different metabolic pathways (C₃ and CAM), and leaf structure (succulent and non-succulent) (Table 1). The plants used were all mature, fully-grown specimens, although we avoided flowering individuals.

We measured CER on individual leaves, still attached to the plant, using an ADC LCA-3 portable infrared gas analyzer (*Analytical Development Company*, Hoddesdon, U.K.), attached to a leaf chamber. As the plants grew in the open under natural light conditions, irradiance varied during the study. However, with respect to day-time carbon exchange (C₃ plants), only CER measured at irradiances in excess of 900 µmol m⁻² s⁻¹ are reported here as PAR-saturated CER. Initially, CER were measured in the morning (between 8.00 and 11:00 AM) to avoid stomata closure due to high temperatures and low humidity later in the day. To ensure that stomata closure did not produce artificially low CER during the measurements, we continuously monitored relative humidity in the incoming as well as the outgoing air in the leaf chamber through built-in sensors. If the relative humidity in the outgoing air declined abruptly, this was assumed to reflect stomata closure, and the photosynthesis data were not included in the data analysis. No net CO₂ uptake was detected in some succulent plants during the day, suggesting these to have CAM photosynthesis. The CER of these plants was measured during the night, when the CO₂ uptake of CAM plants generally exhibits maximum values (Nobel 1976, 1977, Kluge and Ting 1978, Osmond 1978).

After CER and thickness had been measured, the leaves (or leaf discs in the case of very large leaves) were harvested and brought to the laboratory to measure Chl and nutrient concentrations. SLA was calculated by measuring the mass of leaf discs of known area following desiccation at 80 °C to constant mass. Chl *a+b* was measured spectrophotometrically on fresh samples following extraction with acetone (Hendry and Grime 1993). Carbon and N were measured on dried subsamples using a *Carlo-Erba* (Milan, Italy) *EA-1108* CHN-analyzer, and phosphorus was determined colorimetrically following wet acid digestion (Korolev 1970).

As succulent CAM plants almost invariably have lower SLA and lower nutrient and Chl concentrations than non-succulent C₃ plants, we used analysis of covariance (*ANCOVA*, Sokal and Rohlf 1981) to test for differences in the slope and elevation of the linear relationships among C₃ and CAM plants. If no significant differences in slope or elevation were found, the total data set, comprising C₃ as well as CAM plants, was analyzed as a single data set (Sokal and Rohlf 1981). The relationships between CER and leaf characteristics (SLA and nutrient concentrations) were described using linear regression analysis (Sokal and Rohlf 1981). All data were log-transformed (base 10) before data analysis to comply with the assumptions of least-squares linear regression analysis.

Table 1. Parameters measured in this study. Leaf thickness [cm], SLA [cm² g⁻¹], Chl *a+b* concentration [% (d.m.)], and concentration of carbon, N and P, all in % (d.m.), as well as photosynthetic N use efficiency (NUE) [mmol(CO₂) mmol⁻¹(N) s⁻¹] and carbon exchange rate (CER) [mmol(CO₂) g⁻¹(d.m.) s⁻¹] ± 1 SD. CER values are means of 4–6 measurements on the same leaf; all other values are means of triple analyses. No estimate of error is given for these parameters as the triple analyses were performed on the same leaf sample for each species, and therefore only would reflect analytical error, not within species variation. Finally, the table gives the range of each parameter and the coefficient of variation [%]. Plants marked with ^c displayed CAM-photosynthesis.

Species	Thickness	SLA	Chl	Carbon	N	P	NUE	CER
<i>Aristolochia grandiflora</i>	0.005	345.3	0.93	44.64	3.28	0.52	1641.92	3849.1 ± 82.1
<i>Asparagus plumosus</i>	0.010	700.5	1.05	44.47	3.27	0.33	4332.82	10131.8 ± 786.2
<i>Aster verticatus</i>	0.010	369.9	1.04	41.63	3.56	0.43	2038.64	5184.0 ± 194.4
<i>Opismenus hirtellus</i>	0.010	203.4	0.27	42.29	2.58	0.67	1334.59	2458.1 ± 23.8
<i>Canna edulis</i>	0.020	428.5	2.52	43.25	3.97	0.25	3758.76	10644.5 ± 740.9
<i>Musa ornata</i>	0.020	179.6	0.81	43.22	2.92	0.19	479.09	1000.1 ± 58.3
<i>Ophiopodon japonicum</i>	0.020	93.1	0.43	45.87	2.54	0.22	719.32	1304.6 ± 56.2
<i>Alocasia odorata</i>	0.030	197.3	0.99	48.97	3.90	0.52	1218.74	3391.2 ± 47.5
<i>Agapanthus africanus</i>	0.050	156.9	0.84	45.91	2.54	0.31	635.51	1153.4 ± 4.3
<i>Liriope muscally</i>	0.060	120.5	0.80	45.17	2.61	0.60	567.90	1058.4 ± 34.6
<i>Yucca aloifolice</i> ^c	0.085	39.3	0.28	45.81	1.48	0.36	267.44	283.0 ± 41.0
<i>Yucca gloriosa</i> ^c	0.095	39.1	0.22	46.60	1.92	0.30	406.98	557.3 ± 41.0
<i>Clivia nobilis</i>	0.100	168.9	0.92	51.32	2.14	0.23	634.79	969.8 ± 58.3
<i>Gibasis geniculata</i>	0.100	220.1	1.18	37.77	4.79	0.38	715.25	2447.3 ± 30.2
<i>Agave micracantha</i> ^c	0.290	26.9	0.10	41.31	1.18	0.45	261.18	220.3 ± 28.1
<i>Agave filifera</i> ^c	0.340	14.8	0.08	46.48	1.26	0.27	61.92	56.2 ± 4.3
<i>Euphorbia mauritanica</i> ^c	0.440	32.7	0.12	44.84	1.00	0.32	282.20	203.0 ± 51.8
<i>Agave weberi</i> ^c	0.470	11.5	0.12	40.73	1.12	0.22	67.97	54.0 ± 2.2
<i>Opuntia lagopus</i> ^c	0.570	32.2	0.13	36.28	2.10	0.42	353.74	503.3 ± 116.6
<i>Agave ferox</i> ^c	0.645	8.6	0.21	41.26	1.43	0.29	55.91	56.2 ± 8.6
<i>Opuntia columbina</i> ^c	0.895	39.5	0.18	35.53	1.87	0.25	491.15	656.6 ± 38.9
Range	0.050–0.895	8.6–700.5	0.08–2.52	35.53–51.32	1.00–5.97	0.19–0.67	55.91–4332.82	54.0–10644.5
CV	127.1	107.5	92.7	9.9	38.7	38.7	118.9	138.9

Results

SLA varied over two orders of magnitude within this data set, from 8.6 cm² g⁻¹ in *Agave ferox* to 700.5 cm² g⁻¹ in *Asparagus plumosus* (Table 1). Nutrient concentrations varied three to four fold, compared with a 32 fold variation in Chl concentration (Table 1). There was little or no overlap in the measured parameters between the C₃ plants and the CAM plants (Table 1, Fig. 1), suggesting that the data for these two plant groups could not *a priori* be assumed to constitute a single data set. We therefore used analysis of covariance (ANCOVA) to analyze for differences in the relationships between SLA as the independent parameter and N concentration and Chl concentrations as the dependent parameters. Analysis of covariance showed that there was no significant differences in the slope or elevation among the relationships for the two groups between neither SLA and N concentration ($F_{\text{slope}} = 0.068$, $F_{\text{elevation}} = 1.917$, $P > 0.05$) nor SLA and Chl concentration ($F_{\text{slope}} = 0.733$, $F_{\text{elevation}} = 4.282$, $P > 0.05$), indicating that the data for C₃ and CAM plants could indeed be treated as one data set with regard to these parameters. Both N concentration and Chl concentration increased significantly but non-linearly with increasing SLA, so that $N \approx \text{SLA}^{0.3}$ and $\text{Chl} \approx \text{SLA}^{0.7}$ (Table 2, Fig. 1), indicating that plants with low SLA tends to have low N and Chl concentrations. Dimensional analysis showed that if N and Chl content was constant under a given surface area and the scaling to SLA therefore caused by a dilution effect, then N and Chl should scale to $\text{SLA}^{1.0}$. To examine this possibility, we tested the found slopes against slope = 1.0 using Student's test. In both cases was $P < 0.01$, so that the obtained slopes were significantly different from 1.0 in both cases. P and carbon showed no correlation ($P > 0.05$) with SLA in neither C₃ nor CAM plants.

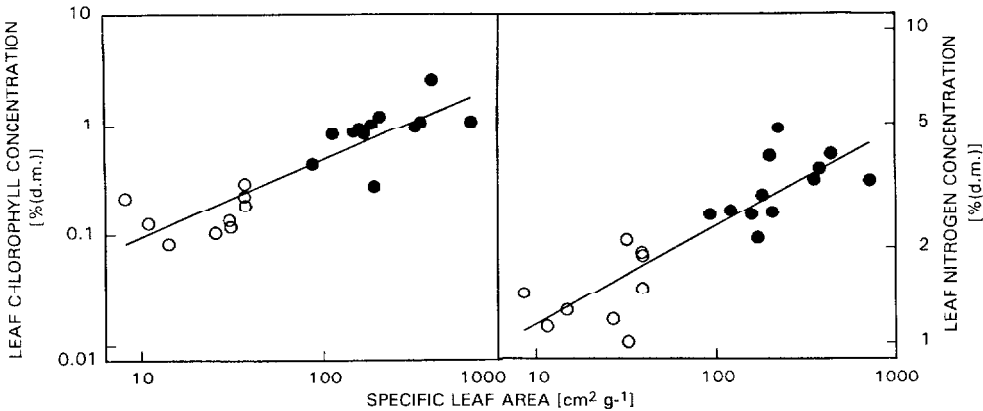


Fig. 1. Leaf N concentration and leaf Chl *a+b* concentration as a function of SLA in C₃ species (closed circles) and CAM species (open circles). The lines represent the fitted regression equations (Table 2).

The PAR-saturated CER varied over two orders of magnitude (Table 1), but also this parameter showed little overlap between C₃ and CAM plants. ANCOVA did

Table 2. Regression equations ($\log_{10}(Y) = a + b \times \log_{10}(X)$) describing the more important relationships between the parameters measured in this study. All parameters are in the same units as in Table 1. The constant (a) and the slope (b) are given $\pm$ SE. Also shown are coefficients of determination (r^2), variance-ratio (F), and probably level (P). $N = 21$ in all cases.

Y	X	a	b	r^2	F	P
N	SLA	-0.258 ± 0.081	0.313 ± 0.040	0.761	60.61	< 0.00001
Chl	SLA	-1.744 ± 0.174	0.699 ± 0.087	0.774	65.15	< 0.00001
CER	SLA	0.599 ± 0.155	1.202 ± 0.077	0.927	241.96	< 0.00001
Chl	N	-1.072 ± 0.095	1.958 ± 0.238	0.781	67.90	< 0.00001
NUE	N	1.987 ± 0.141	2.119 ± 0.355	0.653	35.67	0.00001
CER	N	1.841 ± 0.141	3.119 ± 0.355	0.803	77.28	< 0.00001
CER	Chl	3.442 ± 0.114	1.319 ± 0.196	0.704	45.28	< 0.00001

show, however, that there was no significant differences in slope or elevation among the relationships for the two plant group for CER vs. SLA ($F_{\text{slope}} = 0.509$, $F_{\text{elevation}} = 3.323$, $P > 0.05$), CER vs. N ($F_{\text{slope}} = 0.048$, $F_{\text{elevation}} = 1.036$, $P > 0.05$) or CER vs. Chl concentration ($F_{\text{slope}} = 0.027$, $F_{\text{elevation}} = 1.772$, $P > 0.05$), once again indicating that the data for C₃ plants and for CAM plants could be treated as one data set. PAR-saturated CER increased with SLA^{1.2} (Table 2, Fig. 2). If the scaling of CER to SLA was due to a dilution effect, CER would scale to SLA¹ according to dimensional analysis. Using Student's t -test we tested this possibility by comparing the found slope of 1.2 against the hypothetical slope of 1.0 and found $0.02 < P < 0.01$, so that the scaling coefficient (slope) found was significantly different from the one expected if the scaling was caused by a dilution effect. CER increased much faster with increasing N concentration ($\text{CER} \approx \text{N}^{3.1}$) (Table 2, Fig. 2) than with increasing Chl concentration ($\text{CER} \approx \text{Chl}^{1.3}$) (Table 2, Fig. 2). This suggests that the photosynthetic N use efficiency (NUE) is disproportionately higher for plants with high N concentration than for plants with low N concentration ($\text{NUE} \approx \text{N}^{2.1}$, $r^2 = 0.65$) (Table 2). As for SLA, P and C did not show any correlation with CER in neither plant group.

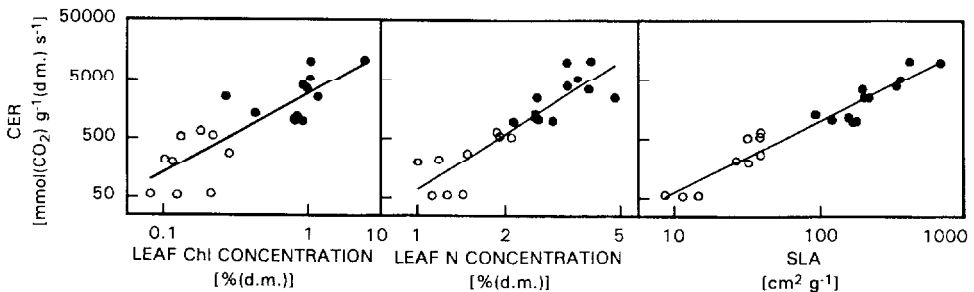


Fig. 2. Carbon exchange rate (CER) in C₃ species (closed circles) and CAM species (open circles) as a function of SLA, leaf N concentration, and leaf Chl $a+b$ concentration. The lines represent the fitted regression equations (Table 2).

Discussion

The nutrient concentrations of the plants examined were relatively uniform and high, particularly for P; P and N concentrations were not correlated, deviating from their positive correlation in wild plants (Garten 1976, Duarte 1992). This probably results from the nutrient supply the plants experienced. Yet, plants with low SLA tended to have lower N concentrations than thin plants, despite growing under the same nutrient supply and environmental conditions. This provides evidence for intrinsic differences in resource use. Thick plants also had lower Chl concentrations than thin plants. Hence, thin plants, with high N concentrations, allocated a significant larger fraction of their N to Chl production than thick plants did.

The increase in CER with increasing N concentration indicates, consistent with previous observations (Field and Mooney 1986, Chapin *et al.* 1987), a tendency towards increasing CER per unit N (NUE) with increasing leaf N concentration. The relationships between CER and leaf N concentration was strong among the plants examined, as observed before for herbs, shrubs and trees (Evans 1983, 1989, Field and Mooney 1986, Chapin *et al.* 1987, Körner 1989, Reich *et al.* 1992).

CER was also positively related to Chl concentration, in contrast to previous findings that differences in Chl concentration across species are only able to account for some 12 % of the variation in CER (Björkman 1981, Nielsen and Sand-Jensen 1989). In our study we also found a significant relationship between SLA and CER. This could, however, be a spurious correlation if, as indicated by earlier work (Field and Mooney 1986, Reich *et al.* 1992), CER and nutrients (and Chl, Evans 1983, 1989) are uncorrelated when all are measured on a surface basis. In that case, the scaling of CER (mass based) to N, Chl (also mass based) and SLA could be caused by dilution of photosynthetically active compounds with storage carbon in thicker leaves. If such a dilution effect was the cause behind the observed patterns, then N, Chl and CER would all scale to $SLA^{1.0}$. This is, however, not the case. Although carbon concentration was independent of SLA, indicating that decreasing SLA (increasing thickness) is indeed caused by increasing carbon content per unit leaf surface, scaling of all three parameters to SLA were significantly different from 1.0, indicating an independent role for SLA in controlling CER. The importance of SLA possibly derives from the fact that increasing SLA leads to increased light absorption per unit biomass (Agusti *et al.* 1994) and possibly also to improved gas exchange (Parkhurst 1986). The close scaling of the PAR-saturated CER to SLA is not limited to terrestrial plants. Similar relationships have been found for marine macroalgae, both within a single species (Hanisak *et al.* 1988) and among species (Littler 1980, Enriquez *et al.* 1995) as well as for phytoplankton (Taguchi 1976, Blasco *et al.* 1982, Glover *et al.* 1987), and has recently been demonstrated across a wide range of phototrophic organisms (Enríquez *et al.* 1996). This suggests that the close scaling of CER to SLA may be a general feature of photosynthetic organisms, independent of photosynthetic pathways, as demonstrated here.

Terrestrial plants develop thick photosynthetic structures with low SLA and low nutrient and Chl concentrations as an adaptation to increase water storage capability, as in succulent plants, and to increase mechanical strength and pathogen resistance

and reducing palatability to herbivores in order to increase leaf life span (Mooney and Dunn 1970, Körner 1989, Diemer *et al.* 1992, Reich *et al.* 1992, Reich 1993); these adaptations being more important in resource-poor environments (Chabot and Hicks 1982). Our results, which combine succulent and non-succulent plants, provide evidence that decreasing SLA, whether by increasing carbon concentration or by increasing thickness by increasing the amount of tissue capable of water storage, have important costs in terms of capacity for carbon exchange. Thick-leaved plants maintain lower nutrient concentrations even when grown under conditions allowing thin-leaved plants to attain high N concentrations. The low SLA, which is a consequence of necessary adaptations to resource poor habitats, leads to low Chl and tissue nutrient concentrations and restricts the achievable carbon exchange rate, so that the low CER often found in plants from these habitats, not only is a consequence of environmental resource limitation, but also is associated with inherent, genetically controlled, adaptations which are not subject to change, even when the plant is grown under ameliorated conditions.

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