WILL NITROGEN FIXERS BENEFIT FROM RISING ATMOSPHERIC CO_2? THE RESPONSE OF *PODALYRIA SERICEA* TO ELEVATED CO_2.

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ABSTRACT

To predict the response of communities and ecosystems to rising levels of atmospheric CO_2, the response of individual plants under natural conditions needs to be determined. *Podalyria sericea*, a fynbos legume, was grown in a greenhouse under ambient (35 Pa) and elevated (70 Pa) CO_2 partial pressure for 18 months. Growth, leaf $\delta^{15}N$ and leaf gas exchange was measured to determine whether this N_2-fixer could maintain a positive growth response under conditions of high competition. Results revealed a lack of positive growth response to elevated CO_2, although far more flowers were produced in the elevated treatment. Allocation patterns were slightly different, with relatively higher shoot mass for plants exposed to elevated CO_2. Leaf $\delta^{15}N$ and N concentrations were unaffected. Photosynthetic capacity was greatly reduced and maximum rates of Rubisco carboxylation (Vc_{max}) and light-saturated electron transport (J_{max}) were lower for plants exposed to elevated CO_2. Net CO_2 assimilation (A) at growth CO_2 was higher for the plants from the ambient treatment. Shading appears to have been an important constraint on growth response to elevated CO_2, and plants which received more light had significantly more biomass in the elevated treatment. The potential effect of restricted root growth and low soil nutrient availability are discussed as additional factors which may have negated a positive growth response.

INTRODUCTION

The recent trend of rising atmospheric CO_2 concentrations (Vitousek et al. 1997) has led to a proliferation of studies on the direct effects of elevated CO_2 concentration on plant growth. Palaeobotanical studies have provided some evidence that terrestrial plant distributions and productivity changed in response to past changes in global CO_2 levels. While physiological studies have long established that photosynthesis and transpiration are sensitive to the concentration of CO_2 at the leaf surface (Ca) (Drake et al. 1997), the recent burst of ecophysiological has documented changes at the leaf and plant level which occur when plants are grown under a supply of elevated CO_2. These studies have been motivated by the realization that predicting plant responses to rising CO_2 levels will require an understanding of the physiological processes by which plants respond to elevated CO_2 (Drake et al. 1997). The vast majority of studies at the plant level have revealed that growth in elevated CO_2 leads to increased resource use efficiency and higher growth rates (Drake et al. 1997) However, responses appear to be constrained by the availability of other resources required for growth, especially soil nutrients (Sinclair 1992; Poorter 1998). A number of *in situ* studies have investigated changes at the population level and ecosystem level in response to elevated CO_2 directly. While not as prolific as plant level studies, this research has revealed that under natural conditions, some species (usually legumes) benefit more than others (e.g. Stewart and Potvin 1996; Luscher et al. 1998), as well as a lack of response to elevated CO_2 in many cases (Bazzaz 1990; Bunce 1995; Stock and Midgley 1995; Drake et al. 1997).

THE POSITIVE GROWTH RESPONSE

Underlying increased growth in elevated CO_2, is increased net photosynthesis. Drake et al. (1997) found a mean increase photosynthesis of 58%, for 60 experiments reviewed. Increased photosynthesis occurs because ribulose bisphosphate carboxylase/oxygenase (Rubisco - the enzyme responsible for incorporating CO_2 into the photosynthetic carbon reduction cycle), has a low affinity for CO_2, and is not saturated with CO_2 at Ca under ambient CO_2 levels. In addition, oxygenation of ribulose bisphosphate (RuBP) by Rubisco, and the resulting production and release CO_2 (a process known as photorespiration), is inhibited by increased CO_2 concentration. Increased Ca therefore results in more CO_2 fixed and less fixed carbon lost to photorespiration (Drake et al. 1997).

Photosynthetic acclimation

Most long term elevated CO_2 studies which have measured leaf gas exchange have found that plants in elevated CO_2 have a reduced capacity for photosynthesis relative to plants grown in ambient CO_2. In such studies, measurements of net CO_2 assimilation (A) show reduced A for plants grown in elevated CO_2 and ambient CO_2 when measured at the same Ca (Drake et al. 1997). Acclimation to elevated CO_2 therefore occurs, in the form of negative photosynthetic adjustment. The mechanism behind acclimation was first proposed to be related to sink limitations, as acclimation was found to be common when root growth was restricted by small soil volumes (Arp 1991; Curtis 1996). Sink limitations result in an accumulation of the products of photosynthesis (photosynthates) produced by enhanced photosynthesis under elevated CO_2, triggering a feedback mechanism which reduces the rate photosynthesis (Arp 1991). Low soil N (McGuire et al. 1995; Drake et al. 1997), restricted root growth (Arp 1991; Curtis 1996) and low temperature (Bazzaz 1990) have been found to induce acclimation. These factors are thought to limit the growth of sinks, resulting in an accumulation of photosynthates (Arp 1991; Poorter 1998). The feedback mechanism appears to reduce the concentration of photosynthetic enzymes, particularly Rubisco (Drake et al., 1997, found a 15% reduction in Rubisco activity in eight studies) but also chlorophyll (Arp 1991). As acclimation has occasionally been reported for growth when sink expansion (in the form of root growth) was not restricted, it appears that low levels of available N alone may trigger acclimation (Drake et al. 1997). Increased starch concentration in the leaf often accompanies acclimation, but does not correlate with acclimation, indicating that starch accumulation is a consequence, rather than a cause, of acclimation (Arp 1991). Anatomical changes in the leaf have also been associated with acclimation (Harley et al. 1992).

Improved resource use efficiency

Reducing Rubisco concentrations through negative photosynthetic adjustment improves N use efficiency, since the same amount of CO_2 can be fixed with lower Rubisco activity under elevated CO_2 (see above). More N, relative to carbon (C), can then be allocated to the growth of sinks. Plants supplied with elevated CO_2 thus maintain a favourable C-N balance and generally showed increased growth rates, despite a reduction in photosynthetic capacity (Drake et al. 1997). Increased sink production may occur in the form of increased root production, resulting in increased absorption of N or other limiting nutrients. It is not surprising then that acclimation is more common when plants are grown under nutrient-limiting conditions (Drake et al. 1997; Poorter 1998).

A reduction in stomatal conductance (g_s) which usually accompanies increased Ca can result in increased water use efficiency (Bazzaz 1990; Stewart and Potvin 1996; Drake et al. 1997; Arp et al. 1998). This reduces transpiration but appears to have little effect on the ratio of Ci/Ca (Drake et al., 1997, found a ratio of about 0.7 for both ambient and elevated plants in 33 studies). More C can therefore be fixed per unit of water lost to transpiration under elevated CO_2. However, reduced transpiration may also reduce xylem flow and therefore nutrient ion uptake by the roots (Poorter 1998).

Enhanced rates of net assimilation of CO_2 are predicted by Rubisco kinetics, as photorespiration occurs even under-light limited conditions, and increased Ca should increase rates of carboxylation (Drake et al. 1997). Elevated CO_2 supply may therefore also result in increased light use efficiency, although empirical support for this is limited (see Drake et al. 1997).

NITROGEN FIXERS

By means of symbiotic N_2-fixation, leguminous species are capable of accessing the practically unlimited source of atmospheric N_2. For this reason, they have been identified as a group of plants which could maintain a positive photosynthetic response, even when soil N is limited (Norby 1987). Improved growth relative of legumes relative to non-fixers has been shown to occur under natural conditions for herbaceous legumes, such as *Trifolium repens* (Stewart and Potvin 1996; Luscher et al. 1998). As N_2-fixation is energetically expensive, root nodule growth and activity are likely to create a sink for additional photosynthates produced under elevated CO_2 supply (Arnone and Gordon 1990). A feedback mechanism may therefore develop under elevated CO_2 supply to prevent negative photosynthetic adjustment: increased photosynthesis leads to increased supply of carbohydrates to the nodules, more N_2 fixed, maintenance of the C-N balance and the continued growth of nodules (Arnone and Gordon 1990). This would then result in a greater positive growth response relative to non-fixers whenever there is a shortage of soil N (Vogel and Curtis 1995). Studies on both herbaceous and woody legumes have provided evidence for this feedback mechanism, in the form of increased plant N concentration (Arnone and Gordon 1990; Thomas et al. 1991), nodule biomass (Norby 1987; Arnone and Gordon 1990; Thomas et al. 1991), increased N supply by N_2-fixation (estimated by from leaf $\delta^{15}N$ measurements - Thomas et al. 1991) and increased nodule activity (measured by acetylene reduction assay - Vogel and Curtis 1995).

GAPS IN CURRENT RESEARCH

The majority of studies have focused on short-lived, herbaceous species and usually involve individual plants grown in the absence of competition. Growth conditions are usually optimal and responses only recorded for a short time period (usually a couple of months). While experiments under ideal growing conditions are useful for determining the physiological responses of plants to elevated CO_2, the response of individuals may become highly modified under natural conditions (Bazzaz 1990; Stock and Midgley 1995). These results cannot therefore be extrapolated directly to higher ecological levels. To make predictions about responses to elevated CO_2 at the population, community or ecosystem scale, the response of plants under natural conditions, where many factors may simultaneously limit growth, needs to be determined (Sinclair 1992, Arp et al. 1998). In addition, little is known about the effects of elevated CO_2 on reproduction, both in terms of reproductive output and plant-pollinator interactions, even though changes in reproductive success may be very important at higher ecological scales (Bazzaz 1990). The majority of elevated CO_2 research therefore provides limited insight into changes which may above the plant level as atmospheric CO_2 levels rise.

Research on the effects of elevated CO_2 on woody legumes has the same flaws. Competition has not been taken into account, and resources other than CO_2 that might constrain growth under natural conditions (such as nutrients other than N and shading) are often supplied in abundance. The experiments involved all had a relatively short duration, the longest lasting 160 days (Vogel and Curtis 1995), and do not document long-term responses. In addition, as seedlings have been used, results may not be applicable to adult trees (Thomas et al. 1991).

Finally, the vast majority of elevated CO_2 research comes from North America and Europe (including those on woody legumes). Studies from the Southern Hemisphere, and in particular southern Africa, are rare. The fynbos biome, a low nutrient system, but with high species and growth form diversity, may show strong responses to rising levels of atmospheric CO_2 (Stock and Midgley 1995). However, the response of almost all species from this biome to elevated CO_2 remains unknown.

This project analyses the response of a woody legume *P. sericea* from the fynbos biome to growth under elevated CO_2 under unfavourable growing conditions. Competition, which could constraint the response of plants to rising CO_2 under natural conditions (Luscher et al. 1998), was incorporated by planting a number different fynbos species were grown together in a glasshouse for 18 months. Plants were grown at a high density in pots supplied with either ambient (35 Pa partial pressure) or elevated (70 Pa partial pressure) CO_2. Growth, N dynamics and leaf gas exchange of *P. sericea* are reported.

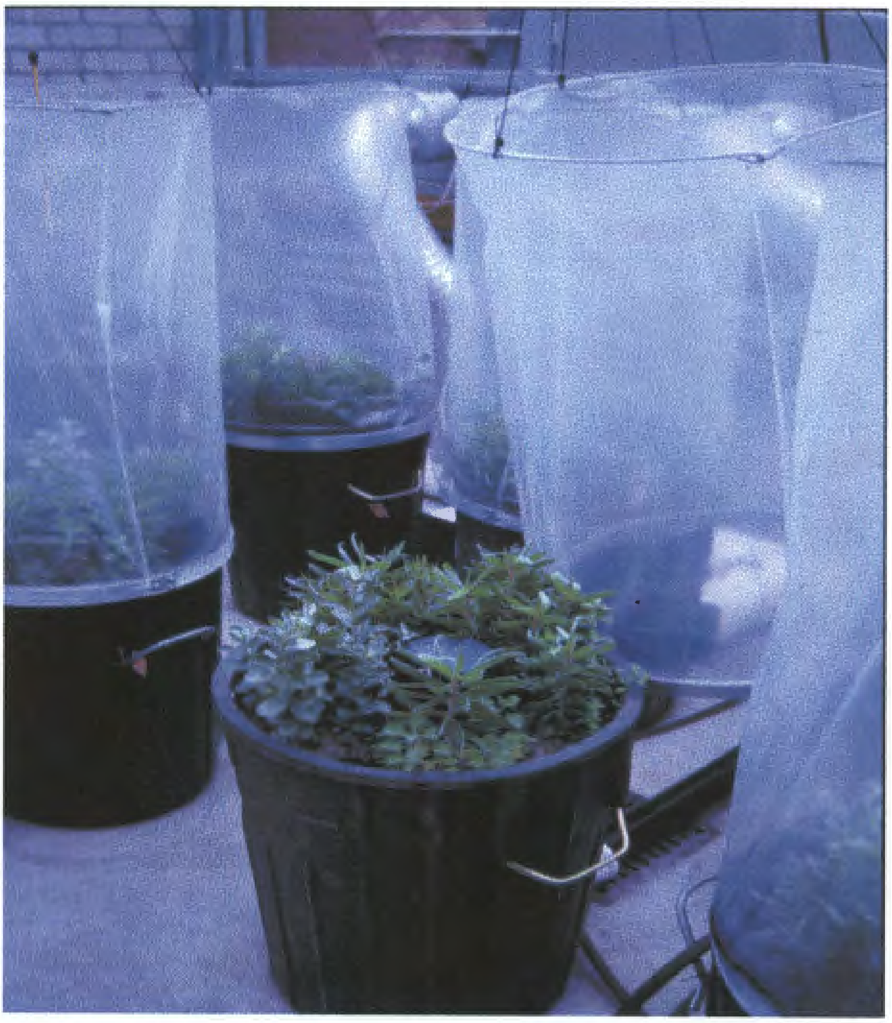


Figure 1: The arrangement of the pots within the glasshouse. The plastic sheath has been removed from one to show the transplanted seedlings. *P. sericea* seedlings are on the left.

METHODS

EXPERIMENTAL DESIGN

Six fynbos species, *Podalyria sericea* (Andr.) R. Br., *Leucadendron laureolum* (Lam.) Fourcade, *Leucadendron xanthoconus* (O. Ktze) K. Schum., *Felicia aethiopica* (Harv.) N. E. Br., and *Salvia africana-lutea* L. were germinated from seed obtained from Kirstenbosch Botanical Gardens. Seedlings were transplanted into pots in the University of Cape Town glasshouse, on the 4 December 1997, after 1 month of growth. Dead seedlings were replaced after two months. The pots were filled with soil from a mountain fynbos habitat (dug from Orange Kloof Reserve, Cape Town), to which soil mix used for cultivating fynbos plants ("Fynbos mix", Kirstenbosch Botanical Gardens, Cape Town) was added. Soil volume was approximately $80dm^3$, and soil surface area approximately 1385 cm^2. The pH and N and P content the final soil mixture is given in Table 1. Pots were watered to saturation once a week. When glasshouse temperatures rose above 25^0C, ventilation pumps limited air temperature to ambient temperature minus 3^0C.

18 pots were arranged in two staggered rows within the glasshouse. Air was supplied via a pipe located vertically in the centre of each pot. The outlet of this pipe was a couple of cm above the soil surface. Clear polyethylene plastic was used to construct a sheath which extended approximately 1m upwards from the rim of the pot (Fig. 1). This sheath contained all the plants and prevented mixing of glasshouse air with that supplied via the pipe. CO_2 at 35 Pa partial pressure was constantly added to the air supply to half of the pots, creating a CO_2 supply of approximately 70 Pa. The remainder received ambient air. Pots supplied with additional CO_2 are referred to as the "elevated" pots hereafter, the others as "ambient pots". The arrangement of elevated and ambient pots was random.

Five individuals of *P. sericea* were planted in each of 17 of the pots (eight ambient, nine elevated). There was therefore a total of 40 *P. sericea* seedlings in the ambient treatment (referred to as "ambient plants" hereafter), and 45 in the elevated treatment ("elevated plants"). Seedlings were arranged in a circle, as part of a larger circle of other species (Fig. 1). The other species in the experiment were planted in the same way. One additional individual of *L. laureolum* was planted in the centre of each of the circle. A total of 30 seedlings were thus planted in every pot.

GROWTH RESPONSE

Plant height, number of branches and number of leaves were counted on 15 July 1997, approximately 7 months after the initiation of the experiment, and 5 months after replanting dead seedlings. Counts were repeated prior to harvesting. Flowers on *P. sericea* were counted regularly from the time flowering began to the time pods had began to form (6 June to 13 July). All plants were harvested 19 months after the initiation of CO_2 supply. Roots of each individual were separated by hand. Roots, stems and leaves were then dried at 70°C for 48 hours and weighed separately. Dead individuals and leaf litter were also weighed. Prior to harvesting, a subsample of mature, healthy *P. sericea* leaves was taken to determine specific leaf weights (SLW = leaf mass/leaf area). Ten leaves were taken from one individual from a pot. Leaf area was determined using a LiCor Li 3000 leaf area meter (Lambda Instruments Corporation, USA) and leaves were weighed fresh.

For leaf N isotopic analysis, dried leaves were taken from one *P. sericea* individual per pot. Leaves from one of the non-legume species, *L. laureolum,* were also analysed, in an attempt to if *P. sericea* was obtaining a significant portion of assimilated N from symbiotic N-fixation. Leaves were ground in a Wiley Mill (mesh size 40). Samples with diseased or dying leaves, identified by a browner colour, were discarded. Samples were then passed through a Carlo Erba CHN elemental analyser (Carlo Erba Instrumentazione, Milan, Italy) connected to a mass spectrometer (Matt Finnigan 252). Helium was used as the carrier to the mass spectrometer, which was operated in continuous flow mode. Isotopic composition was measured as the abundance of ^{15}N relative to atmospheric N_2 in units per mille (‰):

$$\delta^{15}N = (Rsample - Rstandard)/(Rstandard) \times 1000$$

where $R = {}^{15}N/{}^{14}N$ and the standard is atmospheric N_2. Leaf N concentration was obtained from the CHN analyser.

Table 1: pH, and N and P content of the final soil mixture used for the experiment. Values are means (+/- SE) of 5 samples.

	pH	N content (mg N/ g soil)	P content (μg P / g soil)
Top soil	4.87 (0.02)	1.68 (0.14)	101.3 (6.2)
Sub soil	4.70 (0.08)	1.67 (0.350	72.4 (1.69)

PHOTOSYNTHETIC RESPONSE

Nine months after transplantation, net CO_2 assimilation (A) and stomatal conductance to water vapour (g_s) was measured using a LiCor Li 6400 infrared gas analyser (LiCor, Lincoln, Nebraska). Data from these measurements were obtained from Guy Midgley, Kirstenbosch Botanical Gardens. Light was supplied at a photon flux density (PPFD) of 2000 μm PAR m^{-2} s^{-1}, leaf temperature maintained at 25°C, the vapour pressure deficit of between the cuvette chamber and the intercellular airspace maintained between 15 and 17 Pa. To determine the effects of CO_2 availability on A, intercellular CO_2 partial pressure (Ci) was varied between approximately 5 Pa and 950 Pa. Gas exchange measurements were repeated just over 17 months after the initiation of the experiment, using a Ciras 1 portable photosynthesis system (PP Systems, Herts, UK). PPFD was set at 1200 μm PAR m^{-2} s^{-1}, leaf temperature maintained between 20 and 22°C, and vapour pressure deficit maintained between 16 and 19 Pa. For both sets of measurements, mature, fully-expanded leaves most exposed to sunlight and from the tallest plants (but located well within the plastic sheath) were used. Gas exchange was recorded once readings for A and g_s had stabilized (approximately 3-5min.).

Net assimilation versus intercellular CO_2 concentration (A/Ci) data were analysed using Photosyn Assistant v1.1 (Dundee Scientific, 1998). Data for individual plants from were combined for each treatment. Photosyn Assistant uses the mechanistic model of C_3 photosynthesis, proposed by Farquhar et al. (1980) and modified by Harley et al. (1992). The model fits curves of W_c (the rate of carboxylation limited solely by the amount, activation state and kinetic properties of Rubisco), W_j (the rate of carboxylation when RuBP regeneration alone is limiting) and W_p (rate of carboxylation limited solely by inorganic phosphate availability) to the data. From these curves, the photosynthetic parameters of R_d (rate of CO_2 production light resulting from processes other than photorespiration), Vc_{max} (maximum rate of CO_2 fixation by Rubisco), J_{max} (electron transport rate) and TPU (rate of inorganic phosphate turnover) are calculated by an iterative procedure (Harley et al. 1992 provides a more detailed description of the model). Constants used for the calculations are those derived from a range of C3 plants (see Harley et al. 1992). The CO_2 compensation point (A when Rubisco activity is at a maximum, and the point at which RuBP regeneration becomes limiting) is calculated from W_c. TPU was not estimated as A did not appear to reach saturation or decline at the highest Ci values for any of the data sets. Incorporating W_p - the parameter on which TPU is based - resulted in large increases in the standard error (SE) of the other parameters calculated. Limits to W_j were left at the default setting (between 15 and 60

Pa Ci), which provided the best fit in all cases. g_s and Ci at growth Ca were estimated for each CO_2 treatment from the second set of leaf gas exchange measurements. A at growth Ca was then calculated from the A/Ci regressions.

STATISTICAL ANALYSIS

All statistics were performed using STATISTICA 5.12 (Statsoft, '97 Edition). Data were checked for normality and homogeneity of variance. To determine differences between plants grown in each treatment, Student's t-tests (for parametric variables) and Mann-Whitney U tests (for non-parametric variables) were applied.

The arrangement of the pots in the glasshouse resulted in some pots receiving more light than others. To check for any influence of shading on growth, pots were therefore assigned to a high light (H) or low light (L) category. This division was based light saturation estimates for individual plants in different pots. Light saturation was estimated from light response curves, using Photosyn Assistant. Light response curves are fitted according to the AQ equation of Prioul and Chartier (1977). Light response data (A at a range of PPFD) were obtained from the first set of leaf gas exchange measurements. Light saturation results from seven pots were sharply divided, with plants grown in the least shaded corner of the glasshouse having values over 600 μmol CO_2 m^{-2} s^{-1}, and the remainder having values under 375 μmol CO_2 m^{-2} s^{-1}. This distinction occurred between two adjacent pots, which were therefore used as a dividing line to assign pots to the H or L category. A two-way, fixed effects ANOVA (with CO_2 treatment and light category as the effects) was used to identify any interaction between CO_2 supply and shading. Square-root transformations were applied to data which resulted in approximately normal distributions and equal variance for all variables used.

In an attempt to exclude the effects of light (and any other factors which affected growth, besides CO_2 supply) data for individual plants from different treatments category with approximately the same total dry weight, and from the same light, were paired. This reduced the data to between six and eight pairs of plants. Differences in growth variables were then tested using a Student's t-test for paired samples (using log-transformation where necessary) or Wilcoxon's paired sample test (if homoscedasticy or normality could not be obtained by transformation). Differences between total dry weight of these pairs was not significant (paired t-test: $p > 0.515$).

RESULTS

GROWTH RESPONSE

After 5-7 months of growth, seedling survivorship was lower in the elevated CO_2 treatment than the ambient treatment (Fig. 2). By the time of harvesting (another 12 months), only 11 of the 45 seedlings planted in the elevated treatment were still alive, compared to 22 of the 40 seedlings planted in the ambient treatment.

Surviving seedlings in the elevated pots were not taller after 5-7 months, but had more branches and significantly more leaves (Student's t: t = -2.05, df = 49, p = 0.045; Fig. 3). After 17-19 months, differences were significant for both branches (Mann-Whitney: u = 54, p = 0.032) and leaves (Mann-Whitney: u = 55, p = 0.035), but not height. There was no significant difference in leaf area.

In terms of dry weight, total biomass production was not significantly different for the ambient and elevated treatments. Shoot dry weight was slightly higher in the elevated treatments (although not significantly), but root dry weight showed complete overlap and root-shoot ratios were not significantly different. However, there was a large difference in the number of flowers produced. Far more of the elevated plants flowered, and those that flowered produced more flowers per unit biomass (Fig. 4). Based on the maximum number of flowers produced during the flowering period, elevated plants produced significantly more flowers, per unit biomass, than ambient ones (T-test: t = -2.91, df = 17, p = 0.005; Fig. 3). For selected pairs with similar dry weights, this difference was still highly significant (T-test: t = -6.445, df = 5, p = 0.001).

Shading

Shading did not appear to have an effect on seedling survival (Fig. 2). Two-way ANOVA revealed no significant interaction effects for height, number of branches or number of leaves after 5-7 months and 17-19 months. However, 5-7 month old seedlings were taller in the shaded pots ($F_{1,47}$ = 11.03, p = 0.002). Interaction effects were significant for final biomass production, in terms of total dry weight ($F_{1,59}$ = 4.78, p = 0.006) and shoot dry weight ($F_{1,59}$ = 5.62, p = 0.02;

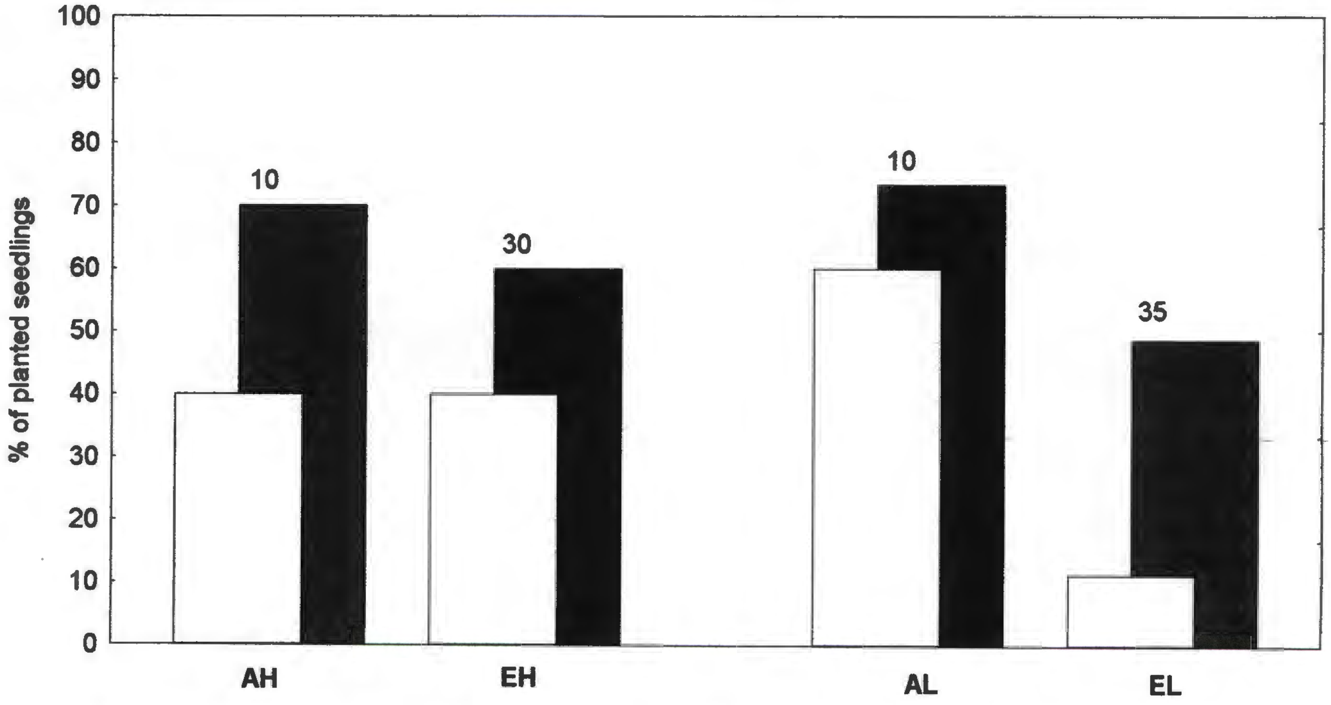


Figure 2: Percentage of *P. sericea* seedlings still alive after 5-7 months (solid bars) and 17-19 months (open bars), in ambient pots which were unshaded (AH) or shaded (AL), and elevated pots which were (EH) or shaded (EL). Numbers above bars show the total number of seedling planted per category.

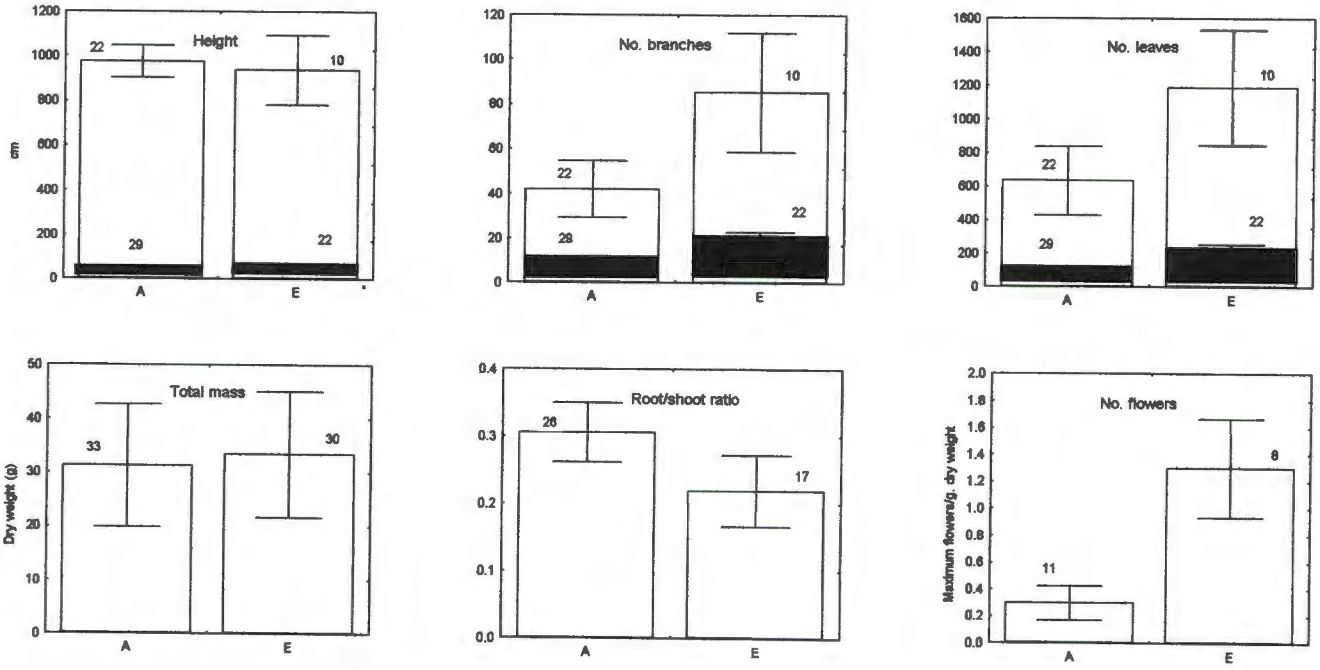


Figure 3: Mean height, number of branches and leaves, biomass, root-shoot ratio, and flower production for *P. sericea* grown under ambient (A) and elevated (E) CO_2. Solid bars are for 5-7 months growth, open bars for 17-19 months growth. Bars show means, whiskers are +/- 1 SE. Numbers above bar show n.

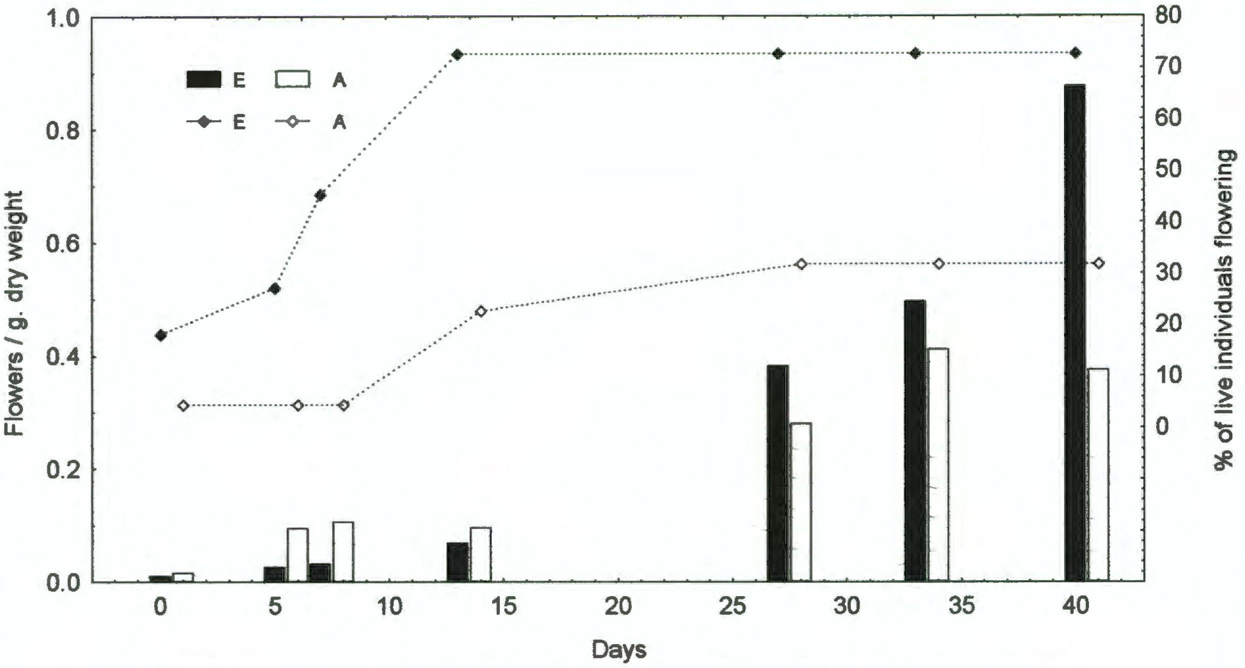


Figure 4: Percentage of live individuals flowering (lines) and number of flowers per total dry weight of flowering individuals (bars), for *P. sericea* grown under ambient (A) and elevated (E) CO$_2$ supply. n = 1-8.

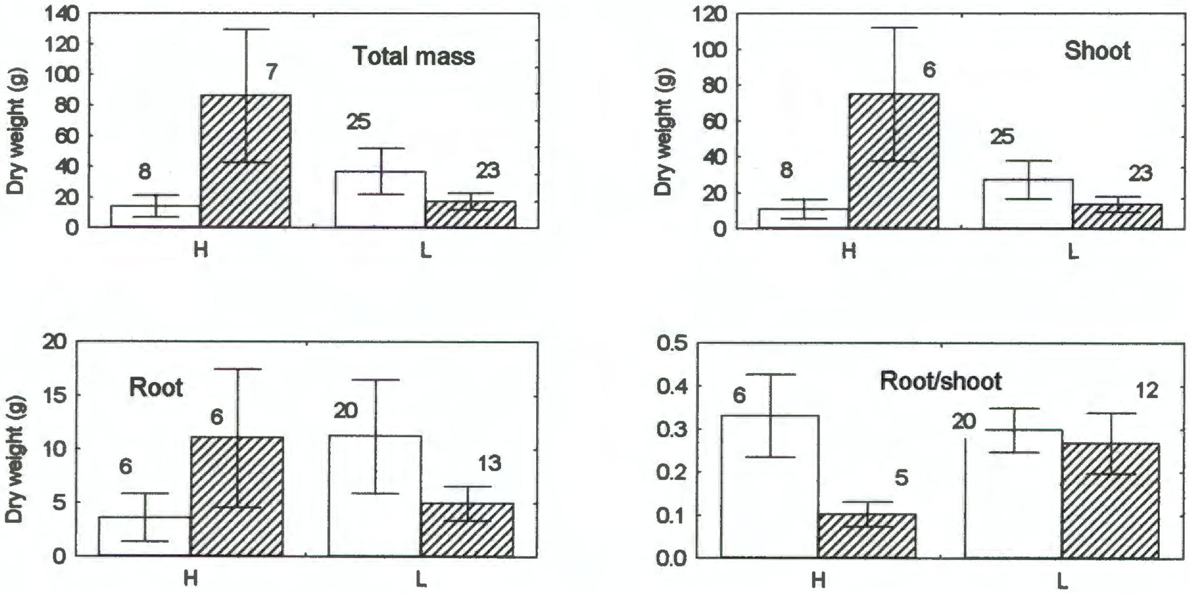


Figure 5: Biomass and biomass allocation for *P. sericea* grown under ambient ( ) □ and elevated ( ) ▨ CO$_2$ supply, in unshaded (H) and shaded (L) pots. Bars show means, whiskers are +/- 1 SE, number above bar shows n.

Fig. 4). Shoot weights were different as a result of differences in both in stem weights and leaf weights, but the difference was only significant for the former ($F_{1,58} = 6.22$, $p = 0.02$). Root weights were similar, and root-shoot ratios did not differ significantly. The effect of shading alone was not significant for any of the variables measured after 17-19 months, including leaf area, SLW and leaf N concentration.

Leaf $\delta^{15}N$

The range of leaf $\delta^{15}N$ values for *P.sericea* was small and values for plants from ambient pots completely overlapped those from elevated pots (Fig. 6). There is therefore no evidence that elevated plants used relatively more fixed-N_2 than ambient plants.

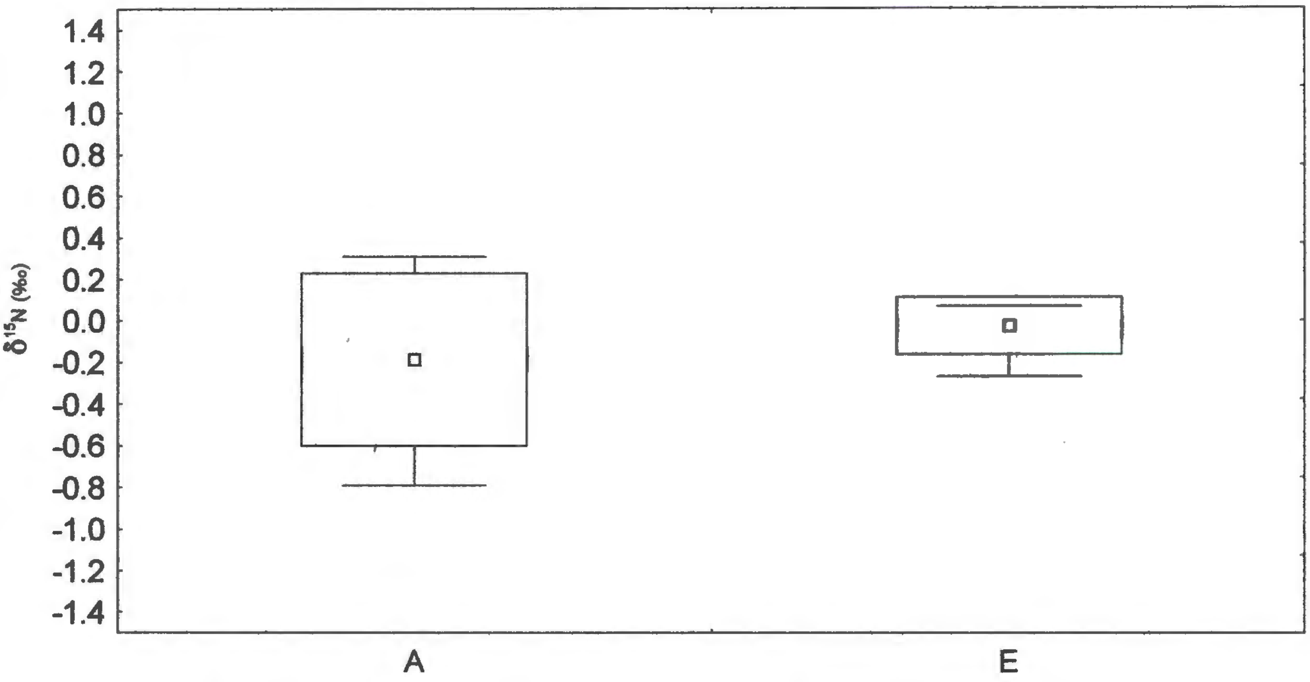


Figure 6: Leaf $\delta^{15}N$ values of *P. sericea* grown under ambient (A) and elevated (E) CO_2 supply. Points are means, boxes =/- 1SD, whiskers show range. n = 5 for both treatments

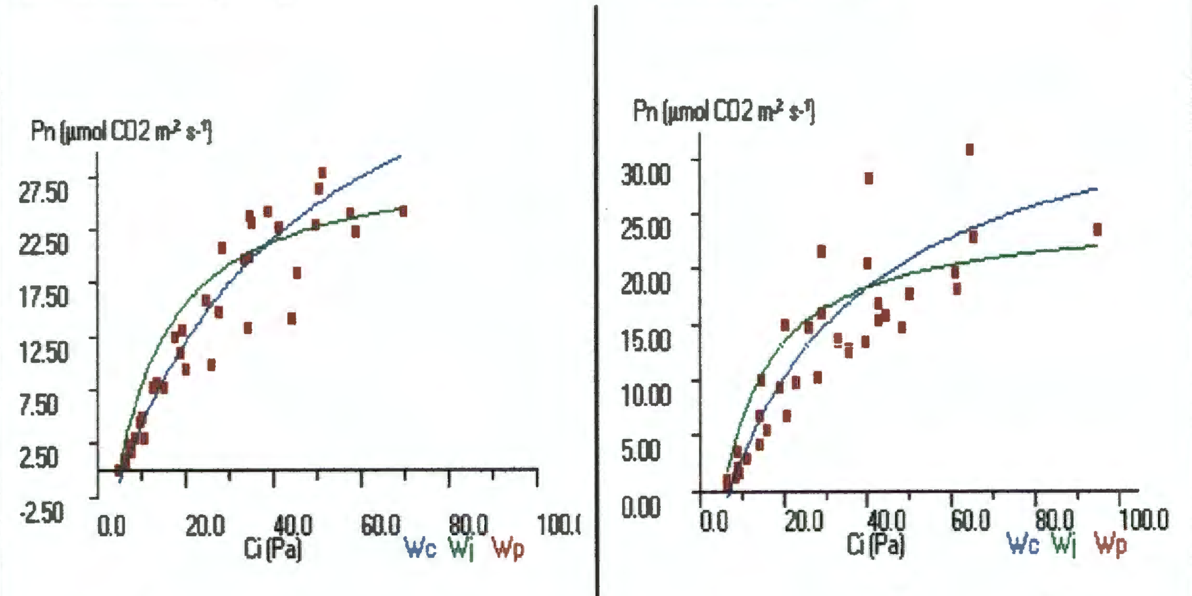
PHOTOSYNTHETIC RESPONSE

Rates of photosynthetic assimilation revealed a reduction in photosynthetic capacity for the elevated plants after 9 months of growth (Fig. 7). These differences increased markedly after another 8 months, revealing substantial negative photosynthetic adjustment in the elevated treatment (Fig. 8). In fact, after 17 months of growth under 70 Pa CO_2, rate of photosynthetic assimilation predicted by the mechanistic model was lower for elevated plants at growth Ca than the ambient plants at growth Ca (Fig. 9). At elevated Ca, A for ambient plants was almost twice that of elevated plants. The decline in photosynthetic capacity displayed by the elevated plants was a result of a reduction on both Vc_{max} and J_{max} (Fig. 7 and 8). In the elevated treatment, Vc_{max} was so low after 17 months that the CO_2 compensation point was almost 10 μmol CO_2 m^{-2} s^{-1}. This value is well below A calculated for growth Ca (4.92 μmol CO_2 m^{-2} s^{-1}) indicating that Rubisco activity alone limited A. For the elevated plants at Ca, the CO_2 compensation point was approximately equal to A at growth Ca, indicating that Rubisco activity was at a maximum. Although g_s was greatly reduced in the elevated treatments, Ci was similar for both treatments at 70 Pa CO_2 (Fig. 9).

The effect of light on photosynthetic capacity was evident after 9 months growth (Fig. 7). Differences in Vc_{max} and J_{max} were greater between shaded and unshaded pots than between elevated and ambient pots. Plants in the unshaded pots had a mean light saturation point of 631.3 μmol CO_2 m^{-2} s^{-1} ($n = 3$), while those in shaded pots had a mean of 326.5 μmol CO_2 m^{-2} s^{-1} ($n = 4$). The differences in slope between the elevated and ambient A/Ci curves may therefore be an artefact of shading effects within each treatment. After 17 months, the effect of shading light was not as clear. Unshaded plants all had high rates of A, and slightly higher Vc_{max} and J_{max}, but the shaded category showed a greater range of responses, and contained the highest and lowest values. CO_2 supply therefore appears to have become more important, in terms of limiting photosynthetic capacity, after 17 months. For all categories combined, Vc_{max} and J_{max} showed a strong linear correlation (Pearson's product-moment: $r = 0.99$, $df = 8$, $p<0.001$).

There was no indication of photosynthetic acclimation from SLW's or leaf N concentration. Neither was significantly different between treatments (Mann-Whitney: $p>0.05$), which suggests that elevated leaves did not contain more non-structural carbohydrates or less N per unit mass.

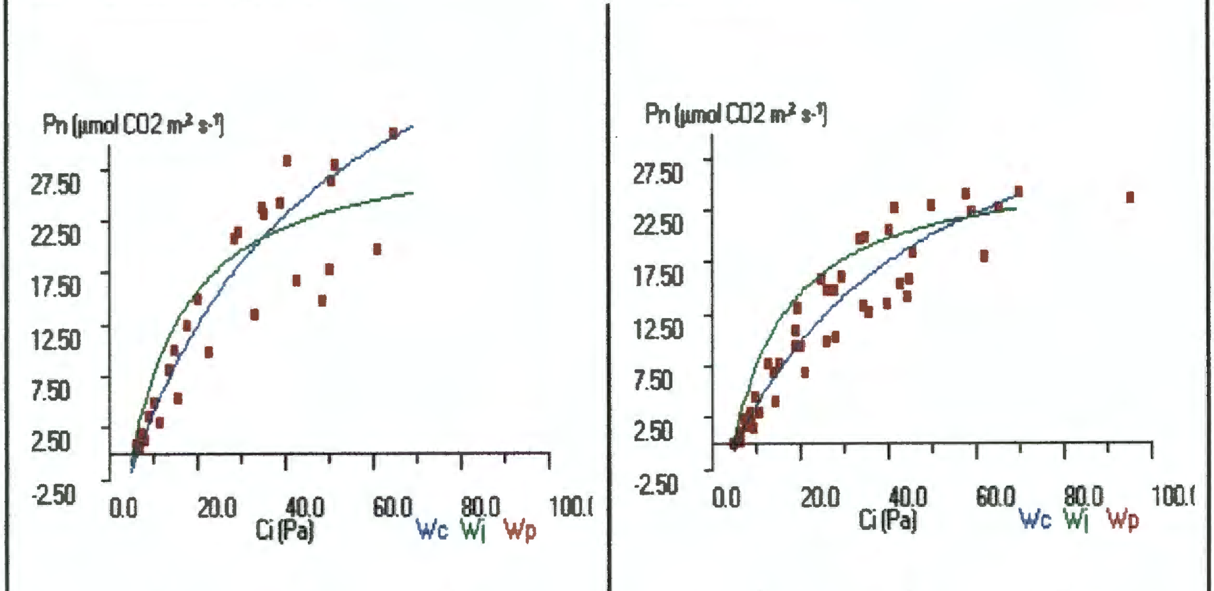
7A **Ambient** (n = 4) **Elevated** (n = 4)



R_d	Vc_{max}	J_{max}	CO_2 comp.
1.69 (0.44)	52.9 (3.73)	130 (7.06)	5.82

R_d	Vc_{max}	J_{max}	CO_2 comp.
2.13 (0.62)	46.4 (0.05)	115 (0.08)	6.51

7B **Unshaded** (n = 3) **Shaded** (n = 5)

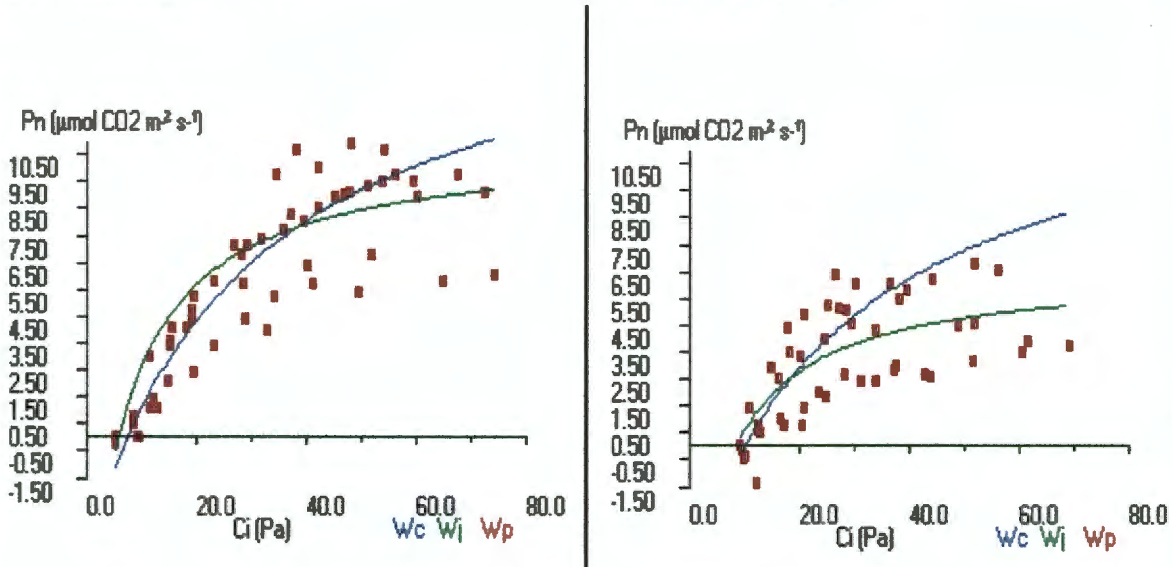


R_d	Vc_{max}	J_{max}	CO_2 comp.
2.5 (0.77)	57.9 (0.09)	138 (0.11)	6.36

R_d	Vc_{max}	J_{max}	CO_2 comp.
1.12 (0.35)	42.3 (0.05)	117 (0.10)	5.56

Figure 7: Photosynthetic parameters calculated from IRGA analysis of *P. sericea*, after approximately 9 months of growth. Plots were fitted using the mechanistic model of Farquhar et al. (1980), on Photosyn Assistant. Data for individuals from separate pots have been combined according to CO2 supply (7A) and shading category (7B). Parenthesis show the number of pots per category. TPU was not estimated as A values did not appear to reach saturation in any of the data sets. Parameters calculated by the model are given in the table below each plot (+/- 1 SE), units =μmol CO_2 m^{-2} s^{-1}.

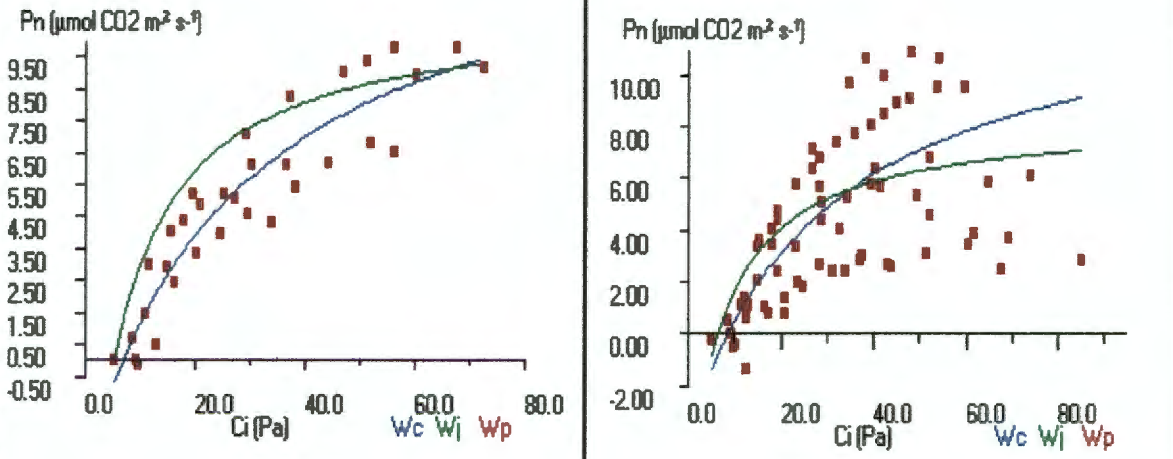
8A **Ambient** (n = 5) **Elevated** (n = 5)



R_d	Vc_{max}	J_{max}	CO_2 comp.
1.82 (0.02)	19.1 (0.02)	51.6 (0.06)	6.87

R_d	Vc_{max}	J_{max}	CO_2 comp.
2.63 (1.3)	17.2 (5.83)	37.1 (7.31)	9.97

8B **Unshaded** (n = 3) **Shaded** (n = 7)



R_d	Vc_{max}	J_{max}	CO_2 comp.
1.27 (0.12)	16.0 (0.04)	50.0 (0.18)	6.77

R_d	Vc_{max}	J_{max}	CO_2 comp.
1.96 (0.27)	15.7 (0.04)	41.6 (0.07)	8.69

Figure 8: Photosynthetic parameters calculated from IRGA analysis of *P. sericea*, after approxiamatley 17 months of growth. Plots were fitted using the mechanistic model of Farquhar et al. (1980), on Photosyn Assistant. Data for individuals from separate pots have been combined according to CO_2 supply (8A) and shading category (8B). Parenthesis show the number of pots per category. TPU was not estimated as A values did not appear to reach saturation in any of the data sets. Parameters calculated by the model are given in the table below each plot (+/- 1 SE), units $=\mu$mol CO_2 m^{-2} s^{-1}.

? - 35 ? 70

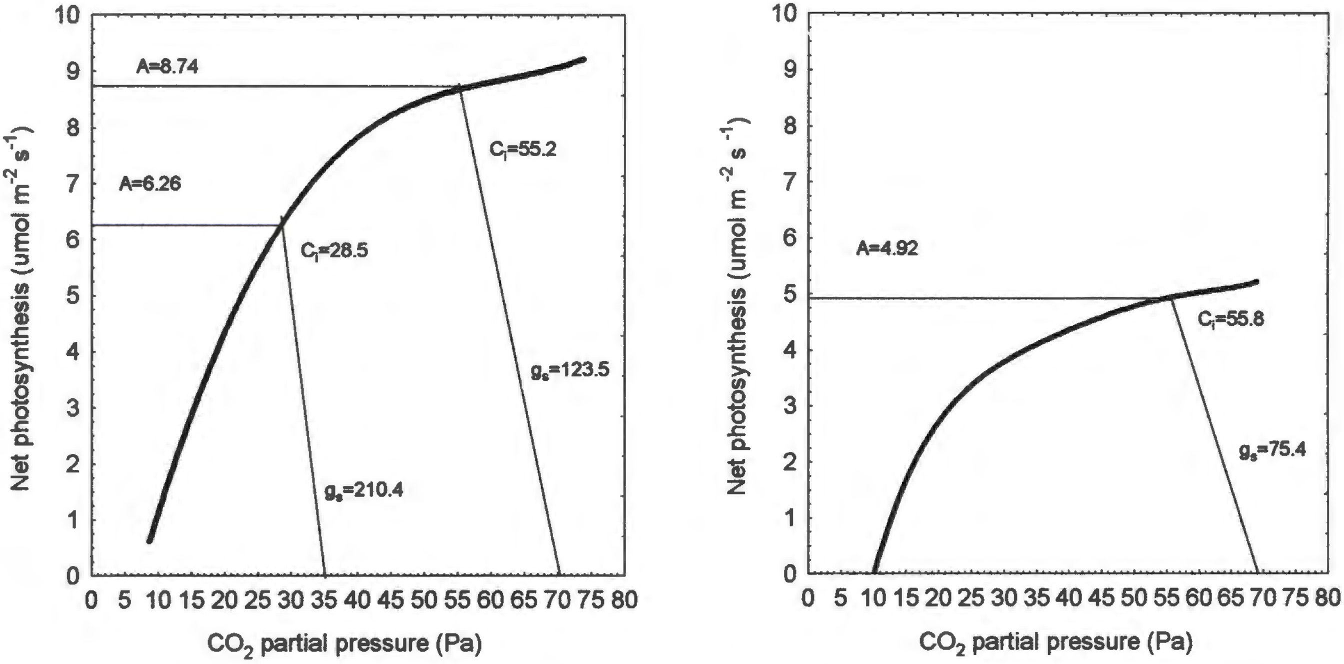


Figure 9: Modelled simulations of net photosynthesis (A) as a function of CO_2 supply. A/Ci data from IRGA set at PPFD of 1200 umol m^{-2} s^{-1}, leaf temperature of 20-22°C and vapour pressure deficit of 16-19 Pa. Parameters for the model are given in Fig. 8. g_s = stomatal conductance to water vapour, C_i = intercellular CO_2 - values shown are means from 5 samples (for measurements at 35 Pa) or 4 samples (for measurements at 70 Pa).

DISCUSSION

The results reveal no overall benefit in growth rates (in terms of biomass production) as well as photosynthetic acclimation for *P. sericea* grown in elevated CO_2. These two results are unusual for a legume, but have been reported for other woody species - a review by Poorter (1998) includes studies in which the biomass of plants from elevated treatments was lower than in ambient treatments, while McGuire et al. (1995) reviews studies which report acclimation for forest trees.

The values obtained from both sets of gas exchange measurements fall within the range found by Wullschleger (1993) in a survey of 109 C_3 species, and in the range found for temperate hardwood trees. However, after 17 months, Vc_{max} and J_{max} were well below the average for temperate hardwoods (Vc_{max} [mean +/- 1SD] = 47+/-33 μmol m^{-2} s^{-1}, J_{max} = 104+/-64 μmol m^{-2} s^{-1}) and sclerophyllous shrubs (Vc_{max} = 53+/-15 μmol m^{-2} s^{-1}, J_{max} = 122+/-31 μmol m^{-2} s^{-1}), and below the lower limit for sclerophyllous shrubs (Vc_{max} = 35 μmol m^{-2} s^{-1}, J_{max} = 94 μmol m^{-2} s^{-1}; Wullschleger 1993). I could find only one published study which reported the results of a leaf gas exchange measurements for a woody legume. Vogel and Curtis (1995) investigated the photosynthetic response of *Alnus glutinosa*, a North American temperate forest species. Leaf gas exchange measurements (PPFD set at 1800 μmol m^{-2} s^{-1}, leaf temperature at 25-27^{0}C) were taken on seedlings approximately 7 months old which had been grown under 35 or 70 Pa CO_2 supply for just under 4 months. Values of A, Vc_{max} and J_{max} at ambient CO_2 were slightly higher than those reported for the first set of gas exchange measurements in this study: mean (+/- SE) for A = 19.6 μmol m^{-2} s^{-1}, Vc_{max} = 57.7 (3.72) μmol m^{-2} s^{-1}, J_{max} = 160.6 (6.75). However, in this study, no negative photosynthetic adjustment occurred and the above values were higher in the elevated treatment.

Photosynthetic acclimation, revealed by the gas exchange results, has been reported for the vast majority of long-term elevated CO_2 studies (Curtis 1996, Drake et al. 1997). For acclimation to be so severe for A at growth Ca to be lower for elevated plants than ambient plants (Fig. 8) is unusual, but not unheard of. McGuire et al. (1995) refer to this process as 'depressed photosynthesis' and found it reported in four of 20 studies investigating the effects of elevated

CO_2 on forest trees. In a meta-analysis of 83 studies involving 43 woody species, Curtis (1996) found a normally distributed range of responses of A to elevated CO_2, with A for elevated plants below that of ambient plants (at growth CO_2) in many cases.

g_s is known to decline in response to increasing Ca (Drake et al. 1997) and has been found in a number of studies involving woody species, although only in experiments where plants were grown under unstressed conditions (Curtis 1996). However, g_s was far lower in the elevated plants that the ambient ones when both were measured at a Ca of 70 Pa. Reduced g_s in the elevated treatment may have been increased by a feedback effect resulting from photosynthetic acclimation - a reduction in A leads to increased Ci which induces stomatal closure (Drake et al. 1997; Arp et al. 1998).

Long-term growth usually results in a decrease in leaf N concentration (McGuire et al 1995; Curtis 1996; Cotrufo et al. 1998). The similarity of leaf N concentrations (specific leaf area) in this experiment is therefore anomalous, especially considering that A/Ci data indicates decreased Rubisco activity in the elevated treatments. As measurement temperatures were constant and Ci was not lower in the elevated plants, reduced Rubisco activity suggests less Rubisco was present. As this enzyme can account for up to 25% of leaf N (Drake et al. 1997), leaves from the elevated plants would be expected to have lower N concentration. It is possible a lack of a significant difference is simply the result of under sampling (the leaf sample was taken from only four individuals for each treatment). However, in a review of 75 studies, Cotrufo et al. (1998) found that leaf N responses to elevated CO_2 supply vary widely, and in many experiments N concentrations remained unchanged. They also found that N_2-fixing species were less responsive than other C3 species, and showed a lower reduction of leaf N concentration.

FACTORS AFFECTING GROWTH RESPONSE

There are two possible reasons for a lack of positive growth response under elevated CO_2. Firstly, genetically based/inherent constraints may have limited growth rates. Secondly, a resource other than CO_2 may have been limiting. Inherently low growth rates seem unlikely for a legume species which can potentially meet all it N requirements through symbiotic N_2-fixation. It is therefore unlikely that growth was constrained by maximum potential growth rates in the elevated treatment. However, a positive growth response to elevated CO_2 supply may have been prevented if maximum potential growth rates were achieved at ambient CO_2. The mean height for both treatments after 17-19 months growth was almost one metre (Fig. 3), well above the range given for mature plants in their natural habitat (50-70cm; Adamson and Salter 1950). In addition, global CO_2 levels are thought to have been about 27 Pa partial pressure when modern plants evolved (Lovelock et al. 1996), and plants under supplied with ambient CO_2 may therefore be experiencing a positive growth response already. The possibility that many of the plants in both treatments obtained maximum potential growth rates or were CO_2 saturated during the experiment cannot therefore be ruled out.

Considering growing conditions, as well as the acclimation revealed by the gas exchange results, it seems more likely that the growth response of the elevated plants was constrained. Results from the leaf gas exchange measurements show some acclimation after 9 months, indicating that some factor was limiting by this stage. By 17 months this acclimation was of a greater magnitude, although all plants showed a large reduction in photosynthetic capacity by this stage. This indicates that whatever factors were limiting growth had become more severe by this time. In previous elevated CO_2 studies, acclimation has been associated with shading (Drake et al. 1997), limited rooting volume (Curtis 1996) and shortages of soil available N (McGuire et al. 1995; Drake et al. 1997).

Light limitations

When the effect of shading was taken into account, an increase in productivity under elevated CO_2 was evident (Fig. 5). Only in the unshaded pots were plants able to significantly more biomass, suggesting that light was more limiting than CO_2 availability in the shaded pots, regardless of CO_2 supply.

It is also possible that plants in the unshaded pots experienced light shortages, due to shading from other species and from the plastic sheaths (see Fig. 1). This would account for the significant increases in the number branches and leaves in the elevated treatment for shaded and unshaded pots combined. In addition, the low root-shoot ratios in the unshaded elevated pots may have been a result of plants allocating all additional carbon gained from growth under elevated CO_2 to shoot production (in an attempt to maximize assimilatory area). However, root mass data should be treated with caution as plants appeared to be root-bound at the time of harvesting, and allocation to roots may have been restricted. In addition, large amounts of fine root material was lost when roots were removed from the soil. Together with low replication for some of the categories used for data analysis (e.g. elevated CO_2), this may have produced inaccurate results for root dry weight.

From the leaf gas exchange results it is not clear whether elevated CO_2 supply or shading, or both, were responsible for negative photosynthetic adjustment after 7 months (Fig. 7). After 17 months negative adjustment was clear for ambient versus elevated plants, but not for shaded versus unshaded plants (Fig. 8). Light may therefore have been an constraint on growth response to elevated CO_2 for the first half of the experiment, when plants were all still more or less the same height and all would have experienced shading from each other. By the end of the experiment, however, some *P. sericea* plants had overtopped other plants. The range of A values obtained in the shaded pots after 17 months (Fig. 8B), may therefore be a result of many of the shaded plants experiencing less shading than before, and comparing across Fig. 8A and 8B it is clear that ambient plants had the higher rates of A than elevated plants, regardless of shading category. A factor other than light is therefore likely to be responsible for the acclimation evident after 17 months. Together with the known potential for a positive growth response under light-limited conditions (Drake 1997), this suggests that another factor was involved in preventing a positive growth response in the experiment.

Water limitations

The high density of plants in each pot (0.35 plants/dm^3 of soil) may have resulted in water shortages. However, pots were watered to saturation once a week, and the plastic sheaths would have limited "canopy" conductance to water vapour. After 17 months of growth, stomatal conductance was far lower in the elevated treatment, but Ci remained higher than for ambient plants (at growth Ca). This translates into considerably higher water use efficiency in the elevated treatments, despite a reduction in A - assuming the values in Fig. 9 are accurate, mmol C fixed per mmol H_20 transpired ($m^{-2}\ s^{-1}$) was 65 for the elevated treatment and 29.5 for the ambient treatment. However, as Arp et al. (1998) point out, improved water use efficiency may not translate into increased biomass if other factors limit growth.

Root volume limitations

Restrictions on root growth may have limited overall growth. Each pot was planted with 30 plants and at the time of harvesting, soil was filled with roots for the entire length of most pots. Restricted root growth may have limited overall sink strength for photosynthates or limited nutrient uptake. A positive growth response in the elevated treatment may therefore have been stifled once the available rooting volume was filled. Higher shoot mass but not root mass in the elevated treatment suggest that this may be have been the case. Reduced root-shoot ratios have been found for plants grown in small pots (Arp 1991), and Curtis (1996) and Arp (1991) found acclimation correlated negatively with rooting volume.

Nutrient limitations

Root volume restrictions are likely to have coincided with soil nutrient shortages. Pot soil was low in N and, particularly, P to start with (Table 1), and below-ground competition was probably. N or P may therefore have limited growth responses to CO_2, especially as the experiment progressed. Curtis (1996) found reduced A at growth Ca for under elevated CO_2 supply for nutrient-stressed plants, while soil N availability has been shown to be an important factor constraining positive growth response in forest trees (McGuire et al. 1996). In an analysis of 87 experiments, Poorter (1998) found that nutrient-stressed plants showed reduced positive growth response relative to unstressed plants. Thomas et al. (1991) found that seedlings of *Gliricida senium*, a woody legume did not show any growth response without N fertilization. On the other hand, Norby (1987) has demonstrated a positive growth response for seedlings of three woody legume species grown in soil with low levels of available N and P.

An analyses of the A/Ci curves did not suggest a limit of inorganic P to photosynthesis. If P was limiting, elevated plants would be predicted to allocate sufficient N to Rubisco production but minimize allocation of P to the triose phosphate production. This would maximize P use efficiency. If this was the case, W_p would be expected to be limit A (Harley et al. 1992). However, this did not appear to be the case for any of the A/Ci curves, as A did not appear saturated (i.e.

values of A did not reach a plateau) around growth Ci. The similarity of leaf N concentration between treatments suggests that N was not limiting either.

N was not expected to be limiting, as *P. sericea* can utilize atmospheric N_2 to overcome shortages in soil N, as has been shown for other woody legumes supplied with elevated CO_2. Nodules were seen on the roots of *P. sericea*, but differences in nodule number, mass or activity were not measured. However, it is likely that the reason *P. sericea* did not show the same positive growth response as other woody legume studies is that N_2-fixation was not enhanced in the elevated treatment. This is suggested by the leaf $\delta^{15}N$ values. An increase in N_2-fixing activity in elevated plants relative to ambient plants would be expected to result in leaf $\delta^{15}N$ values closer to 0‰ (the $\delta^{15}N$ of atmospheric N_2) in elevated plants. The complete overlap of values (Fig. 6) therefore suggests no difference in N_2-fixing activity. This overlap could also result if soil $\delta^{15}N$ was close to 0‰ or if fractionations involved in the assimilation of soil N resulted in leaf $\delta^{15}N$ of 0‰ (Handley and Scrimgeour 1997). Leaf $\delta^{15}N$ from *L. laureolum*, a non-fixing species used in the experiment, had a range of values between 0.7 and 2.3‰, suggesting that this is not the case.

Potential limits to increased N_2-fixation

In all the other studies on legumes, plants have been grown individually and competition, shading and restricted rooting volume was not evident. In addition, the experiments have been relatively short, the longest lasting only about one third of the duration of this experiment (Vogel and Curtis 1995). This experiment differed from previous ones involving woody legumes in that growth-limiting factors other than soil N shortages are likely to have been severe enough to prevent *P. sericea* from achieving increased N_2-fixation and enhanced growth.

N_2-fixation may have been limited by the supply of resources to the nodules even in elevated CO_2 supply. If shading was so severe that assimilation of C was limited even in elevated CO_2, the supply of carbohydrates to root nodules would not have increased. Phosphate shortages may have had the same effect. A shortage of soil N itself may have prevented a positive growth response. Soil N levels may have become so low during the experiment that *P. sericea* relied on fixed-N_2 for almost all of its N requirements in both treatments. This is suggested by the $\delta^{15}N$ values being close to zero for both treatments (Fig. 6). In this case, carbohydrate supply to nodules would not have been a limiting factor, and enhanced photosynthesis under elevated CO_2 would not have enhanced levels of N_2-fixation. However, leaf $\delta^{15}N$ must be treated with caution - Handley and Scrimgeour (1997) point out that leaf $\delta^{15}N$ alone is not a reliable measure of source N.

FLOWER PRODUCTION

The only positive response to growth in elevated CO_2 displayed by *P.sericea* was that of increased flower production. No studies were found on the effect of elevated CO_2 on the reproduction of woody species, and the limited work on herbaceous species has yielded conflicting results. Bazzaz (1990) provides results of a handful of studies investigating reproduction in herbaceous annuals. These show that in elevated CO_2 treatments time to flowering may be reduced, delayed or unaffected, while flower production may decline or remain unchanged. Stewart and Potvin (1996) found that *Trifolium repens* produced more flowers when grown under elevated CO_2. Both Bazzaz and Stewart and Potvin report that growth under higher levels of competition decreased effects of elevated CO_2 on flowering.

It seems unusual that flowers production increased while overall biomass did not. It is possible that increased flower production was simply an artefact of the elevated plants having more branches (the maximum number of flowers/dry weight correlated with number of branches: r=0.51, df=19, p=0.03). More branches in the elevated treatment may have resulted in more points for flower mersitems to develop. Alternatively, flower growth may have created a strong sink for photosynthates and sequested additional carbohydrates produced in the elevated treatment. However, the second leaf gas exchange measurements revealed lower rates of net assimilation of CO_2 just before flowering began. It is therefore unlikely that the elevated plants had more carbohydrates to allocate to developing flowers. However, flowers ~~especially~~ probably have high N contents and it is possible that N, rather than carbohydrates, limited the number of flowers produced. Photosynthetic acclimation may therefore have been a result of the elevated plants allocating relatively more N to developing flower buds than to Rubisco and other photosynthetic compounds. Again, this idea is not supported by the leaf N concentrations.

ECOLOGICAL IMPLICATIONS

Under natural conditions, plants are likely exposed to a number of growth-limiting factors other than CO_2 supply. The lack of a long-term, positive growth in this experiment may therefore be applicable to *P. sericea* in its natural habitat, even if the physiological reasons underlying it are not clear. Leguminous species may not therefore benefit more than other species in elevated CO_2 world. However, while growing conditions in this experiment may been similar to those in nature (in that competition for light and nutrients was high), they were not necessarily the same as those encountered by the of *P. sericea* in its natural habitat. For example, it seems unlikely that shading or rooting restrictions would be as severe in nature. *In situ* studies at the population level are therefore required to validate any predictions about the responses of *P. sericea* and other woody legumes to elevated CO_2. Limited *in situ* research on herbaceous species has produced inconsistent results, with no long term benefit for both legumes and non-fixers in some studies (e.g. Bunce 1995) and increased benefit for legumes relative to non-fixers in others (Stewart and Potvin 1996; Luscher et al. 1998). Final consideration must be given to the increased flower production of elevated plants. If *P. sericea* is limited in its abundance and distribution by seed production, increased flower production could have large benefits for this species at the population level.

CONCLUSION

This study provides unique evidence of a lack of positive growth response of a woody legume to elevated CO_2 supply. However, the physiological processes responsible for this result are not clear. From the results presented, it cannot be determined whether genetic growth potential or the influence of growth limiting factors prevented a positive growth response. The leaf gas exchange results suggest that light was limiting for at least the first part of the experiment, and it is likely that restricted root growth and nutrient shortages were important later in the experiment. There is was also no evidence of increased N_2-fixation in elevated plants, and regardless of the physiological mechanisms involved, it is clear that growth conditions exist in which woody legumes will not benefit from elevated CO_2 in terms of growth rates. However, reproductive success may be enhanced, which could be equally, if not more, significant for responses at the population and ecosystem level.

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