CARBON ALLOCATION IN THE LEAVES OF TWO *LEUCADENDRON* *sp.* GROWN UNDER DIFFERENT CO_2 AND NUTRIENT CONDITIONS

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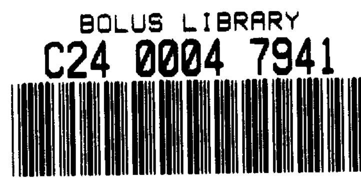
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ABSTRACT

Leucadendron laureolum and *Leucadendron xanthoconus* were grown for two months in growth chambers at two nutrient and CO_2 levels. After being harvested the quantity of crude cell wall material, fats and waxes, polyphenols, soluble carbohydrates, insoluble carbohydrates and lignin present in the leaves and roots of each plant was determined. There were significant differences in the allocation patterns of crude cell wall material, polyphenols and soluble carbohydrates between species, regardless of treatment. Crude cell wall weight was significantly higher in high nutrient treatments in the leaves of *Leucadendron laureolum*. Fats and waxes did not differ significantly between treatments or species. Polyphenol concentration did not differ significantly between the different treatments. Soluble carbohydrates did not differ significantly in the leaves of either species with treatment, however, the roots of *L. laureolum* had higher concentrations of soluble carbohydrates under low CO_2, high nutrients and high CO_2, low nutrient treatments. There were no significant differences in the concentration of insoluble carbohydrates between treatments. In the leaves of *L. xanthoconus* the proportion of lignin in the samples was significantly higher under low CO_2 conditions. The percentage of lignin in the root samples of both species is significantly different in low and high CO2 conditions. *L. laureolum* roots have a contrasting pattern to *L. xanthoconus* roots. In the roots of *L.laureolum* high CO_2 levels result in significantly higher percentages of lignin. In *L. xanthoconus* roots the percentage lignin is significantly lower. The implications of elevated CO_2 levels for the herbivores and nutrient cycle of the fynbos biome are discussed in light of these results and other trends which were found.

INTRODUCTION

The rise in atmospheric CO_2 levels is a well documented fact. Trapped air bubbles in Antarctic ice indicate that in the sixteenth century CO_2 levels were as low as 260 ppm, in 1985 the concentration had risen to 365 ppm and by the year 2050 it is expected to reach 600 ppm. This threefold increase, which has been ascribed to a combination of fossil fuel combustion and the removal of the CO_2 sink through extensive deforestation, is expected to have both direct and indirect effects on plants. Since plants form the basis of almost any ecosystem it is important to understand their response to such changing conditions (Crawford 1989).

Mediterranean ecosystems and the Cape Floral Kingdom:

Recognised as a floral Kingdom, the Cape Floristic region, or fynbos biome, is one of five mediterranean ecosystems in the world (Walter 1973). One of the characteristics of these systems are the extremely nutrient poor soils, particularly with regard to nitrogen and phosphorous (Specht and Moll 1983). The species which occur here cope with these conditions by having both low nutrient requirements and a number of adaptations, such as symbiotic relationships with mycorrhizas, for increasing the efficiency of nutrient acquisition (Stock and Allsop 1992). Related to this is the relatively slow growth rate evident in certain of the indigenous species (Stock and Midgley unpub.).

The nutrient poor conditions lead to low nutrient levels in the leaves which, in conjunction with a high fibre content, results in the leaves having a very low nutritional value for potential herbivores (Stock and Allsop 1992). As a result there are very few large grazers in the fynbos the majority phloem sap suckers, root feeders, pollen or seed eaters (Johnson

1992).

A function of the increased efficiency of nutrient uptake and slow decomposition rates is a compact nutrient cycle. The slow decomposition rates are the result of the small quantity and poor quality of litter. The main mechanism for nutrient release is fire (Read and Mitchell 1983). Fire is also important in the reproductive ecology of the ecosystem, many species being adapted to releasing their seeds during or after a fire. In this context the frequency and intensity of the fire is very important (le Maitre and Midgley 1992).

The manner in which the indigenous species respond to elevated CO_2 levels will determine the adjustments in ecosystem dynamics, specifically in terms of interactions and nutrient cycling.

The effect of elevated CO_2 on photosynthesis and productivity:

A number of experiments have indicated that short term exposure to elevated CO_2 levels results in an increase in photosynthesis, and productivity in C_3 crop plants, especially when resources such as nutrients and water are not limiting (Bazzaz and Fajer 1992). Long term experiments with elevated CO_2 and sufficient resources have shown similar results, with photosynthesis and productivity remaining elevated for a considerable length of time (Grulke et al 1990; Ziska et al 1990). However, where nutrients or water are limited or temperature is unfavourable, photosynthesis and productivity levels soon return to ambient levels (Grulke et al 1990).

The changes to photosynthetic rate and productivity are important because they will affect

the carbon allocation patterns, causing variation in the growth of the whole plant (Poorter and Bergkotte 1992) and altering the C:N and C:P ratio's in the leaves (Stock and Midgley unpub.). According to the carbon-nutrient balance hypothesis, plants growing in low nutrient environments at ambient CO_2, such as fynbos species, are suggested to be able to produce excess carbon. This excess is channelled either into starch, for storage, or into secondary compounds, such as polyphenols, utilised in plant defense (Fajer et al 1992).

Increased productivity in elevated CO_2 conditions will increase the quantity of excess carbon compounds and alter the allocation of these compounds within the plant. For example, an increase in the biomass allocation to the root has been documented under elevated CO_2 conditions when water and nutrients are limiting (Bazzaz 1990). Such alterations to the carbon allocation of the plant can have repercussions for productivity, affecting factors such as net carbon uptake, nutrient uptake, water relations and light capturing ability. Ultimately this affects the competitive ability of the plant (Berryman et al 1993).

Changes to carbon allocation patterns and some consequences:

Slow-growing species, such as those which occur in fynbos, have been shown to accumulate a different proportions of various carbon based compounds, namely hemicellulose, insoluble sugars and lipids, to the fast growing species (Poorter and Bergkotte 1992). Thus it is possible that the changes in carbon allocation in slow growing species, grown under elevated CO_2 conditions, will be different to those of fast growing species.

Modifications in the proportions of carbon compounds with respect to other carbon compounds, nitrogen and phosphorous, has implications for the interactions between the plant

and other organisms in the ecosystem (Oechel and Lawrence 1986). An increase in structural carbohydrates and phenolics, for example, would affect plant-herbivore interactions and decomposition rates, having repercussions for nutrient turnover in the ecosystem. In fynbos it would necessitate adjustments to the fire regime.

Objectives:

The main objective of this study was to determine the manner in which slow growing species from the fynbos biome respond to different combinations of high and low CO_2 and nutrient regimes. Quantifying the specific carbon compounds found in the plants from different treatments provides information on changes and variation of allocation patterns within the species. From this an indication of the possible impact elevated CO_2 will have on fynbos ecosystems, those which are on poor soils and those which have sufficient nutrients, can be obtained. If the indigenous species do not acclimate to elevated CO_2, it is expected that there will be a increase in the litter input. The accumulation of more litter, possibly over a shorter time period, could result in frequent more intense fires (Stock and Midgley unpub.) not only does this have implications for the nutrient cycle, but also for the reproductive ecology of the species concerned.

In a broader context comparisons between allocation patterns in fast and slow growing species can be made.

METHODS:

Two species, *Leucadendron laureolum* and *Leucadendron xanthoconus*, were used. The plants were grown for two months in 3:1 acid washed sand to sterile perlite mix in 2.75 l

pots (0.5 m deep). These pots were situated inside open-top chambers of polycarbonate construction in a polycarbonate-clad green house. Eight plants of each species were grown under the following conditions: elevated CO_2-high nutrients, elevated CO_2-low nutrients, ambient CO_2-high nutrients and ambient CO_2-low nutrients. Ambient CO_2 was 350 $\mu l.l^{-1}$ while elevated CO_2 was ambient + 350 $\mu l.l^{-1}$. Plants grown under the high nutrient regime received 100ml a week of a 20% Long Ashton solution containing 0.20mM nitrogen, while plants grown under the low nutrient regime received 100ml per week of a 5% Long Ashton nutrient solution containing 0.05mM nitrogen in ammonium and nitrate form.

After the plants had been harvested the roots and leaves of each plant were ground through a 40ml mesh and specific compounds extracted sequentially. In the first step, 5ml of petroleum ether was added to 0.25g of plant sample. After 24 hours the supernatant from each specimen was poured out into pre-weighed watch glasses and evaporated. The watch glasses were reweighed to determine gravimetrically the amount of fats and waxes. Ten ml of 50% methanol was added to the residue and left to stand for 24 hours. The supernatant was poured off and centrifuged to remove the lighter residue. The methanol supernatant was stored at 0°C and 10ml 3% HCl was added to the residue. This was placed in a water bath of 100°C for 3 hours. After this the HCl supernatant was centrifuged and then stored, while the residue was placed in an oven at 80°C for 24 hours. This dried residue was then weighed to obtain the crude cell wall weight.

The supernatant from the methanol extraction was analyzed for polyphenol and soluble carbohydrate content.

Polyphenols

Aliquots of 0.2 ml of the methanol extract were made up to 6ml with 50% methanol. To this was added 50 ml of distilled water.

The standard curve was constructed using different concentrations of a 1mM solution of catechin made up with 100% methanol. The sample values were then read off the standard curve and converted into mmol of catechin equivalents per g dry weight (equation 1). This value was then converted to mg catechin/ g dry weight using equation 2:

Equation 1:

$$\frac{Sample conc.}{0.25g} X 50ml X \frac{290,3g}{1000ml} = mmol cat. eqv./g dry weight$$

Equation 2:

$$mmol cat. eqv. X \frac{0.16 mmolar}{1 mmol} = mg cat. eqv./g dry weight$$

Soluble and Insoluble Carbohydrates

The concentration of soluble carbohydrates was determined using aliquots of the methanol supernatant. The extract was blown down to remove the methanol and the remaining solution was made up to 25ml with distilled water. To perform the analyses an 8ml aliquot of each sample was made up to 10ml with distilled water. Ten ml of Reagent 50 (Smith 1981) was added. This solution was then placed in a water bath at 100°C for 15 minutes. Immediately

after being removed the test tubes were cooled in a water bath. Two ml of a potassium iodide-potassium oxalate solution (Smith 1981) was added to the solutions in the test tubes followed by 10ml of 1N H_2SO_4. This was then mixed to dissolve any precipitate which may have been present. This solution was then poured into a Ehrlenmeyer flask and 0.25ml of starch indicator added and a titration against 0.02M Sodium thiosulphate was then performed. Titration results were then converted into mg glucose/ g dry weight according to the formula 3, in Smith (1981). Some changes were made because of the different method of extraction which was used. Since no enzyme was used in the extraction the value for the Reagent 50 was used instead of the value of the enzyme blank so the equation read as follows:

Sugar in the sample aliquot; equation 3:

$$\frac{3ml}{R50blank-Glu.std.} X (R50blank-SampleTitr.)$$

Amount of Total nonstructural carbohydrates in tissue; equation 4:

$$Aliquot'ssugarconc. X \frac{25ml}{8ml} X \frac{1}{0.25g}$$

The same method of titration was used to analyze the acid extracted solutions for insoluble carbohydrates, except that there were differences in the initial treatment of the sample. First the acid was neutralised with 0.05 N NaOH solution and then made up to 50ml with distilled water. A 5ml aliquot was made up to 10ml with distilled water and to this solution 10ml

Reagent 50 was added and from this point the analytical procedure was the same as that described above. For this analysis the amount of sugar in the sample aliquot was corrected using the following equation 5:

Equation 5:

$$Aliquot's sugar conc. X \frac{25ml}{8ml} X \frac{1}{0.25g}$$

<u>Lignin analysis</u>

A 0.04g sample of the dried residue was weighed out and analyzed for lignin. Each sample was placed in a thick walled boiling tube with 5ml acetyl bromide in acetic acid (Morrison 1972). This was then placed in a hot water bath at 70°C for 30 minutes and cooled immediately to room temperature in a water bath. The sample was diluted with 20ml of acetic acid. This solution was shaken and a 5ml aliquot extracted and placed in a 50ml volumetric flask with 0.9ml 2N NaOH in 5ml acetic acid. This was then made up to about 45ml acetic acid and then 1.6ml of a 0.05M hydroxyl-ammonium chloride solution was added before the solution was made up to 50ml. These solutions were allowed to stand for at least an hour so that the sediment could settle. The optical density was read at 280nm in 10mm silica cells.

Using the following equation (6) the optical density values were converted to lignin (% in the sample):

Equation 6:

$$\frac{O.D_S - O.D._B}{c} = A$$

Where $O.D_s$ = optical density of the sample, $O.D._B$ = optical density of the blank, c= concentration of dry organic matter in the final solution ($g.l^{-1}$).

This value was then converted to % lignin in the organic matter using Morrisons (1972) conversion.

Equation 7:

$$(5.12XA) - 0.74 = Lignin(\%organicmatter)$$

The amount of organic matter was determined in the following manner. A 40mg of cell wall residue was placed in a preweighed crucible, was placed in the muffle oven for two hours at 250°C, to burn off all the organic matter. The crucible was then reweighed and the amount of organic matter that had been present was determined.

The original value of %lignin was then divided by weight of organic matter to obtain the amount of lignin on an organic matter basis (% organic matter).

The data from the different analyses were statistically analyzed with a split-plot ANOVA performed in Genstat 5 (Payne et al 1987).

RESULTS

Crude cell wall weight

Crude cell wall weight varies little between the four treatments for both the leaves and the roots (Fig 1), although the combination of high CO_2 and high nutrients consistently resulted in slightly higher weights. If the nutrient treatment is considered alone, then there is a significant difference between the low nutrient and high nutrient treatments in the leaves of *Leucadendron laureolum* (Table 1, $p < 0.01$), with the high nutrient concentrations resulting in higher crude cell weights. The crude cell wall weight is significantly lower in the leaves of *Leucadendron laureolum* than in the leaves in *L. xanthoconus* (Fig 1, Table 1, $p < 0.01$) but a similar difference is not found in the roots of the two species (Table 2).

Fats and Waxes

There were no significant difference between treatments or species in terms of the amount of fats and waxes present in the leaves and roots (Table 1 and 2). However, in Fig 2 it is clear that in the leaves of both species the amount of fats and waxes decreases under low CO_2 and high nutrient conditions. There are no other common trends in the amount of fats and waxes present. In *L. xanthoconus* leaves the amount of fats and waxes increased in high CO_2 and nutrient conditions but in *L. laureolum* it dropped (Fig 2). Similarly, the high CO_2 low nutrient treatment resulted in greater amounts of fats an waxes in *L. xanthoconus* but lower amounts in the leaves of *L. laureolum*. In the roots of both species the treatments had very little effect on to the amount of fats and waxes present.

Polyphenols

Polyphenol concentrations differed significantly in the leaf samples of the two species (Table 1, $p < 0.05$) but not between the roots or the different treatments. *L. xanthoconus* leaves appear to have lower concentrations of polyphenols than the leaves of *L. laureolum* (Fig 3) under all conditions except high CO_2 and low nutrients. In *L. xanthoconus* the concentration of polyphenols in the leaf samples were similar to those in the root samples, in *L. laureolum*, however, the leaf sample contained more polyphenols under all conditions except high CO_2 and low nutrients when the concentrations were virtually identical.

Soluble carbohydrates

The leaves of *L. xanthoconus* had a significantly lower concentration of soluble carbohydrates than the leaves of *L. laureolum* (Fig 4, Table 1, $p < 0.01$). In contrast to this the roots of *L. xanthoconus* had a significantly higher concentration of soluble carbohydrates (Fig 4, Table 2, $p < 0.01$).

Under conditions of low CO_2, high nutrients and high CO_2, low nutrients the concentration of soluble carbohydrates in the leaves of *L. xanthoconus* increased, while at high CO_2 and nutrients the concentration decreased. In the leaves of *L. laureolum* the opposite pattern appeared with the concentration of soluble carbohydrates decreasing under low CO_2, high nutrients and high CO_2, low nutrients. In *L.laureolum* leaves, the high CO_2, high nutrient treatment had soluble carbohydrate concentrations which were equivalent to those of the low CO_2 low nutrient treatment.

There was a marked rise in the concentration of soluble carbohydrates in *L. xanthoconus*

roots under high CO_2, low nutrient conditions (Fig 4). Soluble carbohydrate concentration increased to a lesser degree under the low CO_2, high nutrient and decreased in the high CO_2, high nutrient treatment. In the roots of *L. laureolum* there was a significant difference between the different treatments (Table 2, $p < 0.1$). The concentration of soluble carbohydrates under low CO_2, high nutrients and high CO_2, low nutrients differs significantly from that of the low CO_2, low nutrient and high CO_2, high nutrient treatments (Fig 4).

Insoluble carbohydrates

There were no significant differences in the concentration of insoluble carbohydrates between treatments or species. The leaf samples of both species had higher concentrations of insoluble carbohydrates than the root samples (Fig 5). In the roots of both species three of the treatments, low CO_2 high nutrients, high CO_2 low nutrients, and high CO_2 high nutrients, resulted in a decrease in the concentration of insoluble carbohydrates.

In the leaves a different pattern emerged. There was no difference between the low CO_2 treatments. The high CO_2 low nutrient concentration resulted in the highest concentration of insoluble carbohydrates in the leaves. This concentration was greater in *L. laureolum* than it was in *L. xanthoconus*, as was the difference between the highest and lowest concentrations of insoluble carbohydrates.

Lignin

There was no significant difference between the percentage of lignin present in the leaf and root samples of each species. In the leaves of *L. xanthoconus* the proportion of lignin in the samples was significantly different under low and high CO_2 conditions (Table 1, $p < 0.1$).

Under low CO_2 conditions the percentage of lignin in the *L. xanthoconus* leaf sample is higher than under high CO_2 conditions (Fig 6). In the leaves of *L.laureolum* a indistinct trend seemingly related to nutrients appears, but it is not significant (Fig 6).

The percentage of lignin in the root samples of both species is significantly different under low and high CO_2 conditions. *L. laureolum* roots have a contrasting pattern for the different treatments compared to the leaves, that is the proportion of lignin decreases, to different degrees, in both high nutrient treatments (Fig 6). The pattern related to CO_2 is, however clearer in the roots of *L.laureolum* than in the leaves. High CO_2 levels result in significantly higher percentages of lignin (Table 2, $p < 0.01$).

In *L. xanthoconus* roots the pattern follows that of the leaf with the percentage lignin being significantly lower in the *L. xanthoconus* roots sample from the high CO_2 treatment (Fig 6, Table 2, $p < 0.01$).

DISCUSSION

Interestingly enough the allocation of certain carbon compounds differs significantly between the two species regardless of their treatment. This indicates that variation in the carbon allocation patterns can be expected among species classified as slow growing, even between species of the same genus. For example, the amount of crude cell wall material in *L. laureolum* (Fig 1; Table 1) is significantly lower than in the leaves of *L. xanthoconus*. Polyphenols, compounds utilized in plant defense, are present in significantly lower concentrations in *L. xanthoconus* leaves (Fig 3; Table 1). The different pattern in soluble carbohydrate concentration in the roots and leaves of the two species is very interesting. The

term soluble carbohydrate describes sugars such as respiration and growth (Curtis 1986). In *L. laureolum* leaves these compounds are present in significantly higher concentration than in *L. xanthoconus* leaves (Fig 4; Table 1), while in the roots the opposite pattern is observed *L. xanthoconus* having a higher concentration than the roots (Fig 4;Table 2). Thus, it is reasonable to expect different responses to the nutrient treatments and elevated CO_2 levels may also be expected from the different species.

The first evidence of a differential response to elevated CO_2 between species is found in the photosynthetic response (Stock and Midgley unpub.). Under ambient CO_2 conditions both species had very similar curves. However, in the elevated CO_2 conditions the photosynthetic mechanism of *L. laureolum* down regulated, fixing less CO_2 than under ambient conditions, while that of *L. xanthoconus* remained the same. In cases where photosynthetic rates have increased in elevated CO_2 levels (Chu et al 1992), accompanying changes have been recorded in growth, resource acquisition and partitioning. However, the partitioning of biomass between the roots and the shoots has not altered. It is therefore expected that the result of photosynthetic down regulation will be a reduction in the quantity of carbon compounds produced. While this should directly affect allocation patterns within the plant and, consequently, biological processes such as growth, it is not certain to what extent this will occur.

In terms of predicting changes in litter quantity and perhaps more importantly quality, the changes in crude cell wall material (Fig 1), insoluble carbohydrates (Fig 5) and the percentage of lignin (Fig 6) must be considered. All these compounds are important structural components of the plants cell walls and membranes and alterations in the relative amounts present will either improve or further reduce the quality of the litter.

Elevated CO_2 appears to have no impact on the crude cell wall weight of leaves and roots in either species, except in conjunction with high nutrient levels. Nutrients alone, however, do influence crude cell wall weight. *L. laureolum* leaves had significantly higher crude cell wall weights in high nutrient conditions (Fig 1; Table 1). This increase in crude cell wall weight in *L. laureolum* leaves may be comparable to an increase in biomass accumulation in leaves which has been detected in other experiments involving nutrient regimes (Silvola and Ahlholm 1992).

The insoluble carbohydrates concentration is affected by CO_2 levels increasing in the leaves of both species in elevated CO_2 conditions (Fig 5). This increase, however, is not significant (Table 1) and it is not mirrored in the roots of either species, which exhibit a decrease in the insoluble carbohydrate concentration in all treatments.

Despite the fact that *L. laureolum* leaves have a lower concentration of insoluble carbohydrates at ambient CO_2 and nutrient levels than *L. xanthoconus*, and that the photosynthetic mechanism of *L. laureolum* down regulates under elevated CO_2, *L. laureolum* still has a greater concentration of insoluble carbohydrate in elevated CO_2 conditions. The concentration is especially high in the high CO_2 low nutrient treatment, a response which is also visible in *L. xanthoconus*. This result implies that in the future high CO_2 environment fynbos species will allocate more carbon to structural elements, such as insoluble carbohydrates. This may well result in the decomposition rate becoming even slower.

However, decomposition rates are also affected by the proportion of lignin present. *L. laureolum* and *L. xanthoconus* both differed in their allocation of lignin with regard to

treatment. *L. xanthoconus* showed a significant response in elevated CO_2 conditions with the percentage of lignin dropping unexpectedly in the roots and leaves. In both cases the decrease in lignin was significant (Fig 6; Table 1 and 2).

The drop in the proportion of lignin In *L. xanthoconus* leaves and roots is unexpected for two reasons. Firstly, according to Poorter and Bergkotte (1992) slow growing species invest and accumulate greater amounts of hemi cellulose, insoluble carbohydrate and lignin under ambient conditions. Theoretically this should increase in elevated CO_2 levels. In *L. xanthoconus* crude cell wall weight remains the same and insoluble carbohydrate concentration does increase but lignin exhibits a dramatic decrease. Secondly previous experiments have indicated that there is an increase in the allocation to root biomass in elevated CO_2 conditions (Bazzaz 1990) especially in low nutrient, water stressed conditions.

However, Chu et al (1992) has recorded a decrease in dry matter allocation to the roots under elevated CO_2 conditions and Conroy et al (1990) found contrasting dry matter allocation patterns in two groups of *Pinus radiata* seedlings that were grown under elevated CO_2 conditions with adequate nutrients. This suggests that a decrease in allocation of lignin to the roots is not that unusual. It does appear that in this case there is a greater allocation of structural material to the above ground biomass than to the roots in elevated CO_2.

L. laureolum differs in that the lignin percentage in the leaves do not exhibit a clear pattern related to different CO_2 levels, rather an indistinct trend related to nutrients is apparent. However, elevated CO_2 did affect the allocation of lignin to the roots, there being a slight increase in the proportion of lignin present in the roots at elevated CO_2 levels. This is in

contrast to the decrease in the roots (Fig 6).

Thus, despite the increase in the insoluble carbohydrate concentration in both species at elevated CO_2 levels, litter quality, and thus decomposition rates, may not change because of the decrease in percentage lignin in the leaves of *L. xanthoconus* and a lack of response in terms of lignin, in *L. laureolum*.

Although adjustments to the structural components may affect plant-herbivore interactions, particularly in terms of root feeders, they are unlikely to be as important as the concentration of nitrogen, phosphorous, soluble carbohydrates and polyphenols. This is because the majority of fynbos herbivores are not grazers but phloem sap suckers (Johnson 1992).

The reduction of foliar nitrogen concentrations under elevated CO_2 reduces the quality of the host plant for the insect herbivore, requiring them to eat more to obtain the same level of nutrition (Fajer et al 1989). In both *Leucadendron* species the elevated CO_2 treatment results in a significant decrease in the nitrogen content of the leaves, but no change in the phosphorous concentration (Stock and Midgley pers com.). If the elevated CO_2 levels were also to cause a increase in the amount of soluble carbohydrate in the *Leucadendron* species, similar to that recorded in other cases (Davis and Potter 1989), then the nutrient quality of the leaves would be severely reduced.

However, this trend is not evident in either *L. xanthoconus* or *L. laureolum*. The concentration of soluble carbohydrate in *L. xanthoconus* leaves under low CO_2, high nutrient and high CO_2, low nutrient conditions (Fig 4) is marginally higher than in ambient

conditions, while in *L. laureolum* plants grown under low CO_2, high nutrient and high CO_2, low nutrient conditions exhibit a distinct decrease in soluble carbohydrate concentration.

The polyphenol concentration will also influence the nutritional value of the plant material. High concentrations of these carbon based allelochemicals will reduce the nutritional quality of the plant material substantially. However, previous studies have shown no increase in phenolic production under elevated levels of CO_2 (Bazzaz 1990) and the results for the leaves in this experiment concur (Fig 3). In fact there is a decrease in all treatments relative to the concentration in the plants grown under ambient conditions.

Thus, while the concentration of nitrogen decreases in the leaves the nutritional value of the leaves may not be too adversely affected since there is virtually no increase in the concentration of soluble carbohydrate and a decrease in the concentration of polyphenols.

One set of compounds which have not been considered in this study are the root exudates of the plant. It is possible that the increase in soluble carbohydrate visible in the roots of both species may be translated into root exudates that could affect the micro fauna and flora of the surrounding soil and possibly the root dynamics of neighbouring plants. The release of energy rich compounds into the soil may increase the activity of the organisms involved in decomposition, possibly increasing the rate. This is one more factor to take into account when considering the potential affect of elevated CO_2 on the nutrient cycle in fynbos. The production or increased production of root exudates may also affect the symbiotic relationships between mycorrhizas and their hosts. The conversion of carbon compounds into exudates, if they were not taken into account, could result in an underestimate of the quantity

of carbon compounds allocated to the roots (Silvola and Ahlholm 1992).

CONCLUSION

The allocation patterns of *L. laureolum* and *L. xanthoconus* differ for certain compounds regardless of treatment. This implies that changes in the allocation patterns may also be different between species. In certain compounds this is indeed the case. Crude cell wall material, for example, is higher in *L. laureolum* under high nutrient conditions, while *L. xanthoconus* does not show a response. Similarly, lignin and soluble carbohydrates exhibit contrasting patterns in the species.

The variation in allocation patterns and the changes which occur have important implications for the ecosystem. Changes in the carbon nutrient content of the leaves or roots, due to an increase in the allocation of lignin cellulose, insoluble carbohydrate or polyphenols, would alter the decomposition rate of the plant material and herbivore interactions. Modifications to the allocation of soluble carbohydrates in roots may lead to an increased amount of root exudates which could impact on the microfauna and flora of the soil.

Modifying these aspects of the ecosystem would lead to alterations in the nutrient cycling process which in fynbos has important management implications in terms of fire regimes. Thus in order to speculate more accurately on the effect of nutrients and elevated CO_2 on fynbos ecosystems, investigations into the effect of carbon allocation changes in competitive and herbivorous interactions need to be made.

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Table 1: Split plot analysis of variance, testing the effects of different concentrations of CO_2 and nutrients, on organic compounds found in the leaves of two Leucadendron species. Values are F values, *significant differences at $P < 0.1$, ** $P < 0.05$, *** $P < 0.01$, NS = not significant ($P > 0.05$).

Analysis	Species	CO_2 treatment d.f 6	Nutrient treatment d.f 22	Interaction d.f 22	Between species d.f 1
Crude cell wall weight	*L.xanthoconus*	0.593^{NS}	0.105^{NS}	0.305^{NS}	0.001***
	L.laureolum	0.343^{NS}	0.009***	0.891^{NS}	
Fats & Waxes	*L.xanthoconus*	0.308^{NS}	0.945^{NS}	0.264^{NS}	0.529^{NS}
	L.laureolum	0.923^{NS}	0.104^{NS}	0.629^{NS}	
Polyphenols	*L.xanthoconus*	0.641^{NS}	0.266^{NS}	0.372^{NS}	0.013**
	L.laureolum	0.257^{NS}	0.723^{NS}	0.282^{NS}	
Soluble Carbohydrates	*L.xanthoconus*	0.676^{NS}	0.899^{NS}	0.306^{NS}	0.003***
	L.laureolum	0.951^{NS}	0.953^{NS}	0.117^{NS}	
Insoluble carbohydrates	*L.xanthoconus*	0.343^{NS}	0.509^{NS}	0.479^{NS}	0.604^{NS}
	L.laureolum	0.258^{NS}	0.526^{NS}	0.504^{NS}	
Lignin	*L.xanthoconus*	0.095*	0.982^{NS}	0.965^{NS}	0.761^{NS}
	L.laureolum	0.955^{NS}	0.635^{NS}	0.768^{NS}	

Table 2: Split plot analysis of variance, testing the effects of different concentrations of CO_2 and nutrients, on organic compounds found in the roots of two Leucadendron species. Values are F values, *significant differences at P < 0.1, ** P < 0.05, *** P < 0.01, NS = not significant (P > 0.05).

Analysis	Species	CO_2 treatment d.f 6	Nutrient treatment d.f 22	Interaction d.f 22	Between species d.f 1
Crude cell wall weight	*L.xanthoconus*	0.718^{NS}	0.639^{NS}	0.389^{NS}	0.842^{NS}
	L.laureolum	0.612^{NS}	0.720^{NS}	0.381^{NS}	
Fats & Waxes	*L.xanthoconus*	0.781^{NS}	0.753^{NS}	0.427^{NS}	0.832^{NS}
	L.laureolum	0.843^{NS}	0.377^{NS}	0.981^{NS}	
Polyphenols	*L.xanthoconus*	0.471^{NS}	0.781^{NS}	0.317^{NS}	0.269^{NS}
	L.laureolum	0.838^{NS}	0.356^{NS}	0.253^{NS}	
Soluble Carbohydrates	*L.xanthoconus*	0.188^{NS}	0.827^{NS}	0.122^{NS}	0.001^{***}
	L.laureolum	0.594^{NS}	0.626^{NS}	0.094^{*}	
Insoluble carbohydrates	*L.xanthoconus*	0.771^{NS}	0.479^{NS}	0.918^{NS}	0.699^{NS}
	L.laureolum	0.471^{NS}	0.337^{NS}	0.474^{NS}	
Lignin	*L.xanthoconus*	0.002^{***}	0.551^{NS}	0.750^{NS}	0.789^{NS}
	L.laureolum	0.038^{**}	0.299^{NS}	0.860^{NS}	

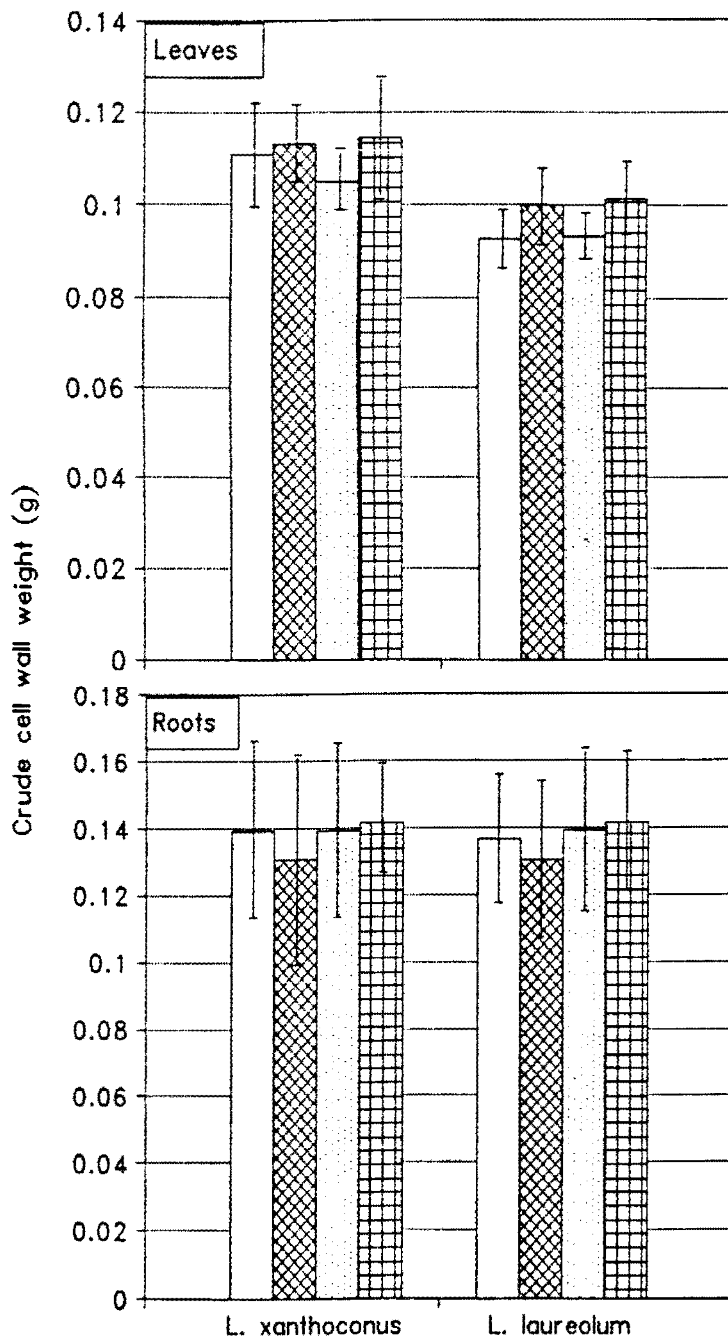


Fig 1: The variation in the crude cell wall weight in the leaves and the roots of *L.xanthoconus* and *L.laureolum*.

low CO_2 low nutrients, low CO_2 high nutrients,

high CO_2 low nutrients, high CO_2 high nutrients

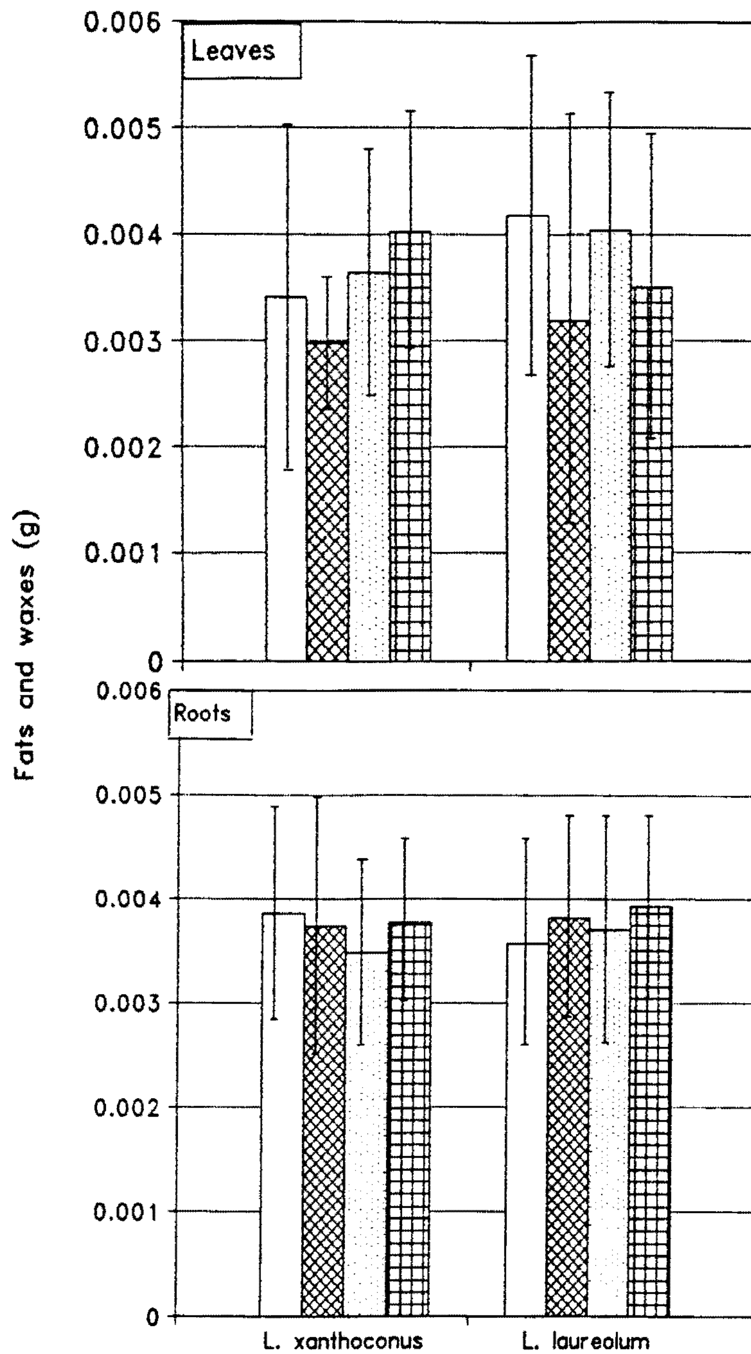
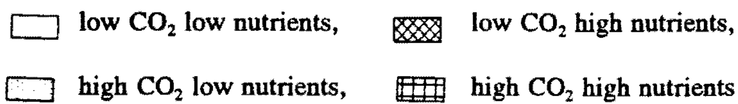


Fig 2: The variation in the fats and waxes in the leaves and the roots of *L.xanthoconus* and *L.laureolum*.



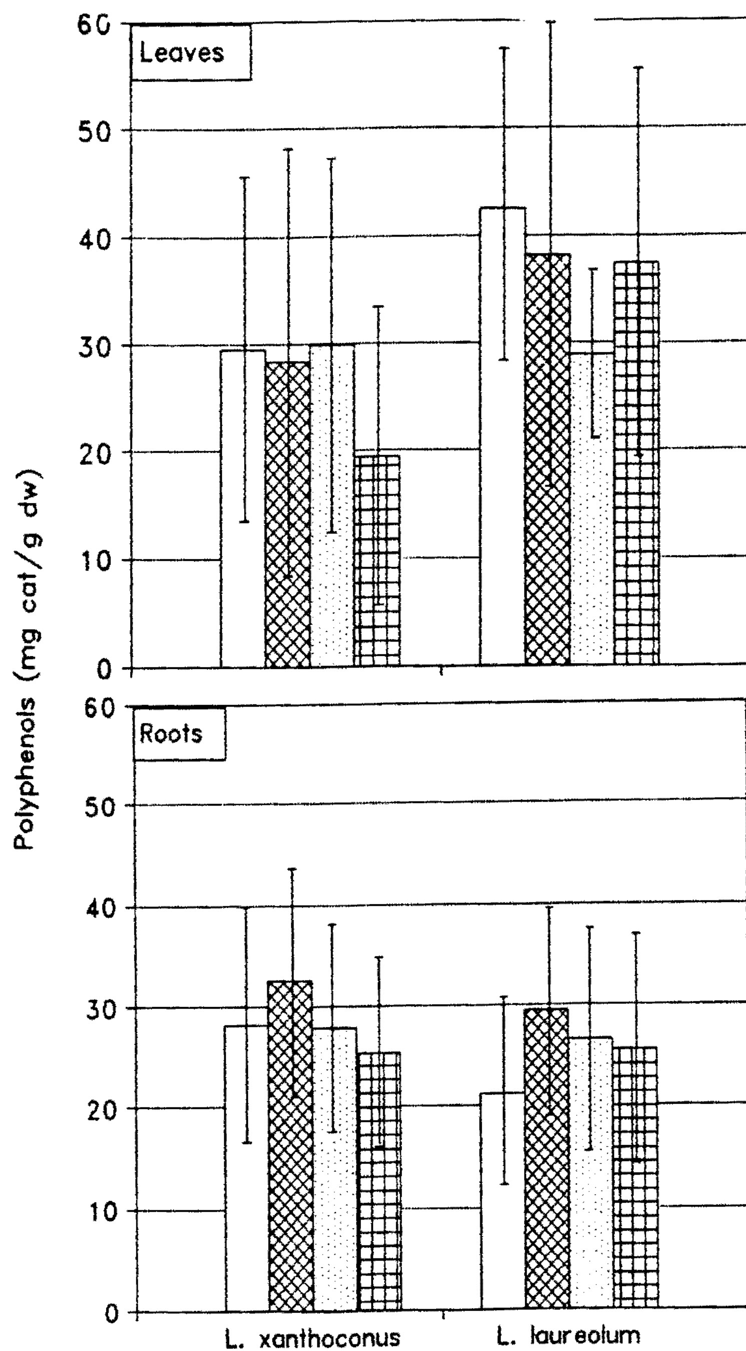


Fig 3: The variation in the polyphenol concentration in the leaves and the roots of *L.xanthoconus* and *L.laureolum*.

low CO_2 low nutrients, low CO_2 high nutrients,

high CO_2 low nutrients, high CO_2 high nutrients

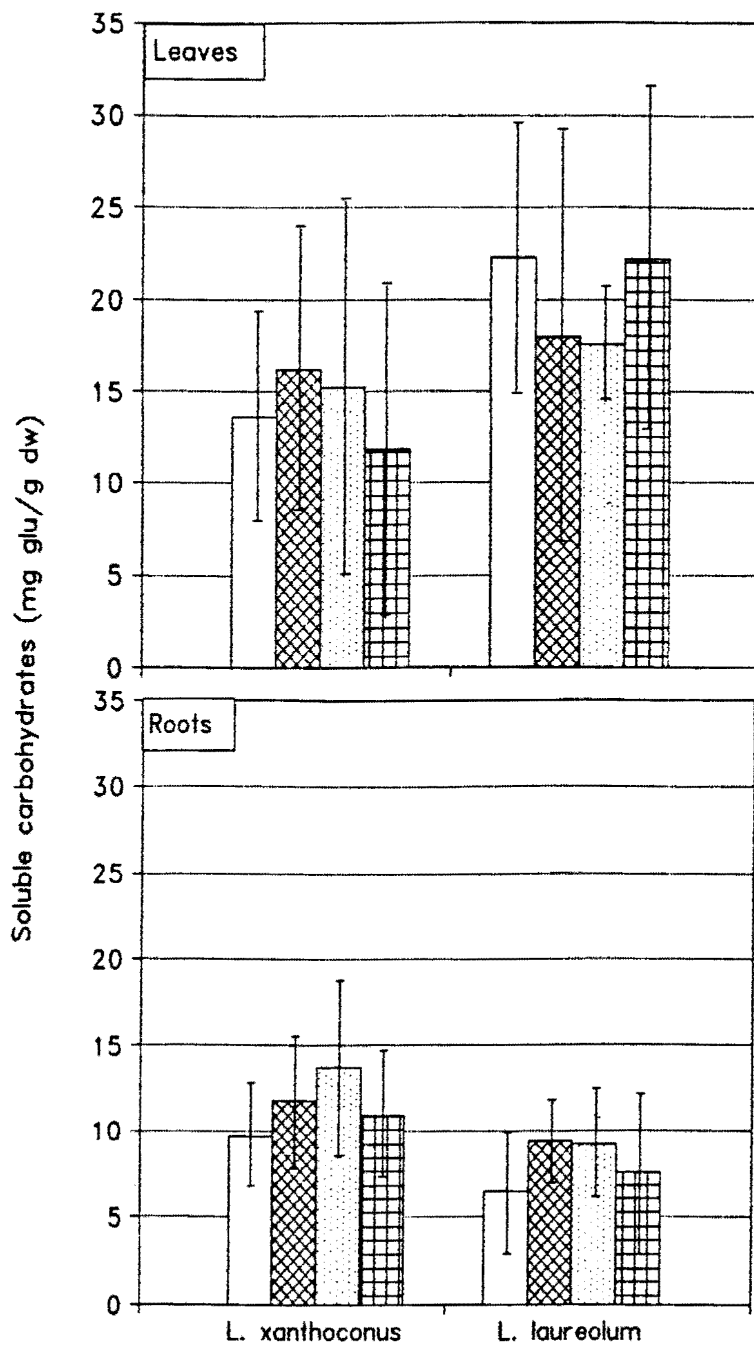


Fig 4: The variation in the soluble carbohydrate concentration in the leaves and the roots of *L.xanthoconus* and *L.laureolum*.
low CO_2 low nutrients, low CO_2 high nutrients,
high CO_2 low nutrients, high CO_2 high nutrients

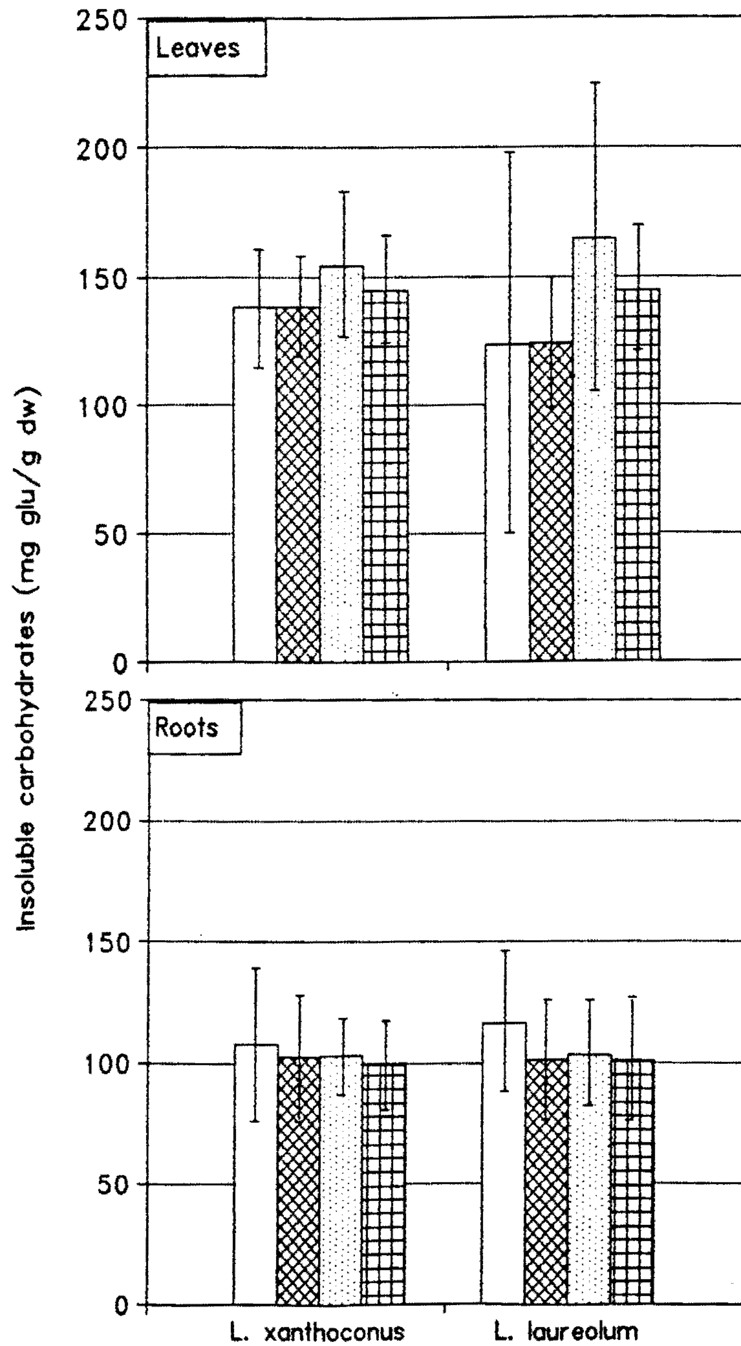
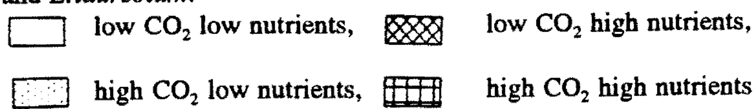


Fig 5: The variation in the insoluble carbohydrate concentration in the leaves and the roots of *L.xanthoconus* and *L.laureolum*.



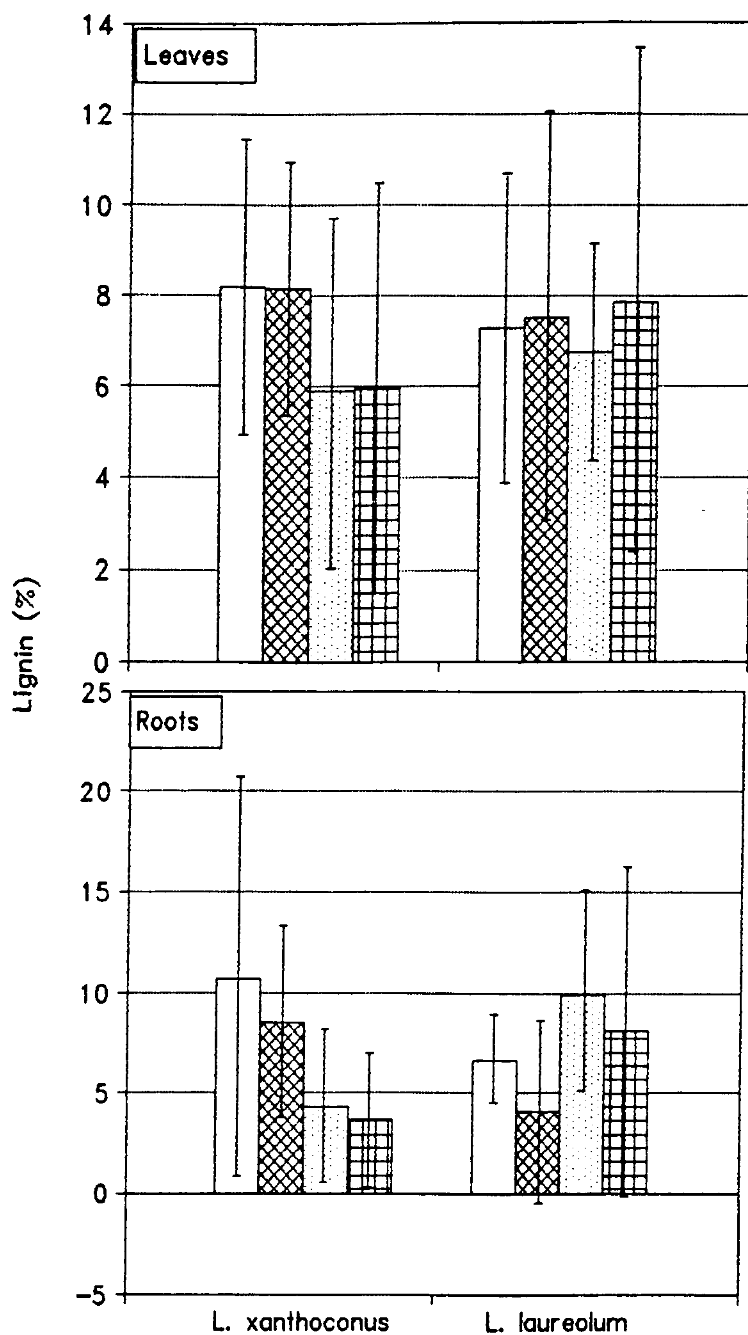


Fig 6: The variation in the proportion of lignin in the leaves and the roots of *L.xanthoconus* and *L. laureolum*.
low CO_2 low nutrients, low CO_2 high nutrients,
high CO_2 low nutrients, high CO_2 high nutrients

REFERENCES:

Bazzaz F. A. 1990. The response of natural ecosystems to the rising global CO_2 levels. Annu. Rev. Syst. 21:167-196.

Bazzaz F. A. and Fajer E. D. 1992. Plant life in a CO_2 rich world. Scientific American p18-24

Berrymen C. A., Eamus D. and Duff G. A. 1993. The influence of CO_2 enrichment on growth and nutrient content and biomass allocation of *Maranthes corymbosa*. Aust. J. Bot. 41:195-209.

Chu C. C., Coleman J. S. and Mooney H. A. 1992. Controls of biomass partitioning between roots and shoots: Atmospheric CO_2 enrichment and the acquisition and allocation of carbon and nitrogen in wild radish. Oecologia 89:580-587.

Conroy J. P., Milham P. J., Mazur M. and Barlow W. R. 1990. Growth, dry weight partitioning and wood properties of *Pinus radiata* D. Don after 2 years of CO_2 enrichment. Plant, Cell and Environment 13:329-337.

Crawford R. M. M. 1989. Studies in Plant survival. Blackwell Scientific Studies. Oxford.

Curtis H. 1986. Biology. Worth Publishers Inc. New York.

Davis T. D. and Potter J. R. 1989. Relations between carbohydrate, water stress and adventitious root formation in leafy pea cuttings rooted under various levels of atmospheric CO_2 and relative humidity. Physiologia plantarum 77:185-190.

Fajer E. D., Bowers M. D. and Bazzaz F. A. 1989. The effects of enriched Carbon dioxide atmospheres on Plant-Insect Herbivore Interactions. Science 243:1198-1200.

Fajer E. D., Bowers M. D. and Bazzaz F. A. 1992. The effect of nutrients and enriched CO_2 environments on production of carbon-based allelochemicals in *Plantago*: A test of the carbon/nutrient balance hypothesis. The American Naturalist 140(4):706-723

Grulke N. E., Riechers G. H., Oechel W. C., Hjelm U., and Jaeger C. 1990. Carbon balance in tussock tundra under ambient and elevated atmospheric CO_2. Oecologia 83:485-494.

Johnson S. D. 1992. Plant-animal relationships. In The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed. Cowling R.

le Maitre D. C. and Midgley J. J. 1992.Plant Reproductive Ecology. In: The Ecology of Fynbos: Nutrients, Fire and Diveristy. Eds. Cowling R.
TREE vol. 5(3):69-70.

Morrison I. M. 1972. A semi-micro method for the determination of lignin and its use in predicting the digestibility of forage crops. J. Sci. Fd Agric. 23;455-463.

Oechel W. C. and Lawrence W. 1986. Carbon Allocation and Utilization. In: Resource use by Chaparral and Matorral: A comparison of vegetation function in two mediterranean type ecosystems. Eds. Miller P.C.

Payne R. W., Lane P. W., Ainsley A. E., Bicknell K. E., Digby P. G. N., Harding S. A., Leech P. K., Simpson H. R., Todd A. D., Verrier P. J., White R. P., Gower J. C. and Tunnicliffe Wilson G. 1987. Genstat 5: Reference Manual. Clarendon Press. Oxford.

Poorter H. and Bergkotte M. 1992. Chemical composition of 24 wild species differing in relative growth rate. Plant, Cell and Environment 15:221-229.

Read D. J. and Mitchell D. T. 1983. Decomposition and Mineralization Processes in Mediterranean type Ecosystems and in Heathlands of Similar structure. In: Mediterranean-Type Ecosystems: The role of nutrients. Eds. Kruger F. J., Mitchell D. T. and Jarvis J. U. M.

Silvola J. and Ahlholm U. 1992. Photosynthesis in willows (*Salix x dasyclados*) grown at different CO_2 concentrations and fertilization levels. Oecologia 91:208-213.

Smith D. 1981. Removing and analyzing total nonstructural carbohydrates from plant tissue. University of Wisconsin. College of Agriculture and Life Science Publication

Specht R. L. and Moll E. J. 1983. Mediterranean-Type Heathlands and Sclerophyllous Shrublands of the world: An overview. In: Mediterranean Type Ecosystems: The Role of

Nutrients Eds. Kruger F. J., Mitchell D. T. and Jarvis J. U. M.t

Stock W. D. and Allsop N. 1992. Functional perspective of ecosystems. In: The Ecology of fynbos: Nutrients, Fire and Diversity. Eds. Cowling R.

Stock W. D. and Midgley G. F. Ecosystem response to elevated CO_2: Nutrient availability and nutrient cycling. unpub.

Walter H. 1973. Vegetation of the Earth: In relations to Climate and the Eco-physiological Conditions. Springer-Verlag. New York.

Ziska L.H., Drake B. G. and Chamberlain S. 1990. Long-term photosynthetic response in single leaves of a C_3 and C_4 marsh species grown at elevated CO_2 in situ. Oecologia 83: 469-472.