

# Southern Hemisphere Conifers: distribution and history interpreted from a physiological perspective

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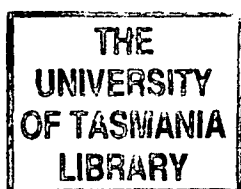
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This thesis contains no material which has been accepted for the award of any other higher degree or graduate diploma in any tertiary institution and, to the best of my knowledge and belief, it contains no material previously published or written by any other person, except where due reference is made in the text of the thesis.

A handwritten signature in black ink, appearing to read 'T. J. Brodribb', written in a cursive style.

T. J. Brodribb

## Abstract

An examination of the morphology and photosynthetic physiology of the two dominant families of conifers in the southern hemisphere, the Podocarpaceae and Cupressaceae, was undertaken here in order to describe the physiological limitations which constrain their distribution. A species specific index of drought tolerance was devised which quantified the maximum attainable water-use efficiency of photosynthesis during imposed drought. This index,  $(c_i/c_a)_{\min}$ , measured the potential of leaves to photosynthesize under water stress, comparing the maximum draw-down of internal  $\text{CO}_2$  concentration ( $c_i$ ) below ambient ( $c_a$ ). When operating at  $(c_i/c_a)_{\min}$ , the leaf was at the maximum water stress before leaf damage (indicated by an irreversible loss of variable fluorescence and photosynthetic rate) occurred.  $(c_i/c_a)_{\min}$  was found to be well correlated with the minimum rainfall within the natural range of the 12 species investigated, and this is strong evidence that the distribution of these species is largely determined by their drought tolerance. Further support for the drought limitation hypothesis came from a comparison of photosynthetic rates (as measured by chlorophyll fluorescence) of conifers in the field. The apparent photochemical electron transport rate (ETR) was found to be significantly reduced in a dry site when compared with a wet site. Most Podocarpaceae, and many Cupressaceae were found to be highly drought sensitive, and probably became increasingly susceptible to extinction during the substantial increase in aridity which occurred in Australia during the Tertiary. The influence of features considered as possible morphological drought adaptations on water loss were also examined. Wax plugs and imbricacy were found to substantially decrease maximum stomatal conductance, with the combination of wax plugs, imbricacy and epistomy reducing conductance to 17% of the value expected on leaves with exposed, unplugged stomata. Competition for light is believed to be another area where conifers suffer due to their lack of broad-leaves. Many podocarp genera appear to have converged with angiosperms, producing discrete, bilaterally flattened short shoots which seem to function as broad-leaves. The degree of shoot flattening, as measured by shoot width, was correlated with the leaf saturation light requirement, broad shoots requiring substantially lower light intensities for saturation. From this it was inferred that the bilaterally compressed short shoot was an adaptation to light competition with angiosperms.

Conifers in the southern hemisphere form a surprisingly distinct group which has retained its character since the earliest Tertiary. The late Cretaceous- early Tertiary also saw the radiation of angiosperms, and it has been suggested that this event resulted in the global decline of the Coniferales. By determining how distributions of these taxa are limited today, it was possible to shed some light on whether conifers in the southern hemisphere were simply overrun and replaced by the more competitive angiosperms, or if other factors such as climate change are likely to have played a part. A brief summary of the evolutionary history of conifers in the southern hemisphere leads to the conclusion that decreasing rainfall and fire have been the main influences on conifer extinction.



## Acknowledgements

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**- Light response characteristics of a morphologically diverse group of southern hemisphere conifers as measured by chlorophyll fluorescence. (*Oecologia*)**

**- Imbricacy and stomatal wax plugs reduce maximum conductance in southern hemisphere conifers. (*Australian Journal of Botany*)**

## CHAPTER 1

### Introduction

When conifers were first encountered by Europeans in the southern hemisphere, the greatest araucarian to the smallest creeping podocarp were christened, in European tradition, as "pines and cedars". Perspectives have changed since those early days, and work begun by Buchholz & Gray (1948) and de Laubenfels (1953) has led us to the situation today which sees naturalised southern botanists parochially refer to "the southern hemisphere conifers" as a group distinct from the northern hemisphere taxa. Although discrimination of "the southern conifers" is geographically convenient (Fig. 1.1), an historical examination of the genera which make up the vast majority of the southern conifer flora reveals a group of plants unified by ancient phylogenetic roots extending back 100 million years and beyond, to the time of the southern supercontinent "Gondwana".

Two families characterise the southern hemisphere conifer flora, the Podocarpaceae and the Araucariaceae, and although these two groups share similar distributional ranges, they contrast strongly in their morphology, ecology, adaptability and evolutionary history. The Araucariaceae are an ancient and extremely conservative group of plants, exhibiting little variation in vegetative or reproductive morphology within the three extant genera which comprise the family. Fossil *Araucaria* leaves from the Tertiary and cones from deposits dating back to the Jurassic differ very little from extant species (Hill, 1990; Stockey, 1980) and records of leaf and wood fossils from America and Europe provide evidence of a distributional range far more extensive than that of today (see Stockey, 1982). The Araucariaceae appear to be a family of plants which arrived at a formula successful enough for them to remain competitive (while gradually becoming restricted in range) throughout the Tertiary despite an apparent minimal potential for adaptive response. There have been no major studies on the physiology or life history of this family, and the features which have enabled its continued, unchanged existence remain a source of considerable interest. Unfortunately the conservatism exhibited by the Araucariaceae makes them unsuitable for inclusion in a project focussing on adaptive variation in morphology and physiology, and hence discussion here is restricted to the other two conifer families in the southern hemisphere, the Podocarpaceae and Cupressaceae.

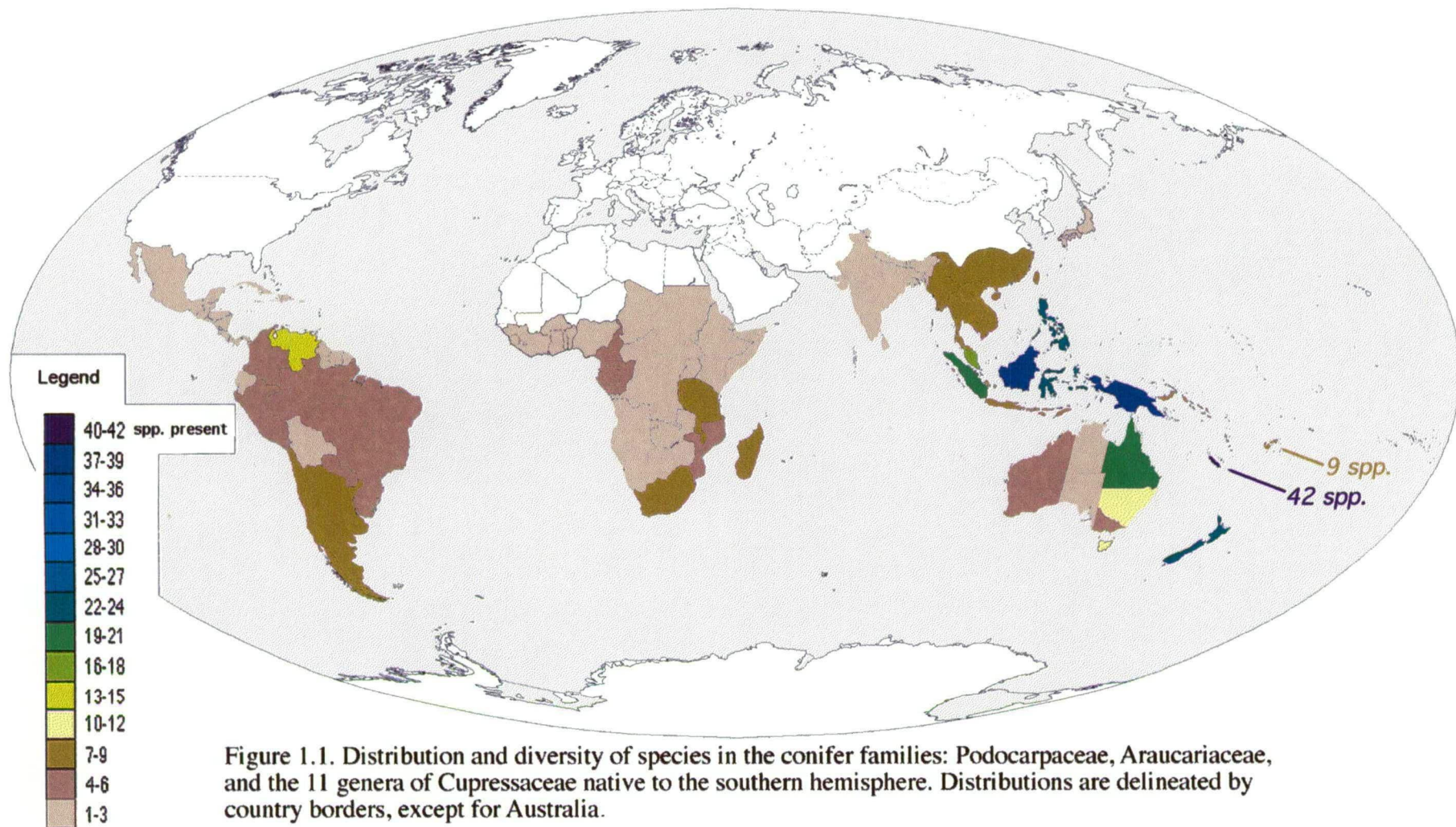


Figure 1.1. Distribution and diversity of species in the conifer families: Podocarpaceae, Araucariaceae, and the 11 genera of Cupressaceae native to the southern hemisphere. Distributions are delineated by country borders, except for Australia.

*Podocarpaceae*

Amongst conifers, the Podocarpaceae are the antithesis of the Araucariaceae, they are without peer in terms of their morphological diversity, and are easily the most speciose family in the southern hemisphere. Because of their unusual morphology, the taxonomic description of this family has been slow to emerge, and the erection of new genera (*e.g.* Molloy, 1995) and proposals to split off some parts of the group (*e.g.* Keng, 1978) are still relatively common. Currently the Podocarpaceae is comprised of 19 genera and 174 species, 124 of which occur in the southern hemisphere, giving the family a strongly southern hemisphere character. Of the 80 species which extend into the northern hemisphere, most are restricted to southern China and northern South America, the most northerly distribution being *Nageia nagi* in southern Japan (de Laubenfels, 1969). This southern hemisphere distributional bias is also apparent in the fossil record of podocarps, with the great majority of confirmed podocarp fossils being from southern localities in Australia, Antarctica, southern Africa and South America (Hill, 1995).

The geographical range of the Podocarpaceae includes all the southern hemisphere continents, with the greatest development of podocarp forest seen in New Zealand, Tasmania, New Caledonia and through Malesia (Fig. 1.1). In cool temperate regions podocarps grow from the sub-alpine zone (Fig. 1.2) down to sea-level (Fig. 1.3), with no obvious altitudinal preference, while in the tropics, the greatest podocarp diversity is associated with montane elevations, although species still occur in forest from around sea-level (Fig. 1.4) to elevations over 3600m (Van Royen, 1979). Despite this apparently extensive environmental range, podocarps are relatively limited in their environmental/ecological niche, with the vast majority of species restricted to cool, wet forest (de Laubenfels, 1988). Water availability and disturbance regime would appear to be the strongest limiting factors in constraining podocarp distribution, and although it is fashionable to suggest that it is angiosperm radiation which has directly displaced conifers from niches they would have previously occupied (Bond, 1989), it seems likely that, in the Podocarpaceae at least, innate limitations in some aspects of their physiology may impose strong limitations on the distribution of conifers in the southern hemisphere. Examination of the fossil history of podocarps in southern Australia provides some support for the notion that conifer extinction is linked to increasing aridity, and in some genera there is



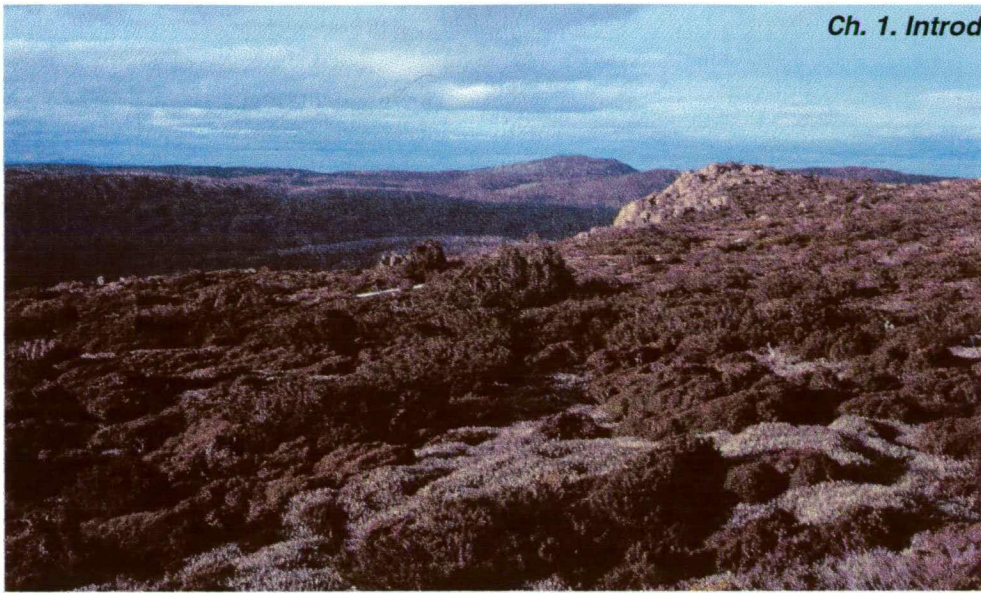


Figure 1.2. Dwarf sub-alpine conifer forest at 1200m on Mt. Field (Tasmania). Species in view include *Athrotaxis cupressoides*, *Diselma archeri*, *Microstrobos niphophilus*, *Podocarpus lawrencii*, and *Microcachrys tetragona*.



Figure 1.3. Lowland mixed conifer forest on the north Island of New Zealand. Species shown include *Agathis australis*, *Dacrycarpus dacrydioides*, *Phyllocladus trichomanoides* (skyline), and *Dacrydium cupressinum* (foreground).



evidence of a shift in leaf morphology over time which may reflect adaptation to diminished water availability (Hill and Carpenter, 1991).

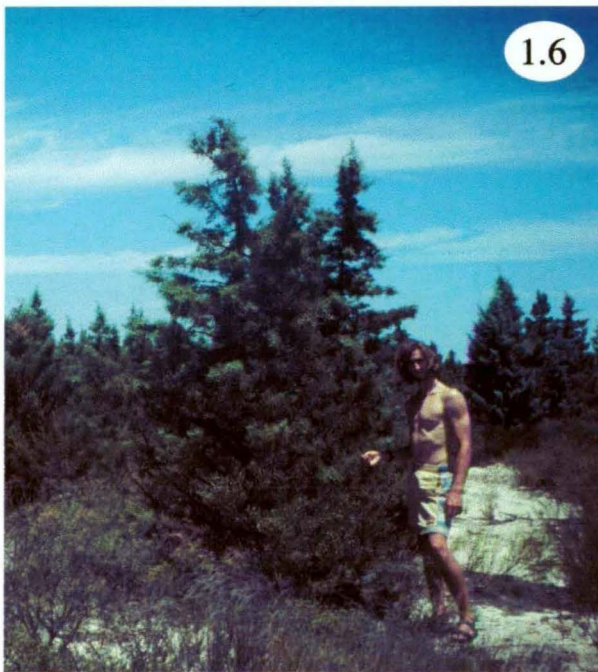
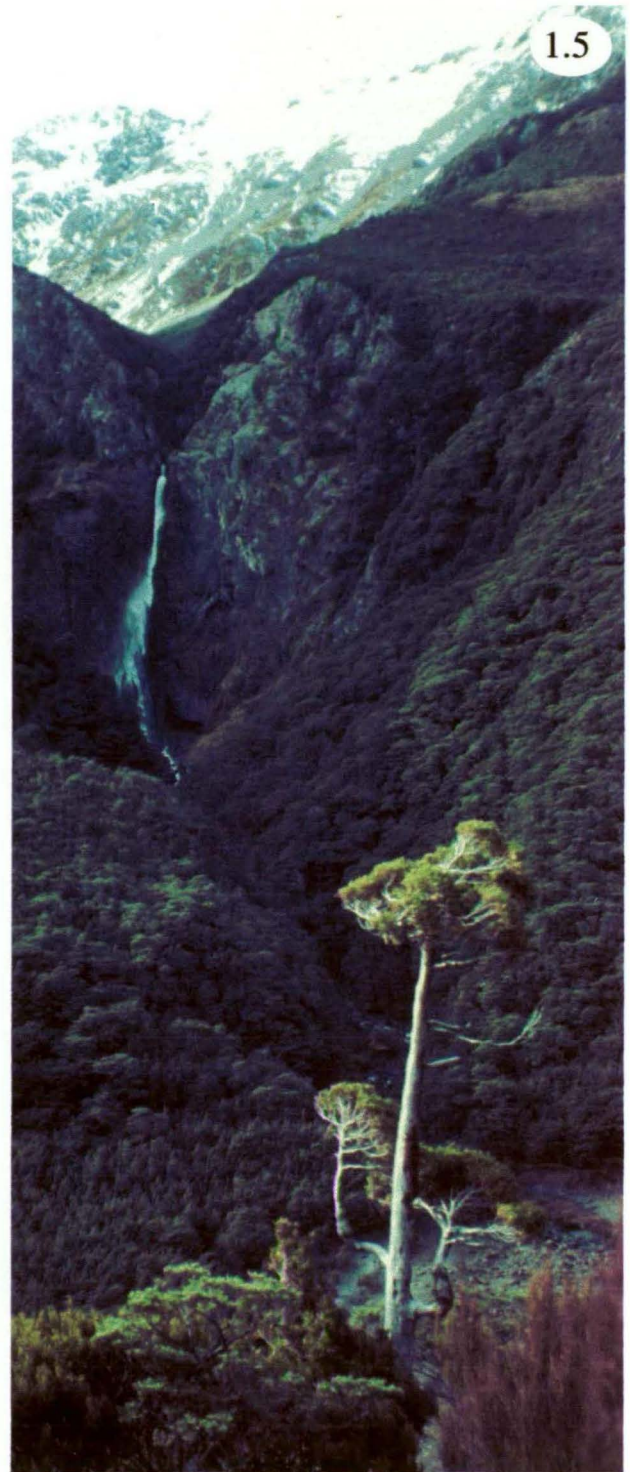
### *Cupressaceae*

The third well represented family of conifers in the southern hemisphere is the Cupressaceae. Although the southern Cupressaceae are quite distinct from their northern counterparts, the family is quite cosmopolitan in its distribution and does not have the "southern character" of the previous two families. Even with the recent merger of the Taxodiaceae and Cupressaceae (Brunsfield *et al.*, 1994), the southern hemisphere Cupressaceae are a less distinct and far less speciose family than the Podocarpaceae, most genera falling within the morphological range of the northern hemisphere Cupressaceae. A total number of only about 42 species from 11 genera have been described in the southern hemisphere. No southern species is known to extend north of the Equator and only a single species of the northern hemisphere genus, *Juniperus* extends south, into southern Africa.

Unlike the Podocarpaceae, the preferred habitat of southern hemisphere Cupressaceae cannot be easily summarised, with genera such as *Libocedrus*, *Papuacedrus*, *Diselma* and *Athrotaxis* being restricted to wet environments, from high latitudes to the tropics (Fig. 1.5), while the genera *Actinostrobus*, *Callitris* and *Widdringtonia* inhabit semi-arid country in Australia and Africa (Fig. 1.6). Apart from a few confirmed records of *Callitris* (Jordan, 1995) only genera currently restricted to rainforest (including northern genera such as *Austrosequoia*, which is probably *Sequoia*; Hill *et al.*, 1993) are found in southern hemisphere fossil deposits. In a similar fashion to the podocarp record it is clear that the abundance and diversity of these taxa has decreased significantly since the early Tertiary (Hill, 1995; Kershaw and McGlone, 1995).

### *Conifer decline*

Many theories have been forwarded to account for the global decline of gymnosperms, most of which hinge on the diversification of angiosperms in the late Cretaceous (Raven and Axelrod, 1974) as providing the ultimate cause. It is proposed that the rise of angiosperms left gymnosperms with relatively inefficient vascular tissue (Carlquist, 1975), a high potential for inbreeding and hence slower speciation (Keister *et al.*, 1984; Raven, 1977),



**Figure 1.4.** *Acropyle pancheri* growing in lowland sub-tropical rainforest at Riviere Bleue (New Caledonia).

**Figure 1.5.** *Libocedrus bidwillii* at an altitude of approximately 950 m on Arthurs Pass (New Zealand).

**Figure 1.6.** Dry forest dominated by *Actinostrobus acuminatus* in Kalbarri (Western Australia).

and an inability to rapidly develop large fully vascularized leaves due to an absence of reticulate venation (Foster and Gifford, 1974). As a result, it is proposed that conifers were out-competed in all but the most marginal environments, where productivity was low enough to allow them to persist (Bond, 1989).

The concept of gymnosperm displacement by angiosperms is supported by evidence of a significant contraction of non-coniferous gymnosperms in the Cretaceous (Crane, 1987), but in general, changes in the relative dominance of the Coniferales in the fossil record are not well correlated with angiosperm radiation. For the southern hemisphere, fossil pollen from Antarctica provides no evidence of conifer decline coincidental with angiosperm radiation, with little change observed in the relative abundance of gymnosperm pollen as angiosperms radiated during the Albian-Maastrichtian (Drinnan and Crane, 1990). The highest recorded diversity of conifer species in south-east Australia (and indeed the world) occurred during the early Tertiary when the angiosperm flora was also diverse and widespread (Hill *et al.*, 1989; Wells and Hill, 1989). Conifer distribution and diversity, in southeast Australia at least, did not show significant evidence of decline until other factors such as seasonality, increasing aridity and increased fire disturbance became apparent (Blackburn, 1994; Douglas, 1994; Kershaw *et al.*, 1994). Recently some authors have suggested that these factors have played the major role in shaping the extant southern hemisphere conifer flora (see Enright and Hill, 1994; Jordan, 1994).

### *Project aims*

The aim of this project has been to examine a large sample of the morphological and physiological diversity in southern hemisphere conifer taxa in order to understand if, and how, species distributions are constrained by their physiology, and if these physiological constraints can explain historical changes in distribution given the climatic changes which are proposed to have occurred during the Tertiary. On a similar theme, an examination of the relationship between external morphology and plant function was undertaken with regard to interpretation of the fossil record. The common focus has been on leaf morphology and photosynthetic physiology, as it is leaf function which will ultimately dictate the ability of a plant to survive under given conditions.

*Thesis outline*

In chapter 2 the foliar morphological spectrum of southern hemisphere conifers is summarised, and sub-groups based on leaf and shoot characteristics are described for the podocarps and southern hemisphere Cupressaceae. This chapter serves as an introduction to the morphology of the southern hemisphere conifers, and provides a reference for later chapters.

Chapters 3 and 4 investigate the drought tolerance of a sample of widely distributed southern conifer species and compare water-use efficiency and some other linked photosynthetic parameters to the environmental range inhabited by these taxa. The implications of lab-based physiological work on plant function in the field are tested in chapter 5. The photosynthetic rates of several endemic Tasmanian conifer species are compared on two mountains which represent wet and dry extremes of the current environmental range for these species.

Ineffective competition for light due to an inability to produce large, bilaterally flattened leaves has been argued as a possible reason for the restriction of conifers from productive lowland forest (Bond, 1989). The growth of podocarp species in such habitats indicates that in the Podocarpaceae, this limitation has been overcome to some extent. In chapter 6 the light response characteristics of podocarp bilaterally compressed foliage is compared with that of other foliage types in order to assess the effectiveness of this morphology in terms of efficient light harvesting. The possibility that the bilaterally compressed foliage may have evolved as an adaptation in response to light competition with broad-leaved angiosperms is discussed. The regulation of gas exchange is also investigated in chapter 6 to determine whether stomatal conductance is optimised, as appears to be the case for most angiosperms studied. It has been suggested that optimal regulation of water loss evolved in response to modern atmospheric CO<sub>2</sub> concentrations (Robinson, 1994), and that plant groups which evolved prior to the Tertiary fluctuations in atmospheric CO<sub>2</sub> (such as many of the genera studied here) may not display optimal stomatal regulation.

The physical relationship between leaf morphology and gas-exchange characteristics is examined in chapter 7. Focussing on the effects of stomatal characteristics and leaf arrangement on the fluxes of water vapour into and out of the leaf, this chapter establishes a quantitative relationship between leaf form and physiological function. The provision of such a relationship greatly enhances the potential for interpretation of morphological trends

observed in Tertiary fossil deposits and what these changes in morphology are likely to represent in terms of physiological adaptation.

Finally, in chapter 8 the nature and implication of the observed morphological trends in the fossil history of conifers in south-east Australia are discussed in the light of the physiological data from preceding chapters.

## CHAPTER 2

### **Variation in the Foliar Morphology of Southern Hemisphere Conifers**

#### **Introduction**

As a study of the physiological adaptation of photosynthesis in the southern conifers, it is important to initially describe the characteristics of foliar morphology. This second chapter describes leaf morphology and arrangement in a subset of southern conifer species from the key genera used in the proceeding chapters and discusses exactly what represents a functional photosynthetic unit. The environmental and photosynthetic fluxes surrounding a leaf are almost always expressed in leaf-area based terms (Jones, 1992), and without a unifying assumption as to the fundamental character of the leaf, interpretation of photosynthetic data in the absence of morphological data is difficult. This is particularly relevant to the inter-species and inter-generic physiological comparisons that follow, as the range of foliar morphologies is very large. Unlike comparative physiological work on angiosperm leaves, where a fundamentally similar foliar formula is usually assumed, *i.e.* bilaterally flattened broad-leaves, or on northern conifers where the vast majority of work has focussed on needle, and imbricate leaved species, assumptions or generalisations about the morphology of southern hemisphere conifer genera cannot easily be made. The reason for this is largely due to the vast range of form exhibited by the most common family in the southern hemisphere, the Podocarpaceae.

Foliar variation in northern hemisphere conifer taxa is, on a broad scale, quite large, with the bilaterally compressed shoots of *Sequoia*, *Metasequoia* and *Taxodium*, contrasting with the imbricate foliage of the Cupressaceae and the needle foliage of the Pinaceae. Numerically however, the northern hemisphere is overwhelmingly dominated by needle-leaved conifers (approximately 224 species) constituting more than 70% of the total number of species in the northern hemisphere, and 82 imbricate-leaved species, amounting to around 26% of the total (Welch and Haddow, 1993). Bilaterally compressed shoots are only found in approximately six species, all from the former Taxodiaceae. No such morphological domination of the conifer flora exists in the southern hemisphere, with approximately 142 species from 13 genera (comprising



about 60% of the total number of conifer species in the southern hemisphere, including Araucariaceae) known to produce distinctly bilaterally flattened foliage, 12 genera producing tightly imbricate adult foliage and the remaining eight genera producing small leaves arranged spirally, or in opposite pairs on the stem. None of these morphologies are substantially more prolific than any other, and, with the exception of the dry conifer forests of arid Australia and Southern Africa, virtually all major conifer floras in the southern hemisphere have representatives of both the bilaterally flattened and three dimensionally arranged foliage types (Enright and Hill, 1995). Very few conifers in the southern hemisphere possess needle leaves as their adult foliage, despite the fact that virtually all species of Cupressaceae, and many Podocarpaceae produce needle leaves during early seedling growth (de Laubenfels, 1953).

Given the large range in foliar morphology and leaf arrangement exhibited by southern hemisphere conifers, it is proposed in this chapter to briefly describe the characteristics of most of the genera in the southern hemisphere to provide a reference point from which to base the physiological comparisons presented later. Broad-scale descriptive work has already summarised the foliar morphology of conifers (de Laubenfels, 1953; Florin, 1931), and detailed descriptions have been made of the imbricate podocarps in the southern hemisphere (Wells and Hill, 1989), and of the morphological variety in the conifers of Australia and New Guinea (Offler, 1984). Thus, the focus of this chapter is at an intermediate scale, dealing with most southern hemisphere conifer genera in order to describe differences and similarities within and among them.

There are two species from the gymnosperm order Taxales in the southern hemisphere, the monotypic *Austrotaxus spicata* from New Caledonia, and *Taxus sumatrana* from Indonesia. The Taxaceae or Taxales are now considered to be an order separate to Coniferales (Stewart and Rothwell, 1993), and thus discussion here is limited to the Podocarpaceae and Cupressaceae.

## Materials and Methods

### *Plant Material*

Podocarpaceae A selection of species from all podocarp genera except *Nageia* and *Parasitaxus*, and *Sundacarpus* was collected from Australia, New Guinea, Indonesia, Borneo, Fiji, New Zealand, New

Caledonia, Africa and South America. This material was propagated from seed or cuttings and included:

-species with imbricate adult foliage: *Halocarpus bidwillii* (Kirk) C.J. Quinn, *Lagarostrobos franklinii* (Hook.), *Lepidothamnus laxifolius* (Hook.f.) C.J. Quinn, *Manoao colensoi* (Hook.) Molloy, *Microcachrys tetragona* (Hook.) Hook.f. and *Microstrobos niphophilus* (Garden and Johnson) C.J. Quinn.

-species with bilaterally compressed adult foliage; *Acmopyle pancheri* (Brongn. and Gris.) Pilger, *A. sahniana* Buchholz and Gray (de Laubenfels), *Afrocarpus falcata* (Thunb.) C.N. Page *Dacrycarpus dacrydioides* (Rich.) de Laubenfels, *Dacrycarpus imbricatus* (Blume) de Laubenfels, *Falcatifolium taxoides* (Brongn. and Gris) de Laubenfels, *Falcatifolium facliforme* (Parl.) de Laubenfels, *Phyllocladus aspleniifolius* (Labill.) Hook.f., *Phyllocladus alpinus* Hook.f., *Phyllocladus hypophyllus* Hook.f., *Podocarpus dispermis* White, *Podocarpus drouynianus* Mueller, *Podocarpus totara* D. Don *Podocarpus gnidioides* Carriere, *Podocarpus grayae* B.Hyland, *Podocarpus lawrencii* Hook.f., *Podocarpus lucenii* de Laubenfels, *Podocarpus neriifolius*, D. Don, *Podocarpus novae-caledoniae* Vieill., *Podocarpus dispermis* B. Gray, *Prumnopitys ferruginea* D. Don, *Prumnopitys ferruginoides* (Compton) de Laub., *Retrophyllum comptonii* (Buchh.) C.N. Page, *Saxagothaea conspicua* (Lindley).

-species with spially arranged, non-imbricate adult foliage; , *Microstrobos fitzgeraldii* (Garden and Johnson) C.J. Quinn., *Dacrycarpus compactus* (Wasscher) de Laubenfels, *Dacrydium araucarioides* (Brongn. and Gris.), *Dacrydium lycopodioides* (Brongn. and Gris.).

A notable omission from the bilaterally compressed species is the genus *Nageia*. Unfortunately, material from this broad-leaved podocarp could not be secured in time for its inclusion here.

Cupressaceae Species from ten genera, including rainforest and arid zone species, were collected for propagation by seed and cuttings; *Actinostrobus acuminatus* Parlatore, *Callitris rhomboidea* R.Br., *Diselma archeri* Marsh, *Widdringtonia cedarbergensis* Marsh, *Fitzroya cupressoides* (Molina) Johnston, *Libocedrus austro-caledonica* Brongn. and Gris., *Libocedrus plumosa* (D. Don) Sargent, *Libocedrus chevalier* Buchholz, *Libocedrus bidwillii* F.Hooker, and *Libocedrus yateensis* Guillaume, *Papuacedrus papuana* (F.Mueller) Li, *Neocallitropsis pancheri* (Compton) Florin, and *Pilgerodendron uviferum* (Pliger) Florin, as well



as the reclassified Taxodiaceae species (Brunsfield *et al.*, 1994); *Athrotaxis cupressoides* D. Don, and *Athrotaxis selaginoides* D. Don and the hybrid between these two species formerly known as *A. laxifolia* (Isoda *et al.*, 1996) were grown.

Unfortunately the monotypic South American genus *Austrocedrus* (D. Don) Florin and Boutelje could not be collected in time for its inclusion here.

### *Propagation*

Plant material was propagated both from seed collected in the field, and by vegetative cuttings taken from field grown specimens and cultivated stock. Seed was the preferred means of propagation; however cone development in conifers is somewhat sporadic, making collection difficult, and the transition from seedling to adult foliage can be slow, limiting the availability of adult foliage, and so cuttings were taken in cases where seed was unavailable or inappropriate.

Seeds from Cupressaceae were easily collected by oven drying semi-mature cones at 40°C for 48-72 hours. Seeds were then germinated in a 50% sand, 50% peat mix, on a heated propagating bed with regular misting. In most cases germination took place in a period of three to five weeks.

Podocarp seed was collected at maturity, and the fleshy ovuliferous scale removed. Germination time in species with woody seed-coats was reduced by sanding most of the way through the seed-coat with fine sandpaper. Seeds were then germinated under the same conditions as described above, and germination times varied from four weeks in *Acropyle* and *Dacrycarpus* to more than 20 weeks in *Prumnopitys ferruginea*.

Cuttings proved to be the most reliable means of establishing plants of both families, although establishment times were considerably longer than for seedlings. Healthy cutting material was capable of surviving for at least two weeks in dark moist conditions prior to potting. For striking, shoots were cut, dipped in 0.1 M indole butyric acid, and placed in a soil mix of 80% sand, 20% peat on a heated misting bed with an artificial 12 h photoperiod to prevent possible bud dormancy during winter. Root formation varied between species and the season which material was struck, but a minimum period of two months was usually required. Some species remained dormant for 18 months before initiating root growth.

Once roots were established, or seedlings had produced their first leaves, plants were transferred to a potting mix containing pine bark, sand and peat in the ratio 8:2:1. Macro and micronutrients were also added to the soil mix, including slow release pellets of N, P and K, dolomite, a commercial micronutrient mix, and chelated iron. Plants were grown under natural photoperiod in a heated glasshouse near sea-level in Hobart, Tasmania.

## Results

### PODOCARPACEAE

Leaf and shoot morphology in the Podocarpaceae falls into three relatively distinct groups; species which obligately produce appressed, imbricate foliage as adults; species with bilaterally flattened foliage as adults; and species with spreading, spirally arranged adult leaves.

#### *Obligate Imbricate Species*

Of the podocarps which produce fully imbricate adult foliage, two distinct morphologies emerge. Tightly imbricate, obligate epistomatic species form one group, and the imbricate amphistomatic species the other.

1. The most distinct type is produced by the Tasmanian species *Microstrobos niphophilus* and *Microcachrys tetragona*, in which leaves are tightly imbricate, and possess a well developed marginal frill which usually completely seals the joint<sup>†</sup> between leaves (Figs 2.1, 2.2). In these species stomata are wholly restricted to the adaxial surface of the leaves which are appressed to the superadjacent leaves on the stem. *Microcachrys* is unusual amongst podocarps in possessing opposite, decussate leaf arrangement (Fig. 2.1a).

The seedling foliage of *Microstrobos niphophilus* is also epistomatic, but unlike adult shoots, the leaves are distinctly spreading in their arrangement (Fig 2.2b). This spreading morphology is gradually replaced by typical imbricate shoots as the seedling matures. The only other *Microstrobos* species described, *M. fitzgeraldii*, retains its lax seedling foliage arrangement at maturity, also producing substantially larger leaves than *M. niphophilus* (Fig 2.3).

Figure 2.1. Scanning electron micrographs of (a) the opposite decussate leaf arrangement in *Microcachrys tetragona*, and (b) the adaxial leaf surface, illustrating the confinement of stomata to the 'inner' leaf surface. The marginal frill on leaves is arrowed. (Scale bar = 1 mm)

Figure 2.2. Spiral leaf arrangement in (a) adult *Microstrobos niphophilus*. Stomata are again confined to the adaxial leaf surface, but become exposed in the juvenile form by the lax leaf arrangement (b) shown here on a shoot from a year old seedling. (Scale bar = 5 mm)

Figure 2.3. Adult foliage of *Microstrobos fitzgeraldii* clearly resembles that of juvenile *M. niphophilus*, again stomata are confined adaxially. (Scale bar = 5 mm)

Figure 2.4. New Zealand species *Lepidothamnus laxifolius* illustrating (a) loosely imbricate adult foliage, and (b) spreading juvenile leaves. The spreading foliage type was also produced in adult plants exposed to deep shade. (Scale bar = 5 mm)

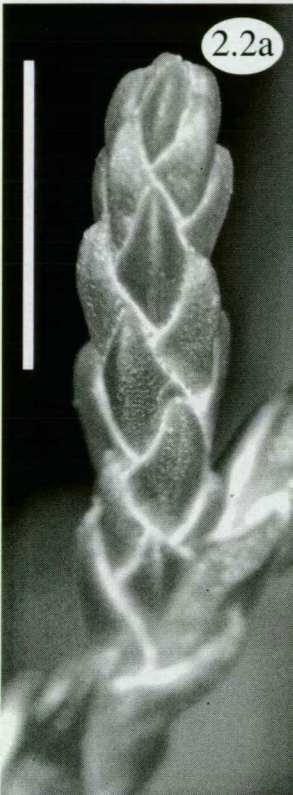
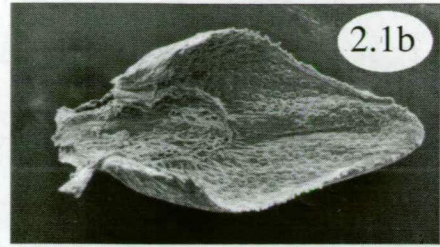
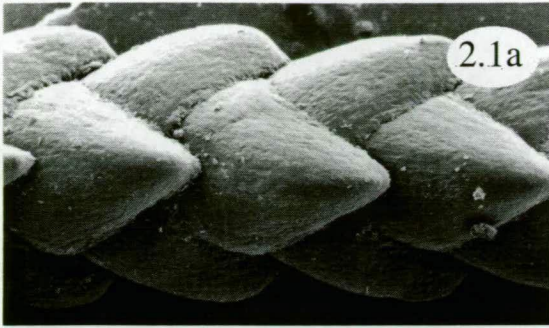


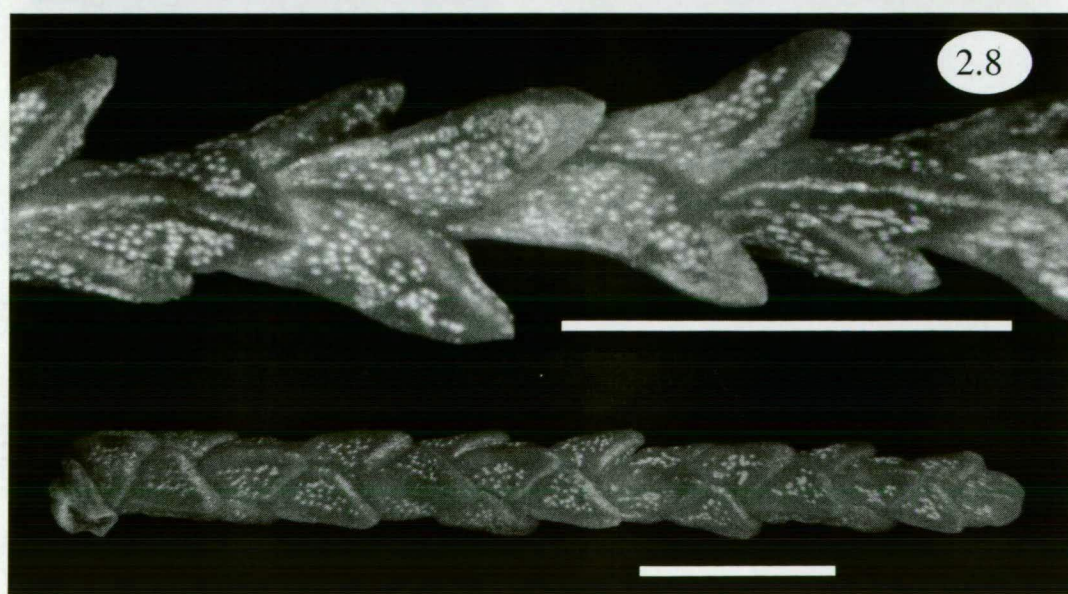
Figure 2.5. A gradual transition is shown, from spreading juvenile type foliage to imbricate adult foliage in a seedling shoot of *Manoao colensoi*. Primary seedling foliage is comprised of needle-like leaves similar to figure 2.30 (b). (Scale bar = 5 mm)

Figure 2.6. Juvenile foliage of *Halocarpus bidwillii* was highly distinct from the adult form. Adaxial (left) and abaxial (right) surfaces of two juvenile leaves are shown, illustrating the hypostomy of the juvenile leaves. Adult foliage is seen to be typically imbricate, with the majority of stomata distributed on the abaxial surface of the leaf. (Scale bar = 5 mm)

Figure 2.7. The transition from juvenile (Juv.) to adult foliage (A) was seen to be abrupt in *Halocarpus bidwillii*, with no evidence of a transitional shoot morphology. Imbricate shoots arose from branches bearing juvenile leaves without the the typical morphological intermediate foliage seen in other species (e.g. Fig. 2.5). Abrupt reversion to "juvenile" foliage was also noted on the shoots of adult plants subjected to deep shade. (Scale bar = 5 mm)

Figure 2.8. The most common response to shade in imbricate species was the production of shoots with a lax morphology as shown here in *Lagarostrobos franklinii*. The uppermost shoot was produced under deep shade, while the lower shoot was grown in full sun. White dots on the leaves are wax plugs in the stomatal pores. (Scale bar = 5 mm)





Unfortunately seedlings of *Microcachrys* could not be propagated, and seedling morphology has never been recorded.

2. The remaining imbricate podocarps all produce spirally arranged, imbricate, unevenly amphistomatic leaves, with stomata generally concentrated on the abaxial leaf surface. Leaves range from loosely imbricate in *Lepidothamnus laxifolius* (Fig. 2.4) to tightly imbricate in *Halocarpus bidwillii* and *Manoao colensoi* (Figs 2.5, 2.6). Seedling foliage in most genera comprises leaves similar to the adult but arranged in a more spreading fashion, which become progressively reduced and more closely appressed to the stem as the plant matures (e.g. *Manoao colensoi* Fig. 2.5). Some species of *Halocarpus*, however, are distinctly dimorphic, producing bilaterally flattened, hypostomatic juvenile foliage which abruptly gives way to mature imbricate leaves (Fig. 2.7). Most species in this group were found to produce more spreading foliage when placed in deep shade (Fig. 2.7, 2.8), and *Halocarpus bidwillii* often completely reverted to its juvenile foliage form (Fig. 2.7).

#### *Bilaterally Compressed Species*

Type 1: The simplest bilaterally compressed foliage in the Podocarpaceae is produced by the genera *Podocarpus*, *Afrocarpus*, *Saxagothaea* and *Sundacarpus*, which all have entire, hypostomatic, single-veined leaves. The size of *Podocarpus* leaves varies from approximately one centimetre in length in highland species such as *P. lawrencii*, to over 20 cm in tropical rainforest species such as *P. dispermis* (Fig. 2.9). Leaves are always narrow-linear in shape, and lamina width appears to be limited by the obligate single vein nature of the leaf. Juvenile and adult foliage are basically identical.

*Podocarpus* is the most speciose genus in the Podocarpaceae, with a relatively high degree of overall variation in leaf size and shape amongst species, relative to most conifer genera. A further example of this potential for variation was noted in a young plant of *P. drouynianus* where two leaves had fused along their margins, effectively forming a multi-veined leaf (Fig. 2.10).

*Afrocarpus*, *Saxagothaea* and *Sundacarpus* leaves all fall within the morphological range of *Podocarpus*, although *Afrocarpus* and *Saxagothaea* both tend to produce leaves in more distinct short shoots than *Podocarpus* (Fig. 2.11).

Figure 2.9. Leaves from nine species of *Podocarpus* ranging in size from largest, *Podocarpus dispersmis* from tropical northern Queensland, to smallest, *Podocarpus lawrencii* from Tasmania. Species shown from left to right are : 2 x *P. dispersmis*, *P. lucienii*, *P. grayae*, *P. neriifolius*, *P. drouynianus*, *P. novae-caledoniae*, *P. gnidioides*, *P. totara* and *P. lawrencii*. (Scale bar = 10 mm)

Figure 2.10. A "multi-veined" leaf of *Podocarpus drouynianus* caused by a mutation whereby two leaves have fused to form a double veined leaf. Only one genus in the Podocarpaceae, *Nageia*, is formally known to produce multi-veined leaves. (Scale bar = 10 mm)

Figure 2.11. Leaf arrangement on an adult short shoot of *Afrocarpus falcata*. Leaves are very similar in appearance to *Podocarpus*. (Scale bar = 10 mm)

Figure 2.12. (a) Adult and (b) transitional phylloclades of *Phyllocladus aspleniifolius*. The fusion and webbing between leaves can be clearly distinguished in the transitional phylloclade. Phylloclades of this species arise as discrete photosynthetic units. (Scale bars = 10 mm and 5 mm respectively)



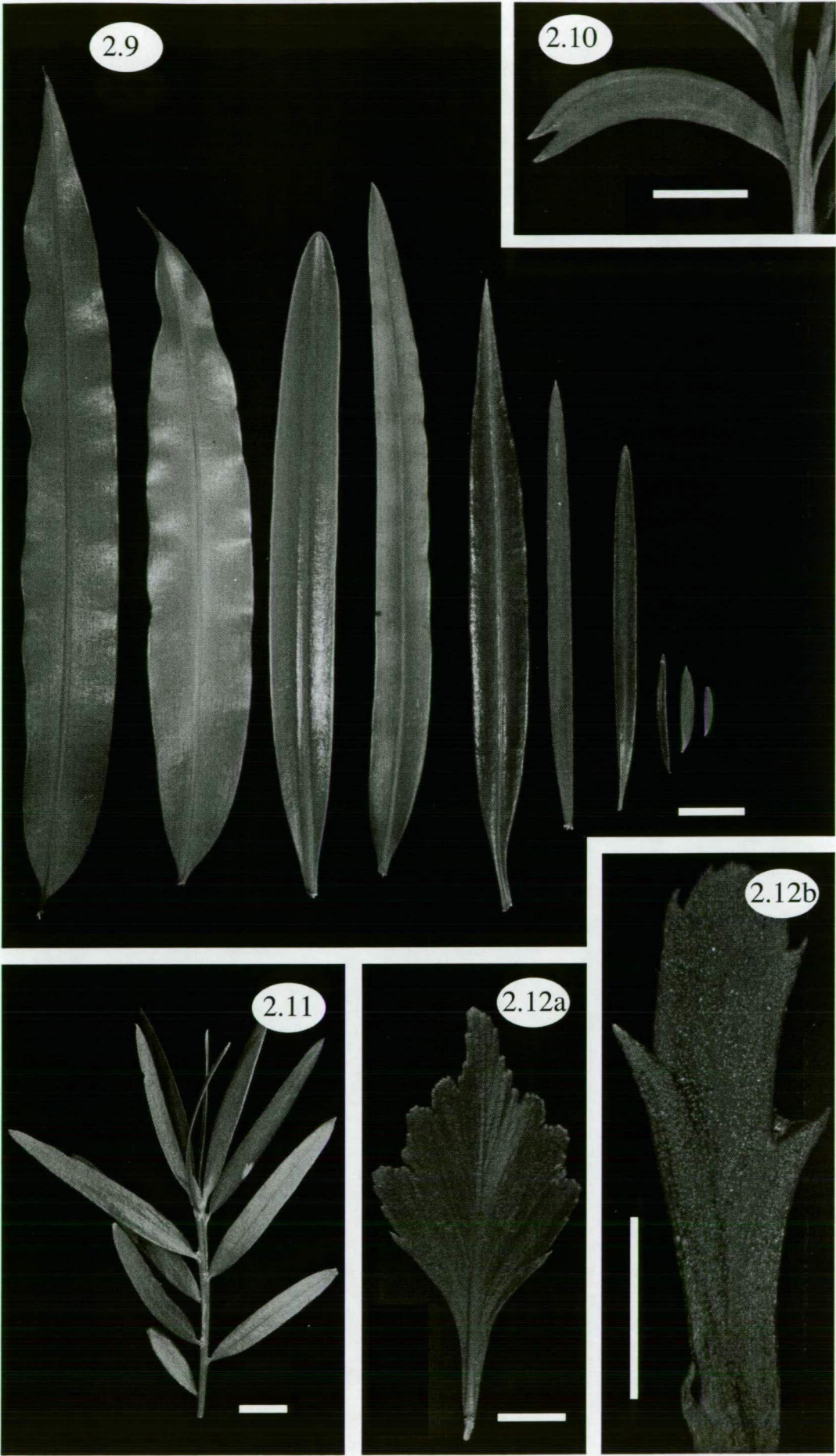


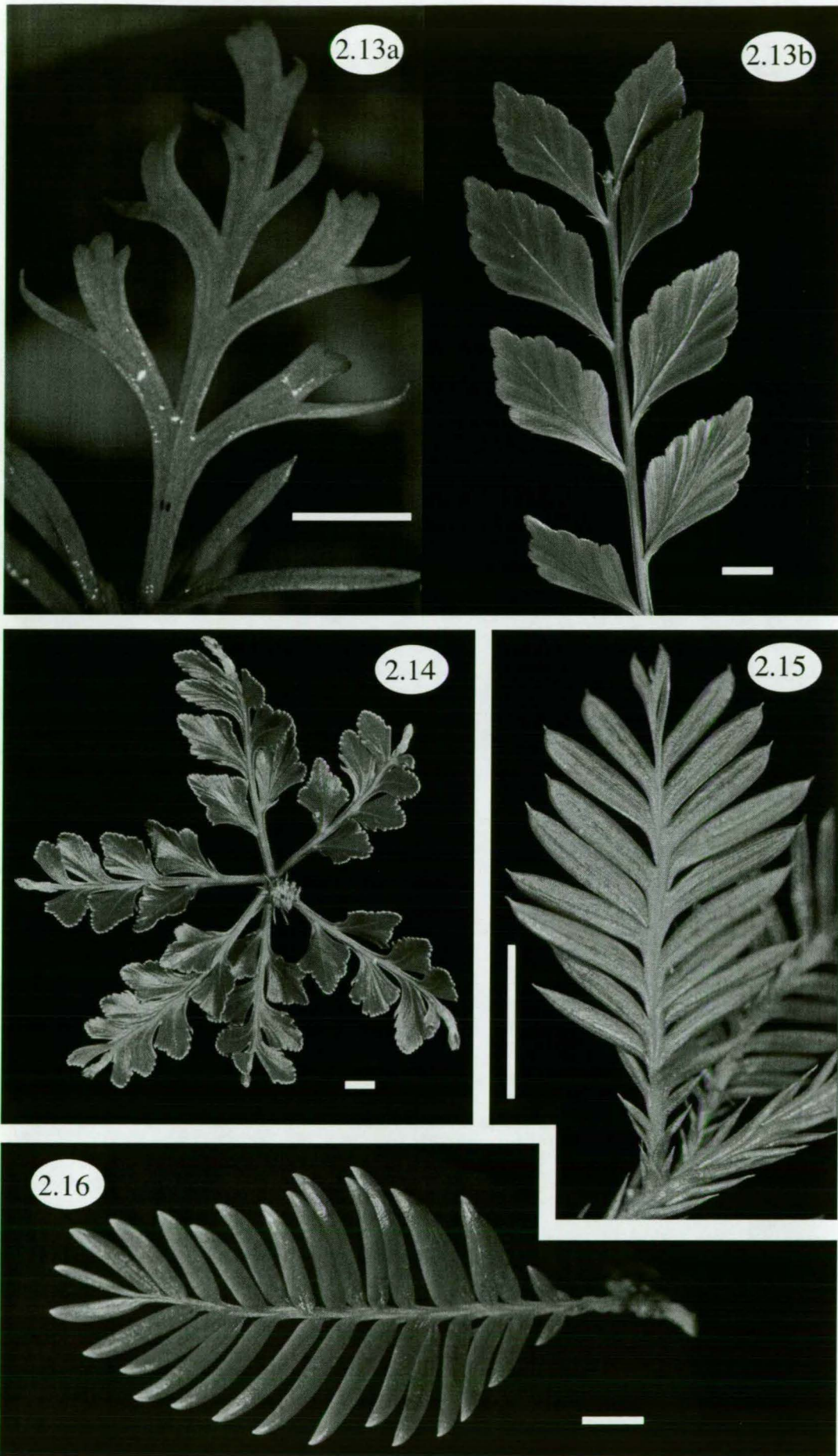
Figure 2.13. (a) A juvenile phylloclade of *Phyllocladus trichomanoides*, and (b) an adult phylloclade of *Phyllocladus hypophyllus*. Both are 'compound phylloclades', and as in figure 2.12, the mechanism of leaf fusion and branch flattening can be visualised in the seedling foliage. (Scale bars = 3 mm and 10 mm respectively)

Figure 2.14. Shoots of *Phyllocladus aspleniifolius* illustrating the degree of variation in foliar morphology produced by this genus. This view, looking down onto the apex of a branch shows phylloclades arranged in a fashion more typical of the compound species. (Scale bar = 10 mm)

Figure 2.15 A bilaterally flattened short shoot of *Dacrycarpus imbricatus*. The arrangement of bilaterally flattened leaves on the short shoot contrasts with the bifacially flattened, spiral leaf arrangement seen on the stem. The analogy between these discrete short shoots and broad leaves is strong (see text). (Scale bar = 10 mm)

Figure 2.16. Short shoot of *Acropyle pancheri*, taken from an angle looking down on the apical bud. The means of leaf and shoot flattening in this genus is the same as that of *Dacrycarpus*. Leaves on the stem are reduced to imbricate scales. (Scale bar = 10 mm)





Type 2: The second mechanism of foliage flattening is employed by the five species of *Phyllocladus* found in Australasia and New Zealand. In this genus whole shoots have been flattened and webbed to form a single, flat, multi-veined phylloclade (Fig. 2.12). Juvenile foliage appears as a typical conifer needle, and as seedlings mature successive shoots develop increasing degrees of flattening and webbing, a process which can be well illustrated by comparing shoots of different ages (Fig. 2.12, 2.13).

Three species of *Phyllocladus* produce "compound phylloclades" (Fig. 2.13) which can be extremely large, and several phylloclades of *Phyllocladus hypophyllum* measured in the glasshouse were approximately 18 cm in length. A high degree of foliar variation within species, and even within individuals characterises this genus, often blurring the distinction between the compound species (*P. hypophyllum*, *P. trichomanoides*, and *P. glaucus*) and other species (*P. aspleniifolius* and *P. alpinus*). A number of examples of *P. aspleniifolius* producing "compound phylloclades" were noted (Fig. 2.14) as were *P. hypophyllum* and *P. trichomanoides* with "simple" phylloclades.

Stomatal distribution on the phylloclades measured on all species ranged from evenly amphistomatic in the New Zealand alpine species, *P. alpinus*, to almost hypostomatic in the tropical forest species *P. hypophyllum*.

Type 3. In two genera, *Acmopyle* and *Dacrycarpus*, a convergent means of producing flat photosynthetic surfaces has arisen. In both these genera, leaves on the short shoots have become compressed bilaterally on an axis parallel to the stem axis (Hill and Carpenter, 1991) and are arranged in two opposite ranks generally oriented perpendicular to the angle of illumination (Figs 2.15, 2.16). On the primary branches, leaves are arranged as a loose spiral, or reduced to imbricate scales, while generally only the leaves on short shoots develop into flattened photosynthetic structures (Fig. 2.15). Seedling foliage in both genera appeared as small needles, with the first bilaterally compressed shoots arising as branches early in the development of the plant (Figs 2.17, 2.18).

Short shoots of glasshouse grown *Dacrycarpus* ranged in size from a less than one centimetre to around 9 cm (Fig. 2.19). In *Acmopyle pancheri*, short shoots grown in Hobart ranged from around 4 cm to

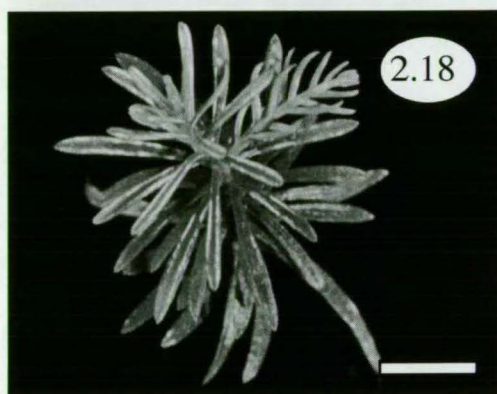
Figure 2.17. Aerial view of a seedling of *Dacrycarpus dacrydioides* illustrating the early development of bilaterally compressed short shoots. Four such shoots can be seen branching out at right-angles, produced after only three nodes. The two cotyledons are clearly visible. (Scale bar = 5 mm)

Figure 2.18. A slightly older seedling of the extremely rare Fijian species *Acmopyle sahniana*, illustrating the initiation of the first bifacially flattened shoot, again as a discrete branch, at about node 30. (Scale bar = 10 mm)

Figure 2.19. Shoots from two species of *Dacrycarpus* representing the full size range observed in glasshouse grown material. The small shoot is from the New Zealand species, *D. dacrydioides*, and the large shoot from the tropical (Fijian) species *D. imbricatus* var. *patulus*. (Scale bar = 10 mm)

Figure 2.20. Detail of stomatal bands on the leaves of *Acmopyle pancheri*. Stomata were extremely abundant on both leaf surfaces and often caused leaves to appear white due to the presence of reflective wax in and around the stomata. (Scale bar = 5 mm)





approximately 18 cm (with a mean length from 30 shoots of 7.1cm) and although *Acmopyle sahniana* did not reach maturity in the glasshouse, short shoots measured in the field for both species were often larger than this (shoots of up to 25 cm were measured in forest in New Caledonia). Leaves of both *Acmopyle* and *Dacrycarpus* were unevenly amphistomatic, with a tendency toward hypostomy in deep shade. Leaves of *Acmopyle* were highly stomatiferous, with stomata densely clustered in distinct waxy bands on leaves (Fig. 2.20).

A third genus, *Falcatifolium*, produces bilaterally flattened leaves in the same way as *Acmopyle* and *Dacrycarpus*, however in this genus short shoots only become flattened and oriented horizontally when exposed to deep shade. In cultivation individuals of both *Falcatifolium taxoides* and *F. falciforme* produced short shoots with leaves arranged in an even, three dimensional spiral (Fig. 2.21) but in the field (in deep shade) leaves were arranged in two ranks producing bilaterally flattened, horizontally oriented shoots (Fig. 2.22).

Type 4. The genera *Retrophyllum* and *Prumnopitys* both produce bilaterally flattened short shoots by twisting each leaf base in such a way as to orient the short axis of leaves parallel to the shoot axis, which is displayed horizontally. Unlike the type 3 morphology, where leaves are flattened bilaterally (parallel to the shoot axis), these genera produce bifacially flattened leaves (flattened perpendicular to the shoot axis) which are then twisted parallel to the shoot axis, resulting in shoots which are ostensibly very similar to type 3 shoots (Fig. 2.23).

The mechanism of leaf twisting differs between *Prumnopitys* and *Retrophyllum*. In *Prumnopitys* leaf bases are twisted such that when the shoot is oriented horizontally, the adaxial surfaces of leaves remain uppermost. In *Retrophyllum*, the leaves on opposite sides of the shoot twist in opposite directions such that the adaxial surfaces of leaves on one side of the shoot are turned down, while on the other side, the adaxial surface remains uppermost (Figs 2.24, 2.25). This probably explains the even amphistomy in *Retrophyllum* compared with the uneven distribution of stomata in *Prumnopitys* (approaching hypostomy).

In all cases, shoot flattening was observed to be related to light availability, in deep shade, shoots were strongly flattened as described above, but in high light environments, leaf twisting was reduced and shoots were oriented more vertically, displaying leaves in a more three dimensional manner (Fig. 2.26). *Retrophyllum minor*, for example, grows

Figure 2.21. A single short shoot of glasshouse grown *Falcatifolium taxoides*, illustrating a three dimensional spiral arrangement of bilaterally flattened leaves around the shoot axis. (Scale bar = 10 mm)

Figure 2.22. Leaf arrangement of *Falcatifolium taxoides* as seen growing in the understorey of a lowland mixed conifer-angiosperm forest in New Caledonia. Under these deep shade conditions shoots were displayed horizontally, and adopted a bilaterally flattened appearance.

Figure 2.23. A short shoot of *Prumnopitys ferruginoides* similar appearance to the short shoots of *Dacrycarpus* and *Acropyle*. In this species, however, leaves were flattened bifacially and twisted through 90°. (Scale bar = 10 mm)

Figure 2.24. Leaf bases on a short shoot of *Retrophyllum comptonii* illustrating the means by which leaves are twisted and reorientated parallel to the shoot axis. Leaves on the left side of the shoot have twisted so the abaxial surface is turned upward, while leaves on the right hand side have also twisted through 90° so that the adaxial surface is facing upward. (Scale bar = 5 mm)





Figure 2.25 A *Prumnopitys ferruginoides* short shoot illustrating that, like *Retrophyllum*, bifacially flattened leaves have been twisted to become oriented parallel to the shoot axis. In this genus, however, leaves on both sides of the shoot are twisted upwards so the adaxial surface on all leaves remains adaxial when the shoot is oriented horizontally. (Scale bar = 10 mm)

Figure 2.26. Foliage of *Prumnopitys ferruginea* as observed growing in an exposed location on Mt. Humboldt, New Caledonia (1200 m). Leaves can be seen to be arranged more or less spirally around the shoot axes, and shoots oriented approximately vertically.

Figure 2.27. The growth form and typical habitat of *Retrophyllum minor* (New Caledonia). The roots of this species contain a large proportion of aerenchyma, enabling it to grow in and around streams. This species is quite rare, and individuals are usually restricted to high light conditions on stream and lakesides.

Figure 2.28. Leaf arrangement in *Retrophyllum minor* where shoots are generally oriented vertically and leaves are displayed in opposite pairs without twisting of the leaf base.





under high light in New Caledonia (Fig. 2.27), and leaves of this species remain opposite and decussate in the field. (Fig. 2.28).

Type 5. The last, and perhaps most significant flattened leaf form is produced by the genus *Nageia*, which has overcome the single vein limitation which most conifers suffer, to develop broad, multi-veined leaves. Leaves are arranged in opposite pairs on compound shoots which are displayed horizontally (Fig. 2.29). Unfortunately this species could not be propagated in time for its inclusion here in the following chapters.

### *Spiral Spreading*

The final type of podocarp foliage is the spreading, spiral leaf arrangement found in the genus *Dacrydium*. Within this group there is a large range of leaf morphologies, from *Araucaria*-like scale leaves in *Dacrydium araucariaoides* (Fig. 2.30), to narrow, densely packed leaves such as those of *Dacrydium lycopodioides* and *Dacrydium guillauminii* (Figs 2.30, 2.31). Virtually all species of *Dacrydium* present this type of leaf arrangement, although there is one species described from the highlands of New Guinea which produces true imbricate apressed foliage (*Dacrydium novoguineense*; Wells and Hill, 1989). One species of *Dacrycarpus*, also from sub-alpine areas in New Guinea (*D. compactus*) produces spreading, spirally arranged leaves as its adult foliage (Fig. 2.32). All species are amphistomatic, although stomata are generally more abundant on the adaxial leaf surface.

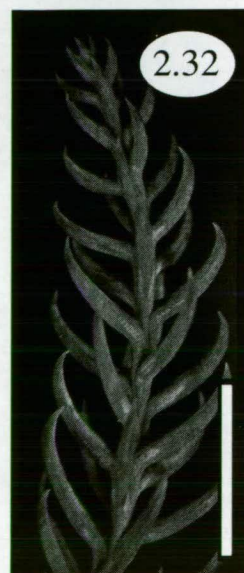
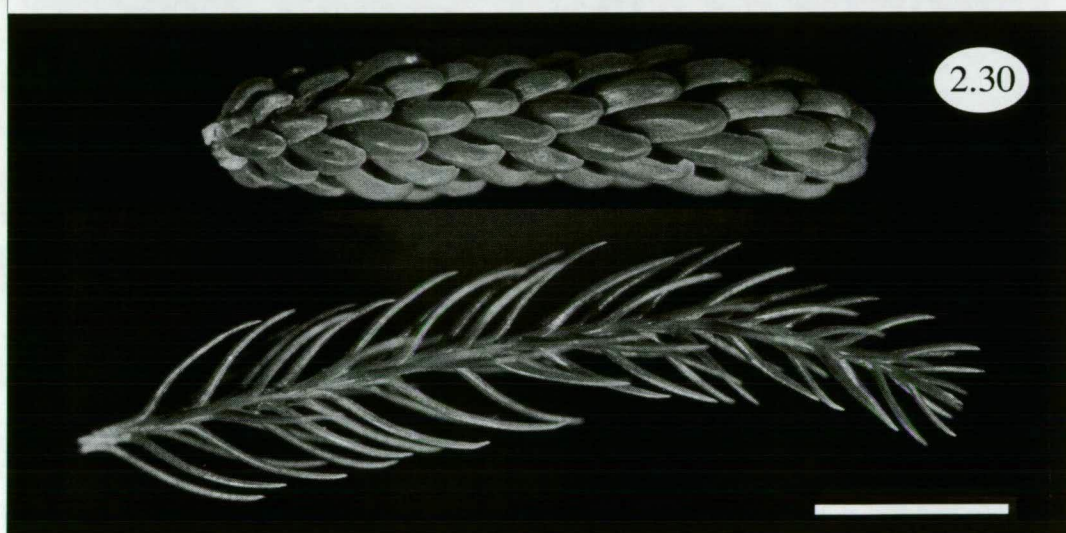
Figure 2.29. Multi-veined leaves on a shoot of *Nageia nagi*. This is the only genus of Podocarpaceae capable of producing multi-veined leaves. (photo R. Hill)

Figure 2.30. Two contrasting types of lax, spirally arranged foliage typical of *Dacrydium* species. Above is the New Caledonian species *Dacrydium araucarioides* overlying a shoot of *D. lycopodioides* also from New Caledonia. (Scale bar = 10 mm)

Figure 2.31. A third example of the three dimensional spiral arrangement of leaves in *Dacrydium*. Shown here is the rare New Caledonian species *D. guillauminii* photographed at Chutes de la Madeleine.

Figure 2.32. A shoot of *Dacrycarpus compactus*, illustrating the loosely arranged, spirally inserted foliage usually typical of *Dacrydium* species. *D. compactus* is the only extant species of *Dacrycarpus* known to produce such foliage in its adult form. (Scale bar = 5 mm)





## CUPRESSACEAE

The foliar morphology of the southern hemisphere Cupressaceae is far less diverse than that of the Podocarpaceae, and can be divided into two broad morphological groups, those with imbricate adult foliage, and those with spreading leaves. Within these groups are the former members of the Taxodiaceae (two species of *Athrotaxis*) which have been reclassified as Cupressaceae (Brunsfield *et al.*, 1994).

*Spreading leaves*

Three genera of southern Cupressaceae produce distinctly spreading foliage as mature plants, those being the Tasmanian species *Athrotaxis selaginoides*, the New Caledonian endemic *Neocallitropsis pancheri*, and the South American species *Pilgerodendron uviferum*.

*Athrotaxis selaginoides* develops epistomatic leaves which are spirally arranged and displayed almost perpendicular to the stem. The angle of leaves to the stem depends on the light regime under which foliage is developed, and under shade conditions leaves are arranged perpendicular to the stem, while in high light the angle becomes more acute (Fig. 2.33). Juvenile foliage is basically identical to the foliage on adult trees, although leaves are usually more spreading in their arrangement.

The second type of spreading foliage is produced by *Pilgerodendron uviferum*, which has leaves arranged in opposite, decussate pairs displayed almost perpendicular to the stem (Fig. 2.34). Leaves are similar to, but smaller than those of *Athrotaxis*, with stomata again restricted to the adaxial surface.

Adult foliage of *Neocallitropsis* is arranged in alternate whorls of four (Fig. 2.35) effectively producing eight ranks of leaves on the stem. Stomata occur on both leaf surfaces of the adult foliage, but juvenile foliage is hypostomatic, and unlike *Athrotaxis* and *Pilgerodendron*, the juvenile foliage of *Neocallitropsis* is morphologically distinct from adult foliage, consisting of long needle leaves again produced in alternate whorls of four (Fig. 2.36).

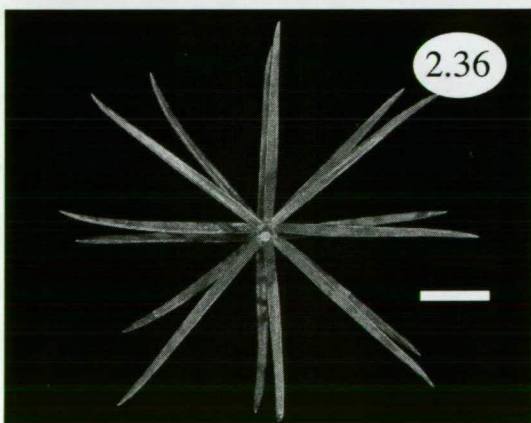
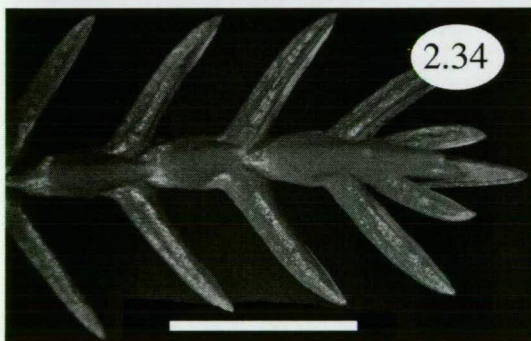
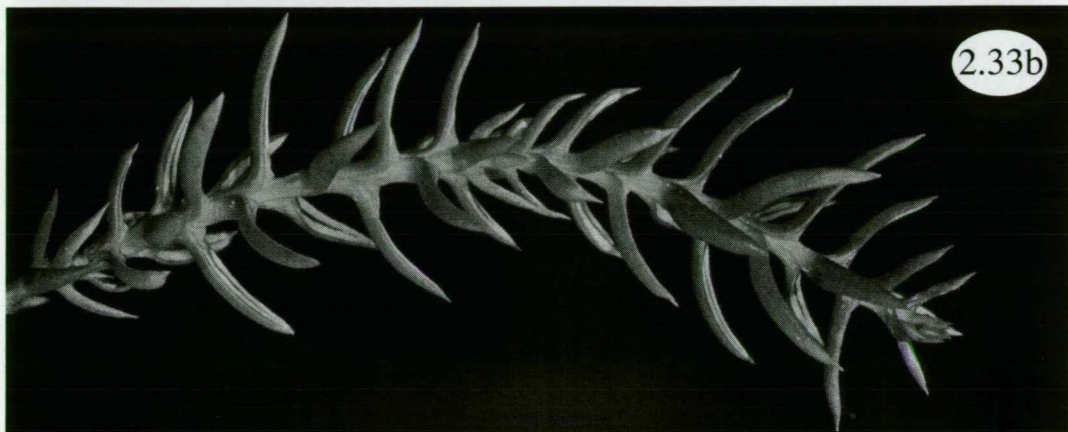
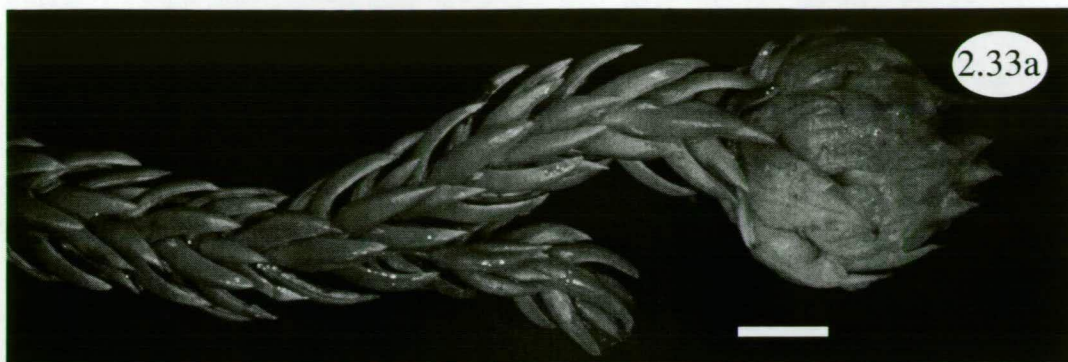
Figure 2.33. Two shoots of *Athrotaxis selaginoides* illustrating - (a) tightly packed foliage collected from a plant growing in an exposed location in Tasmania, and (b) loosely packed foliage collected from a plant grown in deep shade (bottom). In both foliage types stomata are generally confined to the upper (adaxial) leaf surface. (Scale bar = 10 mm)

Figure 2.34. *Pilgerodendron uviferum* adult foliage. Stomata are just visible on the adaxial surface of leaves. (Scale bar = 10 mm)

Figure 2.35. Adult foliage of *Neocallitropsis pancheri* at Chutes de la Madelaine (New Caledonia). Leaves remain loosely arranged around the stem, forming distinct, alternate whorls of four leaves.

Figure 2.36. Juvenile foliage of *Neocallitropsis pancheri* illustrating the needle form of leaves, and the characteristic alternate whorls of four leaves. (Scale bar = 10 mm)





*Imbricate species*

All remaining Cupressaceae produce foliage which is basically imbricate, although there are three types which are fundamentally different:

1. The three dry forest genera in the southern hemisphere, *Actinostrobus*, *Callitris* and *Widdringtonia* form one group. In each of these genera the leaf itself has been reduced, and the leaf base extended to take the major role as the photosynthetic surface.

In *Callitris* and *Actinostrobus* leaves are in whorls of three with the leaf bases fused together, and onto the stem, in such a way as to produce what appears as a three lobed photosynthetic stem (Figs 2.37, 2.38). Juvenile leaves are typical linear needles which become gradually reduced as more mature shoots are formed (Fig. 2.39). The leaf tips of adult foliage, and juvenile leaves are amphistomatic. On the leaf base, stomata are confined to two rows on the edge of each leaf, and in some species the stomatal row becomes encrypted into the leaf margin (Fig. 7.8).

Juvenile foliage of *Widdringtonia*, like *Callitris* and *Actinostrobus*, appears as linear needles which are gradually reduced until the leaf itself becomes a small scale, and the leaf base comprises most of the photosynthetic area. Unlike *Callitris* and *Actinostrobus* however, both the adult and juvenile foliage are spirally arranged (Fig. 2.40), and stomata are present in two patches on either side of the abaxial surface of the leaf.

2. *Diselma archeri*, *Athrotaxis cupressoides*, and *Fitzroya cupressoides* form a second group, all possessing imbricate scale leaves, although *Fitzroya* is only loosely imbricate in its adult form. *Diselma archeri* and *Athrotaxis cupressoides* are restricted to montane and sub-alpine forest in Tasmania where they co-exist with the podocarps of similar morphology (*Microstrobos niphophilus* and *Microcachrys*). *Fitzroya* is found from 100m to 1200m above sea-level in southern Chile (Veblen *et al.*, 1995).

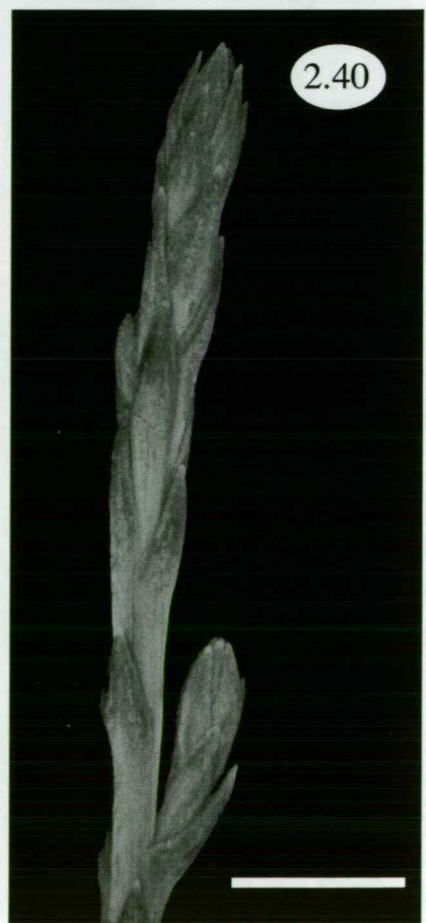
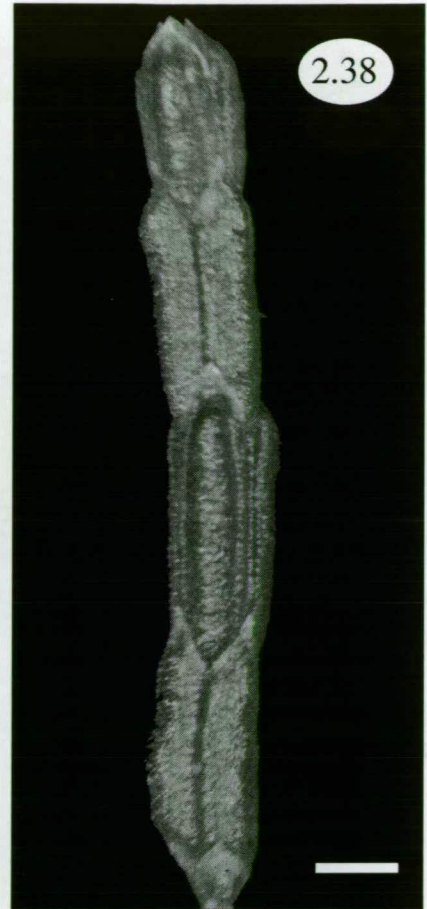
The adult leaves of *Diselma archeri* are tightly imbricate and arranged in an opposite and decussate fashion on the stem (Fig. 2.41). Stomata are almost entirely restricted to the adaxial leaf surface, although two persistent patches also occur on the abaxial leaf surface, which are restricted, in adult sun leaves, to the area of overlap from the underlying leaf pair. The juvenile foliage in *Diselma* is fully spreading and

Figure 2.37. A shoot of *Actinostrobus acuminatus* illustrating the extension of the leaf base as the predominant photosynthetic area. Leaves in this genus and *Callitris* are generally arranged in whorls of three. (Scale bar = 5 mm)

Figure 2.38. In adult shoots of *Callitris rhomboidea* leaves are reduced to small scales with the leaf base assuming the photosynthetic function. The leaf proper contains little mesophyll and is obviously to a large degree vestigial. (Scale bar = 1 mm)

Figure 2.39. The transition from juvenile to adult foliage in *Callitris rhomboidea* sees a reduction in the needle leaves used by the seedling for assimilation to the scales illustrated in figure 2.38. (Scale bar = 5 mm)

Figure 2.40. Adult shoot of *Widdringtonia cedarbergensis* again showing reduction of leaves and the extension of the leaf base, but not to the degree seen in *Callitris* and *Actinostrobus*. Leaves are spirally arranged rather than being in whorls. (Scale bar = 5 mm)





hypostomatic, and there is a gradual foliar progression as the plant matures where leaves become increasingly imbricate and stomata distributed more adaxially (Figs 2.41, 2.42).

*Athrotaxis cupressoides* produces spirally arranged scale leaves which are fused to the stem along much of their length (Fig. 2.43). Only the leaf tip is free of the stem, and hence only a small number of adaxial stomata are found, the bulk of stomata being confined to two patches on the abaxial surface of the leaf. As with the other *Athrotaxis* species, seedling leaves are identical to adult leaves. There are morphological intermediates between *A. cupressoides* and *A. selaginoides*, produced by hybridisation between these two species, and leaves of the hybrid (known as *Athrotaxis laxifolia*) are generally amphistomatic, with slightly spreading foliage (Fig 2.43).

The leaf arrangement of *Fitzroya* is, at best, loosely imbricate in the adult plant, with leaves forming whorls of three (Fig. 2.44). As with most conifer species the juvenile foliage is substantially more spreading, resembling the shoots of type 1 Cupressaceae (Fig. 2.44), and both adult and juvenile leaves are evenly amphistomatic. In deep shade foliage reverts to the spreading 'juvenile' form in adult plants.

3. The final morphotype presented here is found in the genera *Libocedrus*, *Papuacedrus*, and *Austrocedrus*. These plants have leaves arranged in an opposite, decussate fashion, where leaf pairs alternate between a bifacially compressed "hypostomatic" leaf pair, and a smaller, scale leaf pair (Figs 2.45, 2.46). Shoots are oriented horizontally such that the bilaterally flattened leaves are perpendicular to the main stem axis. Because the large leaves are bilaterally flattened, the lower surface of these leaves as they appear on a shoot, is actually comprised of both sides of the leaf, and hence the stomatal arrangement cannot be described as true hypostomy (Figs 2.45, 2.46).

Juvenile foliage in this group is again represented by amphistomatic needles which grade into adult foliage as the seedling matures. Only *Libocedrus* was grown from seed here, and from the two species examined, the process of bifacial flattening and leaf reduction in alternate leaf pairs was clearly evident (Fig. 2.47).

In the highland New Caledonian species *Libocedrus chevalieri*, the bifacially flattened leaf pair is only marginally larger than the alternate pair, and shoots are generally oriented vertically, giving the shoot an intermediate appearance between type 2 and 3 morphologies.

Figure 2.41. From left to right are shown the lax juvenile, intermediate, and tightly imbricate adult foliage of *Diselma archeri*. (Scale bar = 5 mm)

Figure 2.42. Adaxial (left) and abaxial (right) leaves from (a) adult (Scale bar = 1mm) (b) transitional (Scale bar = 5 mm) and (c) juvenile foliage (Scale bar = 5 mm) of *Diselma archeri*. Seedling leaves are hypostomatic with stomata present as two bands parallel to the midvein. Transitional foliage is amphistomatic, with stomata forming adaxially on the leaf tip, while the abaxial stomata become less abundant. In (b) the leaf that was uppermost on the shoot has been removed to expose the adaxial surface of the facing leaf, a small patch of stomata can be seen on the leaf tip. Adult foliage is entirely epistomatic, stomata being confined to the inner surface of the leaf.

Figure 2.43. Tightly imbricate foliage of *Athrotaxis cupressioides* (left), and the intermediate foliage of *A. laxifolia*, a hybrid between *A. cupressioides* and *A. selaginoides* (see figure 2.33). *A. cupressioides* is almost completely hypostomatic and *A. selaginoides* is epistomatic. The hybrid shown here is  $\pm$  evenly amphistomatic, although considerable variation was observed in hybrid morphology. (Scale bar = 10 mm)

Figure 2.44. *Fitzroya cupressioides* loosely imbricate adult, and lax juvenile foliage. Stomatal distribution remains approximately equal between leaf surfaces in both foliage forms. Reversion to the juvenile morphology is also seen under shade conditions. (Scale bars = 4 mm and 10 mm respectively)



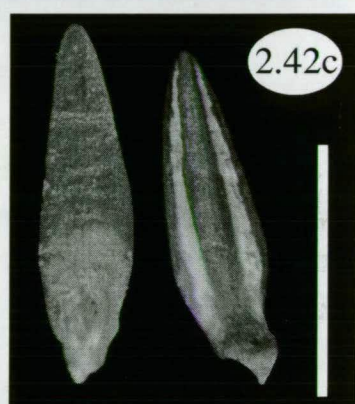
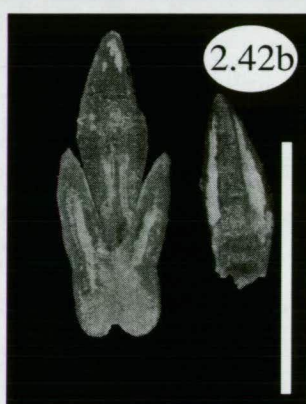
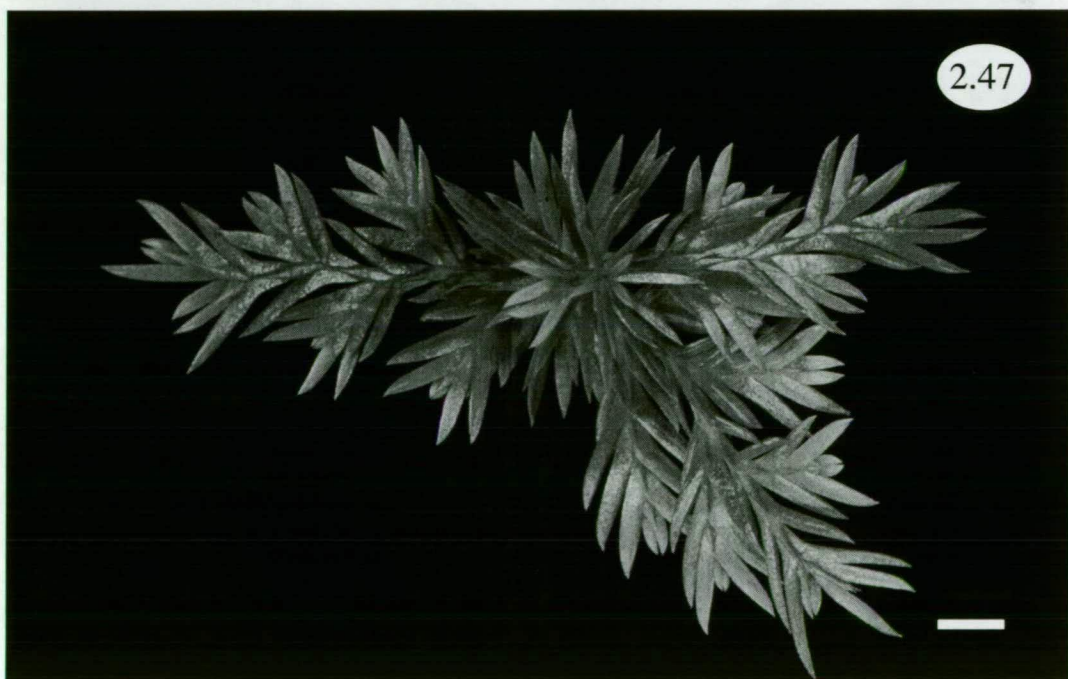


Figure 2.45. Adult foliage of *Libocedrus yateensis* illustrating large bilaterally compressed leaves alternating with small bifacially compressed leaves. Half the shoot is in natural orientation (left), with the other half inverted (right) to show stomatal distribution. Although stomata are largely confined to the lower surface of the shoot, because of the complex arrangement of the stem leaves are not hypostomatic (see text). (Scale bar = 10 mm)

Figure 2.46. *Papuacedrus papuana* showing similar leaf arrangement to *Libocedrus*, again upper and lower surfaces of the shoot are displayed. The lower surface of the shoot is highly stomatiferous, with stomatal patches appearing as white areas largely confined to the bilaterally flattened leaf pair. (Scale bar = 10 mm)

Figure 2.47. Transitional seedling foliage of *Libocedrus yateensis* taken from above, clearly showing the bilateral compression of the major leaf pair, and horizontal orientation of the shoot. (Scale bar = 10 mm)



## Discussion

It is clear that the morphological diversity of the Podocarpaceae far exceeds that of the southern hemisphere Cupressaceae. Although not discussed at length, even the variation within species can be large, with the examples of variability in leaf arrangement of *Phyllocladus* and mutation in *Podocarpus drouynianus* illustrating the high potential for morphological flexibility (relative to other conifers) within this family. Perhaps the most impressive feature of the Podocarpaceae is the polyphyletic evolution of at least five different shoot flattening mechanisms which result in the production of discrete short shoots which closely resemble the broad leaves of angiosperms.

The assumption of a broad-leaf character is obvious in the short shoots (phylloclades) of *Phyllocladus*, and it has been suggested that the bilaterally compressed short shoots of *Dacrydium* and *Acmopyle* also function as discrete photosynthetic units, analogous to broad-leaves (Hill, 1995). Indeed this analogy, which is also applicable to *Retrophyllum*, *Prumnopitys* and *Falcatifolium*, is appropriate for several reasons:

- firstly the size of short shoots is clearly genetically adapted to the temperature regime occupied for different species (see figure 2.19) in a similar fashion to angiosperm leaves, and the variation in shoot dimensions is substantially greater than that of the individual leaves on the shoot. The shoot size of these species falls within the range of leaf sizes of the accompanying angiosperm tree species, whereas the size of individual leaves on the shoot is outside the observed range of associated angiosperms. This is illustrated using a leaf length versus mean maximum temperature for the warmest month regression compiled from leaf litter collected from rainforest throughout eastern Australia (G. Jordan, pers. comm.). Using this regression combined with temperature statistics from 1995-1996 it was calculated that the expected mean leaf length at Rivière Bleue would be 6.9 cm, and this compared favourably with the average shoot length for *Acmopyle pancheri* collected from this location, of  $7.1 \pm 0.05$  cm (as opposed to  $1.7 \pm 0.007$  cm for leaves)

- secondly shoots arise and are mostly shed as discrete units which are usually determinate in growth. However, even when shoots grow for several years, each year's growth retains its discrete appearance, regardless of the presence or absence of seasonality.

- and finally, the occurrence of this shoot morphology is most commonly associated with broad-leaf forest where its bilateral flattening,

horizontal orientation and large surface area clearly serves a similar purpose of maximising light absorbance per unit leaf investment as angiosperm leaves (see chapter 6).

Although some northern hemisphere Cupressaceae have adopted a flattened shoot morphology similar to that of the Podocarpaceae e.g. *Metasequoia* and *Sequoia*, the only genera displaying any degree of shoot flattening in the southern Cupressaceae are the southern "cedars", *Austrocedrus*, *Papuacedrus*, and *Libocedrus*. Shoot flattening in the latter two genera is most pronounced in the first shoots produced by seedlings (see figure 2.47 and Offler, 1984) and clearly also serves to increase light capture during establishment in these rainforest species. In virtually all imbricate species examined, the seedling shoot morphology is more spreading than the adult (e.g. figures 2.4, 2.6 2.8, 2.33, 2.44). In some cases, where juvenile foliage is distinctly different to the adult e.g. *Halocarpus* (Fig. 2.6) and *Callitris*, increased light harvesting seems to be the likely product, but in species such as *Microstrobos niphophilus*, *Diselma archeri* and *Lagarostrobos franklinii*, where juvenile foliage is only slightly more lax than adult shoots, the difference in shoot morphology does not seem enough to significantly alter the area of shoot available for light harvesting. In these plants the lax seedling morphology may act to facilitate gas exchange (see chapter 7).

Where possible, representatives of each of the morphological types mentioned above have been included in experimental work, although in some cases, slow growth rates (particularly in genera such as *Libocedrus* and *Dacrydium*) and delayed collection (*Papuacedrus*, *Austrocedrus*, and *Neocallitropsis*) have prevented inclusion of all morphotypes.



## CHAPTER 3

# **Response to Water Stress in a Sample of 12 Southern Hemisphere Conifer Species and Comparative Drought Tolerance, as Measured Using a New Physiological Index**

## **Introduction**

With few exceptions, most coniferous genera in the southern hemisphere are entirely confined to rainforest or wet alpine areas, implying that water availability places a significant limitation on the distribution of the bulk of southern conifer species. Given that a number of (non-floristic) studies have indicated a decrease in the availability of water throughout the Tertiary in Australia (e.g. Bowler, 1982), it seems a viable hypothesis that conifer extinction and range contraction throughout this period might have been caused by such a reduction in precipitation (Kershaw *et al.*, 1994; Jordan, 1993). The problem with this hypothesis is that accompanying the increase in aridity in the Tertiary is evidence of an increase in the frequency of fire disturbance (Kershaw *et al.*, 1994) and without independent evidence of the drought sensitivity of the conifer taxa in question it can be argued that a decrease<sup>n</sup> in rainfall is simply *prima facie* evidence for drought extinction in Australia. In order to test primarily whether conifers today are limited by water availability, and therefore whether changes in precipitation may have influenced distributions in the past, the drought sensitivity of a large sample of southern hemisphere conifer genera was quantified and compared with the precipitation ranges of the species in question. In this chapter the technique devised for measuring drought tolerance is explained, and in Chapter 4 the relationship between drought tolerance and species distribution is discussed.

Quantification of drought susceptibility is most commonly restricted to crop cultivars, where destructive techniques are used on large numbers of plants (e.g. Premachandra *et al.*, 1995; Hsissou and Bouharmont, 1995; Kolb and Davis, 1994). These techniques were inappropriate for use here as experimental plants were very limited in number, and often extremely slow growing. Similarly, standard techniques used to measure drought effects in the field require the removal of a relatively large number of leaves (e.g. Whitlow *et al.*, 1992; Gebre and Khuns, 1991; Parker *et al.*, 1982), a luxury not afforded by most



of the species grown here. The limitation on available plant material required a technique which did not irreversibly damage experimental plants. As far as its non-destructive nature, the most acceptable method was the commonly used technique of measuring carbon dioxide and water vapour fluxes on a leaf enclosed in a cuvette, allowing calculation of instantaneous leaf gas-exchange. This technique enables photosynthesis and transpiration to be regularly monitored in a leaf, or leaves of a plant subject to drought without any damage being inflicted on either the leaf or plant used. Scrutinising the instantaneous response of photosynthesis and transpiration to drought also provides a far more sensitive means of describing the effect of decreasing soil moisture on the leaf, and therefore plant, than techniques which quantify the damage sustained during drought, as it is most probable that the inhibition of photosynthesis will always precede visual leaf damage, or membrane rupture (e.g. Fellows and Boyer, 1978).

It has long been established that during drought, stomatal conductance ( $g_s$ ) decreases, reducing water loss from the leaf (Slatyer, 1967). In the absence of other effects, such a decrease in conductance will produce a  $\text{CO}_2$  'supply' limitation of photosynthesis (Farquhar and Sharkey, 1982) by causing the intercellular  $\text{CO}_2$  concentration ( $c_i$ ) in the leaf to become depleted. Plotting the course of  $c_i$  depletion and assimilation ( $A$ ) in droughted plants has the potential to provide sensitive means of quantifying the proficiency with which photosynthesis responds to a drought event, and its susceptibility to drought induced photoinhibition. Studies of the  $A$  vs  $c_i$  relationship in plants subject to water deficit have identified two components contributing to the inhibition of assimilation, limitation due specifically to reduced  $g_s$  termed 'stomatal limitation', and photoinhibitory effects, independent of  $g_s$ , termed 'non-stomatal limitation' (Jones, 1985). Many authors have recently described 'non-stomatal' inhibition of photosynthesis as an important influence during drought, attributing this to impaired chloroplast function in leaves subject to water deficits (Boyer, 1971; Graan and Boyer, 1990; Majumdar *et al.*, 1991; Gunasekera and Berkowitz, 1993).

Non-stomatal inhibition of photosynthesis has been commonly invoked to explain results of gas exchange studies showing unchanging or increasing  $c_i$  in the leaves of droughted plants and strong depression in the relationship between  $A$  and  $c_i$  (Ehleringer and Cook, 1984; Wong *et al.*, 1985; Grieu *et al.*, 1988; VanRensberg and Kruger, 1993; Anderson *et al.*, 1995). The suggestion that during drought non-stomatal effects become

important soon after leaf water potential ( $\Psi_l$ ) begins to drop, is not consistent, however, with data obtained in fluorescence studies, which indicate that leaf photochemistry is resistant to moderate drought stress (Sharkey and Badger, 1982; Genty *et al.*, 1987; Cornic *et al.*, 1989) and that during drought an increased flow of electrons to  $O_2$  reflects decreasing  $c_i$  (Cornic and Briantais, 1991). Integrated measures of  $c_i$ , such as carbon isotope discrimination in leaves, also imply that  $c_i$  is lower in water stressed plants (Smith and Osmond, 1987; Hubick *et al.* 1988; Ehleringer *et al.*, 1992; Donovan and Ehleringer, 1994). This disparity between gas exchange data and other measures of  $c_i$  has been attributed to patchy stomatal closure in response to drought.

Implicit in the calculation of  $A$  and  $g_s$  from gas exchange parameters is uniform stomatal aperture on the leaf being measured (Farquhar and Sharkey, 1982). In the case of patchy stomatal closure, the whole  $A(c_i)$  relationship is erroneously depressed, leading to the conclusion that photosynthesis is being directly inhibited (Terashima *et al.*, 1988). Heterogeneity in the distribution of photosynthesis has been observed in most species examined (Pospisilova and Santrucek, 1994) and appears to be associated with the reticulate venation typically found in broad leaved angiosperms. This type of venation tends to subdivide the leaf lamina, especially in the case of heterobaric leaves where bundle sheath extensions from the veins to the epidermis compartmentalise the leaf into small aereoles. Studies of chlorophyll fluorescence (Downton *et al.*, 1988; Cardon *et al.* 1994; Siebke and Weis, 1995) and radiolabelling (Terashima *et al.*, 1988) have shown that during drought or ABA application, groups of aereoles can be actively photosynthetic while adjacent parts of the leaf were not measurably assimilating. This spatial heterogeneity in leaf photosynthesis has prevented an accurate understanding of the dynamics of changing  $c_i$  during drought.

The conifers used here are well suited to study of the drought response of photosynthesis as their morphology suggests a high potential for uniform gas exchange. The narrow leaf width (< 5mm in most species) and universal possession of an unbranched, single vein (the multiveined phylloclades of *Phyllocladus* were not included) in leaves meant that the problem of leaf subdivision was avoided and the potential for rapid lateral diffusion of  $CO_2$  throughout the leaf was maximised. Potential patchy effects were also avoided by slowly and evenly inducing drought (Gunasekera and Berkowitz, 1992; Wise *et al.*, 1992). Given accurate determination of  $c_i$ , it should be possible to graphically compare the

dynamic response of this variable to drought in different species and to use this as an indication of the relative drought tolerance.

## Materials and Methods:

### *Plant material*

A sub-sample of species representing the maximum altitudinal, latitudinal and water availability range was made from the species in cultivation (see chapter 2), including cuttings from *Acmopyle pancheri*, *Dacrycarpus compactus*, *Diselma archerii*, *Lagarostrobos franklinii*, *Podocarpus lawrencii*, *P. drouynianus* and *Microstrobos niphophilus*, and seed grown *Actinostrobus acuminatus*, *Athrotaxis selaginoides*, *Callitris rhomboidea*, *Dacrycarpus dacrydioides*, *Widdringtonia cedarbergensis* and two angiosperms *Eucalyptus tenuiramis* Miq. and *Acacia melanoxylon* R.Br.. Data were always taken from a minimum of 3 replicates per species and in the case of cutting propagated individuals, plants came from at least 2 parents (except *D. compactus* where replicates were cuttings from the one tree).

During droughting, water was withheld from plants, causing a gradual decrease in soil water content (due to the large pot size and relatively small plants). Drought intensification continued until the dark adapted  $F_v/F_m$  was approximately 0.7 or the assimilation rate became negative, at which point plants were at their maximum drought tolerance (see Results). To ensure a relatively even rate of drought intensification, plants with rapid transpiration rates were periodically lightly watered in order to ensure at least a 3 week period between suspension of watering, and maximum drought. During this period gas exchange measurements were taken every one to five days depending on the desiccation rate.

### *Gas exchange.*

Fluxes of  $CO_2$  and water vapour were measured on leaves enclosed in a small cuvette attached to an open-flow gas analyser system. Selection of leaves was important, as there was a large amount of variation in photosynthesis caused by nutrient deficiencies, or age differences in the foliage of each species. Where possible, plants with mature, dark green, sun foliage were selected for measurement. An ADC mk. 3 (Hoddeson Co.) infra-red gas analyser was used to measure  $CO_2$  differentials between

cuvette and reference air, and a modified Dew-10 dew point hygrometer (General Eastern) to monitor water vapour content of pre- and post-cuvette air. Conversions from dew-point temperatures to vapour pressures in mb were made using the equation derived by Tetens (1930).

The leaf cuvette was constructed from UHDPE (ultra high density polyethylene) and glass, and all tubing was stainless steel so as to minimise adsorption of water vapour and CO<sub>2</sub>. Gas flow rates were generally 300-400 mL.min<sup>-1</sup> and the volume of the cuvette was 20 mL. At these relatively high flow rates, the boundary layer conductance of the leaves, which were generally >3 mm in width was at least one order of magnitude greater than the maximum leaf conductance.

Leaf temperature was maintained at 20±1°C by pumping water from a controlled temperature water bath (Contherm. New Zealand) through a jacket surrounding the cuvette, and was measured by a thermocouple placed in contact with the abaxial surface of the leaf. The light source was a 24V, 250W tungsten lamp which provided 1500 μmol photons.m<sup>-2</sup>.s<sup>-1</sup> to the leaf surface. Leaf area was measured using a digital camera (EDC-1000, Electrim Corp.) to determine the projected area of foliage in the chamber. Because of the rarity, slow growth rate, difficulty of propagation, and replication required, it was not feasible to remove leaves in order to measure leaf area, so projected area measurements were employed as a non-destructive alternative. Leaf area measurements were made with the leaf actually in the cuvette, ensuring orientation and positioning were maintained, and that damage to the leaf was minimised.

Air was supplied from compressed air cylinders, and the dew point of the influx air was regulated at either 0°C by passing air over water at 0°C or at around 8°C by passing air through a column of hydrated ferrous sulphate, allowing the leaf to air vapour pressure deficit to be maintained at values of 8-10 mbar.bar<sup>-1</sup>. Different partial pressures of CO<sub>2</sub> were produced by removing different proportions of CO<sub>2</sub> from the air during tank fills. In undroughted plants, the response of A to c<sub>i</sub> was measured by varying the ambient CO<sub>2</sub> concentration (c<sub>a</sub>) from 360 μmol.mol<sup>-1</sup> to 160 μmol.mol<sup>-1</sup> to give c<sub>i</sub> values within the range likely to be found in the field, and photosynthetic CO<sub>2</sub> compensation concentration was measured by recirculating air through the leaf chamber, allowing the leaf to draw down c<sub>a</sub> until net CO<sub>2</sub> uptake ceased. It was assumed that a linear regression approximated this part of the A vs. c<sub>i</sub> response (Farquhar and Sharkey, 1982), and the slopes of these regressions for each species were taken as representing the carboxylation efficiency. The maximum rate of

assimilation ( $A_{\max}$ ) for each species was taken as the average of the five highest assimilation rates at  $c_a = 360 \pm 15 \mu\text{mol.mol}^{-1}$ .

Measurements during drought were made with  $c_a$  constant at approximately atmospheric concentration ( $360 \pm 15 \mu\text{mol.mol}^{-1}$ ). As drought was intensified, assimilation rate and leaf conductance were measured regularly on the five replicates which produced the maximum rates of gas exchange when undroughted (due to shortages of material, only 2 replicates were available for *Acmopyle pancheri*, and three for *Widdringtonia cedarbergensis*). Water was withheld until the  $\text{CO}_2$  flux became negative (net gas exchange was respiratory) at which point plants were watered to avoid death.

Calculation of gas exchange parameters was made according to von Caemmerer and Farquhar (1981) with  $c_i$  corrected for water vapour efflux from the leaf.

#### *Water potential*

Measurements of leaf water potential ( $\Psi_l$ ) were carried out immediately after gas exchange readings using a Scholander pressure bomb. Due to the destructive nature of the Scholander technique (Scholander *et al.*, 1965), and the small amount of plant material available,  $\Psi_l$  could only be determined once or twice per plant. These measurements were made as  $c_i$  approached a minimum, enabling the  $\Psi_l$  to be defined at minimum  $c_i$ .

#### *Fluorescence*

In one species (*Podocarpus lawrencii*), the optimum quantum efficiency ( $F_v/F_m$ ) was measured to determine non-reversible photochemical inhibition during drought. Measurements were made on every second plant after the completion of gas exchange readings. This involved dark adapting whole plants for 3 hours (to ensure complete oxidation of the plastoquinone pool) before  $F_v/F_m$  readings were taken. Fifteen leaves from each plant were measured, using a PSM mk. 2 fluorometer (Biomonitor, Sweden). For a full description of the principle behind using fluorescence as an indicator of plant stress see chapters 5 and 6.

### *Stomatal aperture*

Stomatal aperture was measured every three days during the drought of a single *Acacia melanoxylon* plant. It was not possible to use *Eucalyptus* or any of the conifers as the sunken nature of the stomata did not allow accurate impressions to be made of the guard cells. Leaves were detached after measurement of gas exchange and epidermal impressions made by immediately coating the abaxial surface of the leaf with nail varnish (cellulose acetate). Closed stomata were easily distinguished from open or partially open stomata when impressions were examined under the light microscope, so it was possible to measure the percentage of closed stomata in leaves subject to increasing drought.

## Results

### *Variation in Photosynthesis*

A considerable amount of variation in the response of assimilation rate to  $c_i$  was expressed in all species, which appeared to be largely due to selection of leaves which were not fully mature, or plants which were nutritionally slightly deficient. Variation around the maximum carboxylation efficiency was small however, and the 'optimal carboxylation efficiency' was represented by using data from the five individuals producing the highest photosynthetic rates (Figs. 3.1, 3.2). Linear regressions were fitted to the undroughted  $A$  vs  $c_i$  data from the five maximally photosynthesizing plants of each species. These linear regressions were highly significant for all species (the lowest  $r$  value being 0.96) and the slopes of these regressions were taken to represent the optimal carboxylation efficiency (Table 3.1). Because the compensation  $CO_2$  concentration (CCP) was measured directly, the average of the measured values was used rather than taking the intercept of the  $A$  vs  $c_i$  regression, and in watered plants this ranged from  $43 \mu\text{mol.mol}^{-1}$  in *Actinostrobus*, to  $80 \mu\text{mol.mol}^{-1}$  in *Dacrydium dacrydioides*. In unstressed plants, photosynthesizing at optimal rates,  $c_i$  at an ambient  $CO_2$  concentration of  $360 \mu\text{mol.mol}^{-1}$  (approx. atmospheric), ranged from  $191 \mu\text{mol.mol}^{-1}$  in *Podocarpus drouynianus* to  $258 \mu\text{mol.mol}^{-1}$  in *Acropyle pancheri*.



Table 3.1. Physiological and ecological descriptions for the 12 conifer species included in this chapter. The parameters; carboxylation efficiency, CO<sub>2</sub> compensation concentration (CCP), A<sub>max</sub> and c<sub>i</sub>/c<sub>a</sub> at c<sub>a</sub> = 360 μmol.mol<sup>-1</sup>, were all measured on unstressed plants, with values representing averages from at least 3 replicates. The other parameters; (c<sub>i</sub>/c<sub>a</sub>)<sub>min</sub> and Ψ<sub>l</sub> at (c<sub>i</sub>/c<sub>a</sub>)<sub>min</sub> were measured in droughted plants. (c<sub>i</sub>/c<sub>a</sub>)<sub>min</sub> represents the average value from 3-5 replicates, and the water potential reading taken closest to (c<sub>i</sub>/c<sub>a</sub>)<sub>min</sub> was used as Ψ<sub>l</sub> at (c<sub>i</sub>/c<sub>a</sub>)<sub>min</sub>.

Species	Habitat <sup>a</sup>	Carbox. efficiency	CCP μmol/mol	A <sub>max</sub> μmol/m <sup>2</sup> /s	c <sub>i</sub> /c <sub>a</sub> at c <sub>a</sub> = 360 μmol/mol	(c <sub>i</sub> /c <sub>a</sub> ) <sub>min</sub>	Ψ <sub>l</sub> at (c <sub>i</sub> /c <sub>a</sub> ) <sub>min</sub> MPa
<i>Actinostrobus acuminatus</i>	Dry sandy heathland	0.200	43	39.8 ± 0.9	0.63 ± 0.002	0.11 ± 0.007	-4.50
<i>Widdringtonia cedarbergensis</i>	Dry heathland	0.192	51	25.3 ± 0.5	0.54 ± 0.009	0.16 ± 0.008	-3.50
<i>Podocarpus drouynianus</i>	Open woodland	0.147	53	15.8 ± 0.6	0.53 ± 0.009	0.19 ± 0.003	-3.00
<i>Callitris rhomboidea</i>	Open woodland	0.199	55	27.5 ± 0.3	0.68 ± 0.002	0.21 ± 0.008	-3.20
<i>Podocarpus lawrencii</i>	Wet forest - alpine forest	0.143	53	22.9 ± 0.3	0.59 ± 0.012	0.23 ± 0.011	-3.00
<i>Diselma archerii</i>	Wet montane - alpine forest	0.182	60	19.1 ± 0.2	0.57 ± 0.014	0.26 ± 0.005	-2.90
<i>Microstrobos niphophilus</i>	Wet alpine forest	0.172	60	20.7 ± 0.4	0.60 ± 0.010	0.25 ± 0.004	-2.70
<i>Athrotaxis selaginoides</i>	Cool-temperate rainforest	0.158	60	20.0 ± 0.3	0.66 ± 0.008	0.27 ± 0.011	-2.10
<i>Lagarostrobos franklinii</i>	Cool-temperate rainforest	0.117	73	16.8 ± 0.2	0.65 ± 0.007	0.31 ± 0.013	-2.30
<i>Dacrycarpus dacrydioides</i>	Cool-temperate rainforest	0.109	80	17.1 ± 0.4	0.68 ± 0.006	0.34 ± 0.005	-1.90
<i>Dacrycarpus compactus</i>	Tropical sub-alpine	0.096	63	16.7 ± 0.2	0.65 ± 0.009	0.35 ± 0.009	-1.60
<i>Acropyle pancheri</i>	Tropical rainforest	0.093	67	13.4 ± 0.9	0.67 ± 0.020	0.36 ± 0.0	-1.75

<sup>a</sup> Habitat descriptions from Enright and Hill (1995)

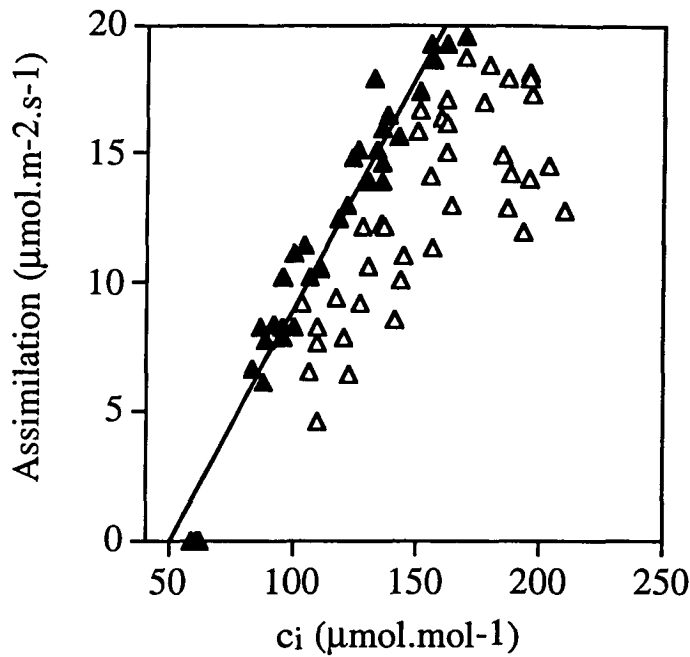


Figure 3.1. The response of net. assimilation rate ( $A$ ) to changes in the internal  $\text{CO}_2$  concentration ( $c_i$ ) in *Diselma archeri* -  $\Delta$ . Changes in  $c_i$  resulted from varying the ambient  $\text{CO}_2$  concentration between the  $\text{CO}_2$  compensation point (CCP) and ambient atmospheric concentration. Optimal Carboxylation efficiency was taken as the linear regression through data from the 5 individuals producing the highest rates of  $A$  -  $\blacktriangle$ .

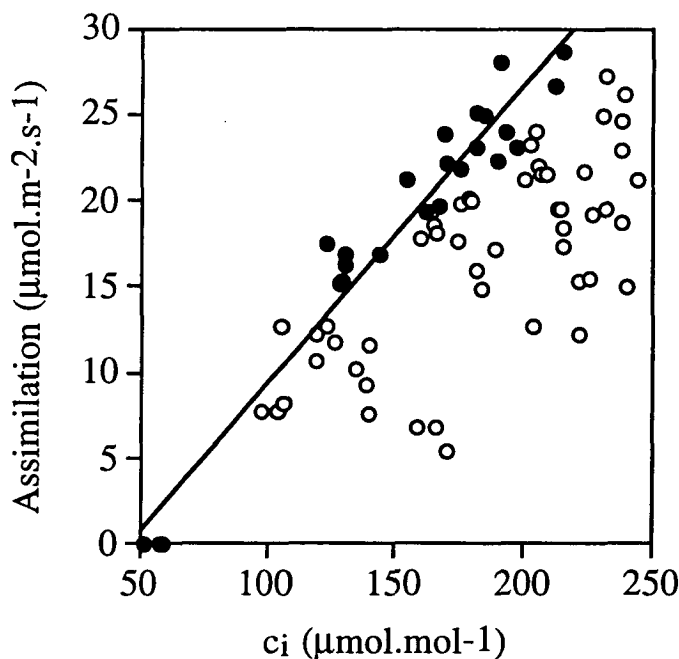


Figure 3.2.  $A$  vs.  $c_i$  response for *Callitris rhomboidea* -  $\circ$ , again the 5 individuals producing the highest maximum rates of assimilation were used in the carboxylation efficiency regression -  $\bullet$ .

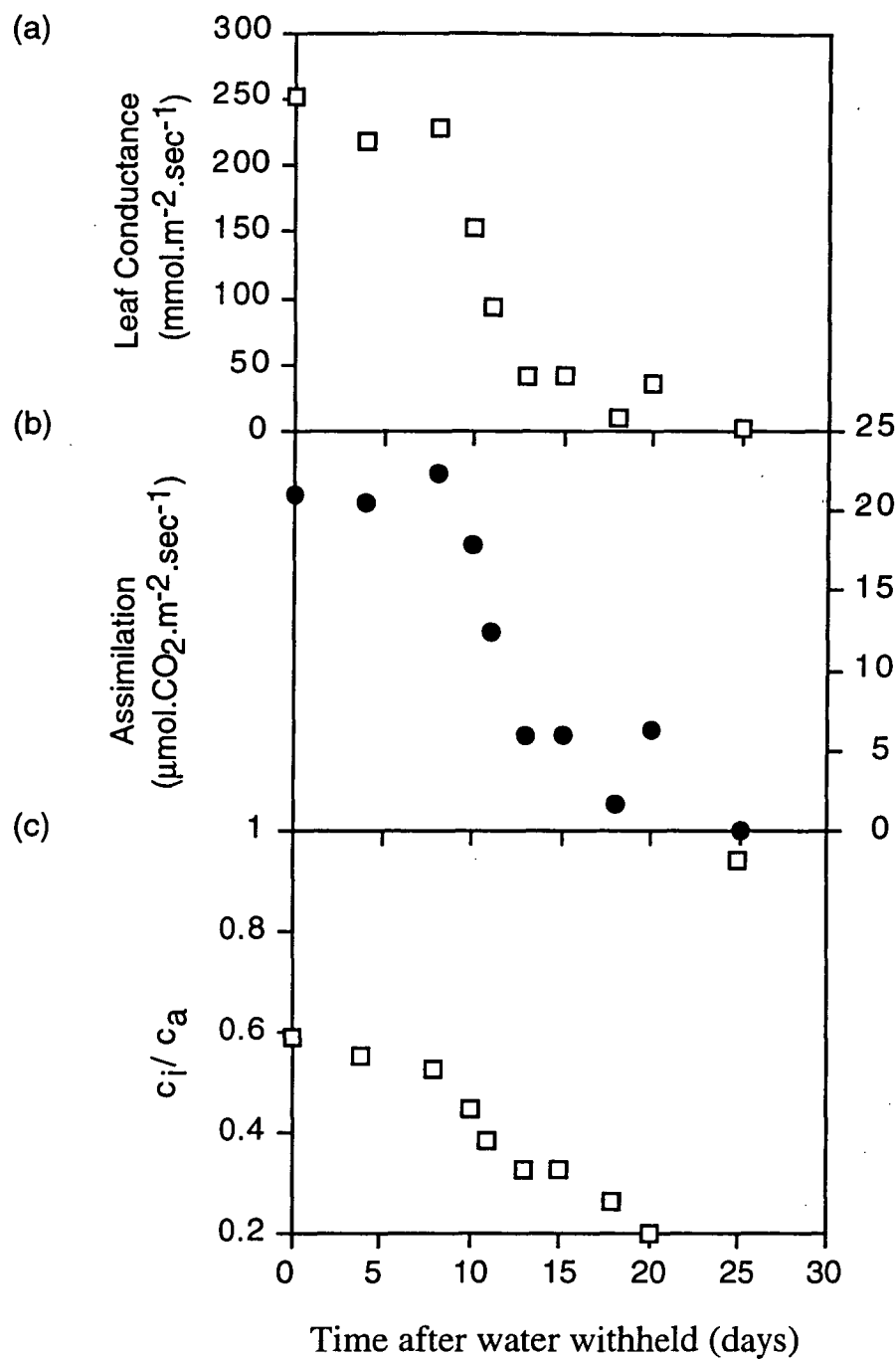


Figure 3.3. Decreasing- (a) conductance ( $g_s$ ), (b) assimilation ( $A$ ) and (c) ratio of internal to atmospheric  $\text{CO}_2$  concentration ( $c_i/c_a$ ) in a single replicate of *Podocarpus lawrencii* during droughting. Minimum  $g_s$  in plants was generally reached 25-30 days after water was withheld.

The range of carboxylation efficiencies, and maximum assimilation rates among the 12 conifer species examined here was large (Table 3.1), with the dry forest Cupressaceae genera such as *Actinostrobus*, *Callitris*, and *Widdringtonia*, producing the highest rates of assimilation (39.8, 27.5 and 25.3  $\mu\text{mol.m}^{-2}.\text{sec}^{-1}$  respectively) and the podocarps, *Podocarpus drouyanus*, and *Dacrycarpus compactus*, producing the lowest (15.8 and 16.7  $\mu\text{mol.m}^{-2}.\text{sec}^{-1}$  respectively).

Once water was withheld, all species responded with a reduction in  $g_s$  and  $A$  as soil water content decreased (Fig. 3.3). The rate of water loss was modulated in species which were prone to faster drying, by very light watering of the soil, resulting in a relatively even rate of soil drying for all species. The dynamics of  $c_i$ ,  $A$  and  $g_s$  during desiccation were similar for each species, with a rapid decline in gas exchange and  $c_i$  in the first days of water stress, followed by a more gradual decrease of  $c_i$  to minimum values of between 126  $\mu\text{mol.mol}^{-1}$  ( $c_i/c_a = 0.35$ ) in *Acmopyle* and 42  $\mu\text{mol.mol}^{-1}$  ( $c_i/c_a = 0.11$ ) in *Actinostrobus*. Figure 3.3 shows a typical example of decreasing  $c_i$ ,  $A$  and  $g_s$  during drought of *Podocarpus*.  $c_i/c_a$  is expressed in preference to  $c_i$  as it accounts for small variations in  $c_a$ . In most species, minimum values of  $c_i$  were typically reached after 20-28 days without water, at which point  $\Psi_1$  ranged from -1.75 MPa in *Acmopyle* to -4.5 MPa in *Actinostrobus*.

As  $c_i$  was forced down by reduced  $g_s$  in droughted plants, the relationship between  $A$  and  $c_i$  was maintained at the same level as that of unstressed plants up until  $c_i$  reached a minimum point. This minimum value was consistent in all replicates of a species (Figs 3.4, 3.5). In plants droughted beyond this minimum level there was a significant deviation from the optimal carboxylation efficiency expressed in undroughted individuals (Figs 3.4, 3.5). This indicates that during most of the drought period non-stomatal limitation of photosynthesis was negligible, and that only when  $A$  and  $g$  reached very low levels did non-stomatal limitation become important. Typical responses are shown for *Podocarpus lawrencii* and *Callitris rhomboidea* in figures 3.4 and 3.5 where  $c_i$  approaches, and then exceeds  $c_a$  as assimilation approaches zero. This rapid increase in non-stomatal limitation occurred when  $\Psi_1$  fell below the critical value measured at minimum  $c_i$  (around -3.0 MPa for *Podocarpus lawrencii* and -3.2 MPa in *Callitris rhomboidea*).

Figure 3.6 shows the response of  $c_i/c_a$  to decreasing  $g_s$  during drought in three representative species with contrasting values of ( $c_i/c_a$ ).

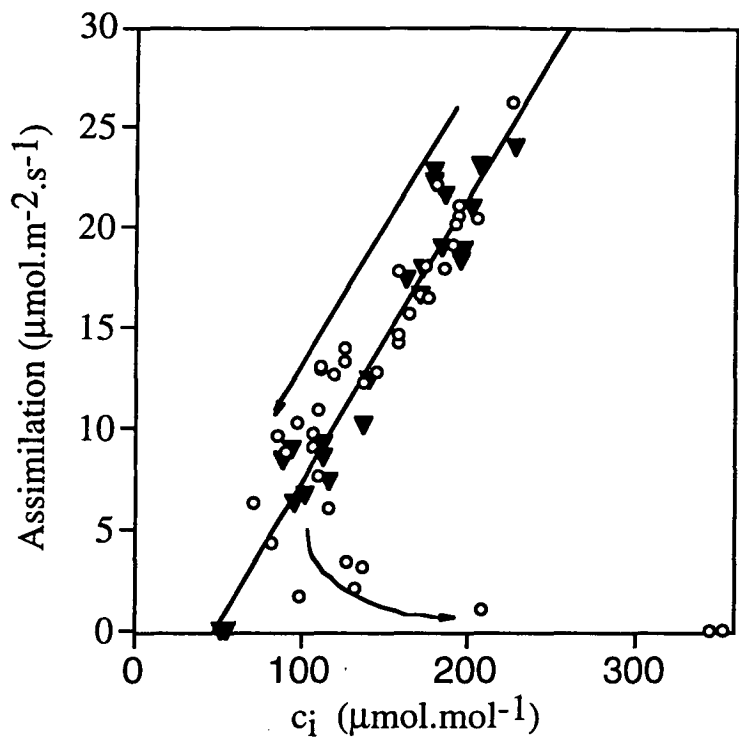


Figure 3.4. The effect of decreasing  $g_s$  in response to drought on  $c_i$  and  $A$  in *Podocarpus lawrencii*- ○. Arrows indicate the progression of  $A$  and  $c_i$  as drought was gradually intensified. Also shown is the  $A$  vs  $c_i$  relationship for undroughted plants- ▼ , and the carboxylation efficiency regression.

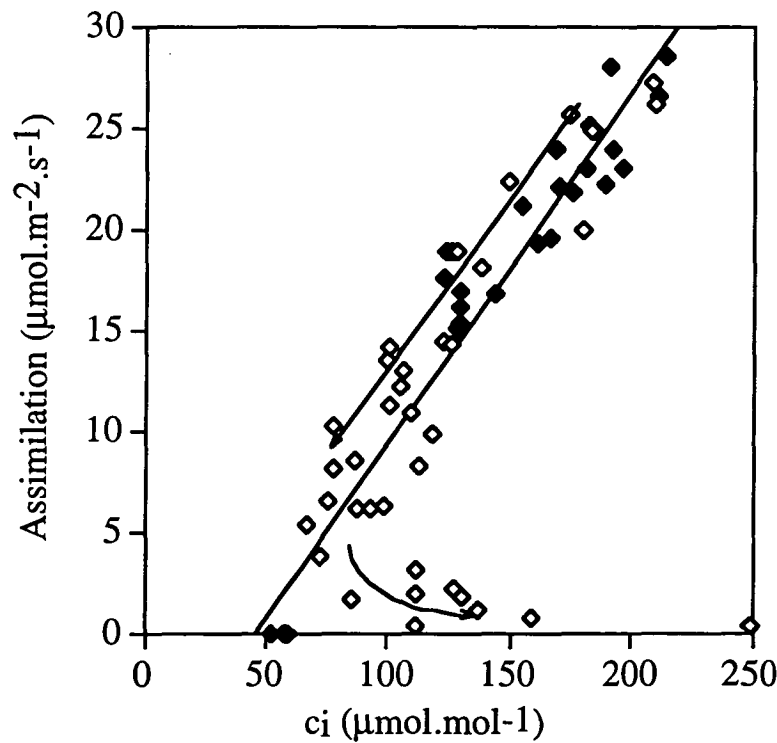


Figure 3.5.  $A$  vs  $c_i$  as in fig. 3.4, for droughted - ◇ , and undroughted- ◆ *Callitris rhomboidea*.



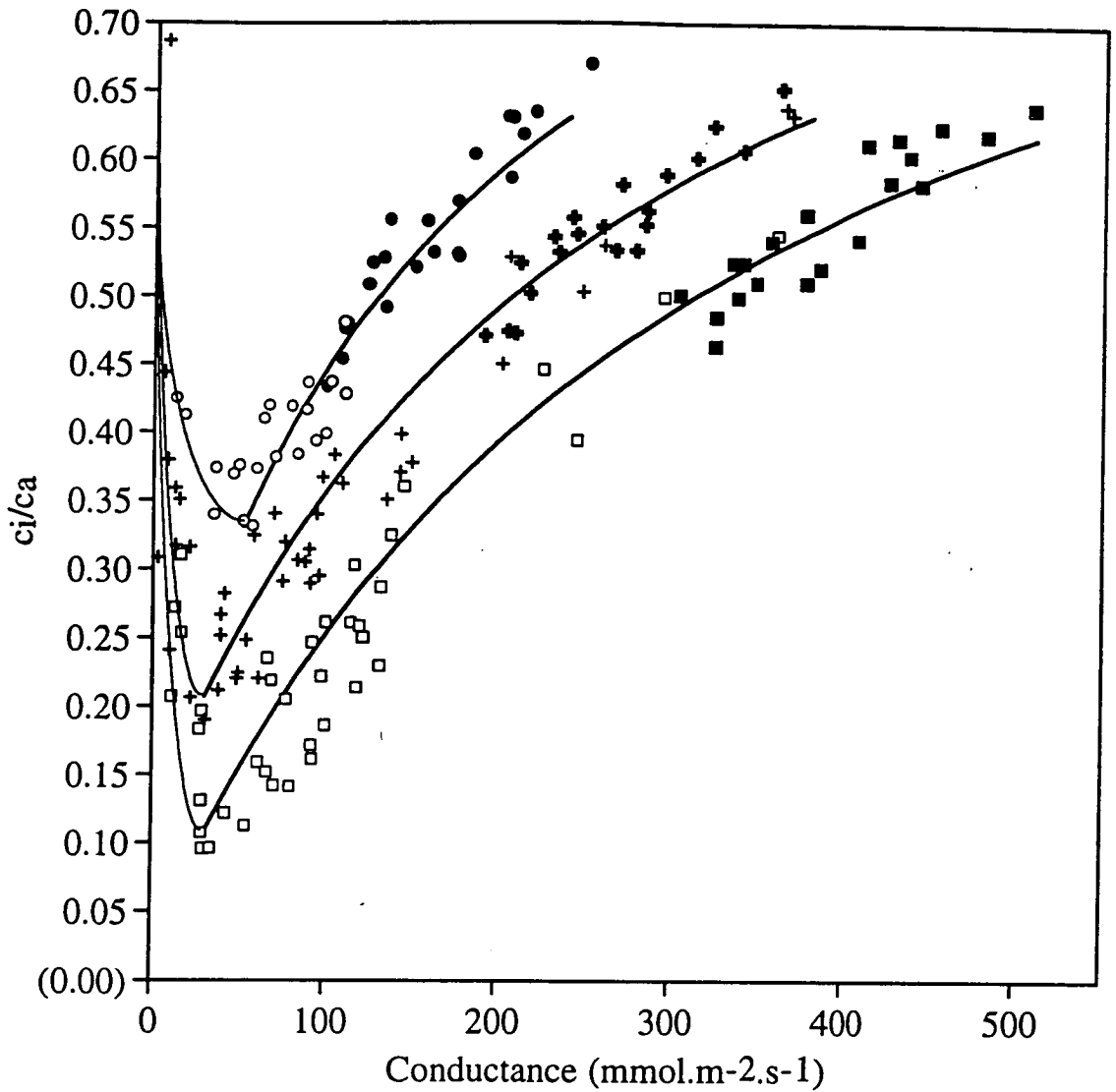


Figure 3.6. Decreasing  $c_i/c_a$  in three species, *Dacrycarpus compactus*-  $\circ$  *Callitris rhomboidea*-  $+$  and *Actinostrobus acuminatus*-  $\square$  subject to gradually intensified drought. Also shown are data for undroughted plants from each species-  $\bullet$ ,  $+$ ,  $\blacksquare$ , respectively. The curves drawn represent the modelled responses of  $c_i/c_a$  to  $g_s$  if decreases in  $A$  were purely a function of decreasing  $g_s$  (*i.e.* non-stomatal limitation of  $A$  was minimal). These curves were calculated using the carboxylation efficiency regressions for each species. During drought, in each species the  $c_i/c_a$  response closely followed the theoretical curve, illustrating that  $A$  is primarily controlled by reduced  $g_s$ .  $c_i/c_a$  decreased until reaching a minimum value  $(c_i/c_a)_{\min}$  after which a rapid increase was observed.

The bold curves shown in figure 3.6 represent the expected response if assimilation was only limited by  $g_s$ , i.e. non-stomatal factors were insignificant. These model values are derived from the carboxylation efficiency equations for undroughted plants (e.g. Figs 3.1, 3.2) and are only continued to the point of minimum  $c_i/c_a$  ( $(c_i/c_a)_{\min}$ ). The y-intercepts for these curves are the  $c_i/c_a$  at the compensation  $\text{CO}_2$  concentration. Points lying at increasing distances above these curves reflect an increasing component of non-stomatal limitation. The faint lines represent an interpolation through the points falling below  $(c_i/c_a)_{\min}$  and indicate the progression of  $c_i/c_a$  during the final and most intense period of drought. Two distinct phases were apparent in the drought response of all plants, an initial stomatal limitation phase which occurred between the optimal  $c_i/c_a$  and  $(c_i/c_a)_{\min}$  and a non-stomatal limitation phase which affected leaves droughted beyond the  $(c_i/c_a)_{\min}$ . The initial phase of drought response followed the model curve closely in each species, although in *Actinostrobus*, the droughted data appeared to produce systematically lower values of  $c_i/c_a$  than expected, suggesting an increase in carboxylation efficiency in drought stressed leaves (Fig. 3.6). The point of minimum  $c_i/c_a$  was well defined, and averages (calculated from 5 replicates) were accompanied by low standard errors (Table 3.1). Leaf conductances at  $(c_i/c_a)_{\min}$ , despite large differences in maximum  $g_s$ , were very similar, ranging from  $30 \text{ mmol.m}^{-2}.\text{sec}^{-1}$  in several genera (*Actinostrobus* and *Callitris*) to  $52 \text{ mmol.m}^{-2}.\text{sec}^{-1}$  in *Dacrycarpus compactus*. The second phase of the drought response in all species was a rapid increase in  $c_i/c_a$  as conductance fell below the  $(c_i/c_a)_{\min}$  level. Values rapidly increased to a  $c_i/c_a$  of around one, but due to the production of a net respiratory loss of  $\text{CO}_2$  there was no well defined y intercept at 1.  $c_i/c_a$  continued to increase above 1 as respiration became progressively more significant.

In all plants droughted beyond  $(c_i/c_a)_{\min}$  rewatering generally saw an increase in photosynthetic rate, but assimilation rates did not recover to pre-stress levels. New foliage produced after rewatering was, in most cases, found to be equivalent in photosynthetic characteristics to leaves measured prior to the imposition of water stress.

#### *Fluorescence in Podocarpus lawrencii*

Changes in the characteristics of the average quantum efficiency ( $F_v/F_m$ ) of *P. lawrencii* indicated a link between the photochemical processes in

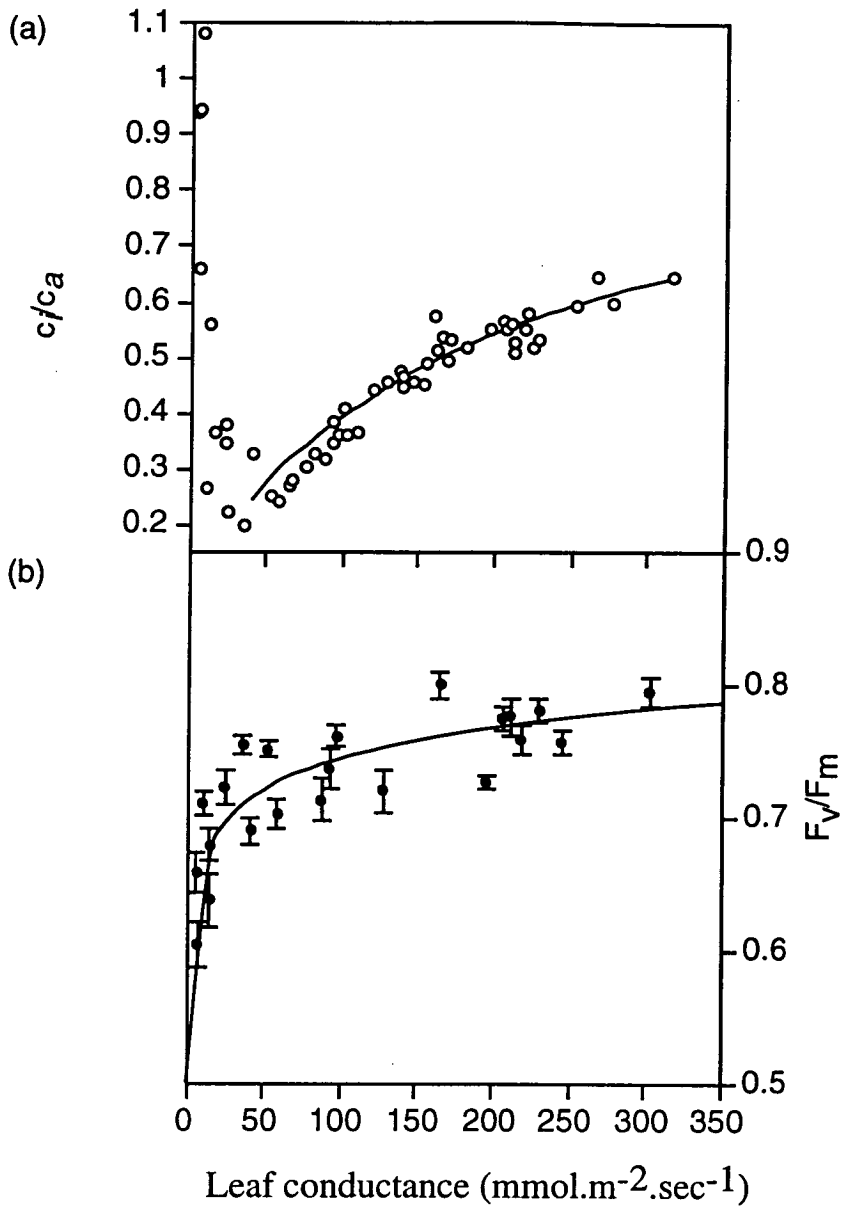


Figure 3.7. Changing ratios of (a)  $c_i/c_a$  and (b)  $F_v/F_m$  as conductance ( $g_s$ ) declined during drought in *Podocarpus lawrencii*. Each value of  $F_v/F_m$  represents the average of 15 leaves. A log function has been used to fit the  $F_v/F_m$  data ( $r^2 = 0.696$ ). As in figure 3.6,  $c_i/c_a$  decreases logarithmically before reaching a minimum value,  $(c_i/c_a)_{\min}$ , increasing rapidly thereafter. The curve through  $c_i/c_a$  represents a theoretical response calculated from the carboxylation efficiency.

leaves during drought, and changes in  $c_i/c_a$  (Fig. 3.7). Values of  $F_v/F_m$  in unstressed plants was found to be  $0.78 \pm .008$ , falling within the normal range for unstressed plants (Björkman and Demmig, 1987), and during the initial phase of droughting as  $g_s$  dropped from 320 to 45  $\text{mmol.m}^{-2}.\text{s}^{-1}$ ,  $F_v/F_m$  decreased only slightly. Below this  $g_s$  however,  $F_v/F_m$  was found to fall rapidly to values below 0.70 (Fig. 3.7). In all plants where the average  $F_v/F_m$  fell below 0.7 leaves became necrotic or were shed after rewatering.

#### *Water potential and $(c_i/c_a)_{\min}$*

A strong linear correlation ( $r = 0.96$ ) was found between  $(c_i/c_a)_{\min}$  and the water potential measured at  $(c_i/c_a)_{\min}$  (Fig. 3.8). No standard error bars are shown for  $\Psi_1$  at  $(c_i/c_a)_{\min}$  as this number represented the single  $\Psi_1$  reading which most closely corresponded to  $(c_i/c_a)_{\min}$  in each species. Species which developed low  $(c_i/c_a)_{\min}$  during droughting did so at low water potentials, so the  $\Psi_1$  of *Actinostrobilus* leaves at a  $c_i/c_a$  of 0.10, was -4.5 MPa, whilst at the other end of the scale,  $c_i/c_a$  in leaves of *Dacrycarpus compactus* began to increase below a  $\Psi_1$  of -1.6 MPa.

#### *"Broad-leaved" angiosperms*

In contrast to the conifers, both *Acacia melanoxylon* and *Eucalyptus tenuiramis* showed only a minor decrease in  $c_i/c_a$  during the drought period. There was, however, a similar rapid increase in  $c_i/c_a$  as  $g_s$  approached zero. As in figure 3.6, the curves in figures 3.9 and 3.10 represent the expected response if decreasing  $A$  was purely a function of  $g_s$  (insignificant non-stomatal component). The early divergence from this curve as  $g_s$  decreased would typically be interpreted as indicating an increase in non-stomatal limitation during the early stages of drought.

Despite a general decrease in stomatal aperture as leaves were affected by drought, closed and open stomata were easily distinguished in stomatal impressions from *A. melanoxylon* (Brodribb, 1991). This allowed the percentage of open and fully closed stomata to be determined in leaves subject to gradually intensified drought. A significant proportion of stomata were found to close early during the drought of a single *A. melanoxylon* replicate, with approximately 50% of stomata found to be closed at approximately 60%  $g_s$  (Fig. 3.9). The proportion of closed stomata increased in a roughly linear manner with respect to  $g_s$ .

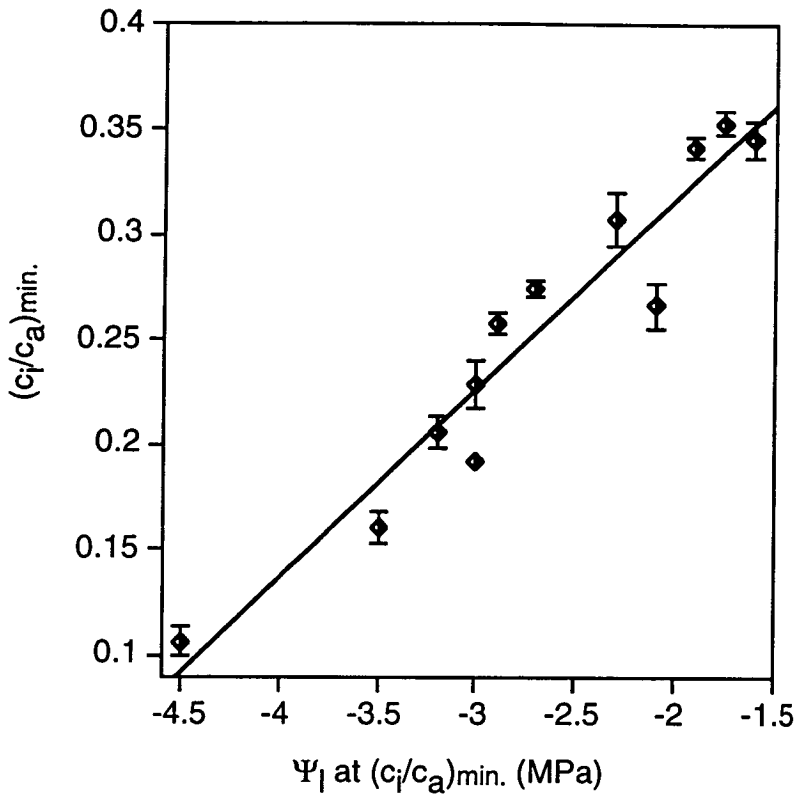


Figure 3.8. Minimum  $c_i/c_a$  for the 12 species examined was strongly correlated with the leaf water potential measured at  $(c_i/c_a)_{\min}$ . A highly significant linear regression ( $y=0.09x + 0.495$ ) is shown ( $r=0.959$ ). The standard error in  $(c_i/c_a)_{\min}$  comes from three to five replicates.



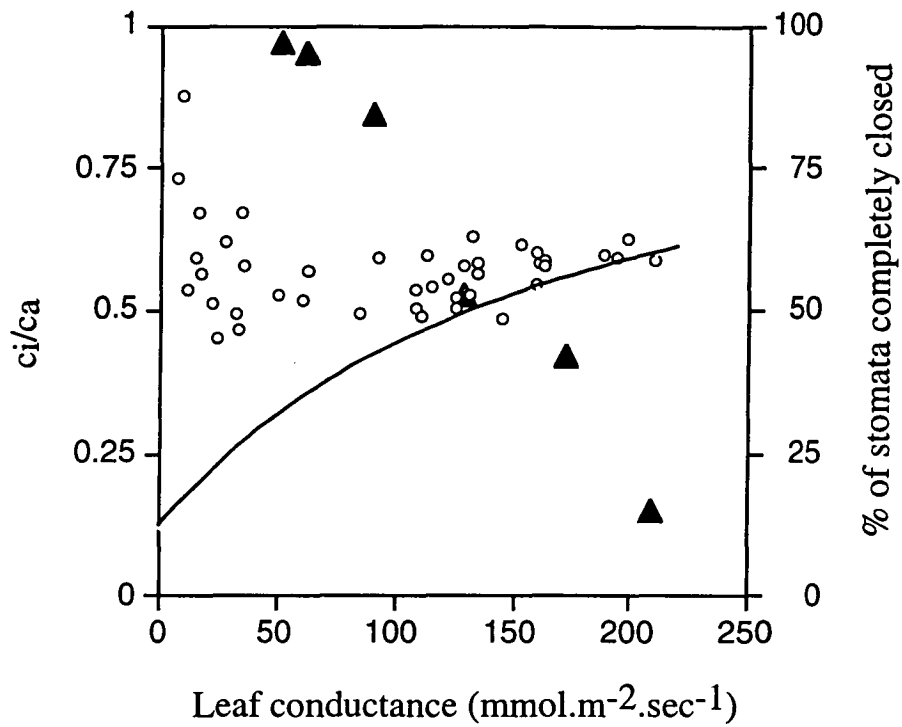


Figure 3.9. Response of  $c_i/c_a$  to decreasing  $g_s$  during drought in *Acacia melanoxylon* -  $\circ$ . The curve shown represents the theoretical response if decreasing  $g_s$  explained the decline in photosynthesis *cf.* fig. 3.6. Also shown is the percentage of stomata seen to be completely closed at 6 times during drought intensification in a single *A. melanoxylon* plant -  $\blacktriangle$

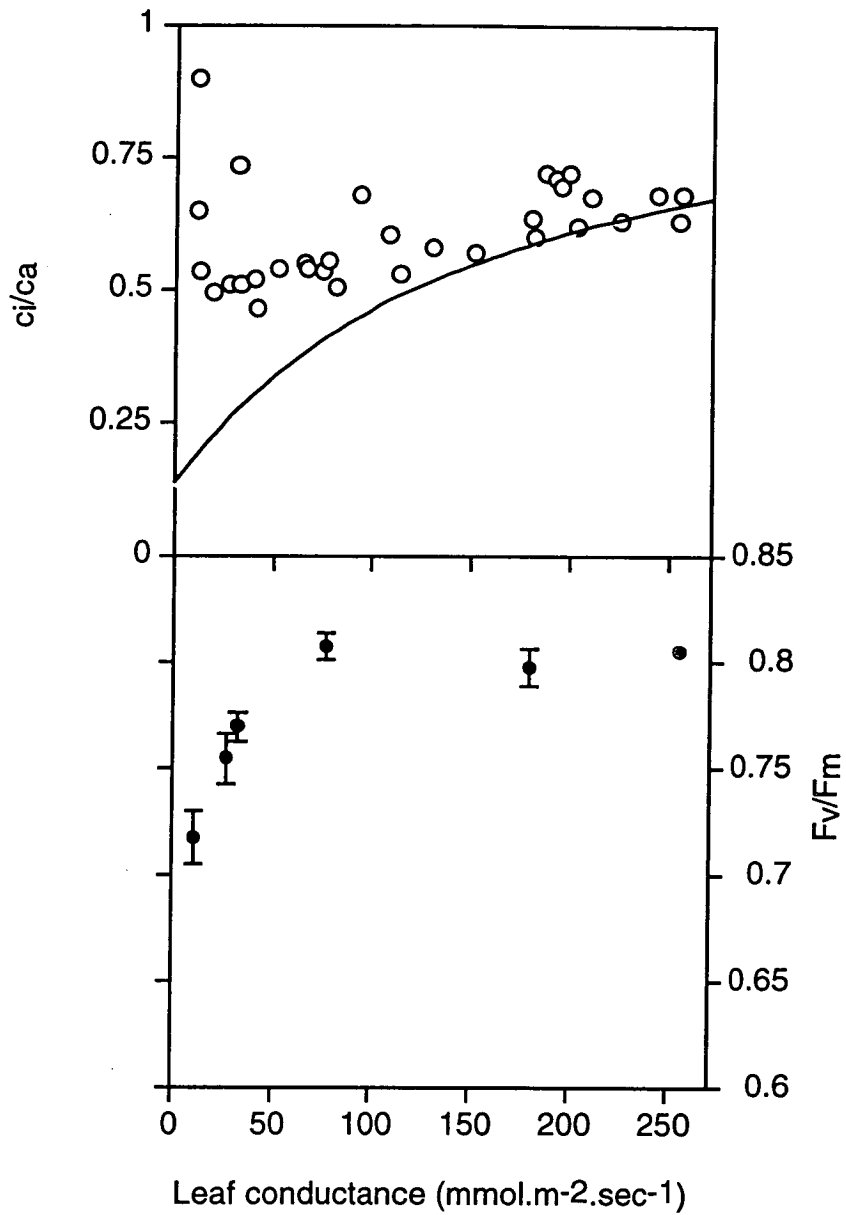


Figure 3.10. Drought response of  $c_i/c_a$  and  $g_s$  in *Eucalyptus tenuiramis*. Again the curve drawn represents the theoretical response if decreasing photosynthesis was stomataly driven. Also shown are  $F_v/F_m$  data for a single replicate during drought.

Changes in  $F_v/F_m$  in *E. tenuiramis* during drought were similar to those seen in *Podocarpus lawrencii* with no significant decrease in optimal quantum yield until  $g_s$  fell below about  $70 \text{ mmol.m}^{-2}\text{.sec}^{-1}$ , at which point a pronounced depression in quantum efficiency was found to occur (Fig. 3.10). Like *Podocarpus*, the rapid decrease in  $F_v/F_m$  was accompanied by a similar increase in  $c_i/c_a$ ; however the apparent increase in non-stomatal limitation suggested by the maintenance of a relatively constant  $c_i/c_a$ , early in the drought response, was not confirmed by a concomitant decrease in  $F_v/F_m$ .

The water potential of leaves immediately prior to the rapid increase in  $c_i/c_a$  was  $-2.9$  and  $-3.20 \text{ MPa}$  respectively in *A. melanoxylon* and *E. tenuiramis*.

## Discussion

The application of area based gas exchange measurements as a means of quantifying the drought response of multi-veined, broad-leaved plants is complicated by the effects of non-uniform stomatal closure. This phenomenon was not found to significantly affect the leaves of southern hemisphere conifers investigated here presumably as a result of their typical narrow, single vein leaf morphology, and the gradually intensified drought regime applied. This minimisation of patchy stomatal closure and photosynthetic heterogeneity, adds a high degree of confidence to the  $c_i$  calculations presented here. In contrast to previous studies (Wong *et al.*, 1985), large variations in  $c_i$  were found to occur during drought, with an initial draw-down of  $c_i$  to between 25 and 55% of unstressed levels, followed by a rapid increase as leaf water potential fell below a critical level. The magnitude of this initial reduction in  $c_i$  provides an excellent measure of the drought sensitivity of the leaf, and plant in question.

During the initial "draw-down" phase there was no evidence of non-stomatal limitation in any of the conifers studied. This is illustrated for *Podocarpus lawrencii* and *Callitris rhomboidea* in figures 3.4 and 3.5, the data showing no systematic deviation from the theoretical curve produced if photosynthesis were responding only to increasing stomatal limitation of gas exchange (insignificant non-stomatal component). Non-stomatal factors did, however, become important in plants droughted beyond  $(c_i/c_a)_{\min}$ , producing a sharp increase in  $c_i/c_a$  as  $g_s$  approached zero (Fig. 3.6). The fluorescence data for *P. lawrencii* also illustrate these

two phases in drought response. Initially  $F_v/F_m$  was found to slowly decline in parallel with  $c_i/c_a$ , presumably as a result of increased non-photochemical energy dissipation as Calvin cycle activity was inhibited by limiting  $\text{CO}_2$  concentration in the chloroplast (Cornic *et al.*, 1989; Cornic and Briantais, 1991). As  $g_s$  approached zero and  $c_i/c_a$  began to increase there was a concomitant steep drop in  $F_v/F_m$  (Fig. 3.7). This indicates a rapid loss of fluorescence from PS II for some reason other than  $\text{CO}_2$  limitation and may represent direct inhibition of photochemistry, Calvin cycle activity, or both. The most likely explanation for the rapid onset of photoinhibition is that plants droughted beyond  $(c_i/c_a)_{\min}$  sustained damage to the photosynthetic apparatus. This damage was not linked to the loss of cell turgor, as genera which produced leaves soft enough to wilt visibly (*e.g.* *Widdringtonia* and *Callitris*) were noted to wilt substantially before attaining  $(c_i/c_a)_{\min}$ . In *Widdringtonia cedarbergensis*, plants were severely wilted at a  $\Psi_l$  of -2 MPa, whereas  $(c_i/c_a)_{\min}$  was not reached until -3.5 MPa. Plants droughted beyond  $(c_i/c_a)_{\min}$  did not recover pre-drought photosynthetic rates (unpublished data), and after 2-4 days continued drought also showed visual signs of leaf damage upon rewatering. Leaf water potential measured at  $(c_i/c_a)_{\min}$  therefore represents the minimum  $\Psi_l$  prior to the onset of irreversible photoinhibition. Values of  $\Psi_l$  at  $(c_i/c_a)_{\min}$  ranged widely from -1.75 MPa to -4.5 MPa, reflecting the wide range of habitats from which different species were collected. In comparison, initial cell damage in rapidly desiccated *Helianthus* leaves was found to occur at a  $\Psi_l$  of -1.5 MPa, with the majority of mesophyll cells damaged at -2.6 MPa (Fellows and Boyer, 1978). The leaves of the conifer species used here were clearly more robust than those of *Helianthus*, a fact which is hardly surprising considering the perennial woody nature of these species.

The dynamic response described for the narrow-leaved conifers was not found to occur in the broad-leaved angiosperm species used here. Both species exhibited relatively constant  $c_i/c_a$  during drought, as described in many other studies (Wong *et al.*, 1985; Anderson *et al.*, 1995). This type of response has classically been interpreted as showing a gradual increase in non-stomatal limitation of photosynthesis during drought. The data presented here do not, however, show any evidence for non-stomatal inhibition in *Eucalyptus tenuiramis* until leaf conductance had declined to around 25% of the level found in undroughted plants (Fig. 3.10), indicating that the leaves had responded to drought well before non-reversible photoinhibition became significant.

The fact that both species are relatively drought tolerant, with *Acacia melanoxylon* foliage found to withstand  $\Psi_1$  of around -3.5 MPa (Brodribb and Hill, 1993) and *Eucalyptus tenuiramis* capable of surviving soil water potentials in the field of around -2.9 MPa without leaf damage (M. Battaglia and N. Davidson, pers. comm.), also suggests it is unlikely that direct inhibition of photosynthesis should have occurred soon after water was withheld. Patchy stomatal closure provides a better explanation for the responses illustrated in figures 3.9 and 3.10, and this is confirmed for *A. melanoxylon* where the number of stomata found to be fully closed increased rapidly with the onset of drought (Fig 3.9). This type of pre-emptive stomatal closure may be explained by fluxes of ABA which are released from the roots and translocated in the xylem (Khalil and Grace, 1993; Jackson *et al.*, 1995), as it has been shown that initial translocation of ABA occurs at leaf water potentials equivalent to only mild drought (Correia and Pereira, 1994).

At some stage during drought it is inevitable that direct inhibition of photosynthesis will occur either by physical effects, such as turgor loss or cell damage, or by loss of chemical function in the chloroplasts. The important question is whether non-stomatal inhibition occurs early during drought, depressing photosynthesis while significant rates of gas exchange are taking place, or whether this effect occurs only in highly stressed plants, as  $g_s$  approaches a minimum. All narrow-leaved conifers in this study exhibited the latter response to drought, resulting in substantial reductions in  $c_i/c_a$  prior to any expression of non-stomatal inhibition. This result supports  $^{13}\text{C}$  discrimination and fluorescence data, indicating a dynamic response of  $c_i/c_a$  to drought, and a minimal non-stomatal influence on assimilation until plants are subject to a severe water stress.

Being a direct function of the ratio of  $A/g_s$ ,  $c_i/c_a$  is inversely proportional to the 'intrinsic' water use efficiency of photosynthesis, and hence  $((c_i/c_a)_{\min})^{-1}$  represents the maximum water use efficiency attainable during drought. Intuitively this seems a highly appropriate index by which to quantify the drought tolerance of photosynthesis, and this suggestion is strongly supported by the fact that species producing high maximum water use efficiencies, or low  $(c_i/c_a)_{\min}$  did so at low leaf water potentials. The highly significant correlation between  $(c_i/c_a)_{\min}$  and  $\Psi_1$  at  $(c_i/c_a)_{\min}$  (Fig. 3.8) indicates that, at the leaf level at least, the magnitude of  $(c_i/c_a)_{\min}$  is indeed inversely proportional to drought tolerance.



Variation in  $(c_i/c_a)_{\min}$  appeared to be a function of both the carboxylation efficiency, and the  $\text{CO}_2$  compensation concentration, and both displayed significant linear correlations with  $(c_i/c_a)_{\min}$  ( $r = -0.86$  and  $-0.88$  respectively). Very little variation was expressed in either the  $g_s$  at  $(c_i/c_a)_{\min}$  or the minimum  $g_s$  measured in each species, indicating that there was no significant relationship between drought tolerance, and the facility for stomatal closure. The passage of decreasing  $g_s$  was the same for each species, and the nature of the drought response was, therefore, wholly shaped by the carboxylation characteristics of the leaf. The carboxylation efficiency determines the rate of decrease of  $c_i/c_a$  in response to reduced  $g_s$ , and the  $\text{CO}_2$  compensation point theoretically places a limit on minimum  $c_i/c_a$ , by defining the intercellular  $\text{CO}_2$  concentration expected with stomata closed. Carboxylation efficiency is directly related to the concentration and activation of Rubisco in the leaf (Farquhar and Sharkey, 1982) and therefore it appears that the concentration of Rubisco (per unit silhouette area) in the leaves of these species, gives an indication of the drought tolerance of foliage. Obviously the amount of Rubisco per unit area in the leaf is largely proportional to the amount of leaf mass per unit area (SLA) and the possibility arises that differences in Rubisco concentration, and thus carboxylation efficiency may be due to morphological differences related to drought tolerance. In angiosperms the relationship between xeromorphy and high SLA is well known (see Fahn, 1974); however this is clearly not the explanation for variation in the drought response in conifers here, as there was no relationship between SLA and either carboxylation efficiency, or  $(c_i/c_a)_{\min}$  ( $r = 0.02$  and  $0.2$  respectively). Differences in carboxylation efficiency must therefore be related to anatomical differences in the leaves, with drought tolerant species producing a denser mesophyll area than drought intolerant species. Transverse sections through the leaves of drought tolerant *Actinostrobus acuminatus*, and drought intolerant *Lagarostrobos franklinii*, confirmed this (Fig. 3.11, also Fig. 7.9). The thickness and density of the palisade mesophyll in sun-grown *Actinostrobus* was substantially higher than that of *Lagarostrobos* grown under identical conditions.

The association between tolerance of water deficit and high maximum assimilation rate is illustrated in figure 3.12. Clearly species which came from dry forest produced higher assimilation rates than rainforest species. Why this is so is probably linked to the conditions under which maximum photosynthesis takes place in the dry and wet

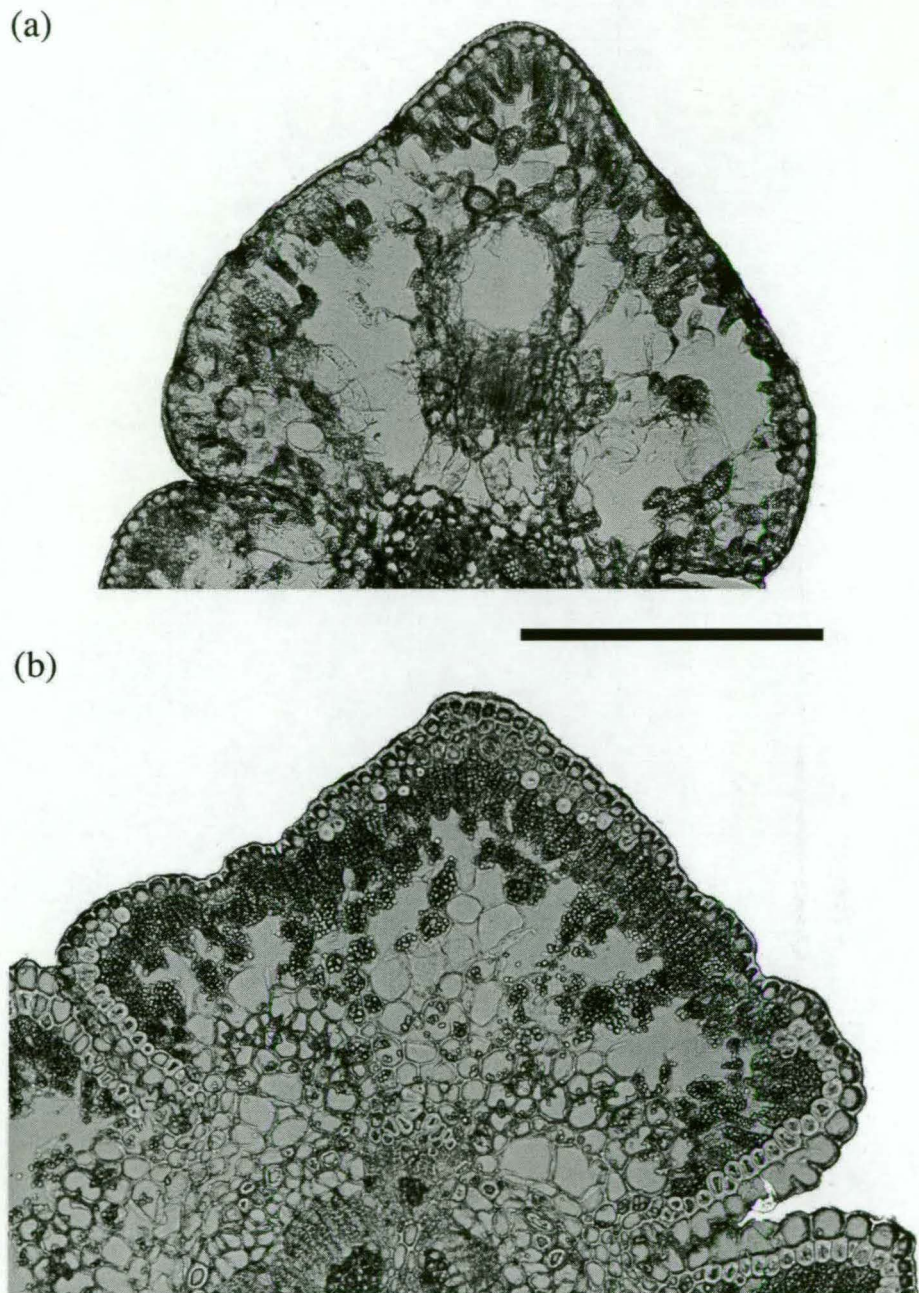


Figure 3.11. Transverse sections of leaves of (a)- *Lagarostrobos franklinii* and (b)- *Actinostrobus acuminatus*. Palisade tissue is well developed in *Actinostrobus acuminatus* compared with the poorly differentiated mesophyll in *Lagarostrobos franklinii*. Both specimens were grown under identical, fully exposed, glasshouse conditions. (Scale bar = 500  $\mu\text{m}$ )

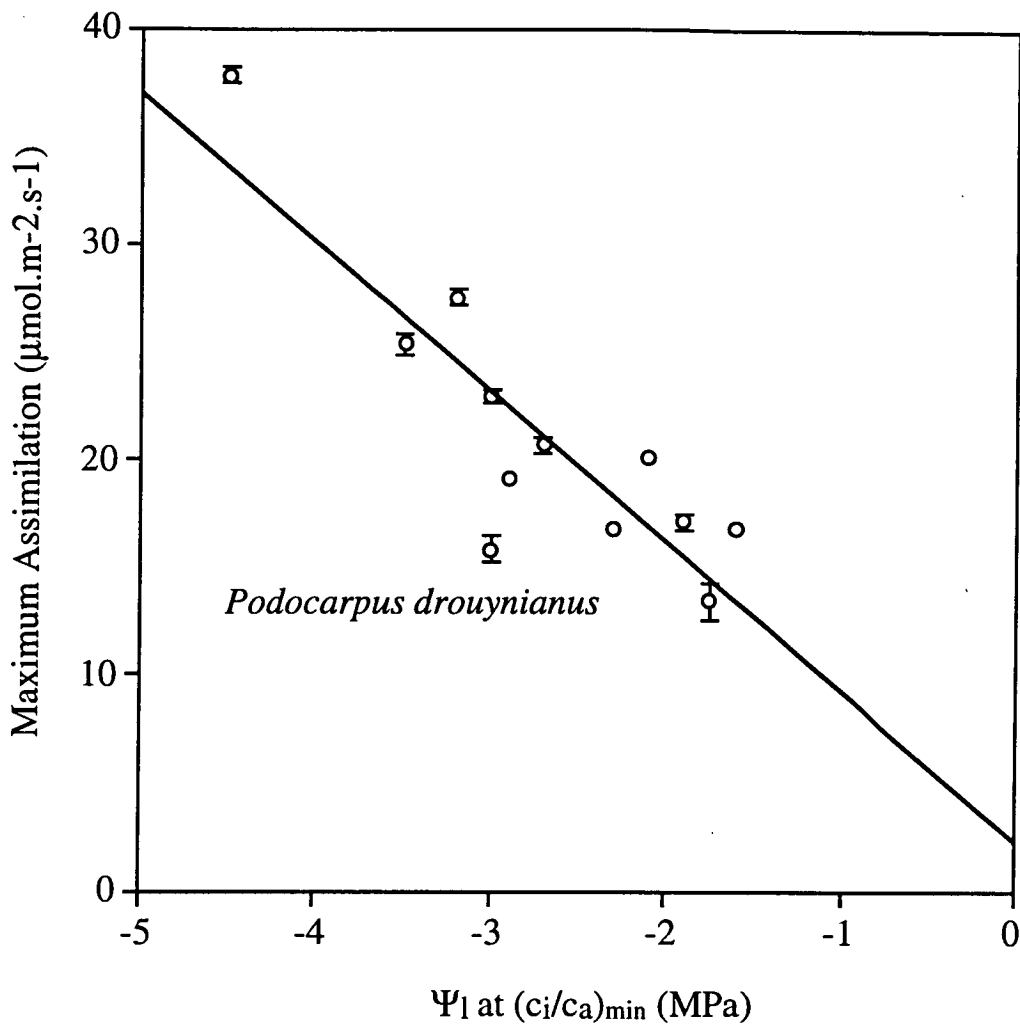


Figure 3.12. The association between maximum net assimilation rate and leaf water potential at  $(c_i/c_a)_{\min}$  illustrates that species which were tolerant of low leaf water potentials produced high maximum rates of assimilation.

*Podocarpus drouynianus* was an outlier both here, and in relationships shown in chapter 6. The implications of this are discussed in detail in chapter 6.

forest species considered here. The dry forest conifers (*Widdringtonia cedarbergensis*, *Actinostrobus acuminatus*, and *Callitris rhomboidea*) all experience highly seasonal rainfall and under optimum field conditions have abundant water, high temperature, and minimal competition for light, thus allowing the potential for high maximum photosynthetic rates. In contrast, the wetter forest species are likely to have either temperature limitations (many are from sub-alpine environments) or light limitations (due to intense competition in lowland forest) on maximum assimilation in the field. *Podocarpus drouynianus* is an obvious anomaly as it produces a relatively high drought tolerance, and yet its maximum assimilation rate was low. The  $c_i/c_a$  measured under optimum conditions was also very low in this species, suggesting that for some reason the maximum  $g_s$  of this species was low relative to its photosynthetic capacity (supported by carbon isotope data presented in Chapter 4). The light response characteristics of this species were also somewhat anomalous, and possible explanations for the unusual physiology of *P. drouynianus* are discussed in chapter 6.

There did not seem to be any systematic relationship between leaf morphology and the ability of species here to survive low water potentials. Amongst angiosperms it is usually easy to distinguish leaves which are tolerant of low water potential from those which are susceptible, simply by their appearance. Mesophytes generally produce large, thin, soft leaves which wilt readily in response to decreased  $\Psi_l$ , while xerophytes on the other hand typically produce small, hard leaves, with rigid cell walls to prevent turgor loss (Fahn, 1974). Indeed the water potential at turgor loss has been used as an indicator of the drought tolerance of angiosperm leaves (Roberts and Knoerr, 1977). Amongst the conifers investigated here there was no such distinction between the foliage of drought tolerant and intolerant species. The leaves of *Actinostrobus* and *Widdringtonia* (see chapter 2) were certainly not appreciably more xeromorphic than the leaves of *Dacrycarpus compactus*, or *Lagarostrobos franklinii*, and in fact, *Widdringtonia* foliage was softer than, and was found to wilt before, either of these species. It is possible that in conifers, the leaves are structurally tolerant of low water potential and that the ultimate cause of leaf damage is not due to a mechanical failure in the leaf, but rather a failure of the vascular system in supplying water to the leaf. There is evidence that xylem embolism plays a significant role in limiting transpiration in trees (Sperry and Pockman,

1993), and this would be exacerbated by the low root pressures (Rüdinger *et al.*, 1994) and absence of xylem vessels in conifers.

In summary this chapter provides a means of quantifying drought tolerance by measuring the minimum  $c_i/c_a$  attained in leaves during an artificially imposed drought. In the absence of patchy stomatal closure the application of  $(c_i/c_a)_{\min}$  as an index of drought tolerance was supported by the strong correlation between the leaf water potential at  $(c_i/c_a)_{\min}$  and the magnitude of  $(c_i/c_a)_{\min}$ . This index can now be used as a means of testing the relationship between drought tolerance and conifer distributions so as to determine whether species are limited by their drought tolerance, or whether other ecological forces play the major limiting role.



## **CHAPTER 4**

# **Relationships Between Conifer Distribution and Photosynthetic Characteristics**

## **Introduction**

The fundamental requirement for genetic survival in conifers, and indeed all plants, is the production of enough photosynthate to allow growth and reproduction. Thus, limitations imposed by the physical environment on the distribution of plant species will usually manifest themselves through their effects on leaf photosynthesis. The environmental parameters most commonly used to explain presence or absence of species are temperature and rainfall regimes, and their inferred effects are almost invariably on the photosynthetic capacity of the species in question. Because of their characteristic longevity (Loehle, 1988), sporadic reproduction, slow rates of dispersal, and short term viability of soil-borne seed, (Gibson, *et al.*, 1994; Veblen *et al.*, 1994) conifers are particularly closely linked to long term environmental characteristics. Unlike the fast growing, highly fecund angiosperms, most conifers do not have the capacity to move in and out of marginal environments in response to short term physical fluctuations, and are thus confined at the physiological extremes of their distributions by the long term survival of adult plants.

Despite the anecdotal and quantitative evidence of environmental constraints on species distributions, few studies have demonstrated the actual mechanisms by which physical limitations affect the survival and reproduction of plant species. Over the last 50 years, research into the characteristics and control of leaf gas exchange has been prolific and the use of leaf photosynthetic characters to explain simple successional changes in the vegetation has become common in the literature, typically using water use efficiency (*e.g.* Meinzer *et al.*, 1984; Dias-Filho and Dawson, 1995), drought response (*e.g.* Kubiske and Abrams, 1993), and light response (*e.g.* Field, 1988; Knapp and Smith, 1991; Ashton and Berlyn, 1994) to account for small scale competitive outcomes. This type of eco-physiological research generally takes the form of comparisons between small numbers of species, and as such does not attempt to make a quantitative link between photosynthetic function and environmental parameters. Indeed, with a few exceptions (*e.g.* Teeri and Stowe, 1976),

large scale relationships between physiology, environment, and species distributions have been overlooked by eco-physiologists.

In order to understand how the distribution of taxa is likely to have been affected by historical climate change, and is likely to be affected by future climatic perturbations, it is important to be able to describe current, known distributions in terms of the most likely physiological constraint. In this chapter I attempt to correlate several quantitative photosynthetic characteristics with the climatic ranges of a group of 13 conifer species (from 11 genera). These species (as used in chapter 3) were taken from a diverse geographical range spanning Australia, New Zealand, New Guinea, South Africa and New Caledonia, and a broad spectrum of habitats from the arid zone to tropical rainforest. Also included were species restricted to high (above 3000m) and low (sea-level) altitudes, ensuring that a wide range of leaf morphologies, and potential physiological adaptations (Körner and Diemer, 1987) were encompassed. The focus of the physiological characterisation of these various species has been on the water-use efficiency and drought tolerance of photosynthesis, as the distribution of most of the conifers included here, and indeed most conifers in the southern hemisphere, qualitatively appears to be constrained by water availability. Conifer forest in the southern hemisphere is generally restricted to areas of high rainfall, with the most extensive, and diverse conifer forests occurring on the west coasts of New Zealand and Tasmania, and in New Caledonia, and highland New Guinea, where rainfall is commonly over 5000 mm per annum (de Laubenfels, 1988). There are, however, several genera which inhabit dry country, including semi-arid regions in Australia, and dry heathland in South Africa and their inclusion here ensures that the entire range of water availabilities for conifers has been covered.

Four indices have been used to describe the physiology of each of the species considered here: the instantaneous water-use efficiency, carboxylation efficiency, maximum water-use efficiency during drought (as described in chapter 3), and integrated water-use efficiency as inferred from carbon isotope discrimination ( $\delta^{13}\text{C}$ ). The first three of these parameters focus on instantaneous gas-exchange at the leaf level, as measured on leaves or small samples of shoot, whilst the last index provides an integrated estimate of the water-use efficiency based on the relationship between carbon-13 isotope discrimination and the ratio of internal to external  $\text{CO}_2$  concentration ( $c_i/c_a$ ) in the mesophyll during  $\text{CO}_2$  assimilation.

The theoretical basis for the use of  $\delta^{13}\text{C}$  as a means by which gas exchange characteristics can be inferred, comes from the fact that the two key processes involved in the entry and fixation of  $\text{CO}_2$  into the leaf, diffusion and carboxylation, have distinct fractionation effects favouring  $^{12}\text{CO}_2$  over  $^{13}\text{CO}_2$ . Diffusion of  $^{12}\text{CO}_2$  is slightly faster than  $^{13}\text{CO}_2$  in air due to its smaller mass, and thus fractionation occurs as  $\text{CO}_2$  moves from the atmosphere into the sub-stomatal cavity (the ratio of diffusivities being 1.0044; Farquhar *et al.*, 1982). The second fractionation occurs due to the preference of the primary carboxylating enzyme (in  $\text{C}_3$  plants), Ribulose-1, 5 b<sup>i</sup>-phosphate carboxylase<sup>oxygenase</sup> (Rubisco) for  $^{12}\text{CO}_2$  over  $^{13}\text{CO}_2$  which produces a fractionation effect of 1.029 (Roeske and O'Leary, 1984). The sum of these fractionations is given by the equation 4.1.

Equation 4.1 
$$\epsilon_T = \epsilon_d \cdot (c_a - c_i) / c_a + \epsilon_c \cdot c_i / c_a$$

where  $\epsilon_T$  is the total fractionation effect;  $\epsilon_d$  is the fractionation due to diffusion (1.0044);  $\epsilon_c$  is the fractionation due to carboxylation (1.029).

Thus it can be seen that when stomata are fully open and  $c_i$  approaches  $c_a$ , fractionation will largely reflect the carboxylation value, but when stomata are almost closed in the light, and  $c_i$  is much lower than  $c_a$ , fractionation will reflect the diffusion component. To determine the concentration of  $^{13}\text{C}$  fixed in the leaf ( $\delta_l$ ) it is necessary to also consider the  $\delta$  value of atmospheric air which is approximately -7.8 ‰ relative to the accepted P.D. Belemnite standard (see equation 4.2). Using a value of -7.8 ‰ for atmospheric air,  $\delta_l$  has a theoretical range from a maximum of -12.2‰ when  $c_i/c_a = 0^a$ , to a minimum value of -34.8‰ when  $c_i/c_a = 1$ . From this relationship it is thus possible to calculate the value of  $c_i/c_a$  during carbon fixation.

The accuracy of equation 4.1 has been experimentally verified by simultaneous measurement of the instantaneous  $\delta^{13}\text{C}$  of air circulating over leaves photosynthesising in cuvettes, and  $c_i/c_a$  calculated from assimilation and leaf conductance (Evans *et al.*, 1986). The  $\delta^{13}\text{C}$  of sugars extracted from leaves has also been found to correlate well with  $c_i/c_a$  measured by gas analyzer (Brugnoli *et al.*, 1988). When  $\delta^{13}\text{C}$  is measured in cellulose or bulk leaf material the discrimination effect is integrated

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<sup>a</sup> In fact the minimum  $c_i/c_a$  attainable is the compensation  $\text{CO}_2$  concentration which would limit  $\delta_l$  to a maximum of around -15.4‰ (Vogel, 1993).

over a longer period of time, combining the effects of changing  $c_i/c_a$  through the course of the day, and further fractionations due to respiration and other metabolic processes (O'Leary, 1993). Despite this, significant correlations have been shown in the relationship between  $^{13}\text{C}$  discrimination in leaf cellulose and both, instantaneous water-use efficiency (Meinzer Goldstein & Grantz, 1990), and whole plant water-use efficiency (Hubick, Farquhar & Shorter, 1989), within species. In these studies the proportionality between  $\delta^{13}\text{C}$  and  $c_i/c_a$  was maintained, but the slope of the relationship was deflected from the value expected using equation 4.2.

Thus, carbon isotope discrimination measured on leaves has the potential to show large scale spatial trends in plant gas exchange, and recently a number of publications have demonstrated local (Ehleringer *et al.*, 1986; Vitousek, Field and Matson, 1990), altitudinal, and global trends (Körner *et al.*, 1988; Körner *et al.*, 1991) in the ratio of  $^{12}\text{C}$  to  $^{13}\text{C}$  in leaves. Other applications of this technique have been in the study of temporal variation in gas-exchange (Marshall and Monserud, 1996) from tree core samples, and even phylogenetic relationships (Read and Farquhar, 1991) in genera.

The interpretation of  $\delta^{13}\text{C}$  depends on information regarding how  $c_i/c_a$  changes during the course of a day, and in response to perturbations such as changing light and water availability, and this constitutes a major limitation, as the calculation of  $c_i/c_a$  is often inaccurate due to patchy stomatal closure, especially during water stress (Terashima *et al.*, 1988). In chapter 3 it was shown that the conifers used here produced uniform stomatal closure, and were thus conducive to accurate calculation of  $c_i/c_a$ , allowing a meaningful interpretation of carbon isotope data.

In this study, all experimental plants were grown under identical conditions so as to emphasise intrinsic differences in gas-exchange among species by minimising environmental variation. Plants here were taken from a wide range of habitats, and field measurements would tend to highlight the response of plants to the prevailing local conditions rather than the effects of genetic variation in morphology and physiology. This is illustrated by the fact that field data are often inversely related to data from plants grown under common-garden conditions (e.g. Marshall & Zhang, 1993).

## Materials and Methods

### Plant material

Data here were collected from the same 13 species used in chapter 3. These species represented the full range of habitats and altitudes for conifers in the southern hemisphere; for details see Table 4.1. Although not used in chapter 3, *Phyllocladus aspleniifolius* (Labill.) Hook.f., was also included in the  $\delta^{13}\text{C}$  analysis.

Table 4.1. Altitude of collection, and forest stratum occupied for the 13 species of conifers investigated.

Species	Region	Forest stratum	Altitude (m)
<i>Acropyle pancheri</i>	New Caledonia	sub-canopy to canopy	200
<i>Actinostrobus acuminatus</i>	Western Australia	canopy	20
<i>Athrotaxis selaginoides</i>	Tasmania	canopy	850
<i>Callitris rhomboidea</i>	Tasmania	canopy	50
<i>Dacrycarpus dacrydioides</i>	New Zealand (South Is.)	canopy	100
<i>Dacrycarpus compactus</i>	New Guinea	canopy	3400
<i>Diselma archerii</i>	Tasmania	forest understorey	1200
<i>Lagarostrobos franklinii</i>	Tasmania	alpine canopy sub-canopy to canopy	400-800
<i>Microstrobos niphophilus</i>	Tasmania	forest understorey	1100
<i>Phyllocladus aspleniifolius</i>	Tasmania	alpine-canopy sub-canopy to canopy	400
<i>Podocarpus lawrencii</i>	Tasmania	forest understorey	1200
<i>Podocarpus drouynianus</i>	Western Australia	alpine canopy forest understorey	50
<i>Widdringtonia cedarbergensis</i>	South Africa	canopy	250

### Gas exchange

Measurement of gas exchange was carried out using the equipment described in chapter 3. Initially,  $c_i/c_a$  and carboxylation efficiency was measured in fully watered plants, photosynthesising under approximately optimal conditions, with leaves at 20°C, leaf-air vapour



pressure deficit at 5-10 mmol.mol<sup>-1</sup>, PPFD at 1400  $\mu\text{mol.m}^{-2}.\text{sec}^{-1}$ , and  $c_a = 350 \pm 5 \mu\text{mol.mol}^{-1}$ . Because the stomatal response of most of the species was very slow, leaves were exposed to full light and 100% relative humidity for a period of 1 to 2 hours prior to the measurement of gas exchange to ensure maximum stomatal conductance. For each species, an average of the maximum readings of  $c_i/c_a$  from 5 individuals was calculated to give  $(c_i/c_a)_{\text{max}}$ . Carboxylation efficiency was taken as the initial slope of the A vs  $c_i$  regression between the CO<sub>2</sub> compensation point and  $c_i = 250 \mu\text{mol.mol}^{-1}$  (Wullschleger, 1993). Following determination of maximum rates of gas exchange, water was withheld from plants for a period of 3-4 weeks, during which time  $c_i/c_a$  was regularly measured to determine the minimum attainable  $c_i/c_a$  ( $(c_i/c_a)_{\text{min}}$ ).

The specific leaf area (leaf mass per unit silhouette area), SLA, was measured on each replicate used for gas-exchange analysis. This involved measuring the silhouette leaf area (as described in Ch. 3) drying the leaf sample at 70°C for 2 weeks, and weighing on a set of digital scales.

#### *Carbon isotope discrimination*

Samples of leaf tissue were taken from three glasshouse grown individuals of each species, ensuring leaves were relatively young, healthy, and had developed under completely unshaded conditions. Samples were all collected at exactly the same time of year, during mid-summer (January), ensuring that possible seasonal effects on leaf  $\delta^{13}\text{C}$  ( $\delta_l$ ) were not contributing to variation among species. Immediately after collection, leaf samples were oven dried at 70°C and ground with a mortice and pestle to a maximum particle size of 0.1mm. The  $^{13}\text{C}/^{12}\text{C}$  ratio in this material was assayed (by the Research School of Biological Sciences at the Australian National University in Canberra) and an average and standard error calculated from the 3 replicates of each species, except *Acropyle pancheri* where only 2 replicates were available. The relationship between measured  $\delta_l$  and  $(c_i/c_a)_{\text{max}}$  was compared with the theoretical relationship between  $\delta^{13}\text{C}$  and  $c_i/c_a$ , calculated using equation 2 (Farquhar *et al.*, 1982).

$$\text{Equation 4.2.} \quad \delta^{13}\text{C} = \delta_{\text{atm}} - a - (b-a) c_i/c_a$$

where :  $\delta_{\text{atm}}$  = atmospheric  $^{13}\text{C}$  ratio relative to PDB standard (-7.8 ‰) (Goodman, 1980),  $a$  = fractionation due to diffusion (4.4‰) and  $b$  = fractionation during carboxylation (net. value = 27‰; Farquhar *et al.*, 1982).

*Phyllocladus aspleniifolius* was included in the discrimination measurements, but not in the drought procedure, as it was the only species with multi-veined foliage, and hence it could not be ensured that uniform stomatal closure was occurring during drought. Unfortunately seed of *Widdringtonia cedarbergensis* did not become available early enough for inclusion of this species in the  $\delta^{13}\text{C}$  measurements.

#### *Selection of environmental parameters*

For the reasons stated above, rainfall was the environmental parameter of most interest, and because  $(c_i/c_a)_{\text{min}}$  appears to reflect drought tolerance (Chapter 3), minimum rainfall figures were considered likely to provide the best correlation.

In attempting to describe the minimum rainfall to which a species is confined, it was necessary to exclude plants that were riparian, or on drainage channels, as these individuals are clearly phreatophytic, not relying solely on precipitation for their water supply. Also estimates were restricted to mature, natural stands of the species in question as information regarding distribution rarely encompasses the scattered, stunted outliers which commonly extend beyond the described limits of a species. Information regarding species distributions was collected either from published material or personal communications, and this was used to approximately locate the driest occurrence of each species. It was necessary to show the rainfall estimate for *Dacrycarpus dacrydioides* as a range, including the driest occurrence for both east and west coast populations, because, although *D. dacrydioides* is present in the dry eastern half of New Zealand, it is invariably riparian, or associated with poorly drained soils (P. Wardle, pers. comm.). Data for other species distributions come from: *Acmopyle pancheri* (de Laubenfels, 1972), *Dacrycarpus compactus* (Wade and McVean, 1972; VanRoyen, 1979), *Athrotaxis selaginoides*, *Callitris rhomboidea*, *Diselma archerii*, *Phyllocladus aspleniifolius*, *Podocarpus lawrencii*, and *Microstrobos niphophilus* (Brown *et al.*, 1983), *Lagarostrobos franklinii* (Gibson *et al.*, 1991), *Actinostrobus acuminatus* (Bowman and Harris, 1995), *Podocarpus*

*drouynianus* (Gibson *et al.*, 1995), and *Widdringtonia cedarbergensis* (Midgley *et al.*, 1995).

Actual rainfall statistics were obtained from the nearest weather station, supplemented in Australia by using estimates from the climatic model BIOCLIM (Nix, 1982). Mean monthly rainfall data were used to calculate the mean annual rainfall, rainfall for the driest quarter (RFDQ), and dry season rainfall (averaged from the four consecutive driest months). RFDQ was used as it has previously been used as the standard index of drought susceptibility, although dry season rainfall provided a better range of values, and was better correlated with the actual period of minimum water availability in dry environments. In all but one case rainfall statistics were averages from periods of >30 years, the New Caledonian data from Yat  only representing averages over 3 years.

Because the 13 species used here represented an altitudinal range of 0 to >3000m, the influence of altitude of origin on  $\delta^{13}\text{C}$ ,  $c_i/c_a$  and carboxylation efficiency was examined to determine whether there was any correlation between measured physiological characters, and the morphological differences observed between alpine and lowland species.

## Results

Mean  $\delta^{13}\text{C}$  values ranged from -28.95‰ in *Acmopyle pancheri* to -24.5‰ in *Podocarpus drouynianus* (Table 4.2). Intra-specific variation in  $\delta^{13}\text{C}$  was generally quite small (< 1.5‰) although the two replicates of *A. pancheri* produced values of -27.8‰ and -30.1‰, resulting in a large standard error. A significant positive correlation ( $r^2 = 0.89$ ;  $p < 0.001$ ) was found between instantaneous  $(c_i/c_a)_{\text{max}}$  and  $\delta^{13}\text{C}$  (Fig. 4.1) in the 12 species investigated. The regression through the data was almost identical to the theoretical relationship between  $c_i/c_a$  and  $\delta^{13}\text{C}$  calculated from equation 1 (Fig. 4.1).

There was no significant correlation between  $\delta^{13}\text{C}$  and any of the other physiological or environmental parameters investigated (rainfall,  $(c_i/c_a)_{\text{min}}$ , or carboxylation efficiency) although there was a tendency for species with higher carboxylation efficiencies to produce higher values of  $\delta^{13}\text{C}$  (Fig 4.2). Despite the observation that, in general, species from high altitudes were more commonly imbricate (pers. obs.), altitude was not correlated with  $\delta^{13}\text{C}$  or any other physiological character measured. Leaf morphology, at least in terms of its effect on leaf area index, was not

found to be related to any environmental or physiological character (Table 4.3).

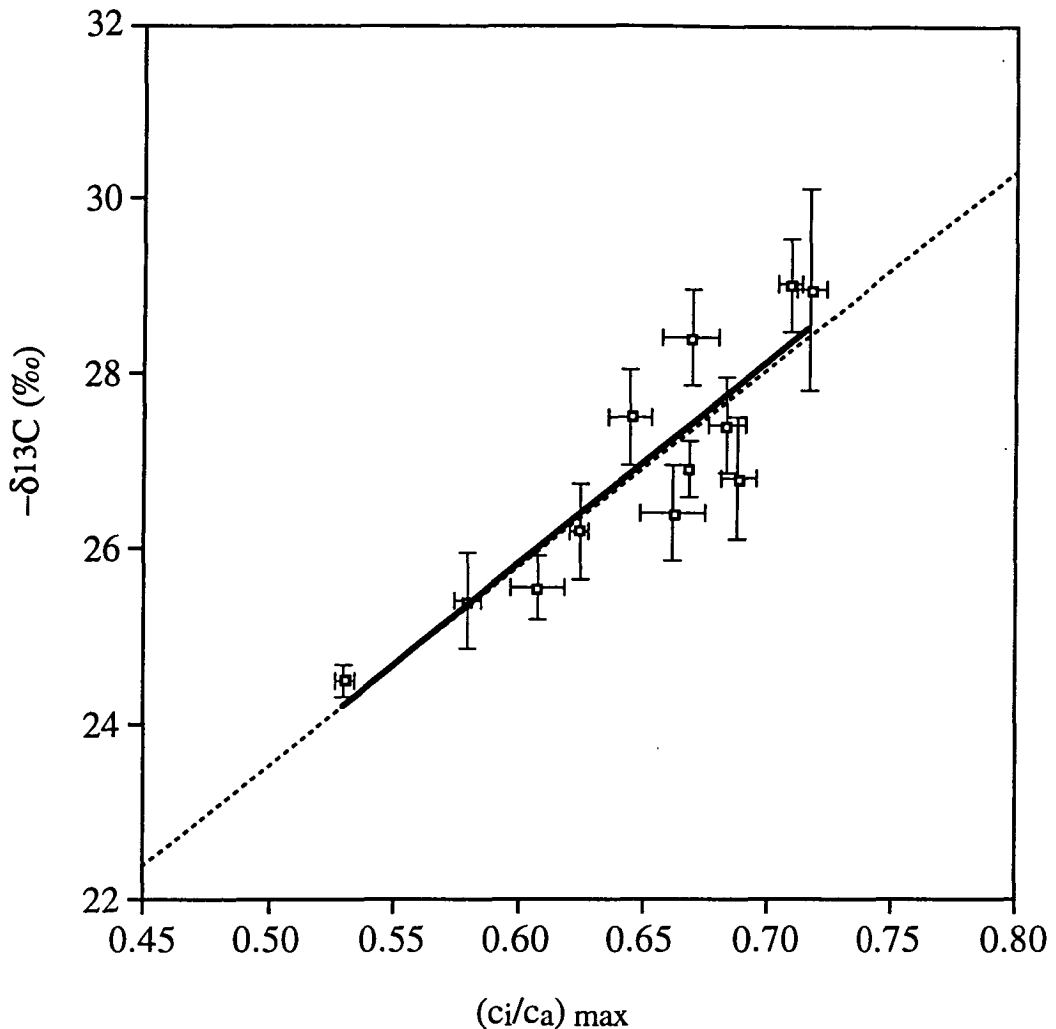


Figure 4.1. Average leaf carbon discrimination ( $n=3$ ), and average maximum  $c_i/c_a$  ( $n=5$ ) measured at  $20^\circ\text{C}$ , with leaf - air vapour pressure deficit at  $5\text{--}10\text{ mmol}\cdot\text{mol}^{-1}$ , light intensity at  $1400\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$ , and  $c_a = 350 \pm 5\mu\text{mol}\cdot\text{mol}^{-1}$ . Species are labelled: 1. *Acropyle pancheri* 2. *Dacrycarpus dacrydioides* 3. *Podocarpus lawrencii* 4. *Dacrycarpus compactus* 5. *Callitris rhomboides* 6. *Actinostrobus acuminatus* 7. *Phyllocladus aspleniifolius* 8. *Lagarostrobos franklinii* 9. *Microstrobus niphophilus* 10. *Athrotaxis selaginoides* 11. *Diselma archeri* 12. *Podocarpus drouynianus* 13. *Widdringtonia cedarbergensis* (Fig. 4.2 only). The regression through the data (in black) is highly significant ( $r = 0.89$  :  $p < 0.001$ ) and the dotted line represents the theoretical relationship between  $\delta^{13}\text{C}$  and  $c_i/c_a$  using equation 4.1.

Plants from drier habitats were found to produce lower values of  $(c_i/c_a)_{\min}$  (or a higher water-use efficiency) and higher carboxylation efficiencies than species from higher rainfall environments. All of the

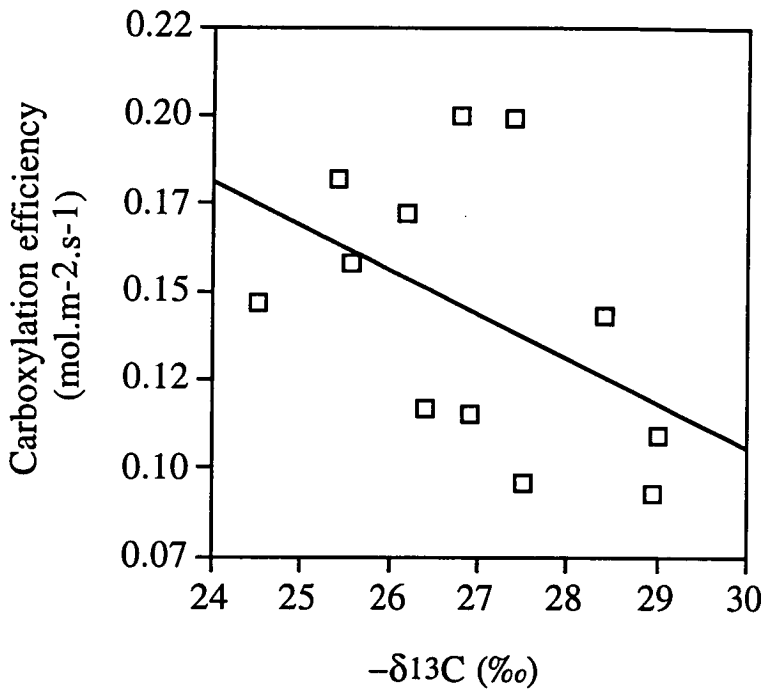


Figure 4.2. Although the correlation between carboxylation efficiency and  $\delta^{13}\text{C}$  was not significant at the  $p < 0.05$  level, there was a tendency for species with high carboxylation efficiencies to produce less negative values of  $\delta^{13}\text{C}$ .

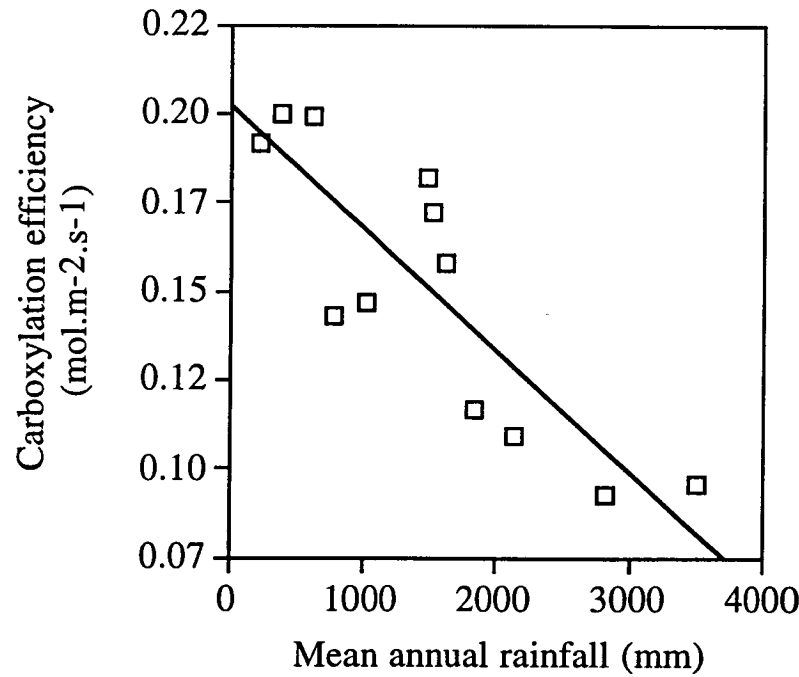


Figure 4.3. A highly significant ( $p < 0.001$ ) linear correlation was found between carboxylation efficiency in the 12 species investigated and mean annual rainfall at the dry end of each species' distribution.

rainfall parameters considered, including the yearly average, dry season<sup>#</sup> average and RFDQ were significantly correlated with carboxylation efficiency (Table 4.3, Fig. 4.3), and strongly correlated with  $(c_i/c_a)_{\min}$  (Figs 4.4, 4.5). Logarithmic regressions provided the best fit for the  $(c_i/c_a)_{\min}$  data ( $r^2 = 0.96$  for dry season rainfall and  $0.92$  for average rainfall with  $p < 0.001$  in both cases). The x-axis in figure 4.4 shows dry season rainfall on a log scale, and the regression fitted is :  $(c_i/c_a)_{\min} = 0.153 \log(\text{dry season rainfall}) - 0.101$ . A highly significant linear regression described the relationship between RFDQ and  $(c_i/c_a)_{\min}$ .

Table 4.2. Maximum  $c_i/c_a$ ,  $\delta^{13}\text{C}$ , average yearly rainfall, and dry season rainfall (driest four consecutive months), for the 13 species of conifers investigated here. The rainfall range shown for *D. dacrydioides* is explained in the text. \* indicates rainfall estimates using the climate model BIOCLIM. *Widdringtonia* was not included in the  $\delta^{13}\text{C}$  analysis due to insufficient adult foliage at the time of leaf harvest.

Species	$c_i/c_a$ maximum	$\delta^{13}\text{C}$ (‰)	Ave. Rainfall (mm)	Dry season rainfall (mm)
<i>Acmopyle pancheri</i>	0.717	-29.0	2825	538
<i>Actinostrobus acuminatus</i>	0.688	-26.8	383	23
<i>Athrotaxis selaginoides</i>	0.608	-25.6	1636	362*
<i>Callitris rhomboidea</i>	0.683	-27.4	630	91
<i>Dacrycarpus dacrydioides</i>	0.709	-29.0	1021-3282	267-958
<i>Dacrycarpus compactus</i>	0.645	-27.5	3501	552
<i>Diselma archerii</i>	0.580	-25.4	1498	327*
<i>Lagarostrobos franklinii</i>	0.662	-26.2	1854	473*
<i>Microstrobos niphophilus</i>	0.625	-26.2	1532	406*
<i>Phyllocladus aspleniifolius</i>	0.668	-26.9	-	-
<i>Podocarpus lawrencii</i>	0.670	-26.9	790	239
<i>Podocarpus drouynianus</i>	0.531	-24.5	1039	96
<i>Widdringtonia cedarbergensis</i>	0.536	-	239	37

Carboxylation efficiency was related to  $(c_i/c_a)_{\min}$  by a simple linear function ( $r=0.84$  :  $p < 0.001$ ), with high carboxylation efficiencies corresponding to low  $(c_i/c_a)_{\min}$  (high maximum water-use efficiency). These two characters are not entirely independent (see chapter 3) and



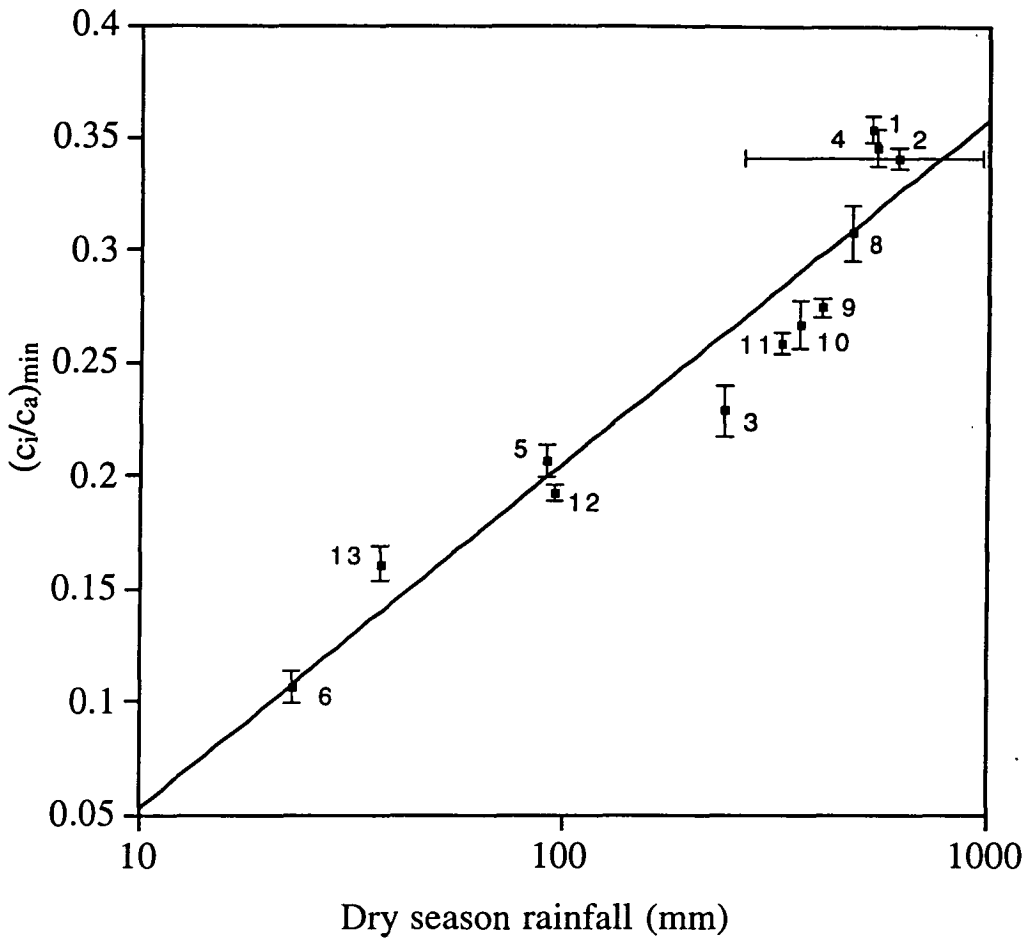


Figure 4.4. Minimum  $c_i/c_a$  (or maximum water-use efficiency) attained during drought, versus precipitation constraints on the distribution of 12 conifer species (species labels the same as fig. 4.1). Average rainfall for the dry season (driest four consecutive months) is shown on a log axis, and the logarithmic regression through the data is highly significant ( $p < 0.001$ ). *Phyllocladus aspleniifolius* has not been included as its broad, multi-veined foliage prevented accurate determination of  $c_i/c_a$  during drought. The precipitation range shown for *Dacrycarpus dacrydioides* is explained in the text.

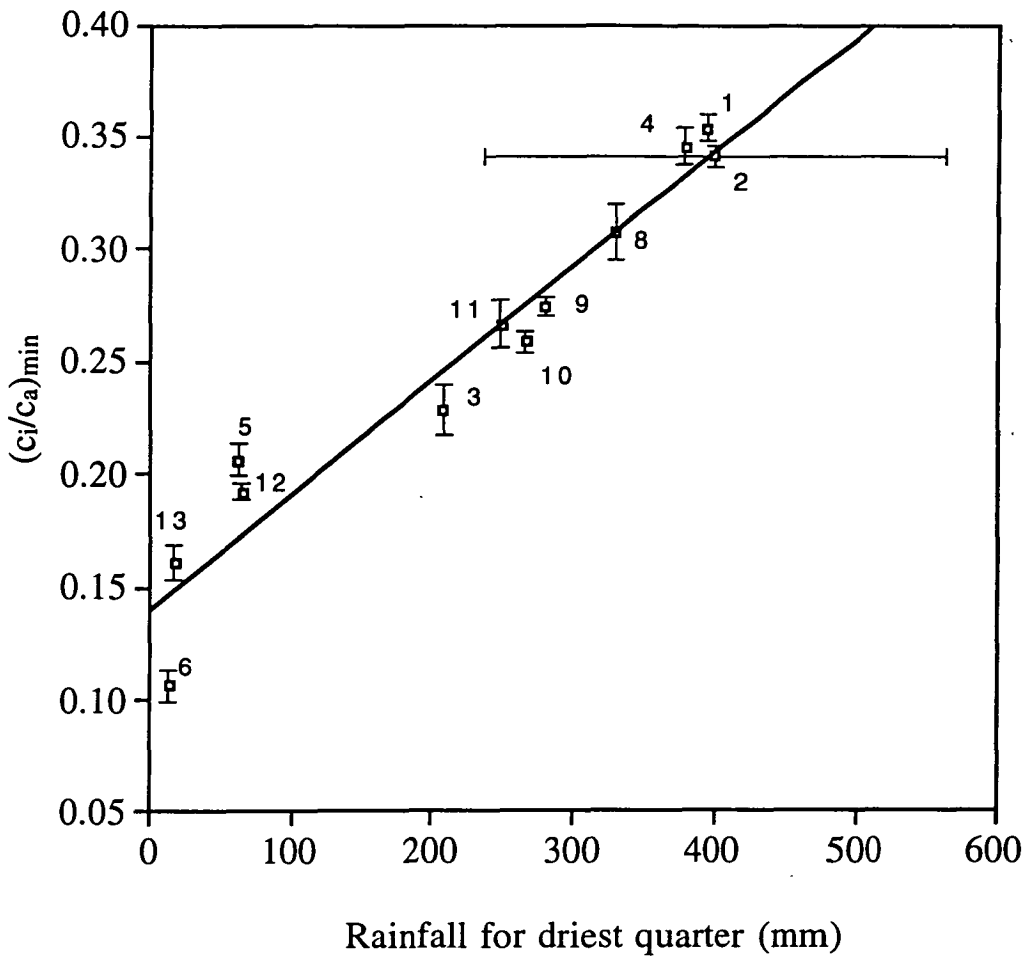


Figure 4.5.  $(c_i/c_a)_{\min}$  is related to the rainfall of the driest quarter (RFDQ) by a highly significant linear function  $(c_i/c_a)_{\min} = 5.10 \times 10^{-4} (\text{RFDQ}) + 1.40$ ; ( $r = 0.969$ ). Species are labelled numerically as in figure. 4.1.

hence similar trends were expected in their relationships with rainfall parameters. Neither carboxylation efficiency nor  $(c_i/c_a)_{\min}$  showed any relationship to  $(c_i/c_a)_{\max}$ .

Correlation coefficients and significance levels are summarised in table 4.3.

Table 4.3. Correlation coefficients ( $r$ ) for regressions between physiological and environmental parameters. All regressions were linear except those indicated.

\* indicates significance at the  $p < 0.05$  level, \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$

	$(c_i/c_a)_{\min}$	$(c_i/c_a)_{\max}$	Carbox. efficiency	$\delta^{13}\text{C}$	Altitude
<b>dry season</b>	0.97***	0.44	0.82***	0.44	0.62*
<b>rainfall (mm)</b>					
<b>RFDQ</b>	0.96 (log)***	0.45	0.82***	0.32	0.60*
<b>Ave. Rainfall</b>	0.92 (log)***	0.31	0.85***	0.33	0.44
<b>SLA</b>	0.02	0.08	0.25	0.48	0.40
<b><math>(c_i/c_a)_{\max}</math></b>	0.39	-	-	-	-
<b>Carboxylation efficiency</b>	0.84***	0.38	-	-	-
<b><math>\delta^{13}\text{C}</math></b>	0.42	0.89***	0.47	-	-
<b>Altitude</b>	0.44	0.10	0.38	0.01	-

## Discussion

It has been demonstrated that although  $c_i/c_a$  measured under uniform, optimal conditions correlated well with leaf  $\delta^{13}\text{C}$ , these leaf characters did not reflect the water availability in the natural range of the 13 species of conifers investigated. There was, however, a good correlation between  $(c_i/c_a)_{\min}$  and rainfall parameters, suggesting that the climatic ranges of these conifers is closely controlled by their photosynthetic and gas-exchange characteristics during drought.

The 13 conifer species investigated produced a large range of leaf  $\delta^{13}\text{C}$  and maximum rates of gas exchange, as would be expected considering the large environmental range represented by these species.

Despite this, a good correlation was found between  $(c_i/c_a)_{\max}$  and  $\delta^{13}\text{C}$ , indicating that in this group of plants, the long term water-use efficiency (inferred from  $\delta^{13}\text{C}$ ) was well represented by the instantaneous water-use efficiency measured by gas exchange. This result also supports the conclusion that, in these species at least,  $c_i/c_a$  measured by gas exchange gives a reasonable representation of the actual ratio of chloroplast to ambient  $\text{CO}_2$  concentration. The near-perfect overlap of the theoretical relationship between  $c_i/c_a$  vs  $\delta^{13}\text{C}$  and the regression through the measured data (Fig. 4.1), while aesthetically pleasing, was not expected, considering that bulk leaf material was used and that secondary fractionations are known to occur during leaf metabolism (O'Leary, 1981) which would tend to modify the relationship between  $c_i/c_a$  and bulk leaf  $\delta^{13}\text{C}$  (Schleser, 1990).

There was no relationship between either  $(c_i/c_a)_{\max}$  or  $\delta^{13}\text{C}$  and rainfall parameters, indicating that in these species, the intrinsic water-use efficiency under well watered conditions does not give any indication of relative drought tolerance exhibited by plants in the field. In fact, the Australian dry forest species *Callitris rhomboidea* and *Actinostrobus acuminatus* were found to produce amongst the highest average  $(c_i/c_a)_{\max}$  and lowest  $\delta^{13}\text{C}$  indicating low water-use efficiencies in both these species under optimal conditions. This refutes the intuitive assumption that plants which produce less negative values of  $\delta^{13}\text{C}$  and high values of instantaneous water-use efficiency are likely to be better adapted to low rainfall and drought (Huc *et al.*, 1994; Read and Farquhar, 1991). The probable reason for this apparent paradox is that species adapted to seasonally dry conditions are likely to produce high rates of assimilation and conductance when conditions are favourable (Table 6.1, see also chapter 3) and thus exhibit relatively low water-use efficiencies when measured under optimal conditions.

The absence of any correlation between altitude of origin and  $\delta^{13}\text{C}$  was also an unexpected result, as several previous studies have shown significant trends between altitude and  $\delta^{13}\text{C}$  in common-garden experiments (*e.g.* Marshall and Zhang, 1994, Read and Farquhar, 1991). However, the fact that in these studies altitude of origin has been correlated with both increasing and decreasing  $\delta^{13}\text{C}$ , suggests that altitude may be a secondary effect. The altitude effect in other studies is often linked to a systematic change in leaf characteristics *e.g.* leaf thickness and nitrogen content over an altitudinal gradient (Vitousek *et al.*, 1990), and the large morphological range of the species described here may have

overridden such an effect. This is supported by the lack of any significant relationship between leaf morphology, as quantified by SLA, and altitude of origin. Altitude has also been linked to high photosynthetic capacity, reflected in a correlation between carboxylation efficiency and altitude (Körner and Diemer, 1987); again, there was no such relationship observed between carboxylation efficiency and altitude here.

In contrast to gas exchange measured under optimal conditions, the minimum value of  $c_i/c_a$  (proportional to [maximum water-use efficiency]<sup>-1</sup>), measured during a slowly intensified drought correlated well with rainfall characteristics at the driest end of the various species ranges (Fig. 4.4, 4.5). This is an important result as it provides a direct link between instantaneous gas-exchange characteristics and species distribution, further supporting the use of  $(c_i/c_a)_{\min}$  as an index of drought tolerance (Brodribb, 1996). This is also emphasised by the fact that a higher correlation was found between dry season rainfall and RFDQ than mean annual rainfall (0.96 and 0.97 vs 0.92), as the former two parameters focus on periods during which the potential for plants to suffer water stress is at its maximum. The logarithmic nature of the regression for average rainfall and dry season rainfall (Fig. 4.4) would be interpreted as reflecting increasing selection for drought tolerance, maximum water-use efficiency, and thus  $(c_i/c_a)_{\min}$ , in drier habitats. The change to a more linear relationship for the shorter RFDQ period reflects the increased seasonality of rainfall in the drier habitats (*i.e.* decreasing the period over which rainfall is averaged will differentially affect low rainfall sites, causing the relationship to become more linear).

The correlation between carboxylation efficiency and rainfall indicates that this character is also related to the distribution of these plants with respect to water availability, such that increasing environmental aridity corresponds with increasing carboxylation efficiency. Carboxylation efficiency effectively describes the rate of assimilation at different levels of  $c_i$  (on the linear part of the  $A$  vs  $c_i$  response) and high carboxylation efficiencies would be of increasing importance for plants faced with water limitation for two reasons. Firstly, carboxylation efficiency provides a far more meaningful expression of the intrinsic water-use capabilities of a plant than instantaneous measures of the ratio of carbon gained to water lost (*e.g.*  $A/E$ ,  $A/g_s$  or  $c_i/c_a$ ) and thus increasing carboxylation efficiency would be expected to accompany decreased water availability, particularly if plants are likely to be operating

at decreased levels of  $c_i$  (Ehleringer *et al.*, 1988). The second reason for a link between high carboxylation efficiencies and decreasing water availability is that species from seasonally dry environments tend to produce high maximum photosynthetic rates when water is plentiful (Chapter 3).

The fact that two water use indices,  $(c_i/c_a)_{\min}$  and carboxylation efficiency, were closely related to the minimum water availability within the environmental ranges of the species investigated, suggests that the distribution of these species is limited by their water economy, and more probably, drought tolerance characteristics. The cost to the plant of maximisation of water-use efficiency, as measured by these two parameters, comes as an increase in the saturation light requirement (discussed in Ch 6) and respiration rate of leaves. Increases in leaf nitrogen associated with higher carboxylation efficiency and photosynthetic capacity in species have been commonly linked to increases in the dark respiration rate, and this has been explained by suggestions of higher tissue maintenance costs, or costs associated with phloem loading and carbohydrate export from the leaf (Bouma *et al.*, 1995; Baxter *et al.*, 1995; Conner *et al.*, 1993). Thus there must be a significant selective pressure on these species to maintain their physiological drought tolerance to a level dictated by the long term water availability in the environment, and an insufficient drought response must become limiting by causing local extinction of a species either by death during periods of extreme conditions, or by competitive exclusion by more efficient species. Either way, the importance of rainfall is emphasised in constraining the distribution of these species.

It should be mentioned that although rainfall has been shown here to produce a significant constraint on species distributions, within the habitat range which is climatically suitable for these various species, other factors play a significant role in further limiting the area available for conifer growth habitation; in Australia the most important of these factors is fire. Virtually all coniferous forest in Australia, from the arid zone to coniferous rainforest has been affected by fire (Gibson *et al.*, 1994; Harris and Kirkpatrick, 1994), and recent anthropogenic changes in fire regimes in Australia (Jackson, 1968) New Zealand (McGlone, 1983), and South America (Armesto *et al.*, 1994) have substantially restricted the distribution of many species. The fact that despite this, species were still found to be distributed according to their drought tolerance, perhaps reflects the more probabilistic nature of fire than rainfall as a limiting



factor, *i.e.* a significant drought is likely to impact on an extremely large area affecting all populations and systematically restricting distribution on marginal sites, whereas an increase in fire frequency may still allow the development of refugia which will, though chance, or location, avoid being burned. From this information it seems that, under natural conditions, fire itself is unlikely to cause extinction of species unless the distribution of that species had been previously diminished by other factors.

The conclusion here, that conifer distributions are commonly water limited, has significant implications for interpretation of the conifer history in the southern hemisphere. Large scale changes in the rainfall and other climatic characteristics are proposed to have occurred throughout the Tertiary, and these changes must have significantly influenced conifer survival. A detailed discussion of the possible effects of climatic change during the Tertiary is presented in chapter 8.

There is also potential for the application of the drought tolerance vs. rainfall link described here, as a predictive tool to model the effects of changing rainfall on species distributions. Obviously further work is required, both in expanding the number of species investigated, and in better quantifying the rainfall parameters (inclusion of temperature to give evapotranspiration estimates) but the fundamental relationship presented here provides a strong foundation for further work.

## CHAPTER 5

### **Inhibition of Photosynthesis Under Summer Field Conditions**

#### **Introduction**

In chapters 3 and 4 it has been shown that the distributions of a sample of southern hemisphere conifers are constrained at a regional scale by water availability. In this chapter the photosynthesis of some endemic Tasmanian conifers is examined in the field to determine to what extent photosynthesis is inhibited under natural conditions, and whether this inhibition is related to habitat at the macro scale.

Tasmania provides a particularly good location for the study of conifer photosynthesis in the field as it possesses a rich conifer flora and a high degree of heterogeneity in habitat. Tasmania appears to have become a refugium for some of the great diversity of conifers that once inhabited south-east Australia, and that are now all but extinct elsewhere in Australia (Carpenter *et al.*, 1994). The extant conifer flora of Tasmania, although depauperate compared with the diversity of species present in the region during the Tertiary, is far more diverse than any other conifer flora in Australia. This small island of only about 67,900 km<sup>2</sup> accommodates 11 species from eight genera (six endemic) as compared with eight genera from the entire remaining Australian continent (approximately 7,686,891 km<sup>2</sup>). The most likely reasons for Tasmania's rich conifer representation relative to mainland Australia are: the high rainfall experienced by the west coast and highland parts of the state; the mountainous topography which provides a high degree of variation in habitat characteristics and inhibits fire spread; and the cool-temperate climate which also reduces the fire disturbance, and which, in combination with the mountainous topography, produces abundant montane and alpine environments, to which conifers are well adapted.

The aim of the work presented here is to determine the rates of photosynthesis of conifers at two locations representing the wetter and drier extremes of conifer forest distribution in Tasmania in order to quantify the inhibition of photosynthesis relative to glasshouse grown standards of each species. Initially it was decided to use portable gas exchange equipment (LCA2, Analytical Development Corporation) to measure CO<sub>2</sub> uptake in leaves, but this method proved inappropriate for several reasons, namely, the low sensitivity of the standard equipment

which resulted in a high degree of error at the low rates of photosynthesis generally produced by the target species, the long period required for stability prior to measurement (preventing sufficient replication for significant inter-species comparisons), and the requirement that each shoot measured be removed for leaf area determination (not suitable for small plants in national parks). Alternatively it was decided to use chlorophyll fluorescence as a means of measuring the yield of photochemistry in the leaf, rather than quantifying the  $\text{CO}_2$  uptake rate of photosynthesis in leaves. The advantages of this technique include the extremely short period of time required for instantaneous measurements of the photochemical reaction rate, the leaf area independence of rate calculations, the ability to measure photosynthesis under ambient conditions (without enclosure of the leaf), and the high degree of sensitivity of the instrument.

The principle of using chlorophyll-a fluorescence as a means of quantifying the rate of photochemical reaction in photosynthesis is based on the fact that excitation of chlorophyll molecules in the reaction centre (P680) of photosystem II (PSII) by absorbed radiation causes energy to be re-emitted in the form of fluorescence. The intensity of this fluorescence is dependent on the intensity of the incident light, and on the amount of energy which passes through the P680 reaction centre on to either the photolysis of water and electron transport, or to be dissipated, in reactions associated with the accessory carotenoid pigments. These two processes of energy removal from P680 compete with the re-emission of energy as fluorescence, and are thus termed photochemical quenching ( $q_p$ ) and non-photochemical quenching ( $q_n$ ) of fluorescence. When a dark adapted leaf is illuminated, a typical fluorescence induction response is produced called a Kautsky curve (Fig 5.1), from which it is possible to measure the minimum fluorescence ( $F_o$ ) and maximal fluorescence ( $F_m$ ) of PSII. Minimum fluorescence corresponds to the ground-state fluorescence produced when the PSII membrane bound electron acceptors (the plastoquinone pool) are fully reduced in the dark, and the maximum fluorescence is produced by PSII when the plastoquinone pool is fully oxidised (and  $q_p$  is reduced to zero). Using these two fluorescence parameters, the maximum quantum yield of the leaf can be simply calculated, as  $F_m$  represents the number of PSII reaction centres present, and  $F_m - F_o$  ( $F_v$ ) measures the quantum efficiency of those reaction centres, thus  $F_v/F_m$  in dark adapted leaves indicates the optimal quantum yield of PSII.

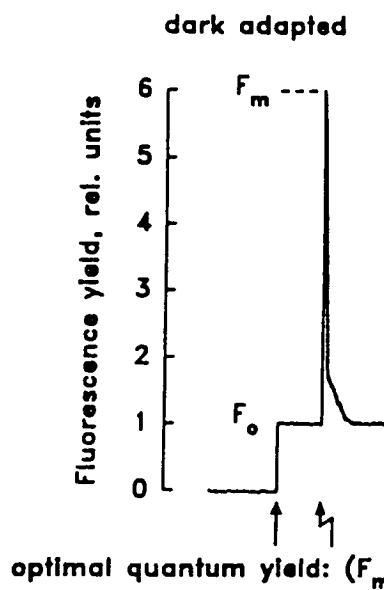


Figure 5.1. A typical fluorescence response from a dark adapted leaf. Minimum fluorescence ( $F_o$ ) is measured when a weak measuring beam is switched on (left arrow), and maximum fluorescence ( $F_m$ ) is measured when a saturating flash of light is applied to the leaf (right arrow). From these two parameters, the optimal quantum yield of PSII ( $\Phi_{PSII}$ ) can be calculated.

The optimal quantum yield has been used extensively as a means of quantifying photoinhibition and other forms of plant stress (Adams *et al.*, 1987; Ferguson & Burke, 1991), but as a measure of dark adapted quantum yield, it cannot give any information about the quantum yield and resultant rate of photochemical electron transport in the light. Measurement of fluorescence in the light is achieved by probing P680 with a weak modulated beam of light and selectively amplifying the resultant fluorescence signal (Schreiber and Bilger, 1992). A series of narrow pass filters remove all wavelengths other than those corresponding to P680 fluorescence, and the specific signal resultant from the measuring light is identified by its modulated nature. Using this technique of modulated fluorescence analysis, determination of instantaneous quantum yield of PS II in fully illuminated samples simply involves applying a saturating flash to a light acclimated leaf and measuring the light adapted maximum fluorescence ( $F_m'$ ). The instantaneous quantum yield of PSII ( $\Phi_{PSII}$ ) can then be calculated using equation 5.1 (Genty *et al.*, 1989).

$$\text{Equation 5.1.} \quad \Phi_{PSII} = \Delta F / F_m'$$

where  $\Delta F$  is the difference between the steady state, and maximum fluorescence. Characteristic fluorescence levels are defined in figure 6.1 (chapter 6).

The photochemical activity in the leaf is empirically coupled to the rate of photosynthetic carbon assimilation by the generation of ATP

which is used in the activation Rubisco prior to the fixation of CO<sub>2</sub> in the first reaction of the Calvin cycle. ATP is formed from a pH gradient across the thylakoid produced by the transport of electrons from water through photosystems I and II to reduce NADP<sup>+</sup>, and hence, calculation of the (non-cyclic) electron transport rate (ETR) from the quantum yield enables a theoretical rate of carbon assimilation to be calculated. ETR is derived from the quantum yield of PSII using equation 5.2.

$$\text{Equation 5.2.} \quad \text{ETR} = \Phi_{\text{PSII}} \cdot I \cdot \alpha / 2$$

where I = incident PPFD (in the waveband 400-700 nm);  $\alpha$  = leaf absorbance, taken here as 0.84 (Björkman and Demmig, 1987); and the factor of two accounts for the fact that two photons are required per electron passed through PSII, assuming linear electron flow, and even distribution of absorbed quanta between PSII and PSI.

Equation 5.2 makes the assumption that only two photons are required per electron passed through the photosystems, and therefore that electron flow is completely linear (Bilger *et al.*, 1995; Loreto *et al.*, 1995). There is evidence that cyclic electron flow occurs through PS I (Butler, 1977), but minimal activity of this cycle has been reported *in vivo* (Edwards and Baker, 1993). ETR calculated from fluorescence parameters was used in this chapter as an alternative to the more conventional gas exchange approach for quantifying the photosynthetic rate of plants in the field, and  $F_v/F_m$  as a measure of the levels of photoinhibition plants were experiencing. Despite impressive results showing a tight linearity between the quantum yield of PSII and quantum yield of CO<sub>2</sub> consumption under a range of conditions in several C<sub>4</sub> grass species ((Edwards and Baker, 1993), the use of fluorescence and ETR has been very limited to date, particularly in the field. The most probable reasons for this are that the development of modulated fluorescence equipment is only relatively recent, and has yet to gain widespread distribution or acceptance amongst researchers, and that there are still reservations about the interpretation of ETR in terms of C<sub>3</sub> photosynthesis (see chapter 6).

The intention of field measurements here was to measure the relative rates of photosynthesis in species which have been shown theoretically to be drought sensitive (Chapter 3), under conditions which were most likely to be stressful in terms water availability in the field. To this end, readings were taken during a two week period in mid-summer

after an extended period without rain. Two field sites of similar altitude and latitude were chosen, one high rainfall site on the west coast, and one lower rainfall site in the south-east to central part of the state. Variation in ETR within and between these sites was examined and compared with data from cultivated glasshouse material.

## Materials and Methods

### *Study sites*

Mt Read- This site was chosen for its extremely high rainfall and well developed conifer forest. Mt Read is located on the west coast of Tasmania (41°51'S 145°32'E; Fig. 5.2) and receives in excess of 3100 mm of rainfall per annum distributed fairly evenly throughout the year (BIOCLIM estimates). The conifer forest present occurs at an altitude of between 800 and 1000 m on the southern slope of the mountain, and is dominated by a mixture of *Athrotaxis selaginoides*, *A. cupressoides* (and a swarm of hybrids between these two species), *Diselma archeri*, and *Lagarostrobos franklinii*, with *Podocarpus lawrencii*, and *Phyllocladus aspleniifolius*, present but in more restricted numbers. The prostrate conifer *Microcachrys tetragona* was also present, but not included in the species studied. Until relatively recently this site has been effectively unburned (Peterson, 1990), and this fact, in combination with the high rainfall, has allowed a particularly luxuriant development of the conifers present. This is expressed in the unusual tree form of *Diselma archeri* (usually only found as a shrub elsewhere in the state), and the dominance of *Athrotaxis selaginoides* - *A. cupressoides* hybrid forest on the mountain, and the apparent persistence of a *Lagarostrobos franklinii* forest since the end of the last glaciation (M.J. Peterson, pers. comm.). Soil composition on Mt Read is mainly comprised of a relatively thin humic horizon above a depauperate quartz substrate.

Mt Field- Situated south-east of Mt Read (42°42'S 146°36'E; Fig. 5.2), Mt Field experiences a substantially lower rainfall than Mt Read (1532 mm; BIOCLIM estimate) while still possessing a significant diversity of conifer taxa. Most species are present as shrubs or small trees occurring from around 1000 m to 1200 m. An extensive conifer shrubbery comprised of *Diselma archeri*, *Microstrobos niphophilus*, *Podocarpus lawrencii*,

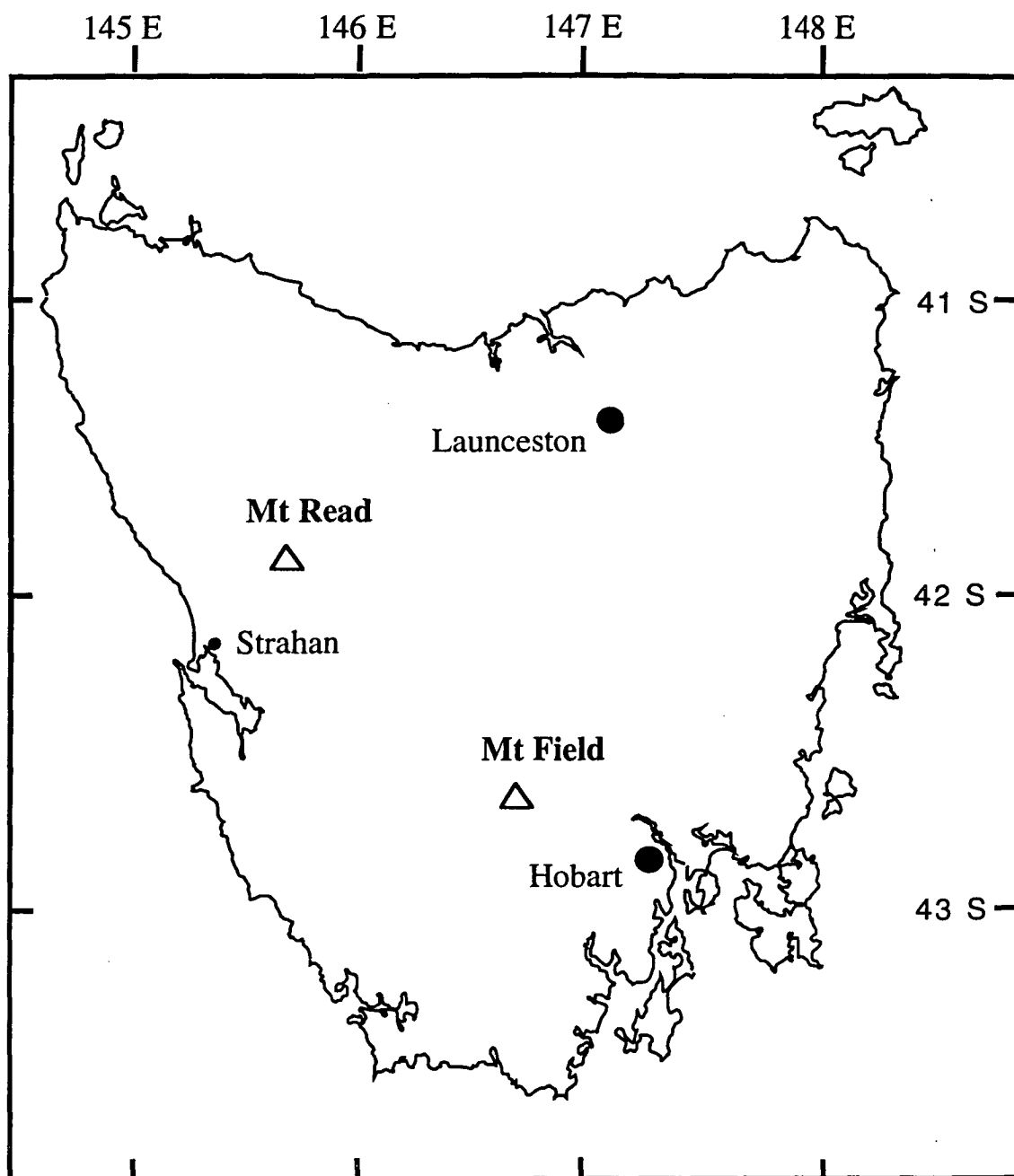


Figure 5.2. Locations of the two study sites, Mt Read and Mt Field in Tasmania. Tasmania lies in the "roaring 40s" a vigorous westerly weather drift, and thus precipitation is concentrated on the west coast. Situated closer to the west coast, Mt Read receives more rainfall than Mt Field.



*Microcachrys tetragona*, *Athrotaxis cupressoides* and *A. selaginoides* is found on, and immediately below, the Mt Mawson plateau, and this area was the focus for data collection. *Phyllocladus aspleniifolius* grows on the lower slopes of the mountain, but does not co-habit with the other species present, and was thus not considered. Being drier, the Mt Field area has been more affected by fire, and eucalypts encroach upon much of the coniferous forest edges. Mt Field lies east of the quartz/dolerite disjunction in Tasmania (Davies, 1965), and thus soil is derived from dolerite bedrock.

### *Field techniques*

#### Inter-site comparison

Field measurements were carried out during the first two weeks of January in 1996 after a dry December period at both sites. Readings were taken from an area of approximately 0.5 km<sup>2</sup> at both locations focussing on patches of vegetation where all species were found to be co-occurring. In order to minimise variation due to ambient atmospheric conditions, readings were taken during the five hours of full sun and maximum temperature between 10.30 am to 4 pm. Field trips were carefully timed to ensure clear skies and mild temperatures during data collection; the leaf temperature range for all readings was between 18.1°C and 26.6°C, and the average leaf temperature was very similar at both sites, ranging from 23.8°C at Mt Field to 23.9°C at Mt Read. Only foliage which was fully exposed to the sun was measured, and hence the variation in light intensities was small, with the photosynthetic photon flux density (PPFD) ranging from around 1100 to 1700  $\mu\text{mol}\cdot\text{quanta}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . The average light intensity of readings taken at Mt Read was 1300  $\mu\text{mol}\cdot\text{quanta}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and at Mt Field was 1180  $\mu\text{mol}\cdot\text{quanta}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .

At each site, 10-20 individuals of each species were sampled, and from each of these a minimum of 40 instantaneous readings of fluorescence were taken from different leaves on the sample individuals. The species investigated were the co-occurring species - *Athrotaxis selaginoides*, *A. cupressoides*, *Diselma archeri*, and *Podocarpus lawrencii*; and species restricted from one site *Microstrobos niphophilus*, *Lagarostrobos franklinii* and *Phyllocladus aspleniifolius*. Also included for comparison were two co-occurring angiosperms - *Richea scoparium* Hook. f., and *Leptospermum rupestre* Hook. f.

Fluorescence measurements were made with a portable amplitude modulated fluorometer (PAM 2000; Waltz, Effeltrich, Germany) using a leaf clip with micro-quantum sensor and leaf thermocouple attached (Bilger *et al.*, 1995). Leaves were clamped into position beneath the fibre-optic cable used for transfer of the fluorescence signal and delivery of saturating pulses and actinic light, ensuring the geometry of the leaf/fibre-optic positions was standardised. Rather than using the ambient light, it was found to be more accurate to use the internal halogen lamp for sample illumination during measurements. Hence it was necessary to measure the ambient light, and set the internal halogen to the appropriate power level to produce a similar intensity of light. Leaves were then shaded, the actinic light switched on for a period of 20 seconds, followed by the application of a saturating flash of light to determine the quantum yield (equation 5.1) and electron transport rate (equation 5.2). Instead of measuring the absorbance ( $\alpha$ ) for each leaf sample,  $\alpha$  was taken as the average value for green leaves of 0.84 as determined by Björkman and Demmig (1987). Thus ETR was calculated using Eq. 5.1

Dark adapted  $F_v/F_m$  was measured on a sample of five individuals of each species. Leaves were shaded with a dark cloth for a period of approximately 20 minutes after which they were placed into the leaf clip and  $F_o$  determined in the dark, a saturating pulse was then applied to allow measurement of  $F_m$ . It was necessary to ensure the exposed surface of the leaf was measured in each case, as considerable variation was observed between  $F_v/F_m$  measured on the exposed upper and shaded lower surfaces of single shoots.

#### Intra-site comparison

Conifers at Mt Field are commonly larger and more abundant along the banks of streams, but rapidly decrease in number and stature at greater distances from the stream edges. In order to determine whether this qualitative gradient was reflected in the instantaneous rate of ETR of individuals, a transect of ETR in *Athrotaxis cupressoides* was measured across a stream at Mt Field. A 30 m tape was laid out perpendicular to the stream edge about 100 m below the outlet of lake Dobson, through a patch of *Athrotaxis cupressoides* trees. At regular intervals along this transect, measurements of ETR were made on the leaves of individuals, with a single value produced for each tree, averaged from at least 20 shoots. Two

transects were made across the same area, one in mid-January and one in early-March.

### *Laboratory techniques*

The maximum rate of electron transport, and the ratio of  $F_v/F_m$  was determined for glasshouse grown individuals of each species using the technique described in chapter 6. These maximum rates were compared with values of ETR and  $F_v/F_m$  collected from plants in the field.

### **Results**

Considerable variation in ETRs was found among species, and between sites, but within species at each site, and in the glasshouse grown plants variation among individuals was low. ETR in the field was substantially lower than rates measured in glasshouse grown individuals, with the largest reduction found in *Phyllocladus aspleniifolius* from Mt Read, where the photosynthetic ETR was reduced from an average of  $117 \mu\text{mol.m}^{-2}.\text{s}^{-1}$  in glasshouse plants to  $63 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ , only 54% of the laboratory maximum (Figs 5.3, 5.4). The minimum reduction in ETR between glasshouse and field plants was in *Athrotaxis selaginoides* from Mt Read, where ETR was reduced from 135 to  $103 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ , only a 24% decrease.

In the four species measured on both Mt Read and Mt Field there was a consistently lower ETR found in plants from Mt Field. On Mt Read the ETR in each of these species was consistently around 75% of the rate measured in glasshouse individuals, while the same species on Mt Field only produced ETRs of between 67% and 56% of glasshouse values. The two species found on Mt Read but not on Mt Field (*Lagarostrobos franklinii* and *Phyllocladus aspleniifolius*) were more inhibited than the co-occurring species, exhibiting more than a 60% reduction in both cases. In *Microstrobos niphophilus*, which does not occur on Mt Read, ETR was around 58.5% of the maximum laboratory rate, which was close to the average depression observed in other species from Mt Field. These differences in ETR were reflected in measurements of  $F_v/F_m$  on dark adapted foliage with glasshouse grown individuals producing high optimal quantum yield, and individuals from Mt Field producing the lowest values (Fig. 5.4). The range of  $F_v/F_m$  in all glasshouse grown species was from 0.79 to 0.82, while  $F_v/F_m$  on species from Mt Field

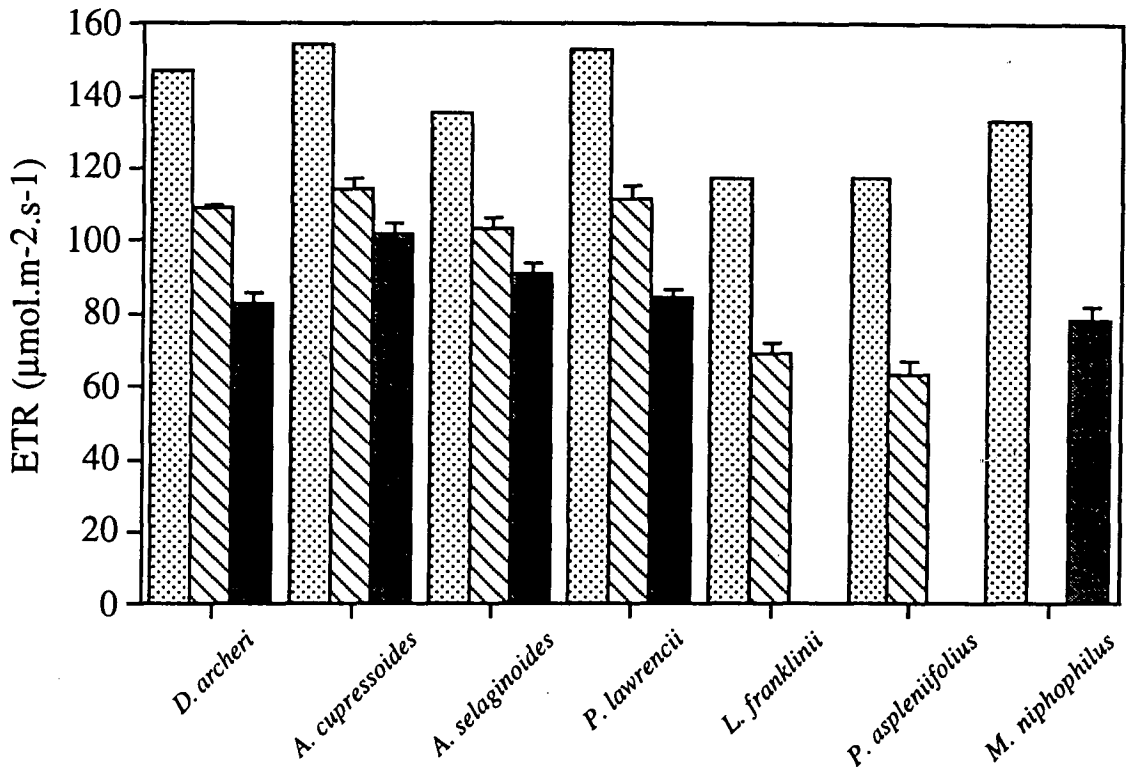


Figure 5.3. Apparent rates of electron transport (ETR) in laboratory plants-  $\square$ , and plants measured at Mt Read -  $\square$ , and Mt Field -  $\blacksquare$ . For all species in the field, values represented averages from at least 10 individuals (at least 40 readings per individual), and for laboratory data averages were taken from 5 individuals. Standard error bars are shown for all species (variation in the laboratory data was low).

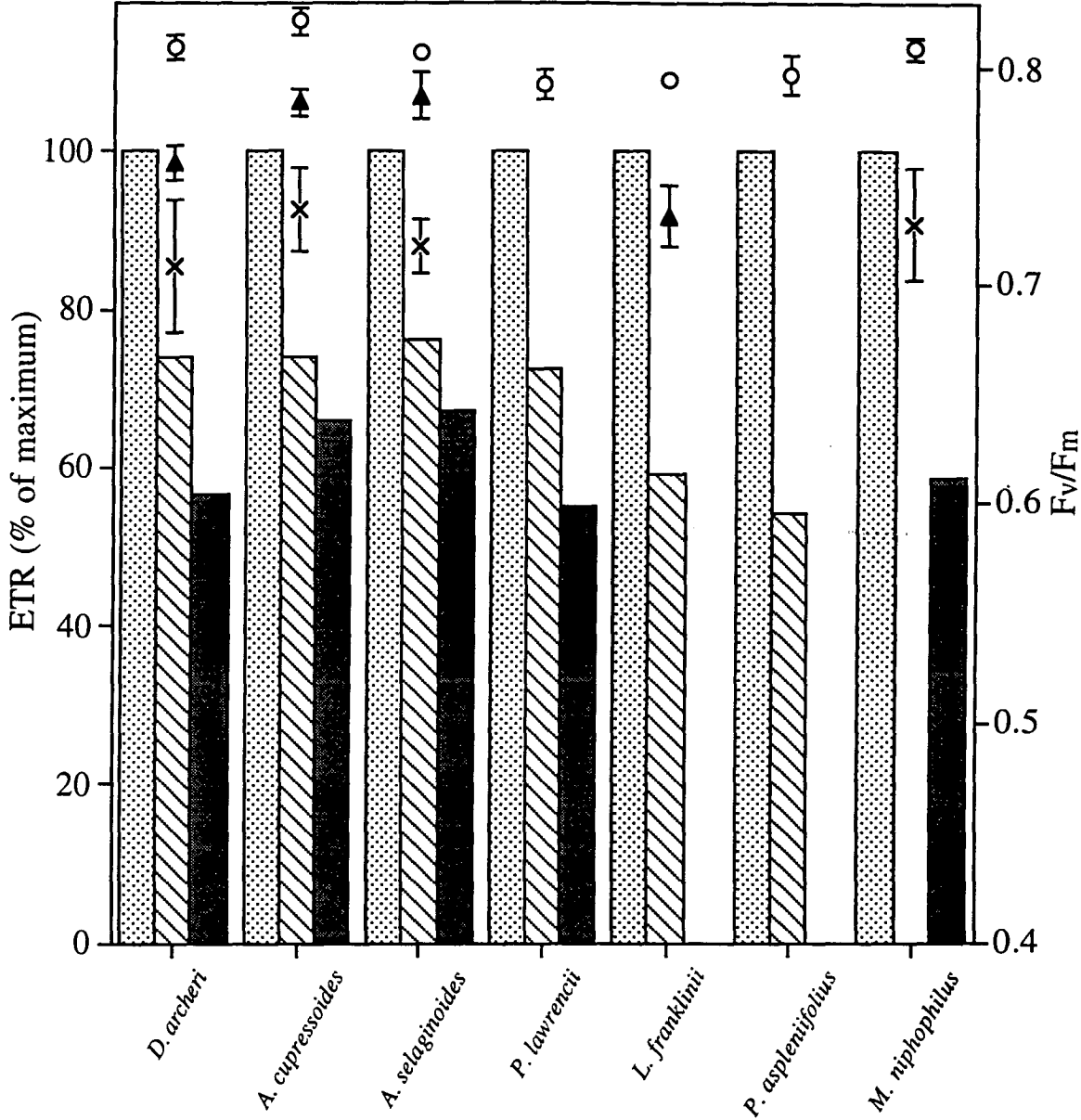


Figure 5.4. Rates of electron transport relative to the average maximum ETR measured for each species grown under glasshouse conditions (100%). Labelling is similar to figure 5.1 with laboratory plants- [dotted], Mt Read plants- [diagonal lines] and Mt Field plants- [solid black] distinguished. Also shown is the average F<sub>v</sub>/F<sub>m</sub> ratio measured for laboratory- x, Mt Read- o, and Mt Field- ▲ individuals.

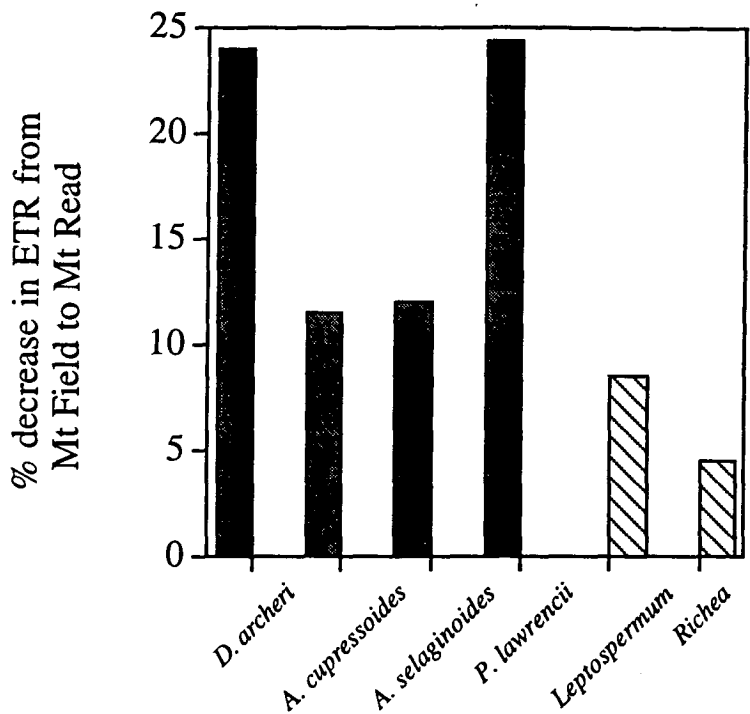


Figure 5.5. Percentage depression in average ETR at Mt Field relative to Mt Read, as measured in four co-occurrent conifer species- ■, and two angiosperm species- ▨ (*Leptospermum rupestre* and *Richea scoparium*).

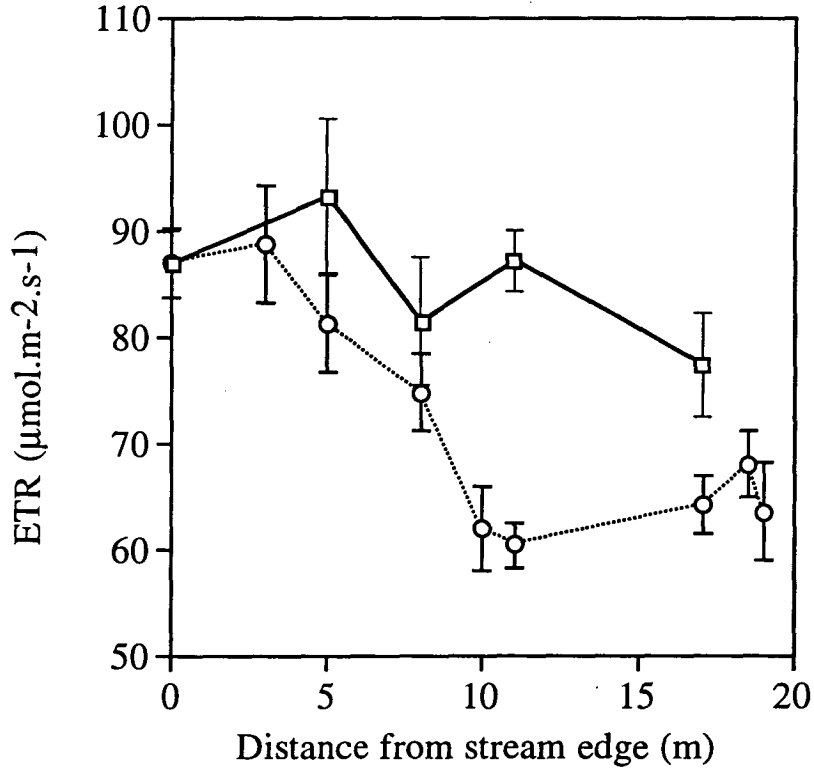


Figure 5.6. Changes in average ETR of *Athrotaxis cupressoides* individuals along a transect running perpendicular to a stream edge at Mt Field. Transects were taken in mid-January- □, and in early March- ○ across the same part of a stream.

ranged from 0.71 to 0.74, and on Mt Read values were between 0.73 and 0.79.

The percentage decrease in ETR from Mt Read to Mt Field was between 11.4% and 24.3% for the co-occurring conifers measured, but in the two co-occurring angiosperms the decrease in photosynthesis between sites was lower, with ETR in *Richea scoparium* reduced by only 4.5% from Mt Read to Mt Field, and *Leptospermum rupestre* reduced by 8.5% (Fig. 5.5).

Contrasting results were obtained in the two stream transects carried out, data collected in mid-January showed little systematic variation in ETR across the transect; however the second transect in March showed a significant trend of decreasing ETR away from the stream edge. In both transects ETR in plants on the stream edge was around  $90 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ , and in the March transect this diminished to values around  $60 \mu\text{mol.m}^{-2}.\text{s}^{-1}$  at a distance of 18 m from the stream edge (Fig. 5.6).

## Discussion

The data presented here illustrate significant differences in photosynthetic rate in the field both among the species investigated, and within species between sites. Such differences were not evident from data collected using gas exchange equipment, and demonstrate the successful application of field based fluorometry.

Substantial decreases in the rate of ETR were observed between glasshouse grown plants measured in the laboratory, and plants grown and measured in the field. Because light conditions were saturating or slightly over-saturating (see Chapter 6) at all times in the field, the cause of this decreased ETR was not related to suboptimal light intensity. The fact that  $F_v/F_m$  was decreased by approximately the same degree as ETR in the field suggests that the reduction in ETR is a result of a decrease in the quantum efficiency of PSII rather than limitations due to the instantaneous prevailing conditions at the time of measurement. The most commonly publicised cause of decreased  $F_v/F_m$  in plants is low temperature photoinhibition (e.g. Öquist and Huner, 1991), but in this case overnight temperatures remained at least  $10^\circ\text{C}$  above freezing and were thus extremely unlikely to have resulted in sustained photoinhibition during the day. High levels of xanthophylls did, however, appear to be present in the leaves of plants in the field, as there



was often a marked difference in the colour of exposed and shaded parts of the plant, with exposed foliage often yellow or red in colour as compared with green shaded foliage, and this is likely to be responsible for a decrease in quantum efficiency. The effect of exposure was also evident on single shoots, and measurements of  $F_v/F_m$  on the exposed upper, and shaded lower surfaces of *Lagarostrobos franklinii* shoots from Mt Read yielded an  $F_v/F_m$  on the shaded side of foliage of  $0.80 \pm 0.005$  as compared with  $0.73 \pm 0.014$  on the exposed side of shoots.

Xanthophyll pigments are the primary means of non-photochemical energy dissipation in the leaf (see Chapter 6), and their importance in the leaves of conifers, particularly in highland areas, would be emphasised by the longevity of foliage in these species. Although cold-induced photoinhibition was not likely to have been directly affecting photosynthesis during the survey period, the regularity of below freezing minimum temperatures all year round suggest that a large xanthophyll pool would be a necessity for long term protection of foliage. The suggestion that decreased quantum yield is linked to long term photoprotection from cold-induced photoinhibition is also supported by the very substantial depression of ETR found in *Phyllocladus aspleniifolius*, a species which is common at lower elevations but is at its altitudinal limit on Mt Read. Under these conditions it would be expected that the production of photoprotective xanthophyll pigments would be particularly important, and hence that the quantum efficiency would be low. Other possible reasons for the decrease in  $F_v/F_m$  in the field include reduced leaf nutrition or super-optimal leaf temperatures, but neither of these are supported by the data here. There was no relationship between leaf temperature and ETR either between or within sites, and the ETRs of all species from Mt Field were lower than those on Mt Read, despite the relatively richer substrate at Mt Field. The consistent differences in ETR and  $F_v/F_m$  between Mt Read and Mt Field are not explained by differences in minimum temperature either, as the mean minimum temperature for the coldest quarter at the Mt Read site, estimated by BIOCLIM is  $-1.37^\circ\text{C}$  compared with  $-1.17^\circ\text{C}$  for the Mt Field site.

The most likely explanation for the decreased leaf ETR and  $F_v/F_m$  observed at Mt Field relative to Mt Read during the mid-summer measurement period is that water stress was affecting plants at Mt Field. The rainfall at Mt Field is approximately 1532 mm per annum compared with 3109 mm per annum at Mt Read (BIOCLIM estimates) constituting a

49% difference in the average rainfall. Anecdotal evidence of comparatively luxuriant growth of conifers on Mt Read (pers. obs.) compared to Mt Field also fits with the suggestion that plants on Mt Field are water limited. Further support for the water limitation hypothesis comes from the decreasing ETR observed in *Athrotaxis cupressoides* growing at increasing distances from a stream edge at Mt Field (Fig. 5.6), particularly as inhibition of ETR only became evident at the end of summer when the expression of water-stress damage is likely to be at a maximum. Associated angiosperms, *Richea scoparium* and *Leptospermum rupestre*, were found to be significantly less inhibited going from Mt Read to Mt Field than the conifers measured. These are both hardy species, widespread in the Tasmanian highlands (Curtis, 1975), and the fact that ETR in both species was inhibited less than in the conifer species, indicates a relatively high degree of sensitivity (presumably to water availability) in the coniferous taxa investigated.

If, as appears to be the case, conifers at Mt Field are being subject to drought induced photoinhibition, then it clearly suggests that this site is marginal for conifer habitation, and indicates the potential for further contraction in distribution in response to decreased rainfall. There is clear evidence of dieback in *Athrotaxis selaginoides* on the lower slopes of Mt Field (around 900m), and this has been qualitatively associated with drought stress (Dwiputranto, 1990). Seedling distributions of *Diselma archeri* and *Microstrobus niphophilis* also appear to be limited by water availability, being largely confined to stream banks and areas of continuous runoff on Mt Field (pers. obs.). These observations are all consistent with the conclusion that water availability is an important constraint on the distribution of conifers, and that the rainfall at Mt Field approaches the minimum requirement for several of the resident conifer species.

## CHAPTER 6

# Light Response and Stomatal Optimisation in Conifer Leaves

## Introduction

The Podocarpaceae is undoubtedly the most successful conifer family in the southern hemisphere in terms of both distribution and diversity, and the extreme variation in foliar morphology among genera illustrates an evolutionary flexibility unrivalled by any conifer family. As discussed in chapter two, the genera of Podocarpaceae produce a spectrum of foliage forms ranging from the typical conifer scale and needle-type leaves to broadly flattened leaves and shoots that appear to have arisen independently on at least five occasions. Podocarps are found in a wide variety of habitats from canopy dominant in dense rainforest, to understorey shrub in open woodland (Enright and Hill, 1995), and it has been suggested that it is the ability to produce functional 'broad-leaves' which has enabled them to compete with angiosperms in the low light environment under forest canopies (Hill, 1995). In this chapter the light responses of species with broad and narrow shoots are compared to determine whether any correlation exists between shoot breadth and light response.

Amongst the southern hemisphere Cupressaceae, only the three closely related genera *Libocedrus*, *Papuacedrus*, and *Austrocedrus* have significant shoot flattening and two of these are restricted to rainforest habitats. Relatively few Northern Hemisphere conifers produce two dimensionally flattened foliage, and those that do are generally associated with dark, wet environments e.g. *Metasequoia*, *Taxodium*, and *Abies* (Leverenz, 1995). The relationship between shoot architecture and light environment is best demonstrated in *Abies*, where shoots developed under high light are typically three dimensional, while those developed at lower irradiance show increasing bilateral compression (increased silhouette area/total leaf area) (Sorrensen-Cothorn *et al.*, 1993). A similar trend was noted in Chapter 2 with the shoot architecture of some podocarp genera grown under glasshouse conditions distinctly more three dimensional than that observed in deep shade under a forest canopy (Figs 1.21, 1.22).

Previous studies of Northern Hemisphere conifers have shown that maximum photosynthetic rate (per unit silhouette area) and

quantum yield of CO<sub>2</sub> fixation in several genera of Pinaceae (*Pinus*, *Abies*, *Tsuga*, and *Picea*) were related to the degree of shoot flattening (silhouette area/total leaf area), and that differences in physiology between flattened and non-flattened shoots were removed by illuminating stems from all directions during measurement (Carter and Smith, 1985; Leverenz, 1995). The variation in leaf and shoot morphology among species of the Podocarpaceae is far more profound than the differences observed in the Pinaceae, and in this study the relationship between leaf/shoot morphology and light response was measured in several genera with distinctly different morphologies. The aim was to determine whether there was a relationship between shoot width and light response in the Podocarpaceae, and specifically whether broad-shoots exhibited a physiological preference for low light intensity.

Instead of applying classical techniques whereby the potential of species to adapt to artificially imposed conditions of sun and shade is used to distinguish species better suited to growth under high or low light environments (e.g. Yun and Taylor, 1986), plants here were all grown and measured under identical conditions of light, nutrient supply, temperature, etc. To ensure no species suffered from lethal extremes of light intensity, the light climate imposed was intermediate between sun and shade. The intention here was not to evaluate the short term adaptive potential (morphological or physiological) of shoots to imposed extremes of sun or shade, but to indicate the relative light climate preferences of different shoot morphologies grown under uniform conditions. From this information it was proposed to test the intuitive association between podocarp "broad shoots" and low light environments. Several species of the conifer family Cupressaceae, and a needle-leaved angiosperm (*Allocasuarina verticillata*) were also included to determine whether relationships between morphology and light response were common among more distantly related species.

The light response of conifers was measured here using a modulated fluorometer (Schreiber *et al.*, 1986) to determine the quantum yield of photosystem II ( $\Phi_{PSII}$ ) of individual leaves, or of a small part of a compound shoot (<1.8cm<sup>2</sup>), avoiding the effects of self-shading. This allowed the response of leaves to be directly measured without the complicating effects of leaf arrangement. The use of chlorophyll *a* fluorescence as a tool for measuring light response is increasing (e.g. McKiernan and Baker, 1992, Bilger *et al.*, 1995), despite the considerable potential of this technique for assaying both the rate of

energy transfer through PS II, and characteristics of the energy dissipating processes such as the xanthophyll cycle (Demmig-Adams *et al.*, 1995) which modulate the response of photochemistry to light (see Chapter 5). Rather than measuring the rate of CO<sub>2</sub> uptake at different light intensities, the response of apparent electron transport rate (ETR) to photosynthetic photon flux density (PPFD) was determined here. As discussed in Chapter 5, ETR expresses the relative rate of electron transport through PSII for reduction of NADP<sup>+</sup>, and is thus closely related to the rate of carboxylation.

In C<sub>4</sub> plants the relationship between ETR and CO<sub>2</sub> assimilation rate has been shown to be linear (Edwards and Baker, 1993); however, in conifers, and indeed all C<sub>3</sub> plants, changes in the internal CO<sub>2</sub> concentration must affect the ratio of Rubisco carboxylations to oxygenations, and hence alter the number of electrons required per mole of fixed CO<sub>2</sub> (Ghashghaie and Cornic, 1994). Under constant conditions of humidity and temperature, stomatal optimisation theory (Cowan, 1982) suggests that  $c_i$  will be maintained constant, and thus that the ETR vs assimilation rate should be approximately linear. Although stomatal optimisation has been reported in a northern hemisphere conifer species (Berninger and Hari, 1993) an examination of the stomatal response to light, and the optimisation of stomatal conductance, was undertaken here to determine whether this physiological trait was common to southern hemisphere conifer genera, and whether there was any association between shoot morphology and photochemical and stomatal response to light. Ultimately, the relationship between the quantum yield of electron transport ( $\Phi_{etr}$ ) and CO<sub>2</sub> uptake ( $\Phi_{CO_2}$ ) was plotted to determine how accurately ETR measured by fluorescence could be used to estimate the assimilation rate.

## Materials and Methods

### *Plant Material*

Eleven species of Podocarpaceae: *Acropyle pancheri*, *Dacrycarpus dacrydioides*, *Dacrycarpus compactus*, *Lagarostrobos franklinii*, *Microstrobos niphophilus*, *Phyllocladus aspleniifolius*, *Podocarpus dispersis*, *Podocarpus drouynianus*, *Podocarpus lawrencii*, *Prumnopitys ferruginea*, and *Retrophyllum comptonii*, encompassing the full range of shoot morphologies from highly flattened to cylindrical in cross-section,

were compared with five morphologically distinct species of Cupressaceae: *Actinostrobus acuminatus*, *Athrotaxis cupressoides*, *Athrotaxis selaginoides*, *Callitris rhomboidea*, and *Diselma archeri*. A needle-leaved angiosperm, *Allocasuarina verticillata*, was included for comparison.

### Fluorescence

Chlorophyll *a* fluorescence was measured using a PAM 2000 fluorometer (Waltz, Effeltrich, Germany). Leaves, or portions of shoot, which had been dark adapted for approximately 30 minutes were clamped into a leaf clip (Bilger *et al.*, 1995), ensuring that no self shading was occurring. Leaf temperature was maintained at approximately 20°C during fluorescence readings.

Initially  $F_0$  (minimum fluorescence with electron chain fully reduced) was measured in the dark, after which a saturating pulse of light was applied to the foliage, allowing calculation of  $F_m$  (maximum fluorescence with electron acceptors fully oxidised) and  $F_v$  ( $F_m - F_0$ ). An actinic light was then switched on, and measurements of light adapted  $F_m'$  carried out again by applying a saturating flash of light.  $F_0$  quenching was determined by illuminating the sample with far-red light for 3 seconds with actinic light switched off, facilitating transfer of excitation energy to PSI while measuring  $F_0'$  (Schreiber *et al.*, 1994). Actinic light intensities were stepped up gradually from 15 to around 2000  $\mu\text{mol.photons}^{-1}.\text{m}^{-2}.\text{s}^{-1}$ , allowing a period of 20 minutes for acclimation to light intensities below 350  $\mu\text{mol.photons}^{-1}.\text{m}^{-2}.\text{s}^{-1}$  and 30-40 minutes allowed for light intensities above this. An external halogen lamp with fibre optic light delivery was used during the acclimation period at PPFDs greater than 350  $\mu\text{mol.photons}^{-1}.\text{m}^{-2}.\text{s}^{-1}$ , as continuous use of the PAM internal halogen caused excessive heating. A significant loss of accuracy became apparent when measuring fluorescence under high external light intensities (Chapter 5) and hence it was necessary to switch off the external light and use the internal halogen during measurements. A period of at least 5 minutes was allowed after the switching of light sources. This also allowed determination of  $F_0'$  at high light intensities, and hence the determination of quenching coefficients.  $\Phi_{\text{PSII}}$ , photochemical quenching ( $q_p$ ), non-photochemical quenching ( $q_n$ ) of fluorescence, and ETR were calculated at each light intensity using equations 5.1 (from chapter 5), 6.1 and 6.2.

Equation 5.2.  $ETR = \Phi_{PSII} \cdot I \cdot \alpha / 2$

where  $I$  = incident PPFD (in the waveband 400-700 nm);  $\alpha$  = leaf absorbance, taken here as 0.84 (Björkman and Demmig, 1987); and the factor of 2 accounts for the fact that 2 photons are required per electron passed through PSII, assuming linear electron flow, and even distribution of absorbed quanta between PSII and PSI. The average value of  $\alpha$  for green leaves of 0.84 (Björkman and Demmig, 1987) was used here, and it was assumed that excitation energy was evenly distributed between PSII and PSI (Bilger *et al.*, 1995; Loreto *et al.*, 1995). The units of ETR are  $\mu\text{mol.electrons.m}^{-2}.\text{s}^{-1}$ , although it should be noted that values of ETR will not be precise as leaf absorbance was not measured. The use of the average  $\alpha$  for leaves, of 0.84, as determined by Björkman and Demmig (1987) was considered a good approximation considering that all leaves were relatively thick and only healthy, green leaves were selected. The one podocarp measured in the survey by Björkman and Demmig (1987) was found to have an  $\alpha$  of 0.835, very close to the average value used here.

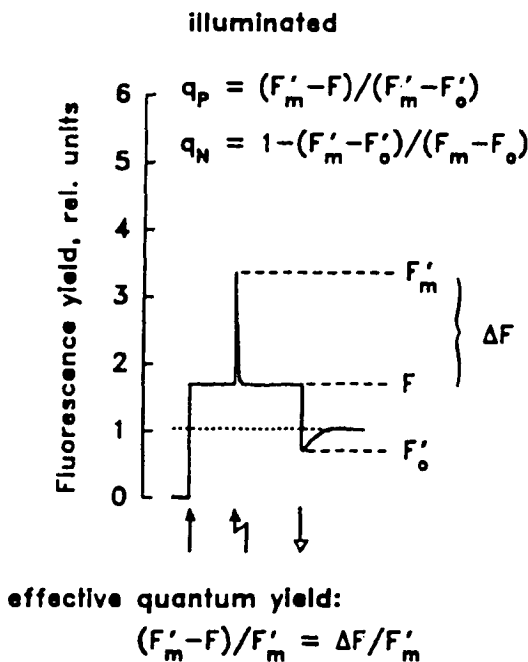


Figure 6.1. Fluorescence parameters as measured with a modulated fluorometer. The dotted line represents  $F_0$  as determined from a dark adapted sample (see fig. 5.1).  $F$  is the fluorescence produced by an illuminated leaf when the modulated measuring beam is switched on (first arrow).  $F_m'$  is the light adapted maximum fluorescence produced by a saturating pulse of light (second arrow). Quenching of  $F_0$  in the light ( $F_0'$ ) is also measured by switching off the illuminating light ( $\downarrow$ ) and applying a far-red beam to excite PS I and fully oxidise PS II. Equations for calculating the quenching coefficients  $q_n$  and  $q_p$  and effective quantum yield are shown. (Adapted from Schreiber and Bilger, 1992).

Equation 6.1.  $q_p = (F_m' - F) / (F_m' - F_0')$

Equation 6.2.  $q_n = 1 - (F_m' - F_0') / (F_m - F_0)$



where fluorescence parameters are defined in Fig. 6.1 and in Schreiber and Bilger (1992).

All fluorescence measurements were carried out at approximately 20°C, and response curves were measured from five replicates of each species, except for *A. pancheri*, *P. ferruginea* and *R. comptonii*, where two replicates were used. Polynomial curves were used to fit the pooled data for each species, and from these curves saturating PPFD ( $\text{PPFD}_{\text{sat}}$ ) and maximum ETR (at  $\text{PPFD}_{\text{sat}}$ ) were read.

### Light Response

In each species, the three replicates which produced the highest ETRs were used to measure the response of net  $\text{CO}_2$  uptake and leaf conductance ( $g_s$ ) to light intensity. The gas-exchange apparatus used is described in Chapter 3, and leaf conditions were maintained at the standard 20°C, leaf-air vapour pressure deficit at 5-10  $\text{mmol.mol}^{-1}$  and ambient  $\text{CO}_2$  at  $350 \pm 5 \text{ } \mu\text{mol.mol}^{-1}$ . Initially, the dark respiration rate was measured, and light intensity was stepped up using neutral density filters (Kodak) until a maximum light intensity of approximately 1400  $\mu\text{mol.photons.m}^{-2}.\text{s}^{-1}$  was reached. At each light intensity  $\text{CO}_2$  uptake and  $g_s$  were measured after readings had stabilised, generally after 30 minutes, but up to 2 hours in some species. This procedure was repeated under gradually decreasing light intensity for each species in order to indicate the amount of hysteresis in the stomatal opening versus closing responses. The small volume of the cuvette (20 mL) and careful selection of foliage allowed leaves to be arranged such that no self shading occurred during measurement of gas-exchange. Following gas-exchange analysis, the silhouette area of the shoot enclosed in the cuvette was determined using a digital camera (EDC-1000, Electrim Corp. Princeton, USA) after which leaves were detached, oven dried at 70°C for 72 hours, and leaf dry weight measured.

Response curves of  $\text{CO}_2$  uptake and ETR to light intensity were matched for each of the three replicates, and the ETR at each measured  $\text{CO}_2$  uptake rate calculated by interpolating from the fitted ETR curve. This was necessary because the light intensity increments produced by the PAM-2000 were not the same as those produced by the light filters for the gas analyser. From these measurements,  $\Phi_{\text{etr}}$  ( $\text{ETR}/\text{PPFD}$ ) and  $\Phi_{\text{CO}_2}$  ( $\{(\text{A} + \text{DRR})/\text{PPFD}\}$ ) were calculated at the 6-7 light intensities used.

### Stomatal Optimisation

Changes in stomatal conductance were measured during light inhibition of photosynthesis as described above. Included for comparison were the broad-leaved angiosperms, *Anopterus glandulosus*, *Nothofagus cunninghamii*, *Eucalyptus tenuiramis* and the "needle-leaved" *Allocasuarina verticillata*. The response of  $g_s$  to photosynthetic inhibition induced by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU),

was also measured in *Eucalyptus tenuiramis* and the conifers, *Actinostrobus acuminatus* and *Lagarostrobos franklinii*. Administration of DCMU into the angiosperm leaf simply involved injecting a 0.001 M solution of DCMU into the petiole of a leaf inserted in the cuvette, allowing the response of  $g_s$  and  $A$  to be recorded over a period of 3 hours as  $A$  approached zero. In gymnosperms the infusion of DCMU required a period of days, presumably because of the less efficient transport of the gymnosperm vascular system. During this period  $A$  and  $g_s$  were measured regularly under saturating light ( $1400 \mu\text{mol.photons}^{-1}.\text{m}^{-2}.\text{s}^{-1}$ ) and the standard leaf conditions of  $20^\circ\text{C}$ , leaf-air vapour pressure deficit at  $5\text{-}10 \text{ mmol.mol}^{-1}$  and ambient  $\text{CO}_2$  at  $350 \pm 5 \mu\text{mol.mol}^{-1}$ . Results were considered in the light of the stomatal optimisation theory of Cowan (1977) which states that the transpiration rate of leaves ( $E$ ) will respond to changes in the assimilation rate ( $A$ ), via regulation of  $g_s$ , such that  $\partial E / \partial A = \text{constant}$ .

### Leaf Characters

Because leaves were arranged to prevent any self-shading, methods of describing shoot morphology, such as silhouette leaf area/ projected leaf area, were not necessary. In all species, short shoots were functionally and morphologically analogous to leaves, in imbricate species, shoot width was less than 2mm, analogous to needle-leaves, and in non-imbricate species, short shoots were analogous to single broad-leaves (chapter 2). For this reason, the width of the short shoot provided the easiest and best means of quantifying its morphological character in the various species.

The width of short shoots was measured at their widest point with a set of digital callipers (measuring to an accuracy of  $\pm 0.01\text{mm}$ ), and for each species, averages were taken from 30-40 shoots (depending on number of plants available).

Specific leaf area (SLA) was also calculated on a silhouette area basis. Values of SLA for each species represented averages from 5 shoots.

## Results

### *ETR and Light Intensity*

ETR increased in response to increasing light intensity, to a well defined maximum ETR ( $ETR_{max}$ ) occurring at  $PPFD_{sat}$ , and declined thereafter. Figure 6.2 shows average values of ETR at each light intensity for three species with different morphological and light saturating characteristics (*Actinostrobos acuminatus* and *Diselma archeri* both produce imbricate shoots, while *Retrophyllum comptonii* produces broad, flat, short shoots). Fourth-order polynomials fitted the data reasonably well, and from these curves,  $ETR_{max}$  and  $PPFD_{sat}$  were measured. The initial slope of the PPFD vs ETR response was virtually identical in all species regardless of  $ETR_{max}$  or  $PPFD_{sat}$ .

Photochemical quenching of fluorescence ( $q_p$ ) decreased in a linear fashion as light intensity increased, with the rate of decrease inversely proportional to  $ETR_{max}$ ; slope  $q_p = 1.19 \times 10^{-6}(ETR_{max}) - 4.5 \times 10^{-4}$ ;  $r = 0.849$ . Figure 6.3(a) illustrates the increasing decay rate of  $q_p$  in species with lower  $ETR_{max}$ , using the same three species as figure 2. The relationship between non-photochemical quenching ( $q_n$ ) and PPFD was more complex (Fig. 6.3b), with a hyperbolic increase in  $q_n$  as light intensity increased. The rate of  $q_n$  saturation in each species was related to its photochemical capacity, with low  $ETR_{max}$  corresponding to rapid saturation.  $q_n$  saturation always occurred at light intensities higher than  $PPFD_{sat}$ , and in several species  $q_n$  did not saturate at the maximum light intensity (approximately  $2500 \mu\text{mol.photons.m}^{-2}.\text{s}^{-1}$ ).

A linear relationship was observed between  $ETR_{max}$  and  $PPFD_{sat}$  in the 16 species investigated (Fig. 6.4), with the maximum  $ETR_{max}$  of  $217 \mu\text{mol.m}^{-2}.\text{s}^{-1}$  (*Actinostrobos acuminatus*) occurring at a PPFD of  $1725 \mu\text{mol.photons.m}^{-2}.\text{s}^{-1}$ , and the minimum  $ETR_{max}$  of  $60 \mu\text{mol.m}^{-2}.\text{s}^{-1}$  (*Podocarpus dispersis*) measured at a PPFD of  $745 \mu\text{mol.photons.m}^{-2}.\text{s}^{-1}$  (Fig. 6.4). This linear relationship indicates that  $\Phi_{PSII}$  at  $PPFD_{sat}$  was almost constant for each species. Measurement of average  $\Phi_{PSII}$  at  $PPFD_{sat}$  for each species indicated that quantum yield was conserved, with an average value of  $\Phi_{PSII} = 0.278 \pm 0.0035$  calculated for all 16 species.

Table 6.1. Average maximum width of shoots, saturating PPFD and maximum electron transport rate in 10 species of Podocarpaceae, 5 species of Cupressaceae, and a single needle-leaved angiosperm.

Species	Average shoot width(mm)	PPFD <sub>sat</sub> ( $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ )	ETR <sub>max</sub> ( $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ )	A <sub>max</sub> ( $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ )
<b>Podocarpaceae</b>				
<i>Acmopyle pancheri</i>	22.2	990	118	12.05
<i>Dacrycarpus compactus</i>	6.50	900	111	37.84
<i>Dacrycarpus dacrydioides</i>	9.16	1025	120	20.04
<i>Lagarostrobos franklinii</i>	1.27	1110	117	21.8
<i>Microstrobos niphophilus</i>	1.34	1170	133	20.66
<i>Podocarpus lawrencii</i>	1.7	1260	153	22.9
<i>Podocarpus drouynianus</i>	4.74	925	121	15.8
<i>Podocarpus dispersis</i>	17.2	745	60	4.67
<i>Retrophyllum comptonii</i>	43.4	740	89	6.93
<i>Prumnopitys ferruginea</i>	19.18	700	84	6.35
<i>Phyllocladus aspleniifolius</i>	16.7	825	117	13.7
<b>Cupressaceae</b>				
<i>Actinostrobus acuminatus</i>	1.08	1725	217	37.84
<i>Athrotaxis selaginoides</i>	2.15	1155	135	20.04
<i>Athrotaxis cupressoides</i>	2.38	1245	154	21.8
<i>Callitris rhomboidea</i>	0.99	1380	150	25.55
<i>Diselma archeri</i>	1.27	1175	147	19.05
<b>Angiospermae</b>				
<i>Allocasuarina verticillata</i>	0.92	1595	202	36.71

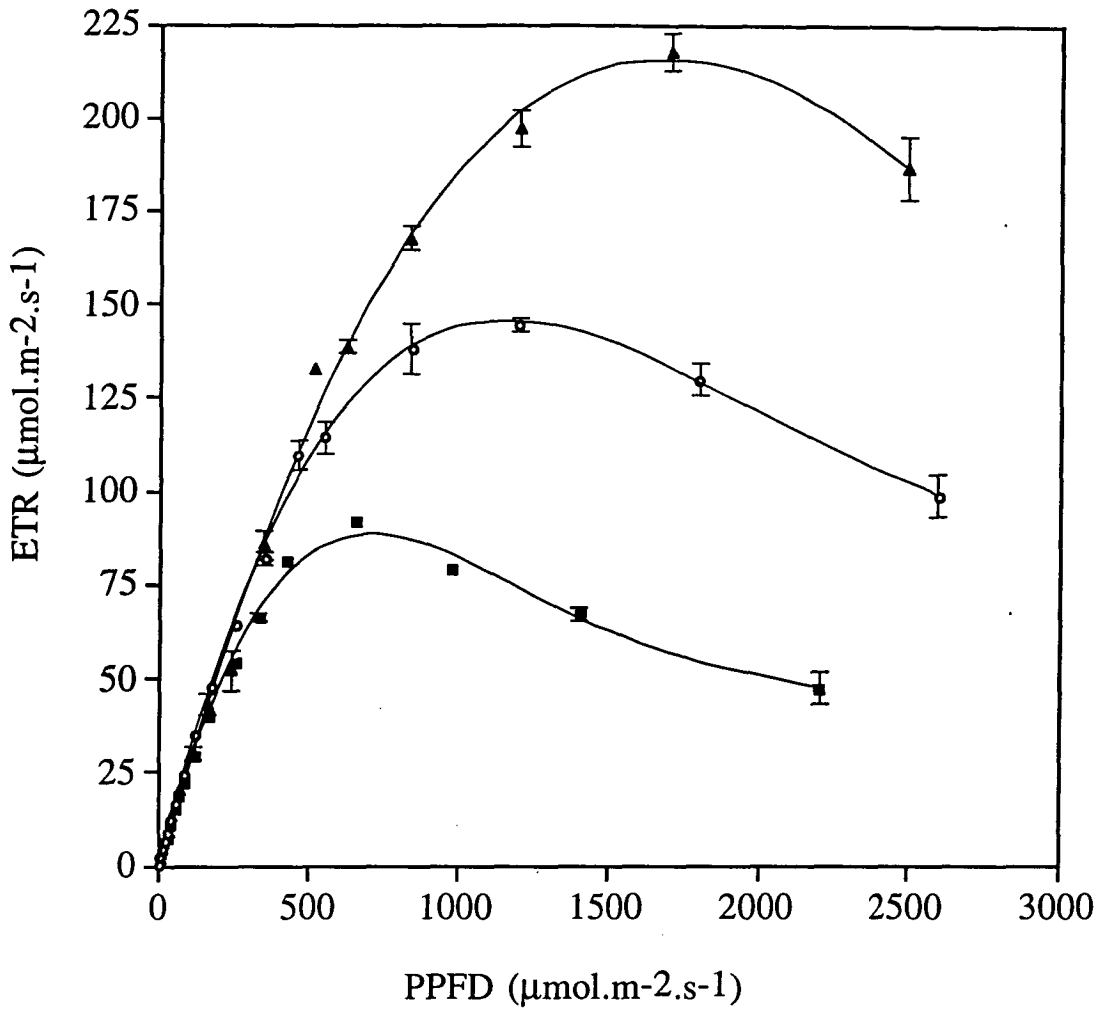


Figure 6.2. The response of apparent electron transport rate (ETR) to PPFD in the conifers *Actinostrobus acuminatus* - ▲ (imbricate shoot), *Diselma archeri* - ○ (imbricate shoot), and *Retrophyllum comptonii* - ■ (broad shoot). Values shown represent averages from 5 plants (except *Retrophyllum comptonii*, where only 2 plants were available). Curves fitted are fourth order polynomials. Values of saturating PPFD ( $\text{PPFD}_{\text{sat}}$ ) and maximum ETR ( $\text{ETR}_{\text{max}}$ ) were taken from these light response curves.

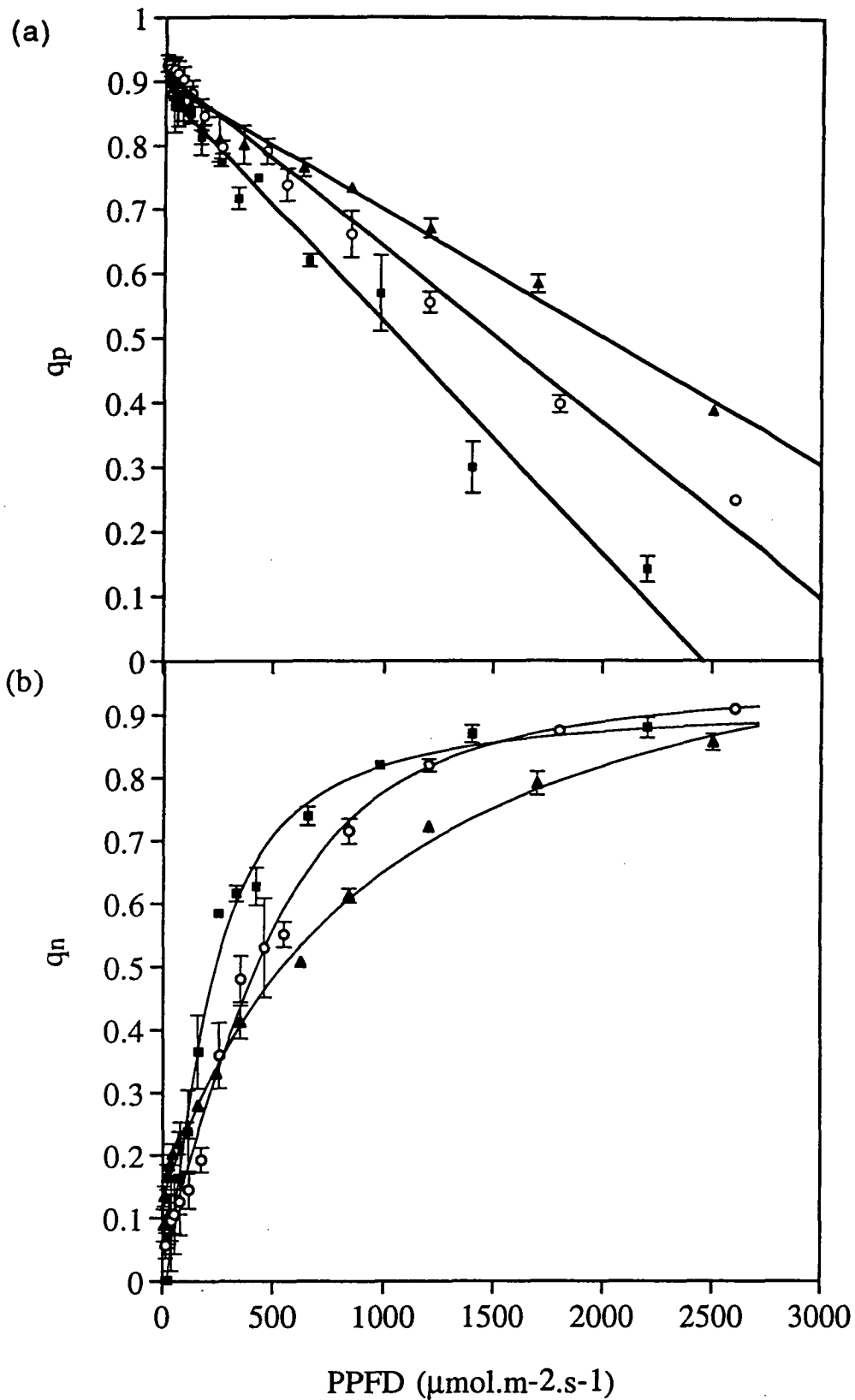


Figure 6.3. Changes in (a)  $q_n$ - photochemical and (b)  $q_p$ - non-photochemical fluorescence quenching in response to increasing PPFD in the same three species as Figure 6.2. Linear regressions were used to fit the  $q_p$  data and rectangular hyperbolae were fitted to the  $q_n$  data. Symbols are as in Fig. 6.2.

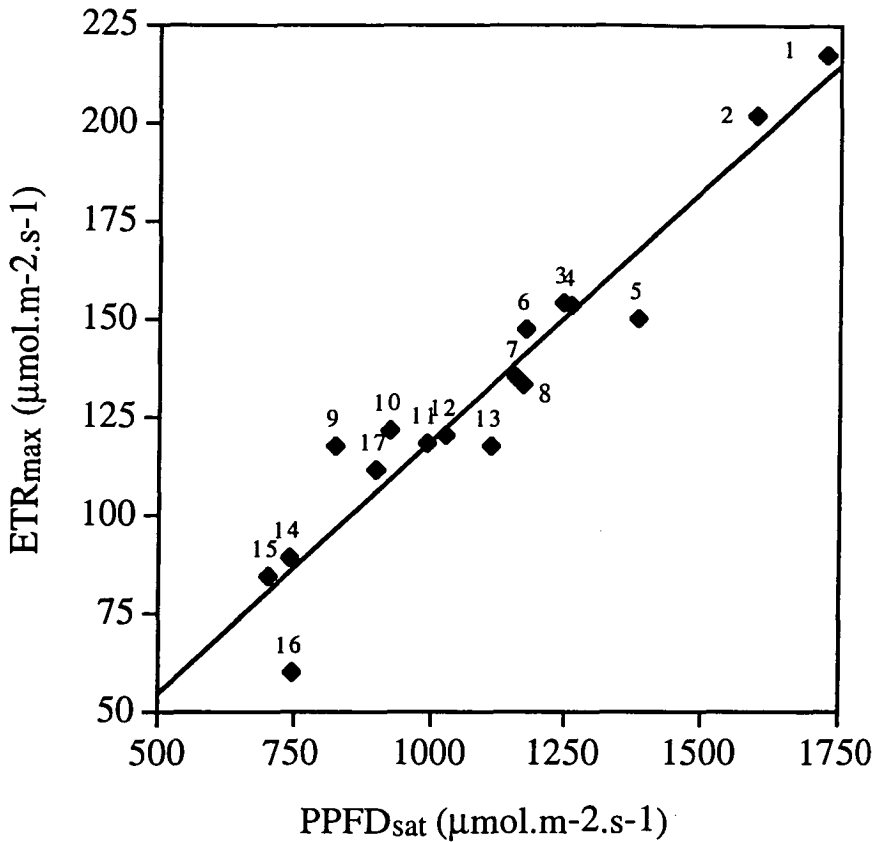


Figure 6.4. A linear relationship was observed between light saturation intensity (PPFD<sub>sat</sub>) and the maximum rate of electron transport through PSII as determined by fluorescence analysis ( $p < 0.001$ ). Among the 16 species shown are: 10 species of Podocarpaceae; 4- *Podocarpus lawrencei*, 8- *Microstrobos niphophilus*, 9- *Phyllocladus aspleniifolius*, 10- *Podocarpus drouynianus*, 11- *Acropyle pancheri*, 12- *Dacrycarpus dacrydioides*, 13- *Lagarostrobos franklinii*, 14- *Retrophyllum comptonii*, 15- *Prumnopitys ferruginea*, 16- *Podocarpus dispersis*. Five species of Cupressaceae; 1- *Actinostrobus acuminatus*, 3- *Athrotaxis cupressoides*, 5- *Callitris rhomboidea*, 6- *Diselma archeri*, 7- *Athrotaxis selaginoides*. And a "needle-leaved" angiosperm; 2- *Allocasuarina verticellata*.



$ETR_{max}$  was also linearly related to the maximum rate of  $CO_2$  assimilation measured in each species by infra-red gas analysis (Fig. 6.5), with the maximum  $ETR$  of  $217 \mu mol.m^{-2}.s^{-1}$  corresponding to an assimilation rate of  $37.8 \mu mol.m^{-2}.s^{-1}$ . This regression was not expected to pass through the origin due to the fact that  $A_{max}$  was a measure of net  $CO_2$  flux, and hence would be negative (due to respiration) at an  $ETR$  of zero.

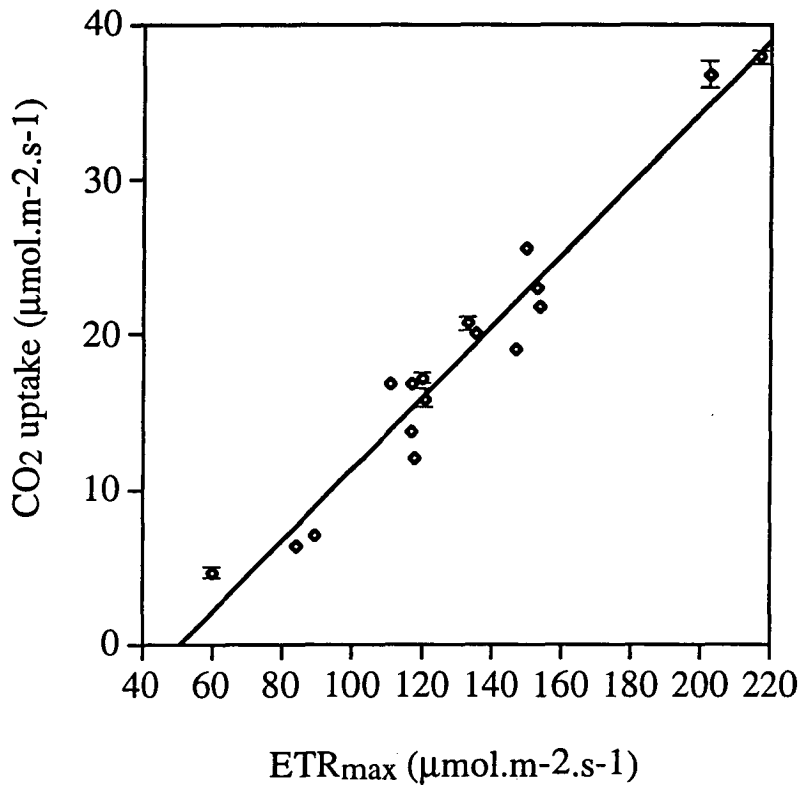


Figure 6.5. The relationship between electron transport rate as determined by fluorescence analysis, and the maximum rate of  $CO_2$  uptake, in all 16 species. A highly significant linear regression is shown ( $p < 0.001$ ). The relationship is not expected to go through the origin considering the fact that  $CO_2$  uptake at 0  $ETR$  will be negative due to respiration. Values of  $CO_2$  uptake are average maximum rates from 5 individuals, except *P. ferruginea*, *R. comptonii*, and *P. dispermis*, which were all represented by 2 individuals.

$PPFD_{sat}$  was significantly correlated ( $PPFD_{sat} = -241 \cdot \log(\text{shoot width}) + 1209$ ;  $p < 0.001$ ) with the log of width of short shoots in the 11 species of Podocarpaceae used here (Fig. 6.6). In narrow (imbricate) shoots,

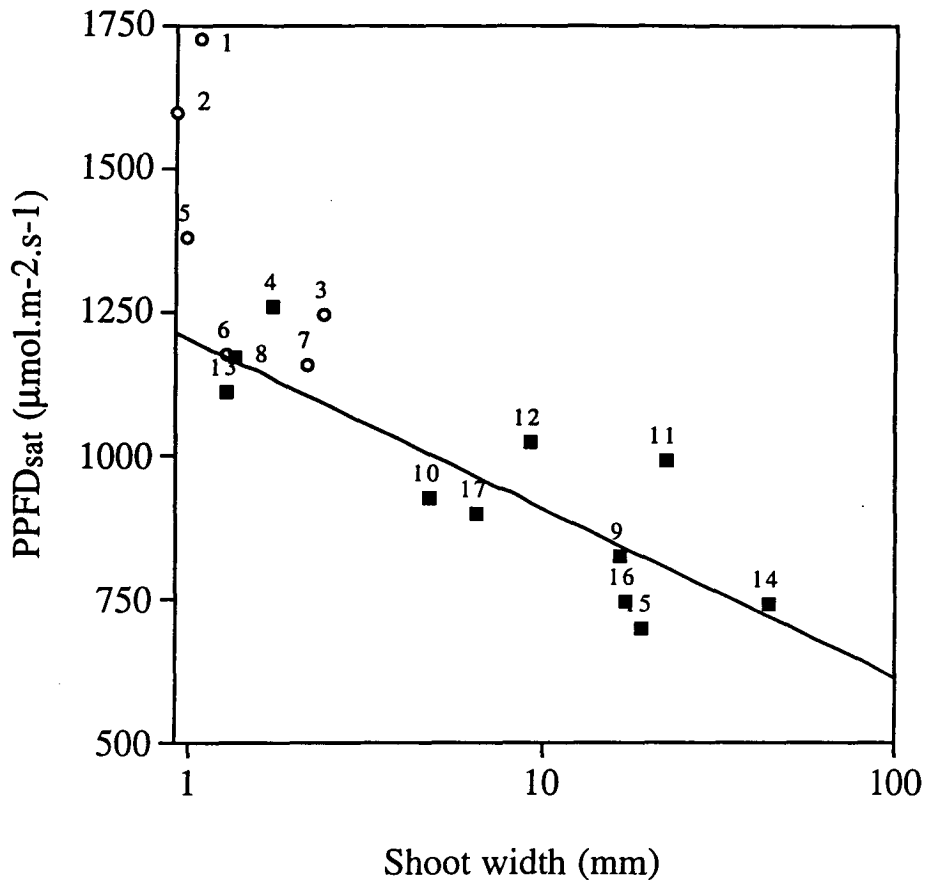


Figure 6.6. The relationship between light saturation intensity and average width of short shoots in 10 species of Podocarpaceae - ■ . A highly significant log regression ( $p < 0.001$ ) is shown. The 6 species of non-podocarps also shown - ○ did not fit on this regression. Species are labelled as in fig. 6.4.

ETR was found to saturate at higher light intensities than in broader, flattened shoots (Table 6.1). Among the five species of Cupressaceae and *Allocasuarina* included in the study, there was no significant degree of shoot flattening, and thus there was no relationship between shoot width and light saturation characteristics in these non-podocarp taxa (Fig. 6.6).

A weak but significant positive linear relationship was present between SLA and  $\text{PPFD}_{\text{sat}}$  ( $r = 0.50$  ;  $p < 0.05$ ).

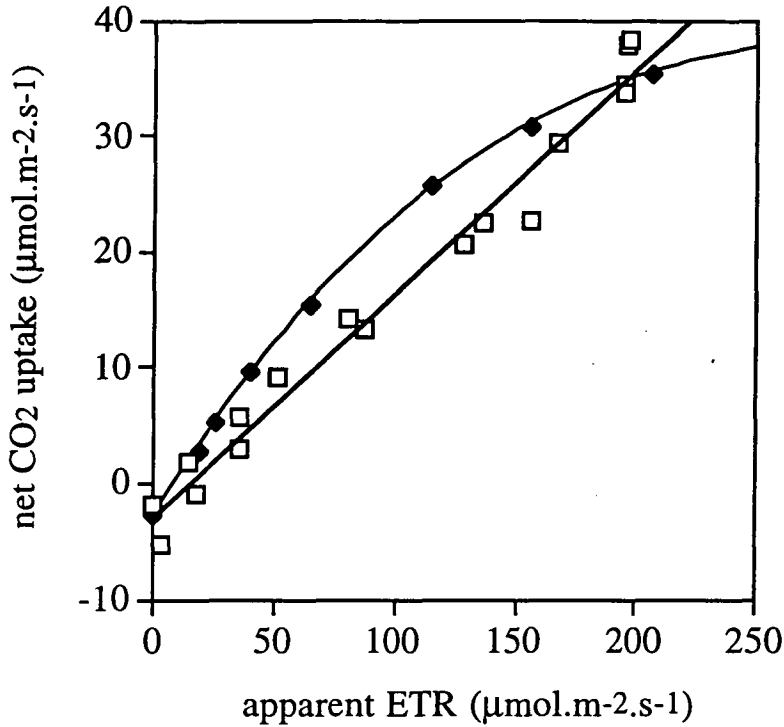


Figure 6.7. ETR versus assimilation rate in the angiosperm *Allocasuarina verticillata* - ◆ and the conifer *Actinostrobus acuminatus* - □. Changes in assimilation and ETR were produced by varying light intensity between 0 and  $1400 \mu\text{mol.m}^{-2}\text{sec}^{-1}$ . A linear regression described the relationship in the angiosperm, whereas in *Actinostrobus* and indeed all conifers, assimilation rate was a non-linear function of ETR.

### Fluorescence and Assimilation

The relationship between ETR and assimilation was linear in *Allocasuarina verticillata*, but in all conifers the relationship was curvilinear, with the CO<sub>2</sub> assimilation rate becoming light saturated faster than the fluorescence yield (Figs 6.7 and 6.8). For all the conifer species measured, polynomial regressions provided good fits to these relationships. The initial slope, or assimilation yield of electron transport, was approximately constant amongst conifers investigated, although the

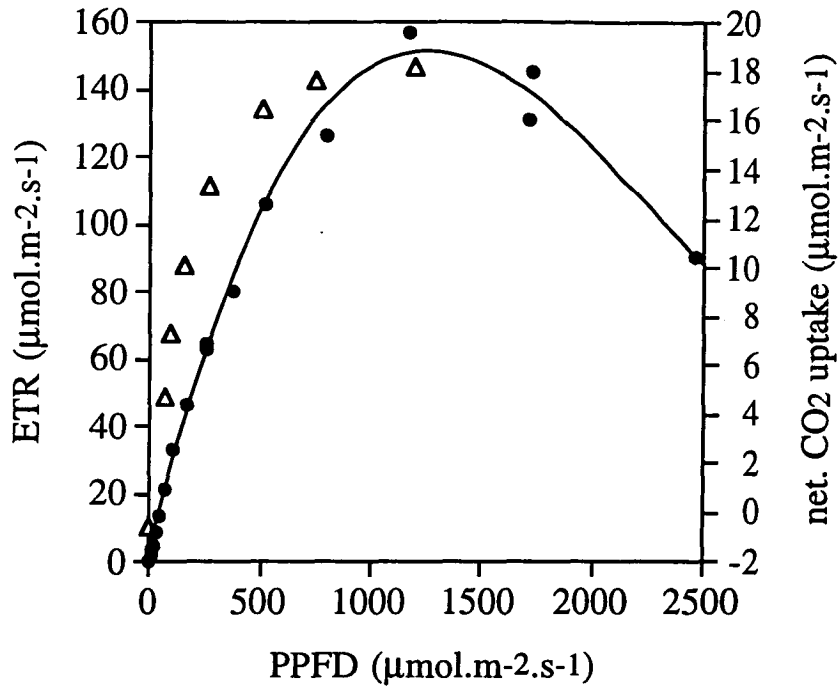


Figure 6.8. The differences in response of ETR- ● and assimilation rate- Δ to PPFD in *Podocarpus lawrencii*. The curvilinearity in A versus ETR relationships in conifers (Fig. 6.6) was produced by relatively higher quantum yield of CO<sub>2</sub> uptake than ETR at low light intensities. Both assimilation and ETR yielded similar values of PPFD<sub>sat</sub>.

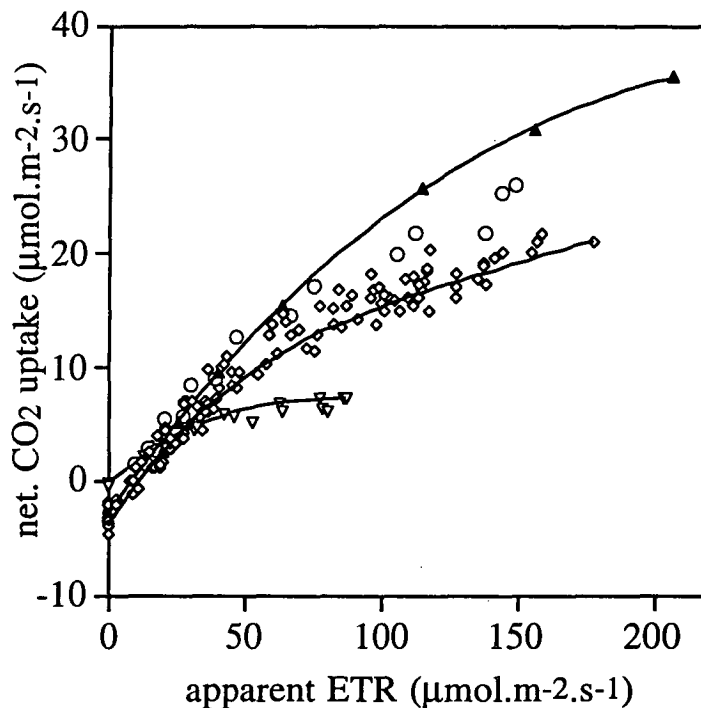


Figure 6.9. The relationship between A and ETR for species with different morphologies: *Actinostrobus acuminatus*- ▲, *Callitris rhomboidea*- ○, imbricate scale-leaf species (*Lagarostrobos franklinii*, *Microstrobos niphophilus*, and *Diselma archeri*)- ◊, and broad-shoot species *Prumnopitys*

saturation of A relative to ETR occurred more quickly in species with low  $PPFD_{sat}$  (Fig. 6.9). Species with similar morphologies/ $PPFD_{sat}$  characteristics produced a very similar shaped response curve of A to ETR (Fig. 6.9).

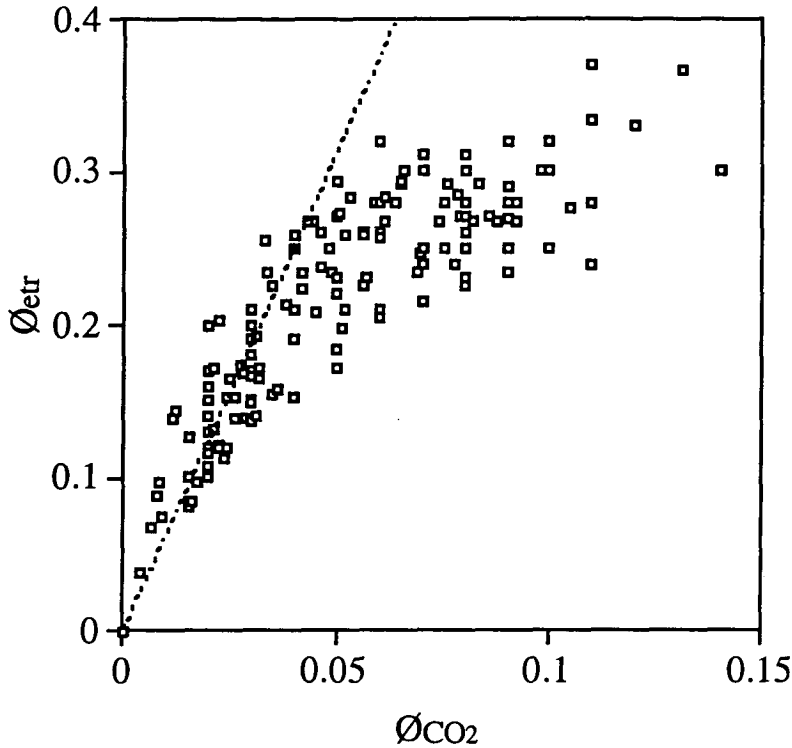


Figure 6.10. Quantum yields of electron transport and  $CO_2$  uptake in eleven conifer species -

*Actinostrobus acuminatus*, *Acmopyle pancheri*, *Athrotaxis selaginoides*, *Athrotaxis cupressoides*, *Callitris rhomboidea*, *Diselma archeri*, *Lagarostrobos franklinii*, *Phyllocladus aspleniifolius*, *Podocarpus lawrencii*, *Prumnopitys ferruginea*, and *Retrophyllum comptonii*. The dotted line indicates the linear relationship observed in Maize (Edwards and Baker, 1993).

The variation in the relationship between  $\Phi_{CO_2}$  and  $\Phi_{e-tr}$  was large, although considerably less than that of A to ETR. Quantum yields of both A and PSII decreased as light intensity increased (see figure 6.3), and the higher initial slope of A vs PPFD than ETR vs PPFD observed in all species (e.g. Fig. 6.8) resulted in the curvilinearity seen in figure 6.10. Also shown in figure 6.10 is the relationship between  $\Phi_{CO_2}$  and  $\Phi_{e-tr}$  as measured in maize (Edwards & Baker, 1993). Maximum values of  $\Phi_{CO_2}$  of the conifer data were considerably higher than for the maize data.

*Stomatal optimisation*

All angiosperms measured produced a stomatal response to light that was consistent with the maintenance of constant  $\partial E/\partial A$  i.e. stomatal conductance changed proportionally with  $A$  as the light intensity increased or decreased regardless of the size of  $A_{\max}$ , or the leaf morphology. In each case the linear regression passed approximately through the origin, and there was no hysteresis in the stomatal response when light was increased gradually from dark to full light, or decreased from full light to darkness (Figs 6.11-6.13).

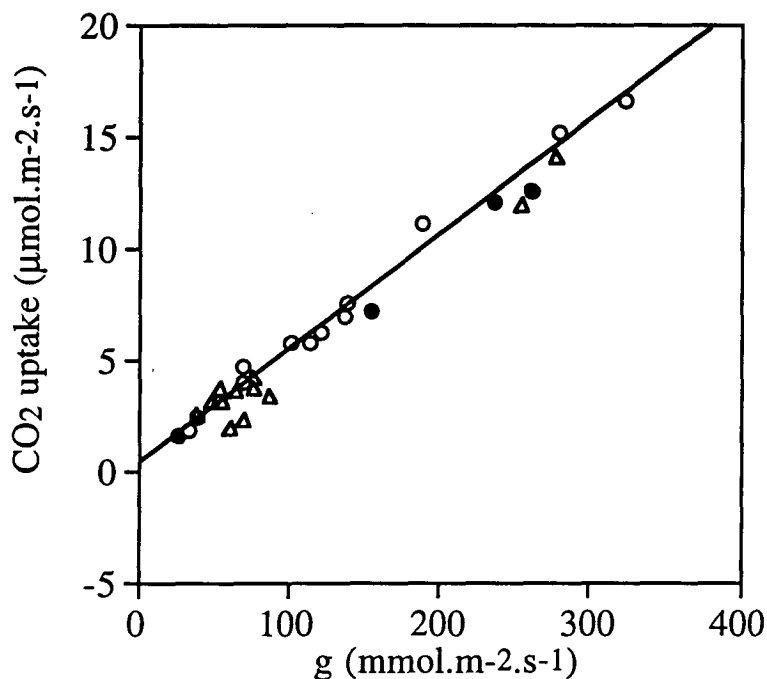


Figure 6.11. Leaf conductance in the angiosperm *Eucalyptus tenuiramis* was found to respond in a linear fashion to changes in assimilation rate induced by decreasing light intensity from 1400 to 0  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$  (○), increasing light intensity from 0 to 1400  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$  (●), and inhibition of photochemistry by injection of DCMU into the leaf petiole (Δ).

The time required for optimal adjustment of  $g_s$  after each light increment was only a matter of minutes for the broad-leaved species, but in the "needle-leaved" angiosperm *Allocasuarina verticillata* at least 20 minutes was generally required for stomatal equilibrium to be reached. The response of stomata to inhibition of photosynthesis by DCMU in *Eucalyptus tenuiramis* was identical to that produced by manipulating  $A$

by altering the light intensity, again all points fell on the linear regression shown in figure 6.11 indicating that stomatal conductance was optimised relative to assimilation rate.

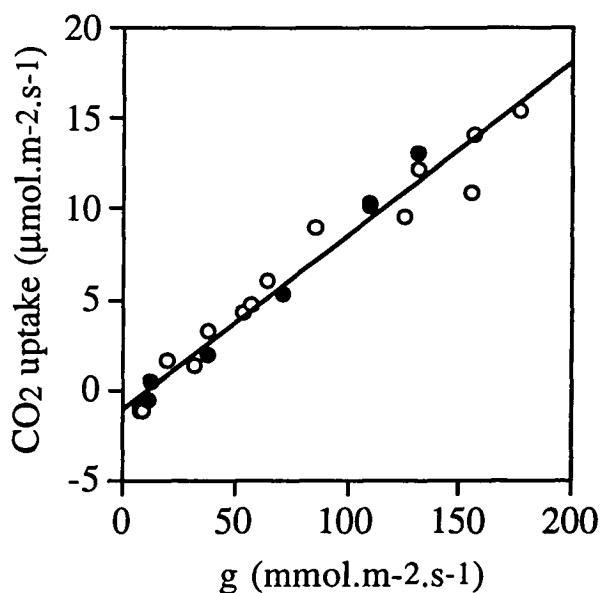


Figure 6.12. *Nothofagus cunninghamii* was also observed to exhibit a close linear regulation of leaf conductance in response to increasing (○) and decreasing (●) light intensity.

The stomatal response of the conifers investigated here was similar to that of the angiosperms in so far as decreases in  $A$  resulted in a concomitant decrease in  $g_s$ ; however, the sensitivity and speed of the response was substantially below that of the angiosperms. Despite the extremely long acclimation time after changing light intensity there was significant hysteresis between the opening and closing responses of some species, often with no significant change in  $g_s$  noted even after a 50% reduction of  $A$  (Fig. 6.14). This was also seen in the response of  $g_s$  to inhibition of  $A$  by DCMU (Fig. 6.14) although in this case plants were taken from shade conditions prior to measurement, and hence non-optimal  $g_s$  was a result of excess stomatal opening.

The non-linear response of  $g_s$  to changes in  $A$  causes a substantial increase in  $c_i$  as  $A$  is inhibited, or a decrease in  $c_i$  as  $A$  increases, this contrasts with the relatively constant  $c_i$  produced during manipulation of  $A$  in the angiosperms investigated (Fig. 6.15).



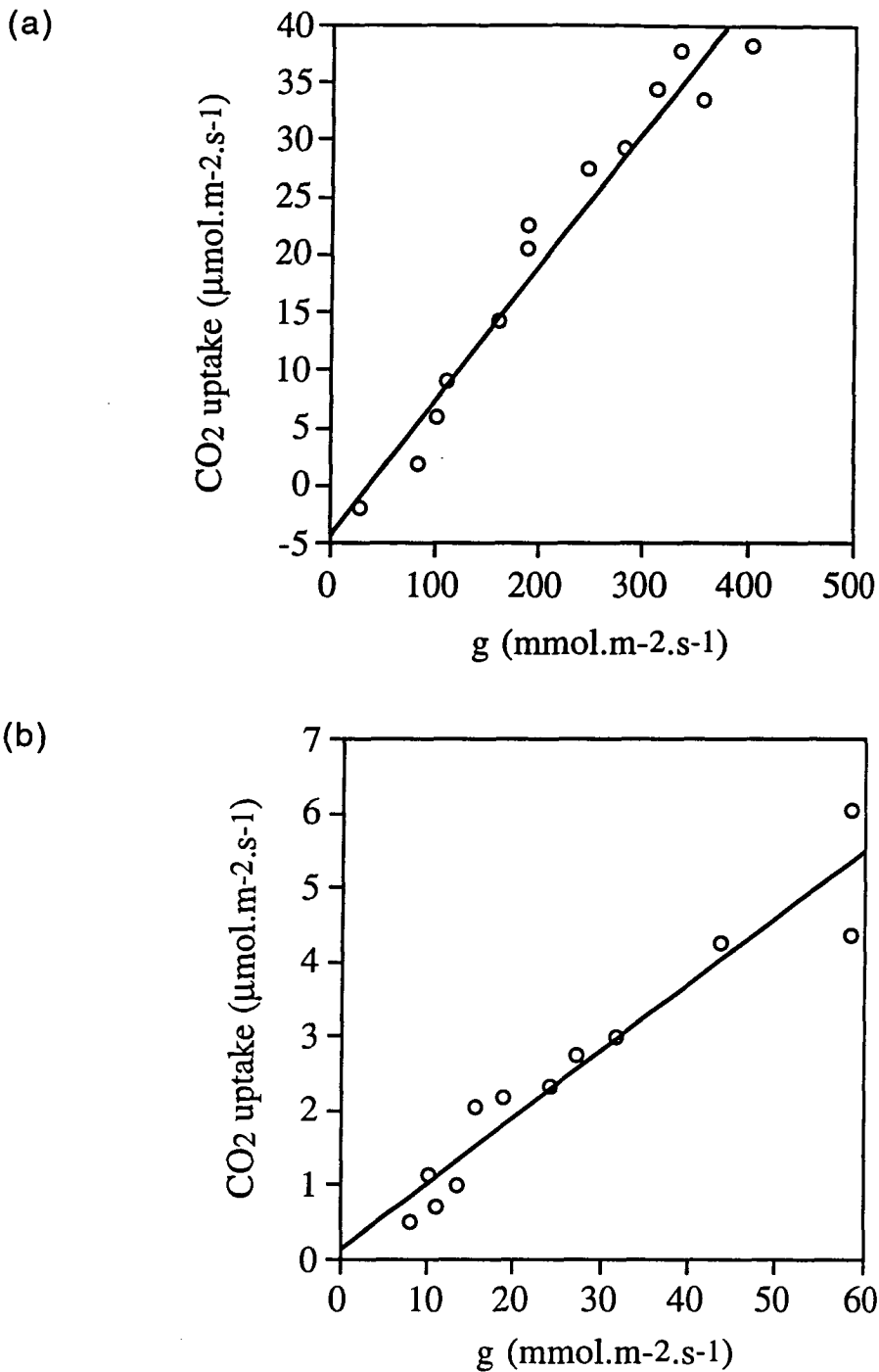


Figure 6.13. Optimisation of stomatal conductance in response to changes in assimilation produced by varying PPFD in two species with gross differences in leaf morphology and ecological niche- (a) the "needle-leaved" angiosperm *Allocasuarina verticillata*, and (b) the broad-leaved rainforest understorey species *Anopterus glandulosus*.

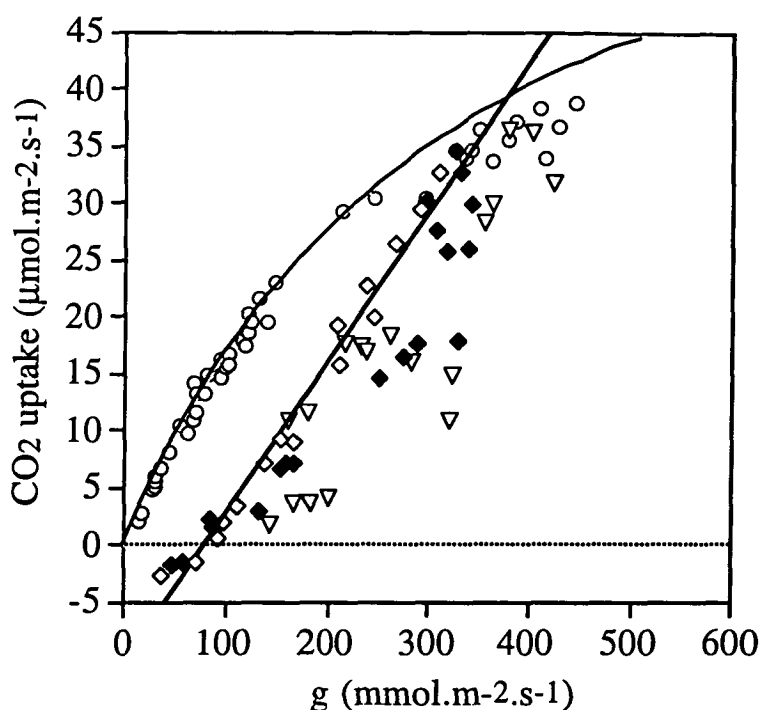


Figure 6.14. Stomatal response to changes in assimilation rate in *Actinostrobus acuminatus*. Similar to figure 6.11 assimilation rate was modified by gradually decreasing PPFD from 1400 to 0  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$  -  $\blacklozenge$ , increasing light intensity over the same range -  $\diamond$ , and by injecting DCMU into the stem -  $\nabla$ . Also shown is the relationship between A and g during drought, with the curved line representing the theoretical relationship assuming that g was controlling A -  $\circ$ . A linear regression is also drawn through the g vs A relationship for gradually opening stomata in response to increasing light intensity.

Species with low rates of assimilation and low  $\text{PPFD}_{\text{sat}}$  tended to produce the most linear stomatal response passing almost through the origin. These species included *Podocarpus drouynianus*, *Prumnopitys ferruginea*, *Retrophyllum comptonii*, *Dacrycarpus dacrydioides* and *Lagarostrobos franklinii* (Fig. 6.16). Species which showed the lowest stomatal sensitivity to light intensity and the highest 'residual conductance' at  $A = 0$  were those with relatively high  $\text{PPFD}_{\text{sat}}$  such as *Athrotaxis cupressoides*, *Actinostrobus acuminatus*, *Microstrobos niphophilus* and *Diselma archeri* (Fig. 6.17).

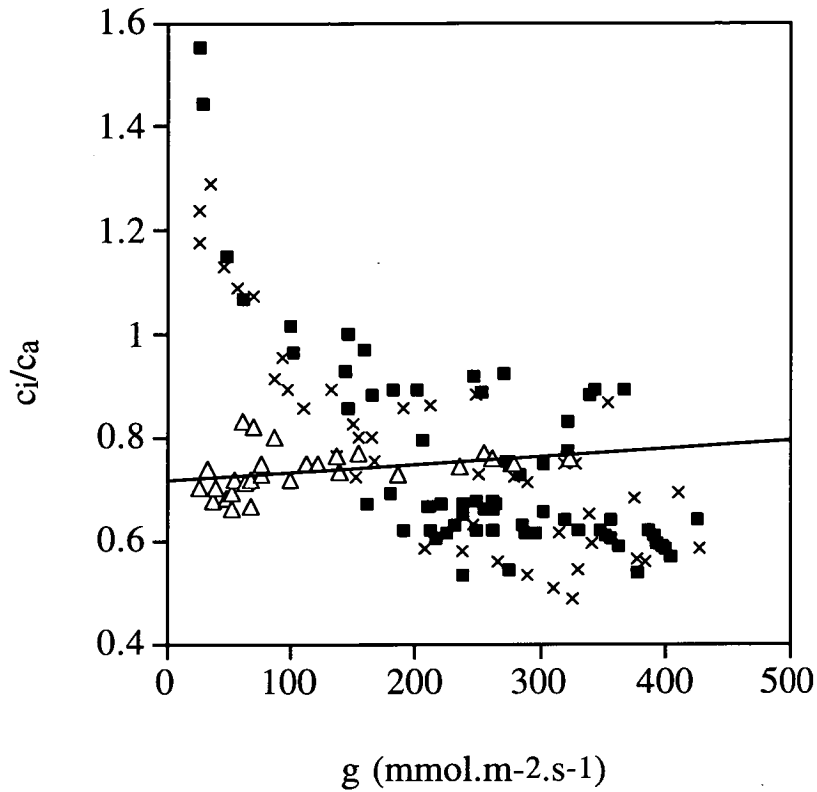


Figure 6.15. Effects on the ratio of internal to ambient  $\text{CO}_2$  concentration ( $c_i/c_a$ ) of optimal and non-optimal stomatal regulation during stomatal closure in response to decreasing photosynthetic rates. Reductions in  $A$  produced by injection of DCMU- ■ , and decreased light intensity- × , in the conifer *Actinostrobus acuminatus* caused  $c_i/c_a$  to increase markedly. In contrast, the angiosperm *Eucalyptus tenuiramis* produced an optimal stomatal response to changes in  $A$ , and thus exhibited a fairly constant ratio of  $c_i/c_a$ - Δ .

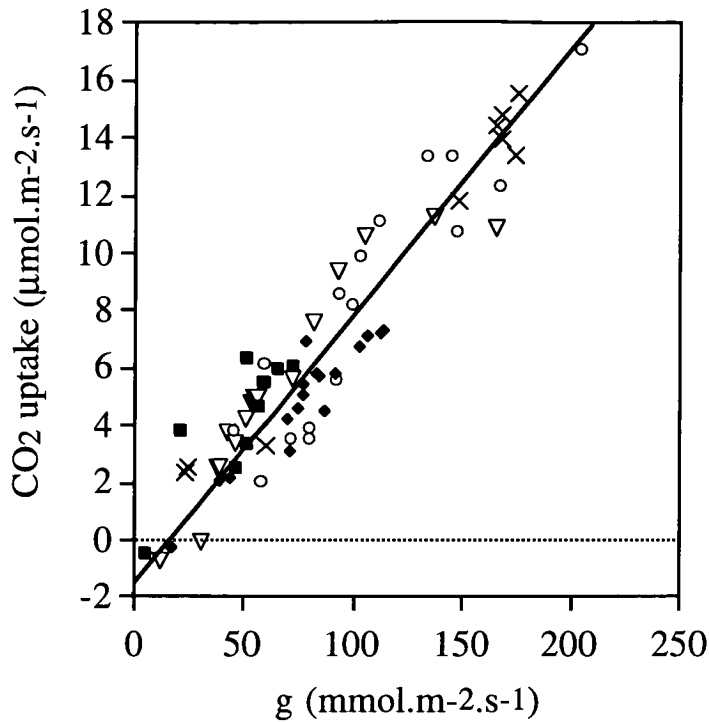


Figure 6.16. Conifers which produced the most optimal regulation of stomata were generally species with low maximum rates of assimilation such as: *Acropyle pancheri*- ○, *Lagarostrobos franklinii*- ×, *Podocarpus drouynianus*- ▽, *Prumnopitys ferruginea*- ■ and *Retrophyllum comptonii*- ◆. A single linear regression is drawn through all data.

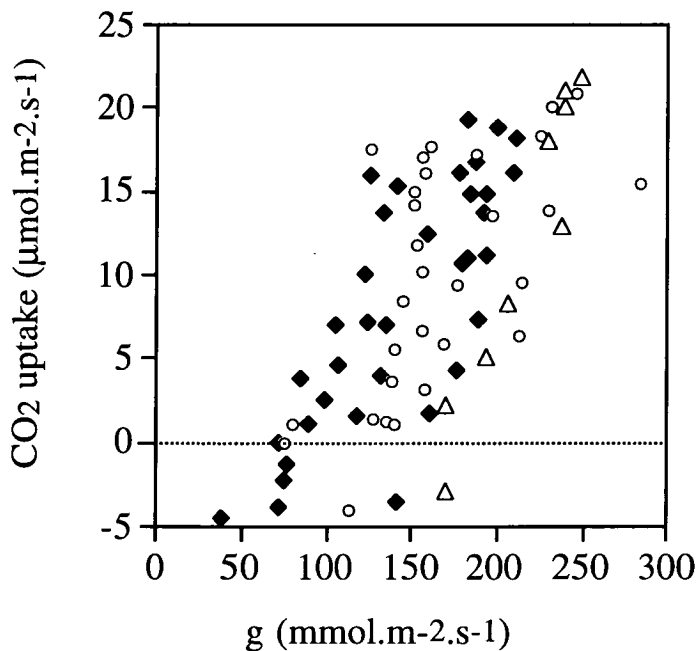


Figure 6.17. Weakest stomatal control in response to changing assimilation rate was observed in species with imbricate morphology such as *Microstrobos niphophilus*- △, *Diselma archeri*- ◆, and *Athrotaxis cupressoides*- ○.

## Discussion

### *Fluorescence technique*

Analysis of chlorophyll *a* fluorescence provides a useful means for comparing light response characteristics among species. The utilisation of fibre optics to deliver light to a small portion of the leaf means that high light intensities can be used without concomitant leaf heating problems. <sup>(This was monitored with a leaf thermometer)</sup> This allows the photochemical response curve to be extended above saturating light intensities, until a combination of high  $q_n$  and photoinhibition cause a decline in ETR. The advantage of this is that the maximum rate of ETR appears as a well defined peak, from which  $PPFD_{sat}$  can be determined precisely. In a previous field study (Bilger *et al.*, 1995), high light intensities were associated with decreased accuracy in the measurement of ETR. This effect was not observed in the data here, and was probably associated with the use of ambient light in the field rather than artificial illumination.

The relationship between  $\Phi_{PSII}$  and  $\Phi_{CO_2}$  has been shown to be linear under a range of conditions in maize leaves (Edwards and Baker, 1993); however, the relationship for  $C_3$  plants has yet to be determined precisely. Data presented here indicate a close correlation between  $ETR_{max}$  and  $A_{max}$  (Fig. 6.5) suggesting that photorespiration was not causing any significant variation in the relationship between  $\Phi_{PSII}$  and  $\Phi_{CO_2}$  under the standard measurement conditions for  $ETR_{max}$  and  $A_{max}$  applied here. The probable reason for this is that only a relatively small variation in the leaf internal  $CO_2$  concentration (between approximately 205 and 250  $\mu\text{mol.mol}^{-1}$ ) occurs in these species under the conditions which  $A_{max}$  was measured (chapter 4). This amount of variation in internal  $CO_2$  concentration would only be expected to produce a maximum variation of approximately 8% (using the stoichiometry of Farquhar *et al.*, 1980) across the range of  $A_{max}$  due to the effects of differing Rubisco oxygenase/carboxylase activities.

Although a linear response was observed between  $ETR_{max}$  and  $A_{max}$ , the relationship between ETR and *A* at light intensities other than  $PPFD_{sat}$  was distinctly non-linear in the conifers examined (Figs 6.7, 6.9) with *A* becoming light saturated at a lower PPFD than ETR. The

reason for this non-linearity is likely to be partly linked to the non-optimal response of stomata in conifers to changes in  $A$ . Sub-optimal stomatal regulation causes a large systematic shift in  $c_i$  from high values ( $>c_a$ ) at low light intensities to low values under saturating light, and the effect of this would be to change the ratio of Rubisco carboxylase/oxygenase activity as light intensity changes (Fig. 6.18). This would certainly contribute to the decreased sensitivity of  $A$  to ETR at increasing light intensity (Ghashgaie and Cornic, 1994); however it does not adequately explain the observed curvature in the relationship, especially considering that the strongest curvature was seen in species with low  $PPFD_{sat}$ , and these species generally produced the smallest amount of variation in  $c_i$ . Other potential problems such as uneven distribution of absorbed quanta between PSII and PSI and cyclic electron flow through PSI may have caused inaccuracies in the relationship between ETR and  $A$ , especially at different light intensities. There is evidence of an increase in the ratio of PSI to PSII produced at low light intensities (Melis and Harvey, 1981; Smith *et al.*, 1993) and this suggests that cyclic electron flow through PSI may be increased at low light intensity.

As a result of this non-linearity it becomes necessary, for the accurate interpretation of  $CO_2$  assimilation from ETR at non-saturating light intensities, that a species specific response curve be determined between ETR and  $A$  at a range of light intensities. Under saturating light however, a single linear relationship described the net  $CO_2$  uptake rate at  $ETR_{max}$  for all species investigated.

#### *Light response characteristics*

The photochemical capacity ( $ETR_{max}$ ) of foliage here was found to be proportional to the light intensity which just saturated photochemistry (Fig. 6.5), indicating that the processes for harvesting and dissipation of radiant energy in the leaves of these species were optimised with respect to the maximum potential for photosynthetic electron transport. Thus, species with a low maximum rate of electron transport (or photosynthesis) became light saturated at light intensities far below those required to saturate species with a high capacity for electron transport. One feature of this optimisation is that the effective  $\Phi_{PSII}$  at  $ETR_{max}$  remained constant, at  $0.278 \pm 0.0035$  in all species investigated. This figure may represent the "optimal quantum yield at light saturation" prior to

the saturation of  $q_n$ , and production of photoinhibitory effects which occur at super-saturating quantum fluxes. The expression of this optimal relationship would have been facilitated by the fact that light conditions during pigment production were uniform, and intermediate between sun or shade, and there was no exposure of plants to light extremes prior to measurement as is often the practice when plants are sun or shade 'acclimated'. The result of this is that the characteristics of the light harvesting proteins would be expected to be closely related to the photosynthetic capacity of leaves, rather than being acclimated to imposed extremes of sun or shade.

The differences in light response observed among species here appear to be related to differences in the characteristics of  $q_n$  (Fig 6.3b). In species with low light requirement for  $PPFD_{sat}$ ,  $q_n$  was found to become saturated at light intensities around 1000-1500  $\mu\text{mol.photons.m}^{-2}.\text{s}^{-1}$ , whereas  $q_n$  in species with high  $PPFD_{sat}$  did not saturate even at the maximum light intensity used (2500  $\mu\text{mol.photons.m}^{-2}.\text{s}^{-1}$ ). This variation in the characteristics of  $q_n$  is most probably affected by differences in the size and characteristics of the xanthophyll pool. The xanthophyll-cycle pigments are responsible for regulating the bulk of non-fluorescent energy dissipation from the photosystems, channelling excess excitation energy away from reaction centres so as to prevent the formation of toxic oxygen radicles. The size and composition of this xanthophyll pool has been correlated with the photosynthetic capacity of leaves of species occupying different light environments in tropical forest (Königer *et al.*, 1995), with species adapted to growth in high light conditions found to possess larger, more dynamic xanthophyll pools (Demmig-Adams *et al.*, 1995). A relatively large xanthophyll pool allows plants to rapidly respond to increasing light intensity (Demmig-Adams *et al.*, 1995), while species adapted to low light environments have no need for efficient energy dissipation. This is consistent with the different dynamics of  $q_n$  shown in figure 6.3(b), the rapid saturation of  $q_n$  in *Retrophyllum comptonii* illustrating a high sensitivity to low to medium light intensity, but a low capacity for responding to light intensities over 1000  $\mu\text{mol.photons.m}^{-2}.\text{s}^{-1}$ . In contrast,  $q_n$  in *Actinostrobilus acuminatus* was able to respond to increasing light intensity even up to values of PPFD greater than full sun.



*Ecological and evolutionary implications of increased shoot width*

The relationship between shoot width and light response illustrated here (Fig. 6.6) has implications for the evolution of conifers and their competitive interaction with angiosperms. One of the explanations used to account for the replacement of conifers by angiosperms is that conifers only have slow rates of foliage production, especially during establishment, making them unable to compete for light with fast growing broad-leaved angiosperms, particularly in more productive temperate-tropical forests (Bond, 1989). As mentioned, several genera of podocarps have evolved bilaterally compressed shoots resembling broad-leaves, providing a foliar morphology that enables a rapid increase in the leaf area available for light capture. The presence of these genera in temperate and tropical broad-leaf forest suggests that this morphological adaptation has contributed to the success of these genera in such habitats (Fig. 1.4). Givnish (1979) discussed the concept of optimal investment in leaf support relative to vascular supply to the photosynthetic unit, and concluded that the most economic strategy for land plants was the production of entire leaves with a large midrib, or compound leaves. Clearly this strategy has been adopted in the bilaterally compressed shoots of conifers here, enabling growth rate per unit of assimilated carbon to be maximised. The bilateral flattening of shoots also allows more efficient harvesting of light by reducing self-shading on shoots, but in the conifer dominated forests of the northern hemisphere, bilateral flattening is confined to the understorey as its presence in the tree crown would severely reduce light penetration into the canopy (Stenberg, 1996). For Podocarps growing in mixed forest with broad-leaved angiosperms, the presence of a broad-leaved canopy emphasises the production of bilaterally flattened foliage as an important strategy. The proposal that competition with angiosperms in the regeneration niche has displaced many conifers from most productive forests (Bond, 1989) is supported by data here, indicating that conifers which successfully compete in broad-leaf forest appear to do so with morphological adaptations (increased shoot flattening) that are linked to low light requirements (low  $PPFD_{sat}$ ).

Although the decrease in  $PPFD_{sat}$  observed in species with greater bilateral compression in shoots is consistent with this adaptation being associated with low light environments, minimal physiological advantage in terms of increased quantum yield of either electron transport or carbon fixation was evident in plants producing this type of

foliage (Figs 6.9 and 6.10). There was a trend towards lower respiration rates in species with lower  $\text{PPFD}_{\text{sat}}$  (Respiration rate =  $0.002 \cdot \text{PPFD}_{\text{sat}} + 0.195$ ;  $p < 0.10$ ; Fig. 6.19) and this produced a slight advantage in net  $\text{CO}_2$  uptake at low light intensity (Fig. 6.20) but apart from this there appears to be little intrinsic difference in the efficiency of light harvesting between species with high and low light requirements. Thus, it appears that the main advantage conferred by bilaterally flattened foliage lies in the economics of light harvest versus leaf investment under forest canopies.

It was suggested in chapter 4 that maximisation of water-use efficiency came at a cost of an increased light saturation requirement, and this is clearly illustrated in figure 6.21 which shows a linear relationship between maximum water-use efficiency and  $\text{PPFD}_{\text{sat}}$ . Another interpretation of this graph is that the evolution of more shade tolerant foliage is linked to a decrease in drought tolerance, possibly due to a decrease in leaf thickness and support in favour of maximal efficiency of light interception per unit leaf investment (Givnish, 1979). A combination of both these effects may come into play, or indeed the relationship may be secondary, linked by a trend of decreasing light competition in progressively drier environments. The anomalous position of *Podocarpus drouynianus* in figure 6.21 seems to support the latter argument, being the only dry adapted species to come from an understorey position, although it could otherwise indicate a recent change in the niche of this species. *Podocarpus drouynianus* was also found to produce an unusually high carboxylation efficiency and drought tolerance relative to its low maximum rate of assimilation (see chapter 3) and it was inferred that perhaps *P. drouynianus* was limited by an unusually low stomatal conductance relative to its photosynthetic capacity, resulting in low  $c_i/c_a$  under optimal conditions (Fig. 4.1). This also seems to suggest a recent shift in niche from open heath to understorey, perhaps in response to anthropogenic changes in fire frequency (Singh *et al.*, 1981), or otherwise may somehow be an artefact of the growth or measurement conditions imposed here.

The palaeohistory of conifers in the Southern Hemisphere demonstrates that the broad, flat photosynthetic unit in the Podocarpaceae has had a long history of co-occurrence with diverse, broad-leaved angiosperm forests with a closed canopy. In Australia, development of such forests had begun by the Late Paleocene, as evidenced by the macrofossil record from southeastern Australia which contains a diversity of broad-leaved angiosperms and conifers including

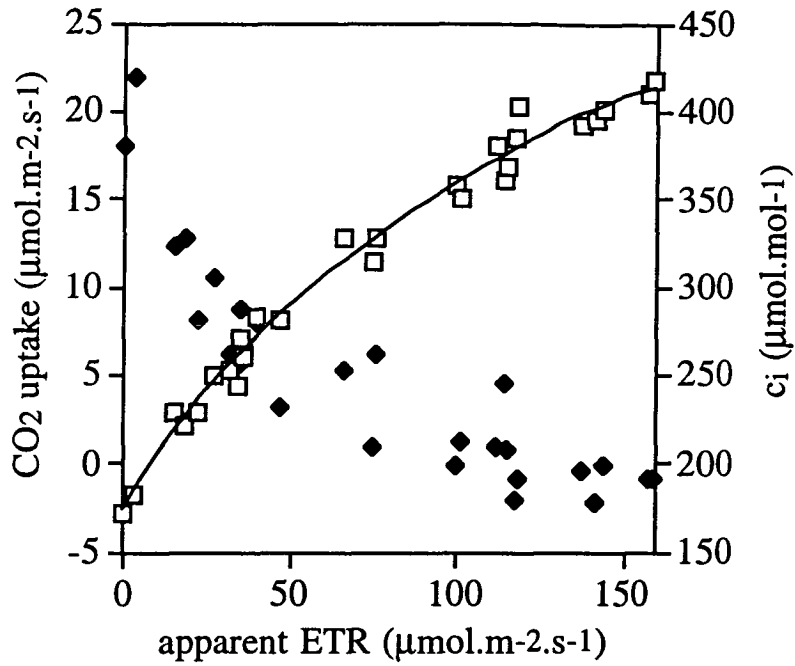


Figure 6.18. Coincidental changes in ETR-  $\square$ , and  $c_i$  -  $\blacklozenge$  in three *Athrotaxis selaginoides* replicates as light intensity was gradually reduced from  $1400 \mu\text{mol.m}^{-2}\text{s}^{-1}$ .

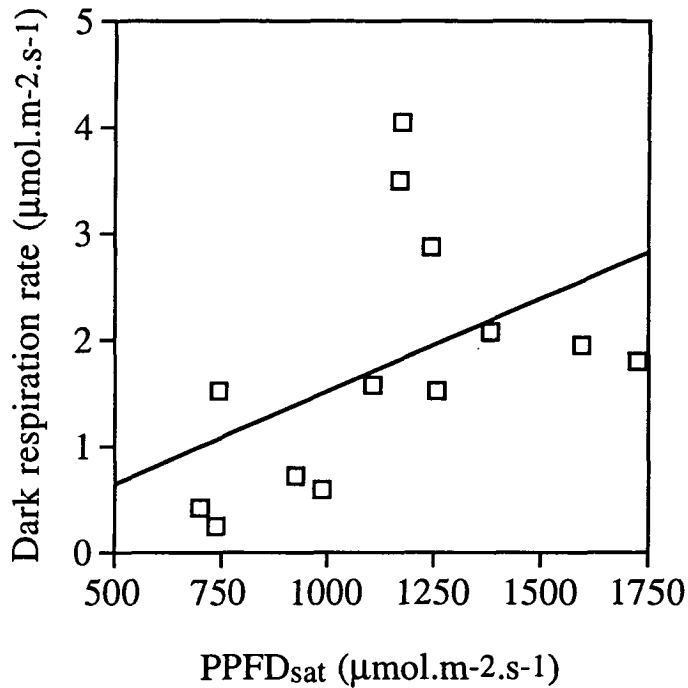


Figure 6.19. A weak association between PPFD<sub>sat</sub> and dark respiration rate is shown for the 13 conifer species from figure 6.4. The regression shown is only significant at the  $0.05 < p < 0.1$  level.

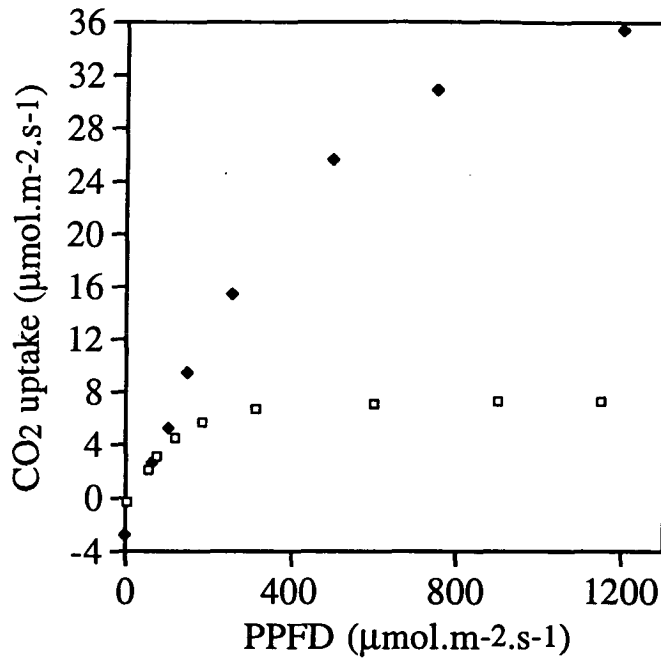


Figure 6.20. The net result of lower respiration rates in species with low  $\text{PPFD}_{\text{sat}}$  is shown here. At very low light intensities the net  $\text{CO}_2$  uptake in *Retrophyllum comptonii*-  $\square$  is seen to be marginally higher than that of *Actinostrobus acuminatus*-  $\bullet$  .

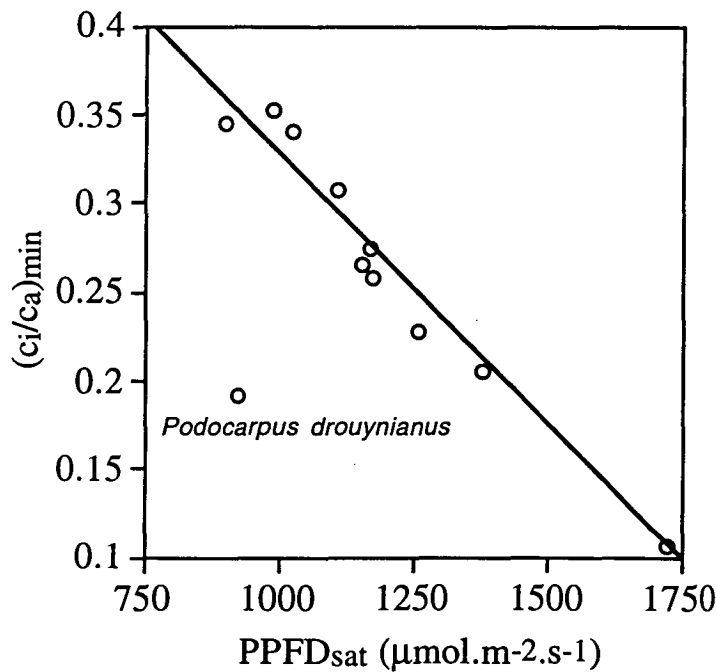


Figure 6.21. A highly significant linear regression ( $r=0.98$ ) was found to exist between minimum  $c_i/c_a$  attained during drought (chapter 3), and saturating PPFD. *Podocarpus drouynianus* was an extreme outlier (see text) and was not included in the regression.

*Acmopyle* and *Dacrycarpus* (Taylor *et al.* 1990, Hill & Carpenter 1991). Complex rainforest associations on the west coast of Tasmania in the Early Eocene contain *Dacrycarpus* and broad-leaved *Podocarpus*, and other extant and extinct genera of podocarps with similar adaptations are present by the Middle Eocene across southern Australia (Hill & Pole 1992, Hill 1995, unpublished data). However, Cretaceous forests in southern Australia and adjacent Antarctica were apparently more open, in response to low sun angles at the prevailing very high latitudes (Truswell 1991, Hill & Scriven 1995), and there is no evidence of podocarps with a broad photosynthetic unit.

It has been shown here that increasing shoot width is associated with a photosynthetic preference for lower light intensity, expressed as a low  $\text{PPFD}_{\text{sat}}$  and a low maximum photosynthetic capacity. This, in combination with ecological and palaeobotanical evidence, suggests that the production of broad short shoots in the Podocarpaceae is an adaptation to low light intensity.

#### *Optimisation of $g_s$*

A final comment should be made with regard to the optimisation of stomatal conductance with respect to the rate of assimilation. It was expected that species from dry environments might, in the interests of water economy, produce the most tightly regulated  $g_s$  under changing rates of assimilation (Cowan, 1982). On the contrary, it has been illustrated here that, in fact, species with low light saturation requirements, from wet, shaded environments, actually produced the most optimal regulation of stomatal aperture (Fig. 6.16). This apparently anomalous result is difficult to explain in adaptive terms, and may be partly due to morphological features such as needle-type leaves which tend to characterise species with high  $\text{PPFD}_{\text{sat}}$ . Strong light gradients are likely to occur across such leaves, possibly reducing the responsiveness of the whole leaf to changing light conditions. Also, species adapted to dry environments such as *Actinostrobus acuminatus* and *Callitris rhomboidea* have been discussed in previous chapters as being "water spenders" under favourable conditions, and this may also explain why the light response of the stomata of these species is not closely optimised under fully watered conditions, despite the fact that stomatal control of water loss is highly sensitive to water availability (Fig. 6.14).

It has been suggested (Robinson, 1994) that optimal stomatal regulation evolved in response to declining atmospheric CO<sub>2</sub> in the late Tertiary, and that the non-optimal regulation exhibited by conifers may have contributed to their decline. The optimisation response was observed in most conifer species tested here, although sluggish stomatal control, and high residual conductances often meant that  $\partial E/\partial A$  was not maintained at a constant level. The only conceivable advantage of maintaining  $\partial E/\partial A$  constant would be to minimize costs associated with transpiration (*e.g.* root growth and drought damage). Thus, if non-optimal stomatal regulation had been an important factor contributing to conifer contraction during the Tertiary, then one would expect that the conifer species which survived in dry habitats should at least show increased responsiveness of stomata to environment. No such association was observed, questioning the importance of maintained  $\partial E/\partial A$  as means of water conservation.

Glasshouse effects may have contributed to the sluggish responses reported above, and a repeat of the procedure in plants grown under lower humidity or lower water potential may resolve inter-species differences not apparent here.

## CHAPTER 7

# The Influence of Leaf Morphology on Gas-Exchange

## Introduction

In previous chapters it has been shown that the physiological characteristics of leaves are related to both species distribution (chapter 4) and leaf/shoot morphology (chapter 6). Despite the correlation between shoot morphology and light response described in the previous chapter, no explanation for this correlation was evident in terms of the physical attributes of the leaves investigated. This chapter examines one of the most fundamental links between morphology and leaf physiology, the association between the stomatal attributes of leaves and their effect on leaf conductance to water vapour and CO<sub>2</sub>.

The photosynthetic characteristics of a leaf are intrinsically linked to the physical characteristics of its stomata. Being the only pathway for gas exchange, the size and density of stomata necessarily dictate the maximum leaf conductance ( $g_{\max}$ ) and hence the maximum photosynthetic rate. A number of theoretical calculations of the physical resistance to diffusion imposed by the stomatal pore ( $r_s$ ) have been determined previously, most of which are based on the diffusion properties of gas through a cylinder (see Jones, 1992). A more comprehensive derivation of  $r_s$  was made by Parlange and Waggoner (1970) expressing resistance in terms of stomatal pore geometry and density. The equation presented by Parlange and Waggoner (1970) has been found to correlate well with measured values of stomatal conductance ( $1/r_s$ ) in leaves which were subsequently cryo-fixed, and stomatal characters measured by electron microscopy (Van Gardingen *et al.*, 1989). Despite this, no general pattern has been described relating the physical characteristics of the stomata to either the physiological or ecological characteristics of species.

The most consistent trend observed between stomatal characters and environment is the relationship between the distribution of stomata on leaves and light intensity. Hypostomy has been qualitatively associated with leaves growing at low light intensities, and amphistomy with species from high light environments which produce high maximum photosynthetic rates and conductances (Peat and Fitter, 1994; Körner *et al.*, 1989; Mott *et al.*, 1982). Stomatal density has also been reported to respond

to short and long term changes in CO<sub>2</sub> concentration (Van der Water *et al.*, 1994; Beerling and Chaloner, 1993; Woodward, 1987) although these results are still somewhat controversial (Körner, 1988).

The difficulty with attempting to describe stomatal trends in the flora is that the size and shape of stomata vary considerably among genera (Meidner and Mansfield, 1968), and differences in leaf morphology have the potential to substantially alter the relationship between pore area and leaf conductance. Common morphological features which must severely affect leaf conductance include the formation of stomatal crypts (common in the leaves of xeromorphic taxa), leaf pubescence, wax plugs (found in a very large proportion of conifers) and imbricacy (common in highland taxa). This chapter focuses on the effects on gas exchange of two of these leaf characters that regularly occur in conifers, wax plugs and leaf imbricacy.

Wax plugs are occasionally found in angiosperms (*e.g.* Mihaich, 1989, Hill and Merrifield, 1993), but are very common in conifers, being present in most southern hemisphere genera and a large number of northern taxa. Their function is not well understood, but antitranspirant (Jeffree *et al.*, 1971), and antipathogen (Giles-Frankle *et al.*, 1993) effects have been observed. The presence of large amounts of wax covering stomatal pores clearly affects the rate of leaf gas exchange by decreasing the cross-sectional area available for diffusion, and by decreasing the diffusion coefficient of the stomatal pore (depending on the size of the spaces between wax crystals). It is unknown whether wax plugs are a primary adaptation to reduce gas exchange, or the resultant inhibition of stomatal conductance is a secondary effect. Imbricacy is also reasonably common amongst conifer genera, and in some species stomata are restricted to the adaxial surface of leaves which are tightly appressed to the superadjacent leaves on the stem, effectively shielding the stomata from the environment (chapter 2). Several of these species also possess a well developed leaf marginal frill, that almost completely seals the remaining gap between leaves, and all have wax plugs in the stomatal pores. Again, this morphology must profoundly reduce the potential maximum leaf conductance, but the extent of this reduction, and the selective force which has resulted in such an unusual morphological response, is unknown.

An understanding of the relationship between stomatal characteristics such as density and morphology, and leaf physiology has considerable application for ecological and climatic interpretation of the



fossil record. Temporal trends of decreasing stomatal density and increased imbricacy have been described for two genera from Tertiary macrofossils, and these changes in leaf morphology have been interpreted as adaptations to restrict water loss as a response to increasing aridity (Hill and Carpenter, 1991). Such conclusions require stringent testing based on an understanding of the effects of these adaptations on the likely rate of gas exchange in living taxa.

In this chapter, the maximum leaf conductances of a group of southern hemisphere conifers with stomata of similar sizes, are compared in an attempt to quantify the effects of leaf morphology and stomatal arrangement on leaf gas exchange.

## Materials and Methods

### *Plant material*

Twenty-three conifer species were investigated, including fifteen species of Podocarpaceae (all with stomatal wax plugs), and seven species of Cupressaceae (three of which possessed wax plugs).

Podocarps included were; *Acmopyle pancheri*, *Dacrycarpus compactus*, *D. dacrydioides*, *D. imbricatus*, *Lagarostrobos franklinii*, *Lepidothamnus laxifolius*, *Microstrobos niphophilus*, *Phyllocladus alpinus*, *P. aspleniifolius*, *P. hypophyllus*, *Podocarpus dispersis*, *P. drouynianus*, *P. lawrencii*, *Prumnopitys ferruginea*, and *Retrophyllum comptonii*.

Cupressaceae were; *Actinostrobus acuminatus*, *Athrotaxis cupressoides*, *Athrotaxis selaginoides*, *Callitris columellaris*, *Callitris rhomboidea*, *Diselma archeri*, and *Widdringtonia cedarbergensis*.

All material was propagated as described in chapter 2, and grown under identical glasshouse conditions. Seed of *Microstrobos niphophilus* could not be germinated, and hence seedling material was obtained by transplanting 2 individuals (<2cm tall) from the field and measuring gas exchange in new foliage expanded after transplanting.

In order to determine the combined effect of imbricacy and epistomy on leaf conductance, juvenile *Diselma archeri*, which produces non-imbricate foliage, was compared with the tightly imbricate adult foliage, and *Microstrobos fitzgeraldii*, a non-imbricate species was compared with the closely related *M. niphophilus* which again possesses tightly imbricate, epistomatic leaves (see chapter 2).

The combination of imbricacy and epistomy was not seen in species without stomatal plugs, and hence *Callitris columellaris* was included to determine the effect of a similar adaptation, the restriction of stomata to grooves in the leaf.

### *Gas Exchange*

Measurement of gas exchange was carried out on leaves or shoots enclosed in a cuvette attached to an open flow infra-red gas analyser and dew point meter, as described in chapter 3. Leaves and shoots were arranged carefully in the cuvette so as to minimise self-shading. Maximum values of leaf conductance were measured for each species in fully watered plants, photosynthesising under approximately optimal conditions, with leaves at 20°C, leaf-air vapour pressure deficit at 5-10 mmol.mol<sup>-1</sup>, light intensity at 1400  $\mu$ mol.photons.m<sup>-2</sup>.sec<sup>-1</sup>, and an ambient CO<sub>2</sub> concentration of 350  $\pm$  5  $\mu$ mol.mol<sup>-1</sup>. Because the stomatal response of most of the species was very slow, leaves were exposed to full light and 100% relative humidity for a period of 1 to 2 hours prior to measurement of gas exchange to ensure maximum stomatal opening. For each species, an average of the maximum readings of leaf/shoot conductance from 5 individuals was calculated to give  $g_{\max}$ .

Both stomatal density and  $g_{\max}$  were expressed as a function of the silhouette area of foliage in the cuvette. This was measured with a digital camera (EDC-1000, Electrim Corp. Princeton, USA) after conductance readings had been taken. Thus, differences in leaf arrangement and shoot morphology affected stomatal density and leaf/shoot conductance in parallel.

### *Stomatal density*

After measuring the silhouette area of a small sample of leaf or shoot (approximately 1 cm<sup>2</sup>), the foliage was detached and stomata counted by one of three methods:

1. Stomata were counted directly using a light microscope with illumination of the sample from above, and a camera lucida attachment to enable counted stomata to be identified. This was employed on small leaves with clearly visible stomata.

2. Epidermal impressions were made using cellulose acetate nail polish, and stomata counted normally using a light microscope. This

enabled replicates of larger leaves to be made and then subdivided for ease of counting.

3. Leaf cuticles were removed by dissolving leaf tissue in 5% aqueous chromium trioxide ( $\text{CrO}_3$ ) and stomata counted under a light microscope. This was employed for leaves with stomata restricted to grooves, making them impossible to count by methods 1 or 2.

In each case, all stomata were counted on the sample, or if leaves were perfectly symmetrical with an even number of stomatal bands, half the stomatal bands were counted and the result doubled. Stomata were counted from leaves of at least two plants of each species, and results given as an average with standard error. All stomatal densities were expressed in terms of number of pores per cm silhouette area.

### *Theoretical maximum conductance*

The theoretical maximum shoot conductance was calculated for samples of imbricate, plugged, and unplugged foliage using equation 1 (Parlange and Waggoner, 1970)

$$\text{Equation 1.} \quad r_s = [(d/\pi.a.b) + \ln(4.a/b)/\pi.a]/D.n$$

where:  $a$ = pore length/2;  $b$ = pore width/2;  $d$ = pore depth (see Fig. 7.1);  $D$ = diffusivity of water vapour in air;  $n$ = stomatal density. Units of  $r_s$  were ( $\text{s.cm}^{-1}$ ).

Measurements of stomatal pore depth and width were carried out by sectioning leaves with a freeze microtome and staining sections with sudan red so as to visualise the stomatal pore and pit. A light microscope with graticule was then used to determine the distance from the inside of the pore to the cuticle surface, and the width of the pit (in all cases the stomatal pit was of very similar dimensions to the actual pore, and was thus considered as part of the pore (Fig. 7.1). Pore length was determined by taking scanning electron micrographs of the inner cuticle surface and measuring the length of the stomatal slit. To compare the calculated and measured conductances it was necessary to add the boundary layer resistance, determined for a standard 2mm wide leaf replica to be  $0.37 \text{ mol.m}^{-2}.\text{s}^{-1}$  (using the standard technique described in Jones, 1993), to the calculated value of  $r_s$ , giving a theoretical leaf or shoot minimum resistance to diffusion, or  $1/g_{\text{max}}$ .

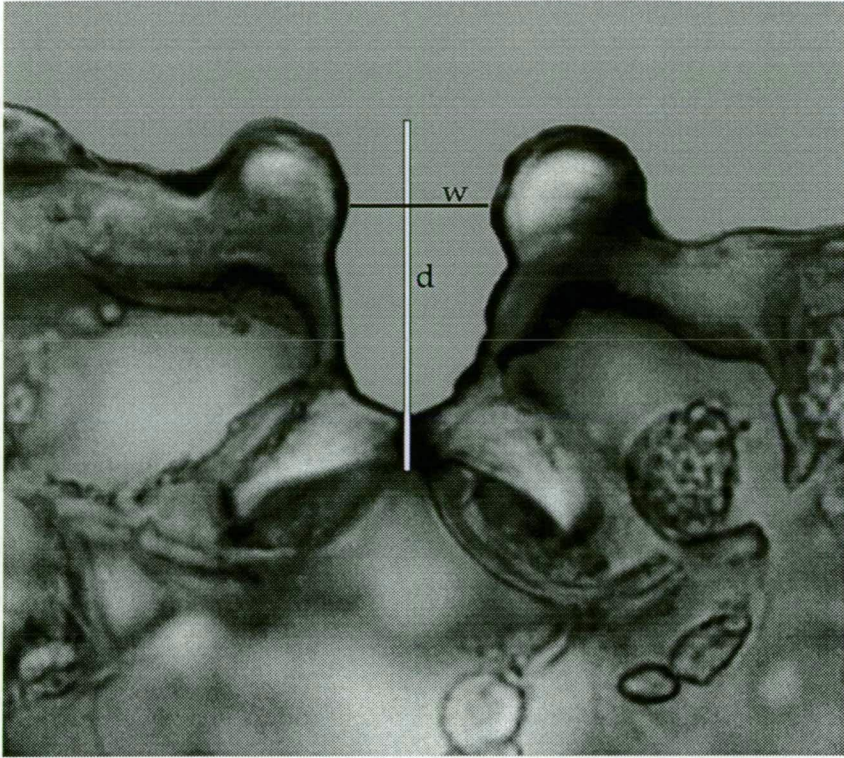


Figure 7.1. Transverse section of an *Actinostrobus acuminatus* stoma, showing the dimensions measured for each species. The depth of the pore (d) was measured from the leaf surface to the inner surface of the guard cell, and the width (w) was the width of the stomatal pit.

## Results

Stomatal density ranged widely from an average of  $4280 \text{ cm}^{-2}$  in *Prumnopitys ferruginea* to  $37,340 \text{ cm}^{-2}$  in adult *Microstrobus niphophilus* foliage (Table 7.1). Variation in the size of the stomatal slit was low, with a maximum range of pore lengths from  $18\text{--}24.5 \mu\text{m}$  (Table 7.2). The most obvious distinction between stomatal pores was the presence or absence of wax plugs occluding the opening of the stomatal antechamber (Figs 7.2–7.5), with plugs present in all genera except *Actinostrobus*, *Callitris* and *Widdringtonia*. The relatively small variation in pore size emphasised the effects of leaf morphology, allowing a clear distinction to be seen between the stomatal density versus measured  $g_{\text{max}}$  relationships for plugged, unplugged, and imbricate foliage (Fig. 7.6). For both plugged and unplugged species, positive linear regressions (significant at the  $p < 0.001$  level) described the relationship between number of stomata and measured  $g_{\text{max}}$ .

The combined effects of imbricacy and epistomy are illustrated for the two species of *Microstrobus*. *M. fitzgeraldii* is epistomatic but the

leaves are not imbricate (Fig. 2.3; chapter 2), and hence its conductance was found to be consistent with other species possessing stomatal wax plugs. Adult *M. niphophilus* on the other hand is entirely imbricate and epistomatic (Fig. 2.2; chapter 2), and clearly displayed a much higher stomatal density relative to maximum conductance than the other plugged species (Fig. 7.6).

The juvenile *M. niphophilus* used here had a shoot morphology intermediate between adult *M. niphophilus* and the spreading foliage of *M. fitzgeraldii*, and was found to be slightly displaced from the regression describing other species with wax plugs (Fig. 7.6). Juvenile *Diselma archeri* has spreading, hypostomatic leaves (Fig. 1.41; chapter 2), and produced a maximum conductance well within the expected range for plugged species. In adult foliage, however, leaves were imbricate, with 79% of stomata found to be restricted to the adaxial (enclosed) surface of the leaf (Fig. 1.42; chapter 2). This foliage also produced a stomatal density which was high relative to maximum conductance, although the magnitude of the difference was not as large as for *M. niphophilus* (Fig. 7.6). Other (plugged) imbricate species such as *Lagarostrobos franklinii* and *Athrotaxis cupressoides*, which had stomata fully exposed to the atmosphere on the abaxial leaf surface, exhibited a similar linear association between stomatal density and maximum conductance to other plugged species.

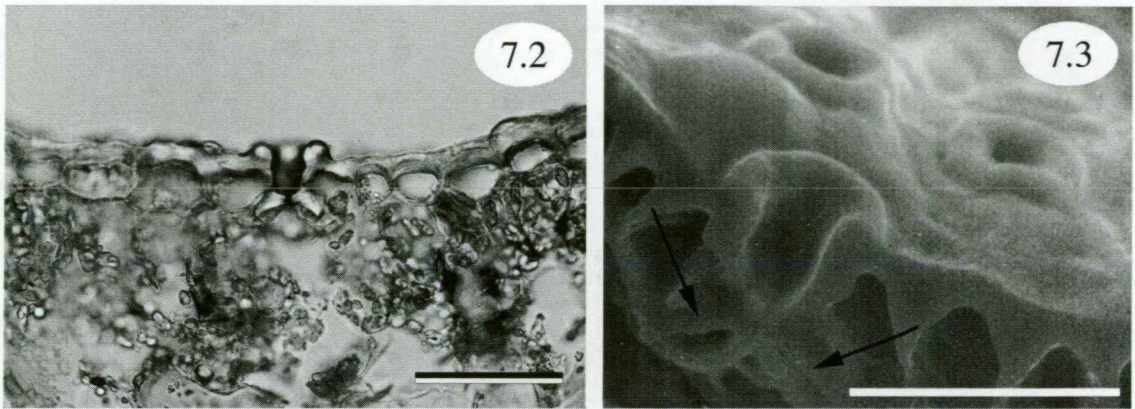
In the three unplugged species, from three genera of Cupressaceae, maximum conductance was clearly a linear function of stomatal density, with these unplugged species producing maximum stomatal conductances more than twice that of plugged species with equivalent densities of stomata (Fig. 7.6).

Calculated values of  $g_{\max}$  using equation 1 were very close to the measured conductances for unplugged species (Fig. 7.7). The linear regression for measured versus theoretical  $g_{\max}$  indicates that measured values of leaf conductance were about 91% of the theoretical value calculated from the stomatal dimensions of these three species. *Callitris columellaris* was included to determine the effect of shoot morphology on  $g_{\max}$  in a species without stomatal plugs. Unlike the other three unplugged species, the stomata in *Callitris columellaris* were restricted to deep invaginations between leaves (Figs 7.8, 7.9) and as a result of this measured  $g_{\max}$  was found to be reduced to about 56% of the expected value (Fig. 7.7).



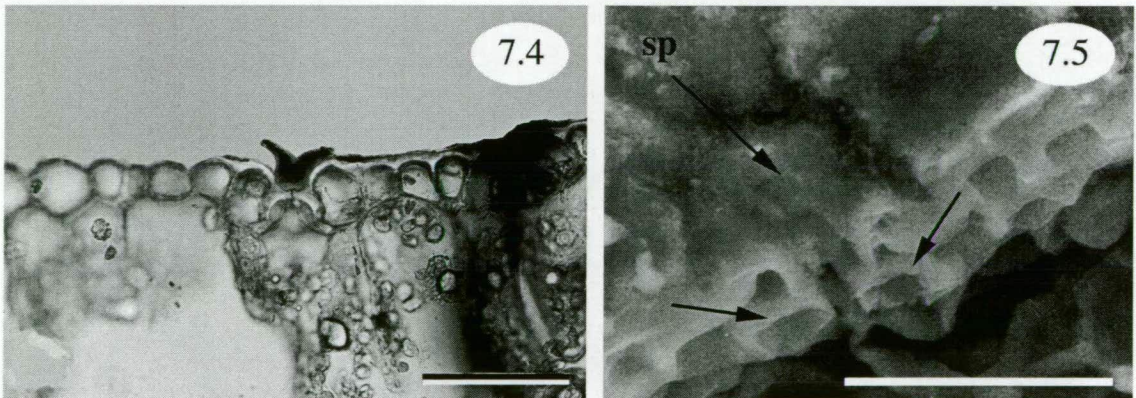
Table 7.1. Morphological details of species investigated, and the ratio of measured, to expected  $g_s$  (expressed as a percentage). Measured  $g_s$  was the average maximum conductance from 5 plants, and theoretical  $g_{max}$  came from measurement of the stomatal pore and application of equation 1 (see Methods section).

Species Family	Leaf Morphology	Stomatal density (cm <sup>-2</sup> )	measured $g_s$ / theoretical $g_{max}$ (%)
CUPRESSACEAE			
<i>Actinostrobus acuminatus</i>	Unplugged stomata, imbricate leaves	11803	90
<i>Athrotaxis cupressoides</i>	Plugged epistomatic	14000	-
<i>Athrotaxis selaginoides</i>	Plugged, hypostomatic imbricate	13897	36
<i>Callitris rhomboidea</i>	Unplugged imbricate	9643	84
<i>Callitris columellaris</i>	Unplugged (stomatal grooves) imbricate	14773	56
<i>Diselma archeri</i>	Plugged, epistomatic, imbricate	24137	24
<i>Diselma archeri</i> (juvenile)	Plugged hypostomatic	16734	35
<i>Widdringtonia cedarbergensis</i>	Unplugged uneven amphistomatic	6360	100
PODOCARPACEAE			
<i>Acmopyle pancheri</i>	Plugged stomata, uneven amphistomatic	12264	38
<i>Dacrycarpus dacrydioides</i>	Plugged amphistomatic	11887	-
<i>Dacrycarpus imbricatus</i>	Plugged amphistomatic	6941	-
<i>Lagarostrobos franklinii</i>	Plugged imbricate uneven amphistomatic	10535	-
<i>Lepidothamnus laxifolius</i>	Plugged uneven amphistomatic	9733	-
<i>Microstrobos niphophilus</i>	Plugged, epistomatic, imbricate	37339	17
<i>Microstrobos niphophilus</i> (juvenile)	Plugged, epistomatic, loosely imbricate	35143	31
<i>Microstrobos fitzgeraldii</i>	Plugged, epistomatic, non- imbricate	28410	32
<i>Phyllocladus alpinus</i>	Plugged uneven amphistomatic	19352	-
<i>Phyllocladus aspleniifolius</i>	Plugged uneven amphistomatic	19811	-
<i>Phyllocladus hypophyllus</i>	Plugged uneven amphistomatic	10350	-
<i>Podocarpus lawrencii</i>	Plugged hypostomatic	18025	-
<i>Podocarpus drouynianus</i>	Plugged hypostomatic	7909	41
<i>Podocarpus dispermis</i>	Plugged hypostomatic	5447	23
<i>Prumnopitys ferruginea</i>	Plugged approx. hypostomatic	4281	-



**Figure 7.2.** Transverse-section of the stomatal pore of *Actinostrobus acuminatus* as seen under a light microscope. In all stomata examined from this species the stomatal antechamber was free of wax. (Scale bar = 50  $\mu\text{m}$ )

**Figure 7.3.** Low vacuum scanning electron micrograph of a transverse-section through the stomatal pore of *Actinostrobus acuminatus* showing the wax free nature of the stomatal pore and guard cells (arrowed). Two neighbouring stomata are also visible on the leaf surface. (Scale bar = 40  $\mu\text{m}$ ).



**Figure 7.4.** Transverse section of a stomatal pore of *Acropyle pancheri* illustrating the wax filled stomatal antechamber. The wax plug has become slightly dislodged during sectioning, showing how closely the plug conforms to the shape of the stomatal antechamber. (Scale bar = 50  $\mu\text{m}$ )

**Figure 7.5.** Low vacuum scanning electron micrograph of a transverse section through the stomatal pore of *Athrotaxis selaginoides* illustrating the position of the stomatal plug (sp) over the pore. The sample was positioned at an angle of 45° and thus half the field of view (upper) is leaf surface, and the other (lower) half is sectioned, showing the inside of the leaf. Guard cells are arrowed. (Scale bar = 40  $\mu\text{m}$ ).

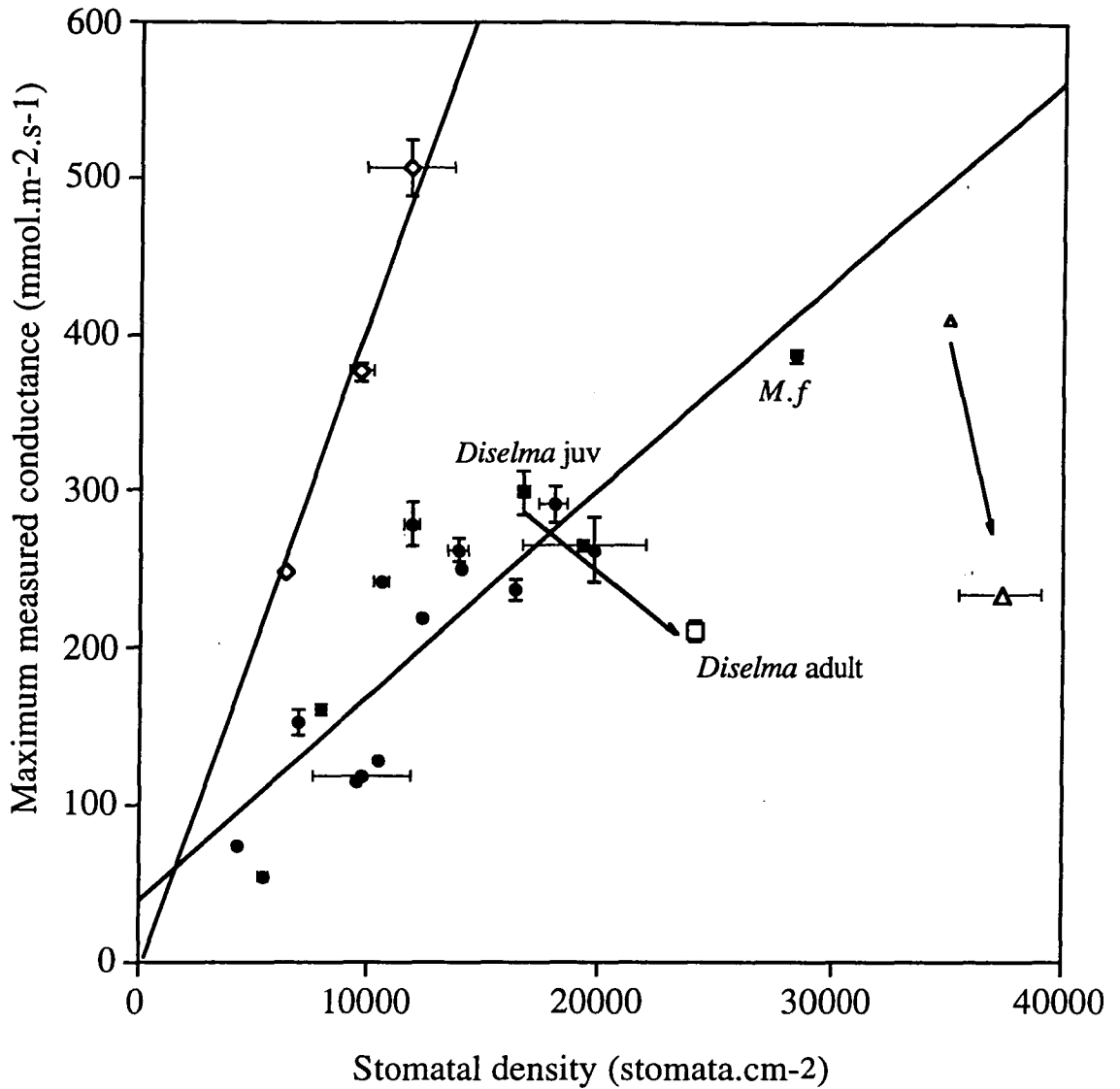


Figure 7.6. The relationship between stomatal density and maximum conductance in conifers with plugged stomata- ● , and without stomatal plugs- ◇ . Also shown are the imbricate epistomatic species *Diselma archeri* and *Microstrobos niphophilus* in adult - □, △ , and juvenile ■, ▲ , form respectively, with arrows indicating the juvenile to adult transition in both species. The fully lax *Microstrobos* species (*M. franklinii*) is labelled (M.f). Regressions have been drawn through the plugged and unplugged species, and both are significant at the  $p < 0.001$  level.



Table 7.2. Stomatal dimensions of the 13 conifer species included in this chapter, each represents the average reading from 5-10 stomata. The theoretical  $g_{\max}$  was calculated using the stomatal dimensions presented here, in equation 1, and a standard boundary layer conductance of  $2.67 \text{ mol.m}^{-2}.\text{s}^{-1}$  measured on a leaf replica.

Species	Stomatal length ( $\mu\text{m}$ )	Stomatal width ( $\mu\text{m}$ )	Stomatal depth ( $\mu\text{m}$ )	Theoretical $g_{\max}$ ( $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ )
<i>Acropyle pancheri</i>	21.6	7.4	11.25	576
<i>Actinostrobus acuminatus</i>	24.5	11.1	22.2	562
<i>Athrotaxis selaginoides</i>	22.2	12.5	17.5	721
<i>Callitris columellaris</i>	19.7	9.9	18.5	590
<i>Callitris rhomboidea</i>	19.6	12.0	20.8	448
<i>Diselma archeri</i>	18.4	8.7	14.8	640
<i>Diselma archeri</i> (juv.)	18.0	8.9	14.2	835
<i>Microstrobus niphophilus</i>	18.0	9.3	9.9	1196
<i>Microstrobus niphophilus</i> (juv.)	22.0	9.1	9.5	1338
<i>Microstrobus franklinii</i>	21.1	9.1	9.9	1379
<i>Phyllocladus aspleniifolius</i>	21.7	9.3	16.1	727
<i>Podocarpus drouynianus</i>	24.5	10.6	22.8	386
<i>Podocarpus dispersmis</i>	18.5	6.2	10.7	235
<i>Retrophyllum comptonii</i>	18.0	7.4	14.8	360
<i>Widdringtonia cedarbergensis</i>	19.8	10.0	24.3	306

A linear relationship was also observed between measured and calculated theoretical maximum leaf conductance in the sub-sample of 8 plugged species (Fig. 7.7). The slope of this regression illustrates that the measured  $g_{\max}$  in species with stomatal plugs was on average 35% of the value calculated from equation 1.

The maximum difference between measured and theoretical  $g_{\max}$  was in the imbricate, epistomatic species *Microstrobos niphophilus*. Adult *Microstrobos niphophilus* foliage produced a maximum conductance of only about 17% of the theoretical maximum expected from the density and size of stomata present (Table 7.1). Gas exchange in *Diselma archeri* was slightly less affected due to its incomplete epistomy, with measured  $g_s$  being about 25% of the maximum theoretical value. In contrast, juvenile *Diselma* foliage clearly fitted on the regression through the other plugged species (Fig. 7.7).

Because of the similar dimensions of the stomatal pores in all species, the relationship between stomatal density and theoretical  $g_{\max}$  (calculated using equation 1) was approximately linear (fig. 7.7). Again the effect of stomatal plugs was clearly evident when observed and expected conductances were compared in species with a range of stomatal densities (fig. 7.10).

## Discussion

The similarity between measured and theoretical  $g_{\max}$  in the three species of conifers used here with fully exposed, unobstructed stomata, further supports the application of the diffusion equation of Parlange and Waggoner (1970) as an indicator of the maximum potential for leaf gas exchange. This also provides good evidence that the assumptions made in measuring the stomatal pores were valid, namely that the width of the stomatal antechamber was approximately equal to the maximum open diameter of the stomatal pore, and that the depth of the stomata included the depth of this pit. The close correlation between measured and calculated  $g_{\max}$  in these species also enables the inhibitory effects of stomatal morphology on maximum potential leaf conductance to be assessed with some confidence that the calculation of maximum theoretical  $g_s$  is accurate.

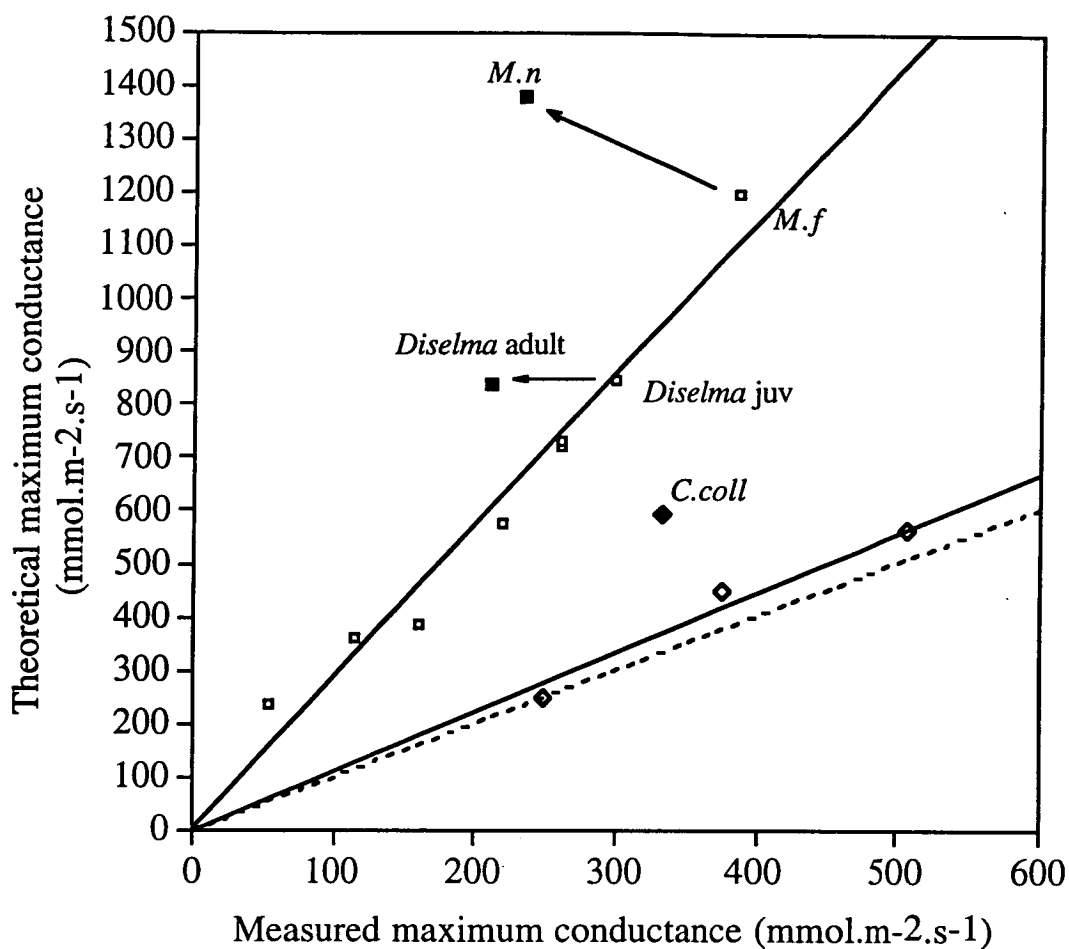
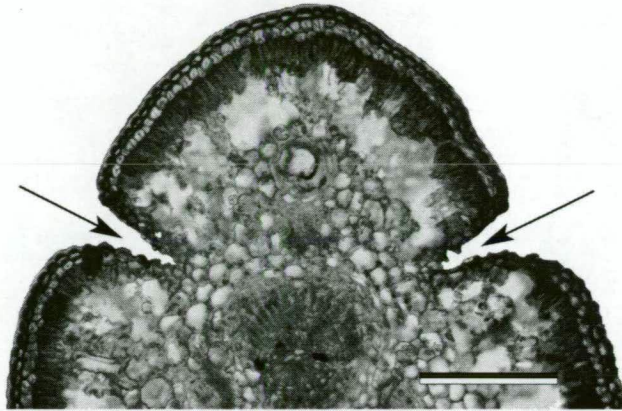
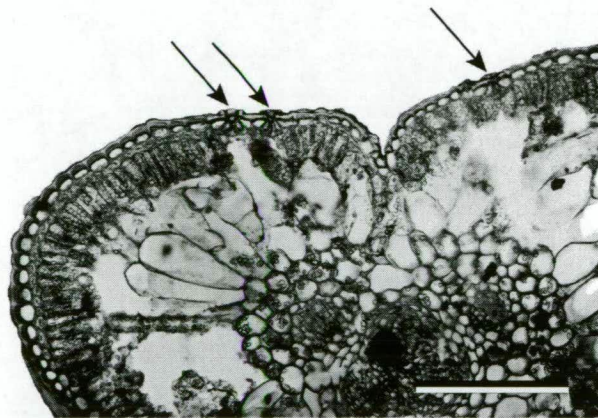


Figure 7.7. Measured versus theoretical maximum leaf conductance in a sample of 8 species with stomatal plugs - ■, three species without plugs - ◆ and two imbricate epistomatic species - ■. Regressions through the plugged and unplugged data are both highly significant ( $r$  for both = 0.98). The dotted line indicates, measured conductance = theoretical conductance. The juvenile to adult comparison for *Diselma* is shown with an arrow, and the comparison between non-imbricate *Microstrobos fitzgeraldii* (*M.f*) and imbricate *M. niphophilus* (*M.n*) is also arrowed. An unplugged species with stomata confined to leaf invaginations, *Callitris columellaris* - ◆ is shown for comparison with the other unplugged species which presented fully exposed stomata.



**Figure 7.8.** Transverse-section of a whorl of three leaves of *Callitris columellaris* where stomata are restricted to invaginations (arrowed) between leaves. (Scale bar = 500  $\mu\text{m}$ ).



**Figure 7.9.** A similar transverse-section for *Actinostrobus acuminatus* where stomata (arrowed) are fully exposed on the leaf surface (Scale bar = 500  $\mu\text{m}$ )

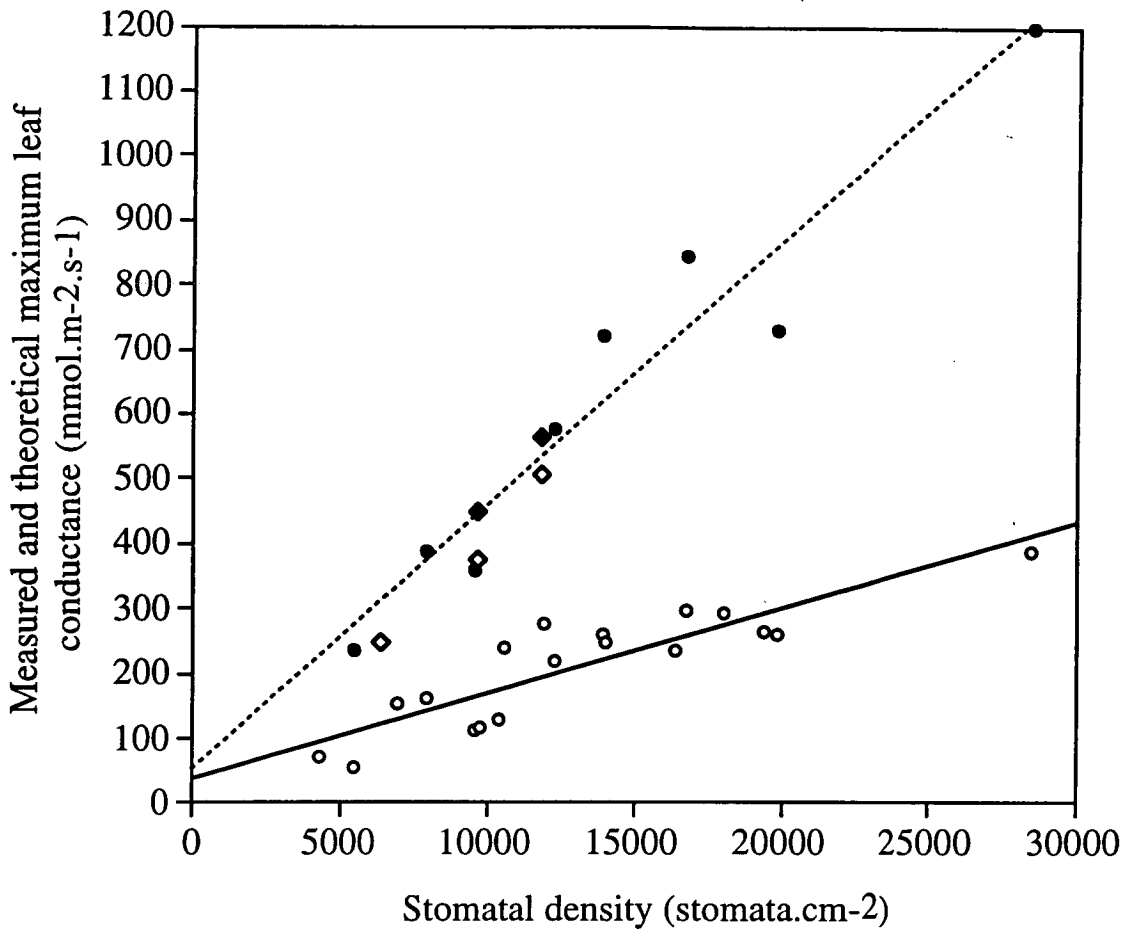


Figure 7.10. The linear relationship between stomatal density, and measured maximum conductance in plugged species - ○ and unplugged species - ◇. Also shown is relationship between stomatal density and theoretical maximum conductance in a sub-sample of plugged - ● and unplugged - ◆ species. Imbricate epistomatic species are not included in the data for plugged species. Regressions shown are theoretical  $g_{\max}$  vs stomatal density (broken line;  $r=0.97$ ) and measured  $g_{\max}$  vs stomatal density in plugged species (unbroken line;  $r=0.88$ )

Maximum conductance in species with exposed stomata occluded by wax plugs was reduced to about 35% of what would occur if the plugs were absent. This inhibition was approximately the same for all species sampled, including five genera of Podocarpaceae and two genera of Cupressaceae. From this it must be assumed that the wax crystals in the stomatal antechamber of each of these species are of similar size and density. A theoretical calculation of the effect on conductance of stomatal plugs in Sitka spruce (*Picea stichensis*) suggested that plugs should reduce leaf conductance by only about 37% (Jeffree *et al.*, 1971). The discrepancy between the 65% reduction shown here and the 37% reduction proposed for Sitka spruce may be due to differences in the nature of the stomatal wax, but is more likely to be due to inaccuracies in the estimate of the pathway of diffusion and hence the diffusion coefficient through stomatal wax plugs made by Jeffree *et al.*

The question remains as to why the bulk of conifers in the southern hemisphere should produce leaves with a high potential for gas exchange, only to occlude all the stomata early during leaf development (Martin and Juniper, 1970). Unlike most plants where wax is distributed approximately uniformly on the leaf avoiding stomatal openings and guard cells, the wax in these conifers is most abundant within the stomatal antechamber (Figs 7.4, 7.5), and in some species wax is almost entirely confined to the stomatal openings. The most common suggestion is that wax plugs serve an anti-transpirant function, a role which they clearly fulfil; however two lines of evidence question restriction of water loss as the primary selective pressure to which they are a response. Firstly, the conifer genera that grow in the driest environments in the southern hemisphere, and hence have the greatest need to reduce water loss, *e.g.* *Actinostrobus*, *Callitris* and *Widdringtonia* (chapter 1) produce leaf wax, but do not possess wax plugs. Secondly, it has been shown that amongst species with stomatal wax plugs the stomatal density is in equilibrium with the maximum rate of conductance. Despite this, the size and nature of wax plugs appears to be unrelated to the magnitude of the maximum leaf conductance. One might expect that if wax plugs were an adaptation to restrict water loss, some variation in the frequency or nature of wax would be evident in species at opposite ends of the conductance spectrum *i.e.* species with high photosynthetic rates and conductances might be expected to produce less wax than species with lower intrinsic photosynthetic capacity and maximum conductance. Such differences were not evident, and in *Diselma archeri* and *Microstrobos niphophilus*,

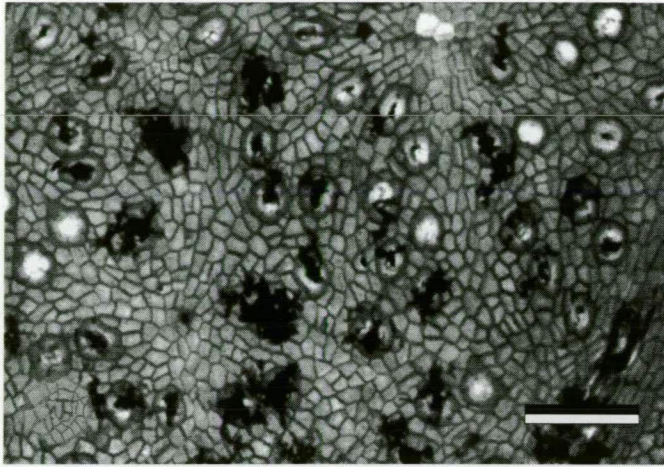
where the conductance of seedling foliage was significantly enhanced relative to adult foliage (Fig. 7.7), increased conductance was produced by large changes in leaf morphology, while the nature of the wax-plugs remained unchanged. In fact, inhibition of photosynthesis, at least in the short term, can result in a decrease in wax deposition (Ylimartimo *et al.*, 1994).

The presence of plugged stomata certainly pre-dates the spread of aridity during the Tertiary (Hill, 1990), and it seems more likely that wax plugs may have evolved as an adaptation to wet conditions. Leaf wetting inhibits photosynthesis both by blocking the stomatal pore, and by directly reducing the capacity of the photosynthetic apparatus (Ishibashi and Terashima, 1995), and decreased leaf wettability has been shown to allow increased conductance and assimilation under wet conditions (Smith and McClean, 1989). The restriction of wax to the stomatal antechamber may facilitate gas exchange under ever-wet conditions, while avoiding the increased leaf reflectance associated with a uniform wax deposition on the leaf.

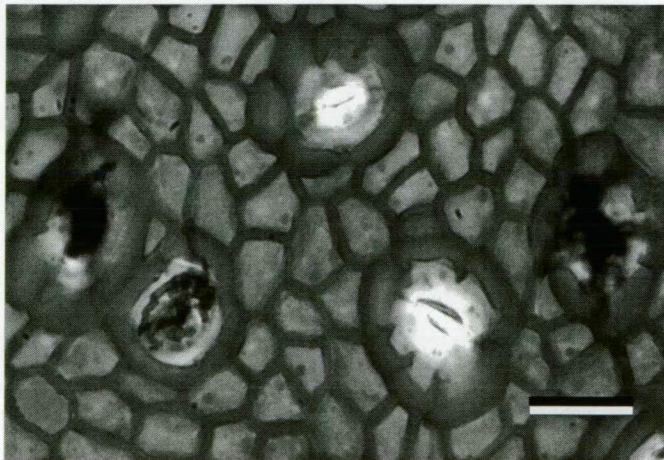
A second advantage associated with obstructing the antechamber is in the prevention of fungal invasion via the stomatal pore. Fungal spores accumulate in the stomatal pits of angiosperms, and germinate there presumably in response to the moist conditions (Figs 7.11, 7.12). This poses a particular threat to conifer leaves due to their generally high longevity (Ewers and Schmidt, 1981). Stomatal wax would prevent spore and water accumulation around the stomatal pore, and provide a physical barrier to the invasion of fungal hyphae. This is supported by the fact that erosion of the stomatal wax plugs by airborne pollutants leaves foliage more susceptible to fungal attack (Meng *et al.*, 1995).

Imbricacy in itself has no effect on the relationship between stomatal density and  $g_{\max}$ , but when combined with epistomy, leaf conductance was found to be substantially reduced. The effect is clearly illustrated in the comparison between *Microstrobos niphophilus* and *M. fitzgeraldii*, two closely related species with very similar leaf and stomatal morphology, but with the latter species possessing spreading rather than imbricate leaf arrangement (Fig. 7.7). In *M. fitzgeraldii*, leaf conductance was reduced to 32% of maximum theoretical conductance, whereas in *M. niphophilus*  $g_{\max}$  was reduced to 17% of theoretical  $g_{\max}$ . 65% of this inhibition can be attributed to the resistance produced by wax plugs (average for all species, see figure 7.7) meaning that the remaining 18% reduction of  $g_{\max}$  was due to the imbricate, epistomatic arrangement of





**Figure 7.11.** Surface view of fungal hyphae in the stomatal pore of a typical broad-leaved angiosperm. Hyphae appear as dark blotches concentrated around the stomatal pores of *Olearia argophylla* (Labill.) Benth. Unaffected stomata appear as clear areas. (Scale bar = 100  $\mu\text{m}$ )



**Figure 7.12.** Detail of hyphae in three stomata. Fungii appear to use the pore as a germination point, and to gain entry into the leaf. (Scale bar = 20  $\mu\text{m}$ ).



leaves. This reduction in conductance was less marked in *Diselma archeri* (Fig. 7.7) due to the fact that only 79% of stomata were on the adaxial surface of the leaf, and no effect was observed in imbricate species such as *Lagarostrobos franklinii* or *Athrotaxis cupressoides* where leaves were not tightly imbricate, with less than 30% of stomata on the adaxial leaf surface.

Considering the large inhibition of conductance (and hence assimilation) affected by this morphology, there must be an equally large benefit conferred. The most obvious advantage is that of physical protection of the stomatal surface. *Microstrobos niphophilus* and *Diselma archeri* (and the only other entirely imbricate epistomatic species, *Microcachrys tetragona*) are most common in sub-alpine shrubberies in Tasmania, where mechanical abrasion of the leaf is likely to be high, and thus dedication of the exposed (abaxial) leaf surface to structural support, by confining stomata to the adaxial leaf surface, would be advantageous in terms of minimising leaf damage from effects such as ice-abrasion. The fossil record provides some support for the protection hypothesis, with the description of several other (extinct) epistomatic, imbricate species from a Tertiary high altitude sites in Tasmania (Hill and Carpenter, 1991). However, the Tertiary fossil history of south east Australia also indicates that this morphology has been far more widespread in the past, with fossil occurrences of these genera also associated with low altitude, broad-leaved forest (Jordan, 1995; Blackburn and Sluiter, 1994; Wells and Hill, 1989; Blackburn, 1985). This indicates that the current restriction to highland areas may only be a recent phenomenon.

The other possibility is that this morphology primarily functions to restrict water loss, a purpose which it obviously serves extremely well. In *Microstrobos*, where imbricacy and epistomy are best developed, the conductance of non-imbricate foliage (juvenile *M. niphophilus* and *M. fitzgeraldii*) is very high (Fig. 7.6), a factor no doubt largely responsible for the distributional restriction of *M. fitzgeraldii* to spray zones of waterfalls (Harden, 1990). It seems plausible therefore that imbricacy may extend the range of *Microstrobos* by decreasing leaf conductance and allowing the genus to grow in drier habitats. It is interesting to note that in virtually all imbricate species the juvenile foliage is much less tightly packed than that of mature plants (chapter 2). From the data presented here, the effect of this must be to enhance the maximum leaf conductance, particularly in species where a large proportion of stomata are adaxial (e.g. *Diselma* and *Microstrobos niphophilus*). Whether this is the primary function of lax

juvenile foliage, enabling higher rates of photosynthesis and growth during establishment, or whether increased light capture also plays a role is unknown.

Restriction of water loss is a good explanation to account for the restriction of stomata to invaginations in the leaf of *Callitris columellaris*. Although not as effective as imbricacy in *Microstrobos*,  $g_{\max}$  was reduced in *C. columellaris* to about 56% of theoretical  $g_{\max}$ . The most common species with this morphology (*C. preissii*) extends into arid environments (Bowman and Harris, 1995), and the effect of this morphology in increasing the resistance to diffusion from the stomatal pore to beyond the boundary layer would enhance water use efficiency during photosynthesis.

In conclusion, it was found that maximum leaf conductance in the species investigated was proportional to the stomatal density, although the nature of the proportionality was dependent on the presence or absence of stomatal wax plugs, or whether leaves were partially or fully epistomatic and imbricate. With such information, maximum conductance can be predicted from extant or fossil conifer leaves with some confidence.

## CHAPTER 8

### **The Fossil history of Conifers in Australia, and Implications of Physiological Data**

Conifers have persisted, and remain successful in Australia in conditions which are cool and wet, or infrequently burned and dry, but are excluded from virtually all other habitats. For an understanding of why extant conifers have been restricted to such a small niche in Australia (and indeed the southern hemisphere) it is informative to consider the fossil history of conifers in Australia.

#### *Evolution of "Broad-shoot" conifers*

The fossil record in Australia suggests that although conifers have been a major component of the southern hemisphere flora since the early Cretaceous, it was not until the Early Tertiary (Eocene to Oligocene), more than 80 million years later, that conifer diversity reached its peak in southern Australia (Hill *et al.*, 1989; Wells and Hill, 1989). Conditions at this time are believed to have been highly equable, with a prevailing wet, temperate climate which apparently enabled the co-existence of a high diversity of coniferous and angiosperm taxa (Quilty, 1994). It is clear that angiosperms also thrived under these conditions, and the shift from conifer dominated forest to largely evergreen conifer/angiosperm mixed forest around this time is likely to have had a profound effect on the light availability, particularly in the understorey of such forests (Stenberg, 1996). The bulk of the coniferous taxa recorded from this period are araucarians and podocarpaceous genera bearing broad shoots, such as *Acmopyle*, *Dacrycarpus*, *Retrophyllum*, *Prumnopitys*, *Podocarpus*, *Phyllocladus* and extinct genera *Willungia* and *Smithtonia* (Hill and Pole, 1992). Given the strong genetic association between broad shoot width and low light saturation requirement illustrated in chapter 6 it is possible that this shoot morphology evolved, or at least radiated, in response to changes in the light availability in the understorey.

Changes in the distribution of stomata in fossil *Acmopyle* leaves have been interpreted as providing evidence of adaptation in this genus to reduce water loss under conditions of diminishing precipitation during the Tertiary (Hill and Carpenter, 1991). This is unlikely to be the explanation considering the data presented here. It has been shown in

chapter 3, and other studies (Körner, 1988) that the maximum leaf conductance is well correlated with the maximum assimilation rate, and in chapter 7 a linear relationship between stomatal density and maximum conductance was observed. Thus the decrease in stomatal density in *Acmopyle* is almost certainly a response to decreased maximum assimilation rate in leaves, and this most likely came about either via a decrease in temperature, or a decrease in light availability.

Evidence supporting a decrease in light intensity comes from the fact that stomatal numbers were found to become differentially reduced on the upper surface of the shoot compared to the lower surface, thus causing a shift from amphistomy to hypostomy in shoots. Hypostomy is the most common stomatal distribution found in shade plants, while amphistomy is more common in species from open habitats (Peat and Fitter, 1994), and the shift from amphistomy to hypostomy in *Acmopyle* fossils would tend to suggest a reduction of the available light in *Acmopyle* habitat. A closure of the canopy by invading angiosperms may have been the cause of such a change in the distribution and availability of light from forest crown to floor. There is also evidence of a decrease in temperature at the last occurrences of *Acmopyle* in Tasmania (R.S. Hill pers. comm.). Although this temperature change apparently did not affect the shoot size of *Acmopyle*, it is possible that this may have contributed to stomatal loss in the latter history of the genus in Australia.

There has been some speculation as to the structure and function of the high latitude conifer/angiosperm dominated forests which apparently extended across much of Australia and Antarctica during the mid-late Cretaceous (e.g. Specht *et al.*, 1992), and this has considerable bearing on the evolution of today's conifer flora. During the mid-Cretaceous Australia and Antarctica were joined, and most of the continent was south of 60°S (Fig. 8.1). Occurrence at such high latitudes has led to the proposal that these forests were dominated by stands of pyramidal crown conifers, as this crown shape most efficiently harvests light at low solar angles (Terborgh, 1985; Specht *et al.*, 1992), forming a forest structure similar to that seen at high latitudes in the northern hemisphere boreal zone today. Evidence suggests that many of the angiosperms (and gymnosperms) present in these Cretaceous forests were deciduous (Pole, 1992; Mc Loughlin *et al.*, 1995; Hill and Scriven, 1995) also conforming with the observed pattern in the boreal forests, where a mixture of deciduous angiosperms and evergreen conifers predominate. Obviously there are difficulties in ascertaining the relative importance of the

deciduous element in these fossil floras, as the taxonomic affinities of the species present are often poorly understood, and the identification of deciduousness is based on fairly meagre evidence; however it is widely accepted that a mixture of deciduous and evergreen angiosperms was present. Extant evergreen broad-leaved taxa with affinities to fossil species have been shown to survive long dark periods such as those that would have prevailed during winter at high latitudes (Read and Francis, 1992). The presence of a significant high latitude evergreen angiosperm flora contrasts with the situation today at high latitudes in the northern hemisphere. Persistence of the evergreen element was probably allowed by the fact that mild winter temperatures (Dorman, 1966) did not cause significant damage to overwintering broad-leaves.

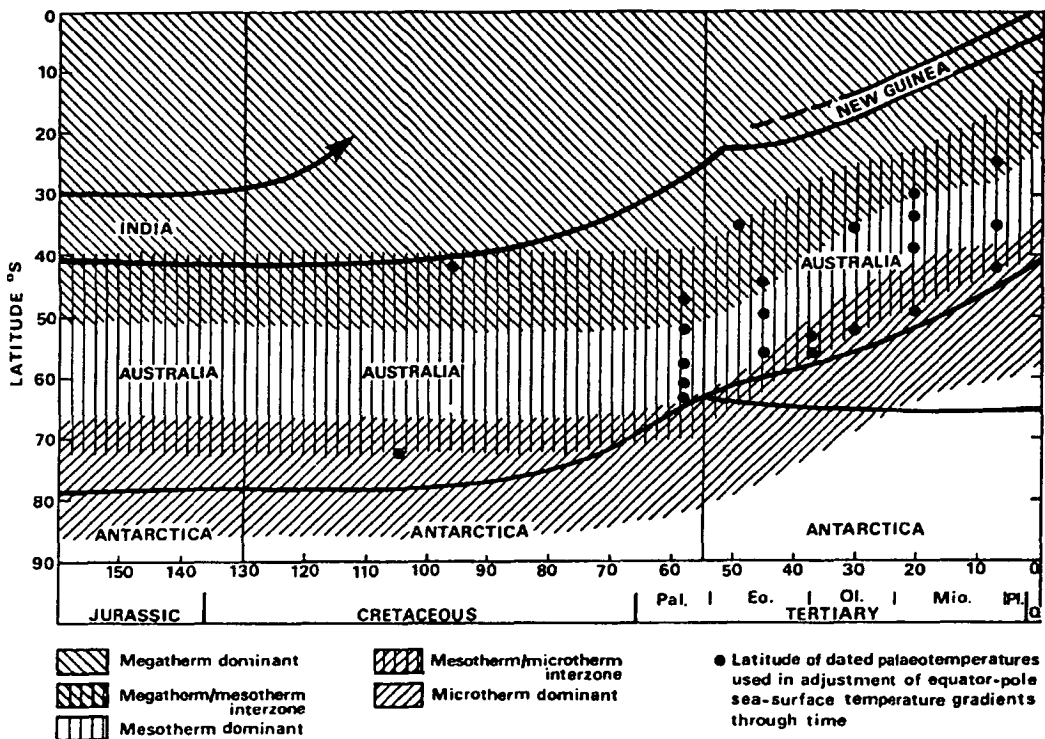


Figure 8.1. Latitudinal drift of Australia from the Jurassic to present, with associated changes in climatic conditions. The cooling phase beginning at about 55 million years B.P. is thought to be caused by the establishment of the circum-polar current after Australia and Antarctica separated. Temperatures corresponding to the various thermal zones are as follows : megatherm  $> 24^{\circ}\text{C}$ : megatherm/mesotherm  $> 20^{\circ}\text{C} < 24^{\circ}\text{C}$ : mesotherm  $> 14^{\circ}\text{C} < 20^{\circ}\text{C}$ : mesotherm/microtherm  $> 12^{\circ}\text{C} < 14^{\circ}\text{C}$ : microtherm  $< 12^{\circ}\text{C}$ . (From Nix, 1982).

The most common conifer shoots from Cretaceous deposits are narrow, imbricate foliage from genera with affinities to *Araucaria*, *Sequoia*, *Dacrycarpus*, and small leaved *Podocarpus*-like species. The first

appearance of podocarps with significantly bilaterally flattened shoots is in southern Australia during the early Tertiary (probably *Acmopyle* in the Palaeocene; Hill and Carpenter, 1992) and it is probable that the podocarp genera with bilateral shoots first evolved under the low solar angle conditions experienced during the Cretaceous / early Tertiary. Shoot arrangement in many conifers from high latitudes in the northern hemisphere today shows a progression from cylindrical in the canopy, to bilaterally flattened in the understorey (Sorrenson-Cothorn *et al.*, 1993), and it is conceivable that a similar pressure to allow penetration of light deep into the canopy (Stenberg, 1996) led to the evolution of flattened shoots in podocarps. Of course once solar angle increased and the winter period became light, as land masses drifted north, the advantages of conical crowns and deciduousness were lost. Higher incident radiation year round would have allowed broad-leaved evergreen angiosperms to flourish in the canopy. Podocarps would have been "exapted" (Gould and Vrba, 1981) to this change, and the expansion of genera with broad shoots at this time would have taken advantage of the change in light conditions. This correlates well with the morphological response seen in most broad-shoot podocarps to light conditions described in chapter 2, and the shade tolerance of bilaterally flattened shoots (chapter 6).

The foliar convergence between broad-shoot podocarps and angiosperm broad-leaves appears to have enabled the persistence, and possibly the radiation of conifer taxa bearing this shoot morphology, as the Tertiary forest structure in southern Australia apparently shifted from high latitude conifer/deciduous to evergreen broad-leaf forest. A significant liability does, however, seem to be linked to this adaptation, that being a low tolerance of water stress. It was illustrated in chapters 3 and 4 that virtually all podocarps exhibited quite low drought tolerance, and that the broad-shoot genera such as *Dacrycarpus* and *Acmopyle* were particularly intolerant of drought. This drought intolerance does not appear to result from suboptimal stomatal regulation of gas exchange (chapter 6), and seems rather to be a result of early damage to leaf tissue under water stress, or perhaps an intrinsically inefficient root or vascular system which is susceptible to early cavitation (see Sperry and Pockman (1993) for discussion of xylem embolism and transpiration). The outcome of this is that while broad-shoots may have allowed podocarps to retain co-dominance in high rainfall, mixed Tertiary forest, these taxa were left extremely vulnerable to decreased rainfall.

### *Decline of Conifer Abundance in Australia*

All indications (e.g. sea sediment cores, stratigraphic information) are that rainfall did decrease through the Tertiary (Bowler, 1982; Quilty, 1994) along with a gradual decrease in sea surface temperature (Wei, 1991). This was probably due to the establishment of the circum-polar current approximately 30 million years ago, and as a result of the shift in climate which followed (Fig. 8.2), widespread extinction of the coniferous element in Australia occurred.

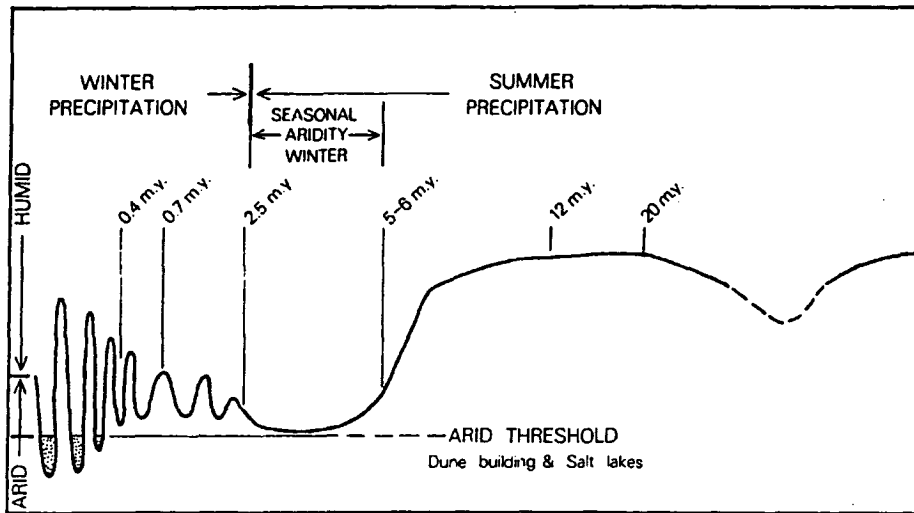


Figure 8.2. Diagrammatic summary of changes in humidity experienced by regions now arid or semi-arid in southern Australia. The horizontal axis represents time, extending from present (far left) back to the lower Miocene.

Evidence for these changes in forest composition mostly comes from micro and macrofossil deposits across southern and southeastern Australia, ranging from Western Australia to eastern Victoria and New South Wales and Tasmania. Such deposits usually only provide a brief 'snapshot' of the surrounding flora, and thus the record of changing forest composition is somewhat discontinuous. What is clear from these deposits, however, is that conifer-angiosperm mixed forest was widespread throughout southern Australia in the late Eocene, and that by today's standards, the conifer diversity represented was extremely high (a Oligocene deposit in Tasmania representing a small river catchment contains between 20 and 25 conifer species; R.S. Hill, pers. comm.).

Interpretation of vegetation successions from coal seams deposited

in the Oligocene to early Miocene in Victoria illustrate the first signs of replacement of conifer rainforest with angiosperm taxa under seasonally dry conditions where fire disturbance was significant (Martin, 1983; Blackburn and Sluiter, 1994). The Tertiary fossil record from Tasmania is relatively comprehensive, with an abundance of well preserved macrofossils, but unfortunately very few sites of Miocene age exist. Bearing this in mind, it can be said that the period between the Oligocene and Pleistocene in Tasmania saw the decline of the bulk of the diverse broad-shoot conifer flora which was in place early in the Oligocene. Almost all species from Tasmanian Pleistocene deposits can be assigned to extant (mostly angiosperm) genera. Many of these, such as *Banksia*, *Epacris*, *Allocasuarina*, *Acacia*, and *Eucalyptus* (Carpenter *et al.*, 1994; Jordan, 1995), are today associated with drier environments.

Decreasing temperature in the later Tertiary is also likely to have been a significant factor contributing to the loss of broad-shoot Podocarps, particularly from Tasmania. The effect of decreased temperature on the abundance of broad-shoot podocarps also becomes apparent in the comparison of concurrent low and high altitude early Oligocene deposits in Tasmania. Low altitude sites of this age contain abundant material from broad-shoot podocarps, whereas localities from higher altitude contain a poor representation of broad-shoot species (R.S.Hill pers. comm.). The relationship between shoot width and temperature is discussed further in chapter 9.

Hill and Carpenter (1991) documented the disappearance of broad shoot *Dacrycarpus* from Tasmania by reconstructing changes in the shoot morphology of this genus in Tertiary deposits from around the state. They described the shift in the predominant morphology, from bilaterally flattened (broad-shoot) species in the early Eocene, to bifacially flattened species in the Oligocene to Miocene. The loss of bilaterally flattened foliage is most likely to be related to decreasing temperatures over this period, correlating with a decreased tendency for extant *Dacrycarpus* species to produce bilaterally flattened foliage in cooler climates/higher altitudes. More significant was the observation of a trend amongst the imbricate fossil *Dacrycarpus* species towards a restriction of stomata to the adaxial (inner) leaf surface. This trend was apparent when all fossil species are pooled together, although even within a single fossil species there was an obvious shift in stomatal distribution, from bifacially flattened amphistomatic leaves in the Eocene, to leaves identical in size and shape, but epistomatic, in the late Oligocene. The restriction of



stomata to the adaxial surface of imbricate leaves has been shown to significantly reduce water loss in living taxa (chapter 2), and the shift towards this morphology in fossil *Dacrycarpus* is likely to be an adaptive mechanism to reduce water loss, presumably in response to a drying climate. *Dacrycarpus* today is commonly riparian, and its abundance in fossilised fluvial sediments indicates that it was common in the riparian flora of south-east Australia during much of the Tertiary. Being therefore on the forest edge, this genus may have responded less to changing light conditions, and more to the availability of water.

*Podocarpus* was found to be anomalous in both its high drought tolerance, and its low light requirement, and it seems that this genus has been able to overcome the drought intolerance which characterises other genera of Podocarpaceae. This being the case, *Podocarpus* provides strong support for the hypothesis that water limitation has been that major force in the elimination of conifers from forests in Australia. The reason for this is that *Podocarpus* is the most diverse, widespread, and common genus in the southern hemisphere today, and the success of *Podocarpus* is likely to be attributed to its relative tolerance of low water potential. *Podocarpus* seems to have persisted in habitats ranging from dry open woodland, to tropical lowland forest, to alpine shrubberies, not through any significant shift in leaf morphology, but rather, by its ability to survive the episodes of drying which are hypothesised to have impinged upon other podocarp (and non-podocarp) genera.

The question still remains as to why the drought adapted coniferous taxa such as the Callitroideae and *Widdringtonia* were not able to continuously occupy areas which became too dry for the broad-leaf podocarps. There is evidence that during the later Tertiary, these taxa grew close together, with a continuum in the floras from Victorian coal deposits between swamp Podocarp/angiosperm forest, and drier forest with *Callitris* co-dominant (Blackburn and Sluiter, 1994). Also in Tasmania, Pleistocene deposits contain *Callitris* which must have been within, or close to the conifer dominated rainforest (Jordan, 1994). In Australia today, *Callitris* and *Actinostrobus* are usually well separated from rainforest, and this can probably be attributed to both competition by broad-leaved angiosperms, and an increase in fire frequency. As shown in chapter 6, all the drought adapted Cupressaceae only reached maximum assimilation rates at very high light intensities, and this high light requirement would certainly compromise the competitiveness of these genera in closed forest. The distribution of *Callitris* in New Caledonia

today seems to confirm this, with species usually restricted to river edges or ridgelines, presumably because of an inability to penetrate the understorey of closed forest. Fire has also been a significant factor in limiting the distribution of drier conifer forest, and there is evidence that an increase in fire frequency in the Holocene, perhaps associated with early human activity in Australia caused large scale changes in the flora, resulting in a replacement of forest dominated by "needle-leaved" Casuarinaceae and *Callitris*, with fire adapted *Eucalyptus* forest (Singh *et al.*, 1981; Hope, 1994). Thus, today these drought tolerant conifer taxa are restricted to areas where the frequency of intense fire is low, either because of low plant density (dry forest), or where landforms provide fire protection.

### Summary

Although gymnosperms dominated forests throughout much of the Cretaceous, the greatest species diversity of conifers in the southern hemisphere seems to have occurred in the early to mid-Tertiary. These new genera, mostly podocarps, arose from ancestors which apparently grew in the high latitude, humid forests of southern Gondwana. While conditions remained wet and humid during the Eocene and Oligocene conifers (mostly broad-shoot podocarps) remained co-dominant with angiosperms in diverse rainforest communities. A drying climate during the latter part of the Tertiary appears to signal the demise of conifers throughout southern Australia. Drought adapted genera (such as the *Callitris* and *Actinostrobus*) may have persisted in dry forests associated with Casuarinaceae, but increases in the fire frequency have largely confined even these taxa to refugial distributions.

## CHAPTER 9

### Conclusions

This work provides explanations for both the distributional limitation of conifer forest, and the influence of foliar morphology on light harvesting and gas exchange. These data provide a quantitative framework from which conifer history can be interpreted using macrofossil records. Implications for the future distribution of conifers in the southern hemisphere are discussed as are key areas for further research.

#### *Physiological characteristics and limitations*

Considering the large foliar morphological range of the conifers examined here it is perhaps not surprising that a correspondingly large range of physiological preferences were observed among species. It was found that these physiological preferences, or limitations, appear to constrain conifer distributions in a well defined manner enabling distributional limits to be described in terms of physiological characteristics.

Drought tolerance was measured by a novel technique whereby the minimum attainable ratio of internal to external leaf  $\text{CO}_2$  ( $(c_i/c_a)_{\min}$ ) was quantified. This index was strongly correlated with rainfall at the driest occurrence within the natural range of each of the conifers sampled, implying that species were confined by their photosynthetic efficiency and tolerance during drought. On the contrary, photosynthetic characters such as instantaneous water-use efficiency under optimal conditions, and integrated water-use efficiency as measured by carbon-13 discrimination in the leaf, bore no relation to the water availability within species distributions, and did not appear to provide any information as to the drought tolerance of species. The conclusion that most of the species investigated were limited in their distributions by the availability of water (chapter 4) was supported by data presented in chapter 5 where photosynthesis in species from comparatively wet and dry locations was compared. Measured in the field under summer conditions, photosynthesis in each co-occurring conifer species was lower at the drier site than at the wet site, suggesting that water limitation was a factor contributing to the inhibition of photosynthesis in these species under

conditions which were fairly typical for summer. These measurements were taken from adult plants, and it is likely that seedlings are even more prone to water shortage. The local distribution of seedlings observed on mountains in Tasmania appears to be strongly influenced by water availability, with seedlings highly restricted to shady sites adjacent to continual running water (pers. obs.). Obviously seedling recruitment is an important determinant of the future distribution of these species and it would be interesting to survey the natural occurrence of seedlings and to quantify the habitat requirements for successful seedling recruitment.

Like drought tolerance, a similarly large range of light response characteristics were noted in the conifers investigated, and this trait appeared to be linked to the width of the short shoot. Short shoot width provided a simple, non-destructive means of approximating the degree of shoot flattening as well as the potential horizontal area of the shoot. Species with broad shoots were found to be suited to photosynthesis under low light conditions, while species with narrow shoots generally required much higher light intensities to achieve maximum photochemical and assimilation rates. In chapter 6 comments were made referring to an apparent link between the production of broad shoots (with a low light requirement) and drought intolerance. There is a danger of circular reasoning here. Drought intolerance may be cause or effect in the confinement of these species to wet forest, and equally, low light requirement may represent an adaptation to light competition with angiosperms, or a product of the confinement of drought intolerant species to wet forest. The close association between drought tolerance and distribution illustrated in chapter 4 supports the conclusion that characteristics of the physiological response of these species to drought are causal in determining distribution. Furthermore, the strong genetic control of light response illustrated in chapter 6 suggests that this character is adaptive, and therefore, that broad shoots are an adaptation to low light.

### *Form and Function*

One of the aims of this project was to determine what information can be gleaned about conifer adaptation to environment, from examination of foliar morphology. Associations between morphology, function and environment are summarised here in light of the data presented in previous chapters.

The link between shoot flattening in podocarps, and preferred light environment was the clearest association between form and function described here. Not only was a quantitative relationship evident between increasing width of shoot shoots and lower light requirement (chapter 6), but variation in shoot morphology among individuals of many species was associated with the production of strongly flattened two-dimensional short shoots in deep shade (chapter 2). A similar relationship has been described for northern hemisphere needle bearing conifers, with needle arrangement observed to become more two-dimensional under shaded conditions (Sorrensen-Cothorn *et al.*, 1993), although the degree of flattening found in the podocarps is far more profound<sup>than</sup> that recorded in needle-leaved northern taxa (see chapter 2). The similar effects of temperature on both the shoot size in conifers and leaf size in angiosperms (chapter 2) illustrate the functional parallel between broad shoots and broad leaves, and unfortunately this complicates the relationship between light environment and shoot flattening. Cool conditions reduce the advantage of producing large, broad-leaves (as is reflected by a decrease in average leaf size; Parkhurst and Loucks, 1972), and thus conifers growing in cool-temperate rainforest tend to possess narrow shoots (*e.g.* Tasmania and New Zealand) regardless of light climate. Thus, for the purposes of reconstructing the palaeo-ecology of conifers at different fossil deposits, it is necessary to consider the leaf size of the associated angiosperm flora in order to indicate whether low temperature or high light explains the absence of conifer broad shoots from mixed conifer-angiosperm forest. At the other end of the temperature scale, it seems that broad-shoot conifers are also mostly excluded from megathermal lowland equatorial rainforest (de Laubenfels, 1988), and it is likely that either mechanical or vascular limitations on the maximum size of short shoots makes them uncompetitive under conditions favouring megaphyll flora. This hypothesis requires further testing.

The relationship between conifer shoot morphology and water availability is complex, and perhaps the most positive conclusion that can be drawn from current day distributions and data in chapters 3 and 4, is that genera such as *Acmopyle* and *Dacrycarpus* with large, bilaterally flattened shoots, are highly sensitive to drought. Although the species found here to be most highly drought adapted all produced extremely narrow shoots, this morphology could not be described as specifically representative of significant drought tolerance, as drought sensitive taxa

from cool and/or high altitude habitats produced similar narrow, imbricate foliage.

One morphological feature of conifers which has previously been described as an adaptation to restrict water loss is wax plugs (Jeffree *et al.*, 1971), a hypothesis which is not supported by the data presented in chapter 7. While wax plugs were found to significantly reduce gas exchange, it was concluded that this was unlikely to be the primary function of the wax, as species most susceptible to drought were found to have wax-free stomata despite the presence of wax on the leaf surface. The alternative functions of plugs proposed in chapter 2 *i.e.* as an anti-fungal agent, and as a means of keeping the stomatal pore free of water, are considered more probable, and better aligned with the observed predominance of species bearing wax plugs in wet forest. More work is needed using both conifers and plug-bearing angiosperms to clarify the primary function of wax in the stomatal pore.

Imbricacy is another morphological feature which has been interpreted as an adaptive measure to restrict water loss, and in chapter 7 it was shown that whilst imbricacy in hypostomatic leaves has no effect on the maximum stomatal conductance, the confinement of stomata to the adaxial surface of the leaf markedly reduces the maximum conductance. Although this morphology would serve to protect the stomatiferous surface of the leaf from damage by forces such as ice-abrasion, it was concluded that, along with encrypting of the stomata within the leaf (as seen in *Callitris columellaris*), the significant decline in maximum leaf conductance produced by these morphologies is the primary advantage derived by the plant. There are examples of apparent systematic changes in the stomatal distribution on leaves of imbricate *Dacrycarpus* species during the Tertiary. The observed shift from amphistomy in early fossils to epistomy later (Hill and Carpenter, 1992) is interpreted here as adaptation to restrict water loss, probably in response to decreased water availability.

### *Conifer Conservatism*

Despite a collectively large range of physiological adaptation, significant functional barriers appear to limit the development of different groups of coniferous taxa today. Podocarps have overcome the leaf size limitation afflicting most other conifer groups, enabling this family to successfully compete with broad-leaved angiosperms. Low drought tolerance,

however, was common to all podocarp genera (with the exception of *Podocarpus*) and this was compounded in the broad-shoot species, restricting the great majority of these species to high rainfall environments. In contrast, some cupressaceous genera (*Actinostrobus*, *Callitris* and *Widdringtonia*) were found to exhibit an impressive potential for survival and water efficient photosynthesis at extremely high water deficits, but apparently lacked the morphological potential for broad shoot development, thus excluding them from most types of dense canopy forest. Virtually all southern hemisphere conifers are highly sensitive to fire, and even the genera with well developed serotiny (*Callitris* and *Widdringtonia*) are unable to survive changes in fire frequency or intensity (Midgley *et al.*, 1995; Bowman and Harris, 1995).

In summarising these apparently insurmountable liabilities in conifer function it seems that one of the universal liabilities among coniferous taxa is a relatively low rate of, or potential for, adaptation (compared with angiosperms). Evidence of the conservatism in conifer genera is abundant in the fossil record, where the majority of well preserved macrofossil remains from Tertiary deposits are confidently placed into living genera. Leaf records of *Acmopyle*, *Dacrycarpus*, and *Podocarpus* extend to the Palaeocene, and genera such as *Araucaria* are commonly identified from deposits aged well into the Cretaceous. The genus identified as *Austrosequoia* by Peters and Christophel (1978) from Cretaceous sediments in Australia is also almost identical to the extant *Sequoia* and a merger into the genus has been suggested (Hill *et al.*, 1993). This slow rate of morphological evolution appears to be mirrored in, and is probably linked to, the apparently low potential for physiological evolution in conifer genera. General coniferous features such as single veined leaves and vessel-free wood are often cited as features which provide a universal "governor" on the competitive ability of conifers (see Bond, 1988); however it would seem that these characters are simply further examples of a minimal adaptive potential of this group of plants. Whether this slow rate of evolution is a feature of the reproductive characteristics such as long generation times, or related to peculiarities of the conifer genome is unknown, but its effects are clear, giving conifers a generalised "refugial" status in the southern hemisphere in particular.

The conservative nature of the southern hemisphere conifers largely explains why these taxa have been particularly susceptible to the recent, very rapid anthropogenic changes in the ecology of many southern hemisphere forests. Many taxa are extremely restricted in

distribution, and species such as *Microstrobos fitzgeraldii*, and *Acmopyle sahniana*, are only represented by known populations totalling only 10-20 individuals. With rapid anthropogenic climate change now a reality, the survival of these and many other species may be severely compromised, and continued work on the propagation, physiological requirements, distribution, history, and ecology of this enigmatic group of plants should be a high priority.



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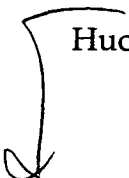
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**Appendix A:       Papers accepted for publication**

# Dynamics of Changing Inter-cellular CO<sub>2</sub> Concentration (c<sub>i</sub>) during Drought and Determination of Minimum Functional c<sub>i</sub>

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Nine conifer species with narrow (<5 mm), single-veined leaves were selected for the purpose of examining changes in intercellular CO<sub>2</sub> concentration (c<sub>i</sub>) during drought. Due to the leaf morphology of the study plants, the confounding effects of nonhomogeneous photosynthesis common to most reticulate-veined angiosperms were largely avoided, giving a clear picture of c<sub>i</sub> dynamics under increasing drought. A characteristic biphasic response was observed in all species, with an initial stomatal control phase resulting in a substantial reduction in c<sub>i</sub> as stomatal conductance (g<sub>s</sub>) decreased. As g<sub>s</sub> reached low levels, a strong nonstomatal limitation phase was observed, causing c<sub>i</sub> to increase as g<sub>s</sub> approached a minimum. This nonstomatal phase was linked to a concomitant rapid decrease in the fluorescence parameter quantum efficiency, indicating the onset of nonreversible photoinhibition. The ratio of internal to atmospheric CO<sub>2</sub> concentration (c<sub>i</sub>/c<sub>a</sub>) decreased from values of between 0.68 and 0.57 in undroughted plants to a minimum, (c<sub>i</sub>/c<sub>a</sub>)<sub>min</sub>, which was well defined in each species, ranging from 0.10 in *Actinostrobus acuminatus* to 0.36 in *Acropyle pancheri*. A high correlation was found to exist between (c<sub>i</sub>/c<sub>a</sub>)<sub>min</sub> and leaf water potential measured at (c<sub>i</sub>/c<sub>a</sub>)<sub>min</sub>. Species developing high maximum intrinsic water use efficiencies (flow (c<sub>i</sub>/c<sub>a</sub>)<sub>min</sub>), such as *A. acuminatus*, did so at lower leaf water potentials (−4.5 MPa) than more mesic species (−1.75 MPa for *A. pancheri*). It is concluded that in the absence of patchy stomatal closure, (c<sub>i</sub>/c<sub>a</sub>)<sub>min</sub> gives a good representation of the drought tolerance of foliage.

It has long been established that during drought, g<sub>s</sub> decreases, reducing water loss from the leaf (Slatyer, 1967). In the absence of other effects, such a decrease in conductance will produce a CO<sub>2</sub> "supply" limitation of photosynthesis (Farquhar and Sharkey, 1982) by causing the c<sub>i</sub> to become depleted. Recently, however, the importance of reduced g<sub>s</sub> in limiting A during drought has been questioned. Many authors have described "nonstomatal" inhibition of photosynthesis as an important influence during drought, attributing this to impaired chloroplast function in leaves subject to water deficits (Boyer, 1971; Graan and Boyer, 1990; Majumdar et al., 1991; Gunasekera and Berkowitz, 1993).

Nonstomatal inhibition of photosynthesis has been commonly invoked to explain results of gas-exchange studies showing unchanging or increasing c<sub>i</sub> in the leaves of droughted plants and a strong depression in the relationship between A and c<sub>i</sub> (Ehleringer and Cook, 1984; Wong et

al., 1985; Grieu et al., 1988; VanRensberg and Kruger, 1993; Anderson et al., 1995). The suggestion that during drought nonstomatal effects become important soon after  $\psi$  begins to drop is not consistent, however, with data obtained in fluorescence studies, which indicate that leaf photochemistry is resistant to moderate drought stress (Sharkey and Badger, 1982; Genty et al., 1987; Cornic et al., 1989) and that during drought an increased flow of electrons to O<sub>2</sub> reflects decreasing c<sub>i</sub> (Cornic and Briantais, 1991). Integrated measures of c<sub>i</sub>, such as carbon isotope discrimination in leaves, also suggest that c<sub>i</sub> is lower in water-stressed plants (Smith and Osmond, 1987; Hubick et al., 1988; Ehleringer et al., 1992; Donovan and Ehleringer, 1994). This disparity between gas-exchange data and other measures of c<sub>i</sub> has been attributed to patchy stomatal closure in response to drought.

Implicit in the calculation of A and g<sub>s</sub> from gas-exchange parameters is uniform stomatal aperture on the leaf being measured (Farquhar and Sharkey, 1982). In the case of patchy stomatal closure, the whole A(c<sub>i</sub>) relationship is erroneously depressed, leading to the conclusion that photosynthesis is being directly inhibited (Terashima et al., 1988). Heterogeneity in the distribution of photosynthesis has been observed in most species examined (Pospisilova and Santrucek, 1994) and appears to be associated with the reticulate venation typically found in broad-leaved angiosperms. This type of venation tends to subdivide the leaf lamina, especially in the case of heterobaric leaves, in which bundle-sheath extensions from the veins to the epidermis compartmentalize the leaf into small aereoles. Studies of chlorophyll fluorescence (Downton et al., 1988; Cardon et al., 1994; Siebke and Weis, 1995) and radiolabeling (Terashima et al., 1988) have shown that during drought or ABA application, groups of aereoles can be actively photosynthetic while adjacent parts of the leaf are not measurably assimilating. This spatial heterogeneity in leaf photosynthesis has prevented an accurate understanding of the dynamics of changing c<sub>i</sub> during drought.

The aim of this study was to measure changes in c<sub>i</sub> and A during drought in species with a low potential for nonuniform gas exchange. By using functionally amphis-

tomatous species with single-veined leaves < 5 mm in width, the problem of leaf subdivision was avoided and the potential for rapid lateral diffusion of CO<sub>2</sub> throughout the leaf was maximized. Potential patchy effects were also avoided by slowly and evenly inducing drought (Gunasekera and Berkowitz, 1992; Wise et al., 1992). These measurements allowed determination of a minimum attainable c<sub>i</sub>/c<sub>a</sub> during drought, a parameter that could be compared between species and that reflects the relative drought tolerance of the species examined.

## MATERIALS AND METHODS

### Plant Material

Cuttings of the conifers *Acropyle pancheri* (Brongn. and Glis) Pilger, *Diselma archerii* Hook.f., *Lagarostrobos franklinii* (Hook.) C.J.Quinn, *Podocarpus laurercii* Hook.f., and *Microstrobos niphophilus* Garden and Johnson, and seed from *Actinostrobus acuminatus* Parlature, *Athrotaxis selaginoides* D.Dun, *Callitris rhomboidea* R.Br., *Dacrydium dacrydioides* (Rich.) de Laubenfels, and two angiosperms, *Eucalyptus leucon* Miq. and *Acacia melanoxylon* R.Br., were propagated in sand in Hobart (Tasmania, Australia). Seedlings and rooted cuttings were transferred to a pine-bark potting mix in large (3 L) pots and grown under ambient glass-house light and temperature. A minimum of three individuals per species were used in experimental work.

During droughting, water was withheld from plants, causing a gradual decrease in soil water content (due to the large pot size and relatively small plants). Water was withheld until the dark-adapted  $F_v/F_m$  was approximately 0.7, at which point plants were at their maximum drought tolerance (see "Results"). This took between 2 and 4 weeks, during which time gas-exchange measurements were taken every 1 to 5 d depending on desiccation rate.

### Gas Exchange

Fluxes of CO<sub>2</sub> and water vapor were measured on leaves using an open-flow system. An ADC mk. 3 (Hoddesdon, UK) IR gas analyzer was used to measure CO<sub>2</sub> differentials between cuvette and reference air, and a modified Dew-10 dew-point hygrometer (General Eastern, Watertown, MA) was used to monitor water vapor content of pre- and postcuvette air.

Air was supplied from compressed air cylinders, and different partial pressures of CO<sub>2</sub> were produced by removing different proportions of CO<sub>2</sub> from the air during tank fills. In undroughted plants, c<sub>i</sub> was varied from 360 to 160  $\mu\text{mol mol}^{-1}$  to give c<sub>i</sub> values within the range likely to be found in the field, and photosynthetic CO<sub>2</sub> compensation concentration was measured by recirculating air through the leaf chamber, allowing the leaf to draw down c<sub>i</sub> until net CO<sub>2</sub> uptake ceased. Measurements during drought were made with c<sub>i</sub> held constant at approximately atmospheric concentration (360  $\pm$  15  $\mu\text{mol mol}^{-1}$ ). The dew point of the influx air was maintained at 0°C by passing air over water at 0°C or at around 8°C by passing air through a column of hydrated ferrous sulfate, resulting in leaf to air vapor pressure deficits of 8 to 10 mbar bar<sup>-1</sup>.

The leaf cuvette was constructed from ultra-high-density polyethylene and glass, and all tubing was stainless steel so as to minimize adsorption of water vapor and CO<sub>2</sub>. Gas-flow rates were generally 300 to 400 mL min<sup>-1</sup> and the volume of the cuvette was 20 mL. At these relatively high flow rates, the boundary layer conductance of the leaves, which were generally >3 mm in width, was at least 1 order of magnitude greater than the maximum leaf conductance.

Leaf temperature was maintained at 20  $\pm$  1°C by pumping water from a controlled-temperature water bath through a jacket surrounding the cuvette and was measured by a thermocouple placed in contact with the abaxial surface of the leaf. The light source was a 24-V, 250-W tungsten lamp that provided 1500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  to the leaf surface. Leaf area was measured using a digital camera (EDC-1000, Electrim, Princeton, NJ) to determine the projected area of foliage in the chamber.

Calculation of gas-exchange parameters were made, according to von Caemmerer and Farquhar (1981), with c<sub>i</sub> corrected for water vapor influx from the leaf.

### $\psi$

Measurements of  $\psi$  were carried out immediately after gas-exchange readings using a Scholander (Commonwealth Scientific and Industrial Research Organization, Hobart, Tasmania, Australia) pressure bomb. Due to the destructive nature of the Scholander technique and the small amount of plant material available,  $\psi$  could be determined only once or twice per plant. These measurements were made as c<sub>i</sub> approached a minimum, enabling the  $\psi$  to be defined at minimum c<sub>i</sub>.

### Fluorescence

In one species (*Podocarpus laurercii*), the optimum  $F_v/F_m$  was measured to determine nonreversible photochemical inhibition during drought. Measurements were made on every second plant after the completion of gas-exchange readings. This involved dark adapting whole plants for 3 h (to ensure complete oxidation of the plastoquinone pool) before  $F_v/F_m$  readings were taken. Fifteen leaves from each plant were measured, using a PSM mk. 2 fluorometer (Biomonitor, Umeå, Sweden).

### Stomatal Aperture

Stomatal aperture was measured every 3 d during the drought of a single *Acacia melanoxylon* plant. It was not possible to use *Eucalyptus* or any of the conifers, since the sunken nature of the stomata did not allow accurate impressions to be made of the guard cells. Leaves were detached after measurement of gas exchange and epidermal impressions were made by immediately coating the abaxial surface of the leaf with nail varnish (cellulose acetate). Closed stomata were easily distinguished from open or partially open stomata when impressions were examined under the light microscope, so it was possible to measure the percentage of closed stomata in leaves subject to increasing drought.

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## RESULTS

Gas Exchange and Fluorescence in *P. lawrencii*

Within the range of  $c_i$  produced by varying  $c_a$  from compensation to atmospheric concentration, a linear regression ( $r^2 = 0.935$ ) described the  $A$  versus  $c_i$  response curve for well-watered *P. lawrencii* (Fig. 1). At an ambient  $\text{CO}_2$  concentration of  $360 \mu\text{mol mol}^{-1}$  (approximately atmospheric),  $c_i$  for *P. lawrencii* was  $205 \pm 5 \mu\text{mol mol}^{-1}$ .

Once water was withheld,  $g_s$  and  $A$  decreased slowly over the 1st week. Generally, gas exchange was found to drop sharply during the 2nd week of drought, continuing at low values during the 3rd and 4th weeks. Figure 2 illustrates this trend in a single replicate. Accompanying the decrease in gas exchange was a steep decline in  $c_i$ , typically from around  $205 \mu\text{mol mol}^{-1}$  ( $c_i/c_a$  of around 0.6) on the 1st d of water stress, to minimum values between 71 and  $88 \mu\text{mol mol}^{-1}$  (Fig. 1).  $c_i/c_a$  is expressed in preference to  $c_i$  since it accounts for variation in  $c_a$  (Fig. 2c). Minimum values of  $c_i$  were typically reached after 20 to 28 d without water, at which point  $\psi_s$  was  $-3.0$  MPa and  $c_i$  was quite close to the compensation value of  $53 \mu\text{mol mol}^{-1} \text{CO}_2$ . Until the point of minimum  $c_i$  there was no difference in carboxylation efficiency between droughted and undroughted plants (Fig. 1), indicating that nonstomatal limitation was not significantly increased. Only when  $A$  and  $g_s$  reached very low levels was there any deviation from the unstressed carboxylation curve, indicating a rapid increase in nonstomatal limitation. This can be seen in Figure 1, where  $c_i$  approaches and then exceeds  $c_a$  as assimilation approaches 0. This rapid increase in nonstomatal limitation occurred when  $\psi_s$  fell below  $-3.0$  MPa.

Figure 3a shows the response of  $c_i/c_a$  to decreasing  $g_s$  during drought. The curve shown in Figure 3a represents

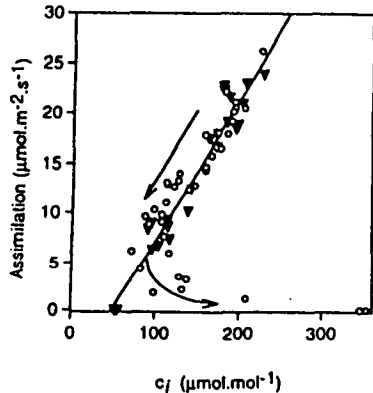


Figure 1. The relationship between  $A$  and  $c_i$  in undroughted *P. lawrencii* (filled circles), with  $c_a$  ranging from compensation to atmospheric concentration, and droughted plants (open circles), with  $c_a$  constant at  $360 \mu\text{mol mol}^{-1} \text{CO}_2$ . A linear regression ( $r^2 = 0.935$ ) was used to fit the data from undroughted plants. Arrows show the progression of  $A$  and  $c_i$  during droughting.

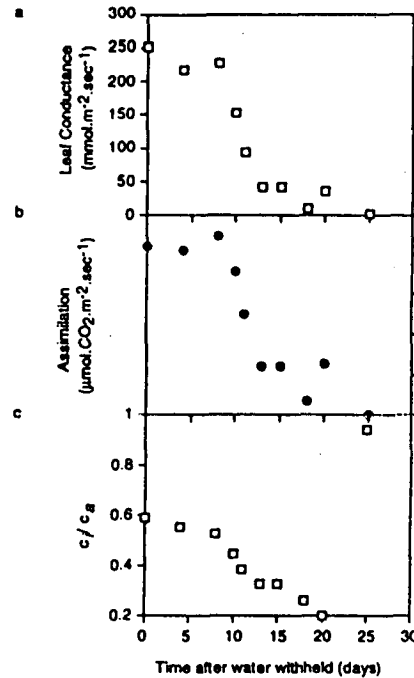


Figure 2. Decreasing  $g_s$  (a),  $A$  (b), and  $c_i/c_a$  (c) in a single replicate of *P. lawrencii* during droughting. Minimum  $g_s$  in plants was generally reached 25 to 30 d after water was withheld.

the expected response if assimilation was limited only by  $g_s$ , i.e. nonstomatal factors were insignificant. Points falling at increasing distances above this curve reflect an increasing component of nonstomatal limitation. During the initial stomatal limitation phase between conductances of 320 and  $45 \text{ mmol m}^{-2} \text{s}^{-1}$ , a curvilinear relationship was observed between  $c_i/c_a$  and  $g_s$ . The lowest value of  $c_i/c_a$  occurred at a  $g_s$  of  $45 \text{ mmol m}^{-2} \text{s}^{-1}$ , with  $c_i/c_a$  (and hence nonstomatal limitation) increasing rapidly at conductances below this level. Photosynthesis in plants that were allowed to reach this phase of rapidly increasing  $c_i/c_a$  did not completely recover after rewetting, and several individuals sustained leaf damage.

The average  $F_v/F_m$  of *P. lawrencii* leaves from unstressed plants was  $0.78 \pm 0.008$ , which falls within the normal range for unstressed plants (Björkman and Demmig, 1987). During droughting,  $F_v/F_m$  decreased only slightly as  $g_s$  dropped from 320 to  $45 \text{ mmol m}^{-2} \text{s}^{-1}$ . Below this  $g_s$  however,  $F_v/F_m$  was found to fall rapidly to values below 0.70 (Fig. 3b). In all plants where the average  $F_v/F_m$  fell below 0.7, leaves became necrotic or were shed after rewetting.

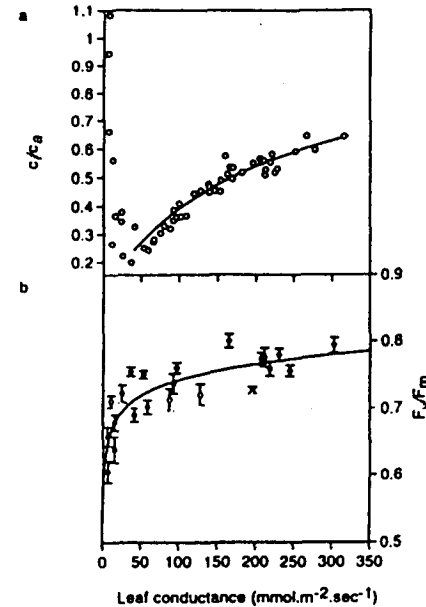


Figure 3. Changing ratios of  $c_i/c_a$  (a) and  $F_v/F_m$  (b) as  $g_s$  declined during drought in *P. lawrencii*. Each value of  $F_v/F_m$  represents the average of 15 leaves. A log function has been used to fit the  $F_v/F_m$  data ( $r^2 = 0.696$ ).  $c_i/c_a$  also decreased logarithmically before reaching a minimum value,  $c_i/c_{a,\text{min}}$ , increasing markedly thereafter. A line representing the theoretical relationship between conductance and  $c_i/c_a$ , under conditions in which  $g_s$  was the primary factor controlling decreasing photosynthesis is shown using the regression in Figure 1. This has been extended only to the  $g_s$  at which  $c_i/c_a$  was a minimum.

## Other Conifers

The response described above for *P. lawrencii* was characteristic of all the conifers measured. In all species  $c_i/c_a$  decreased during drought to  $(c_i/c_a)_{\text{min}}$  at low  $g_s$ . Continued droughting beyond this point resulted in a pronounced increase in  $c_i/c_a$  as direct (nonstomatal) limitation of photosynthesis became significant. The minimum  $c_i/c_a$  for each species was averaged from at least three replicates (Table 1).

A large degree of interspecific variation was observed in  $(c_i/c_a)_{\text{min}}$ , with a maximum value of 0.36 in *A. pantheri* ranging to a minimum of 0.10 in *A. acuminatus*. Minimum  $c_i/c_a$  was strongly correlated ( $r^2 = 0.976$ ) with the  $\psi_s$  measured at  $(c_i/c_a)_{\text{min}}$  (Fig. 4).

## Broad-Leaved Angiosperms

In contrast to the conifers, both *A. melanoxylon* and *E. tenuiramis* showed only minor decreases in  $c_i/c_a$  during the drought period. There was, however, a similar rapid increase in  $c_i/c_a$  as  $g_s$  approached 0. As in Figure 3a, the curves in Figures 5 and 6 represent the expected response if decreasing  $A$  was a pure function of  $g_s$  (insignificant nonstomatal component). The early divergence from this curve as  $g_s$  decreased would typically be interpreted as indicating an increase in nonstomatal limitation during the early stages of drought.

Increasing numbers of closed stomata were observed in stomatal impressions from *A. melanoxylon* leaves as a single plant was subject to drought (Fig. 5). Although average stomatal aperture was found to decrease as plants were droughted, closed stomata were clearly distinguished from partially open stomata, and a high proportion of stomata were found to close early during drought, while leaf conductance was still relatively high.

Changes in  $F_v/F_m$  in *E. tenuiramis* during drought were similar to those seen in *Podocarpus* (Fig. 3b), with no significant decrease until  $g_s$  fell below about  $70 \text{ mmol m}^{-2} \text{s}^{-1}$ , at which point a significant depression in quantum efficiency was found to occur.

The  $\psi_s$  immediately prior to the rapid increase in  $c_i/c_a$  was  $-2.9$  and  $-3.20$  MPa in *A. melanoxylon* and *E. tenuiramis*, respectively.

Table 1. Mean  $c_i/c_a$  at approximately atmospheric  $[\text{CO}_2]$  for the nine species of conifers used ( $c_i/c_{a,\text{min}}$  refers to the mean minimum  $c_i/c_a$  attained during drought.  $\psi_s$  at  $(c_i/c_a)_{\text{min}}$  represents the lowest value of  $\psi_s$  measured at  $(c_i/c_a)_{\text{min}}$ ).

Species	Habitat*	$c_i/c_a$ at $c_a = 360 \mu\text{mol mol}^{-1}$	$(c_i/c_a)_{\text{min}}$	$\psi_s$ at $(c_i/c_a)_{\text{min}}$ MPa
<i>A. acuminatus</i>	Dry sandy heathland	$0.63 \pm 0.0016$	0.10	-4.50
<i>C. rhomboidea</i>	Open woodland	$0.68 \pm 0.0017$	0.21	-3.20
<i>P. lawrencii</i>	Wet forest-alpine forest	$0.59 \pm 0.012$	0.21	-3.00
<i>D. archerii</i>	Wet montane-alpine forest	$0.57 \pm 0.014$	0.25	-2.90
<i>M. niphophilus</i>	Wet alpine forest	$0.60 \pm 0.010$	0.26	-2.70
<i>A. selaginoides</i>	Cool-temperate rainforest	$0.66 \pm 0.0075$	0.29	-2.10
<i>L. franklinii</i>	Cool-temperate rainforest	$0.65 \pm 0.0068$	0.30	-2.30
<i>D. dactyloides</i>	Cool-temperate rainforest	$0.68 \pm 0.0057$	0.33	-1.90
<i>A. pantheri</i>	Tropical rainforest	$0.67 \pm 0.020$	0.36	-1.75

\* Habitat descriptions are from Enright and Hill (1995).

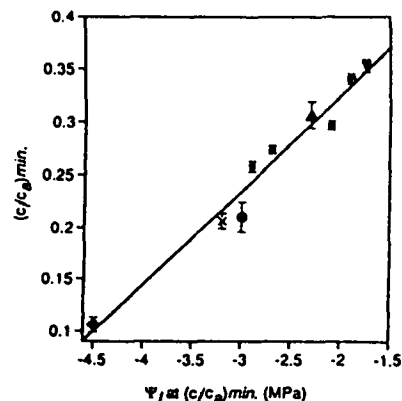


Figure 4. The relationship between  $c_i/c_{a,min}$  and  $\psi$  at  $c_i/c_{a,min}$  in the nine conifer species examined: *A. pantheri*,  $\nabla$ ; *A. selaginoides*,  $\blacksquare$ ; *A. acuminatus*,  $\diamond$ ; *C. rhomboidea*,  $\times$ ; *D. dacrydioides*,  $\ominus$ ; *D. archerii*,  $\oplus$ ; *L. franklinii*,  $\Delta$ ; *P. lawrencii*,  $\odot$ ; *M. nipphophilus*,  $\star$ . A highly significant linear regression is shown ( $r^2 = 0.976$ ). Each point represents the average  $c_i/c_{a,min}$  from at least three individuals, and  $\psi$  at  $c_i/c_{a,min}$  represents the water potential reading that corresponded most closely with  $c_i/c_{a,min}$ .

#### DISCUSSION

The combination of narrow, single-vein leaf morphology and the drought regime used in this study should have minimized patchy stomatal closure and photosynthetic heterogeneity, adding a high degree of confidence to  $c_i$  calculations presented here. In contrast to previous studies

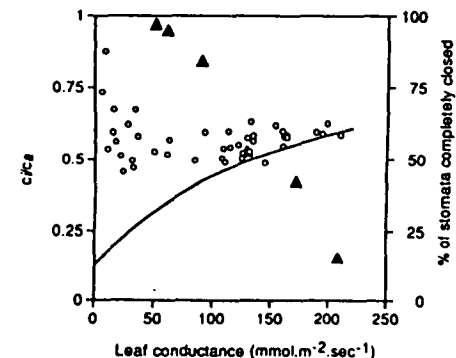


Figure 5. Response of  $c_i/c_a$  to decreasing  $g_s$  during drought in *A. melanoxylon* ( $\Delta$ ). The curve shown represents the theoretical response if decreasing  $g_s$  explained the decline in photosynthesis (cf. Fig. 3a). Also shown is the percentage of stomata seen to be completely closed at six times during drought intensification in a single *A. melanoxylon* plant ( $\odot$ ).

(Wong et al., 1985), large variations in  $c_i$  were found to occur during drought, with an initial draw-down of  $c_i$  to between 25 and 55% of unstressed levels, followed by a rapid increase as  $\psi$  fell below a critical level.

During the initial "draw-down" phase, there was no evidence of nonstomatal limitation in any of the conifers studied. This is illustrated for *P. lawrencii* in Figure 3a, where the data show no systematic deviation from the theoretical curve produced if photosynthesis were responding only to increasing stomatal limitation of gas exchange (insignificant, nonstomatal component). Nonstomatal factors did, however, become important in plants droughted beyond  $(c_i/c_a)_{min}$ , producing a sharp increase in  $c_i/c_a$  as  $g_s$  approached 0 (Fig. 3a). The fluorescence data for *P. lawrencii* also illustrate these two phases in drought response. Initially,  $F_v/F_m$  was found to slowly decline in parallel with  $c_i/c_a$ , presumably as a result of increased nonphotochemical energy dissipation, since Calvin cycle activity was inhibited by limiting  $CO_2$  concentration in the chloroplast (Cornic et al., 1989; Cornic and Briantais, 1991). As  $g_s$  approached 0 and  $c_i/c_a$  began to increase, there was a concomitant steep drop in  $F_v/F_m$  (Fig. 3b). This indicates a rapid loss of fluorescence from PSII for some reason other than  $CO_2$  limitation and may represent direct inhibition of

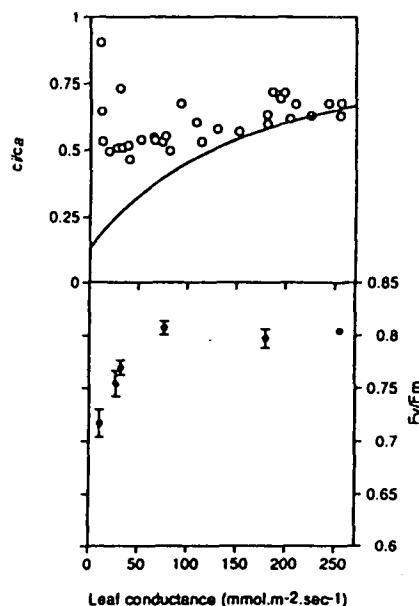


Figure 6. Drought response of  $c_i/c_a$  and  $g_s$  in *E. tenuiramis*; again the curve represents the theoretical response if decreasing photosynthesis was stomatically driven. Also shown are  $F_v/F_m$  data for a single replicate during drought.

photochemistry, Calvin cycle activity, or both. The most likely explanation for this rapid onset of photoinhibition is that plants droughted beyond  $(c_i/c_a)_{min}$  sustained damage to the photosynthetic apparatus. This is supported by the fact that these plants did not recover predrought photosynthetic rates, and after 2 to 4 d of continued drought they also showed visual signs of leaf damage.  $\psi$  measured at  $(c_i/c_a)_{min}$  therefore represents the minimum  $\psi$  prior to the onset of irreversible photoinhibition. Values of  $\psi$  at  $(c_i/c_a)_{min}$  ranged widely from  $-1.75$  to  $-4.5$  MPa, reflecting the wide range of habitats from which different species were collected. In comparison, initial cell damage in rapidly desiccated *Helianthus* leaves was found to occur at a  $\psi$  of  $-1.5$  MPa, with the majority of mesophyll cells damaged at  $-2.6$  MPa (Fellows and Boyer, 1978). The leaves of the conifer species used here were clearly more robust than those of *Helianthus*, a fact that is hardly surprising considering the perennial, woody nature of these species.

$c_i/c_a$  is inversely proportional to the "intrinsic" water use efficiency and, hence,  $(c_i/c_a)_{min}^{-1}$  represents the maximum water use efficiency attainable during drought. Qualitative comparison of  $(c_i/c_a)_{min}$  with habitat indicates that species developing low  $(c_i/c_a)_{min}$  were from drier habitats (*A. acuminatus* grows in dry heathland), and species with higher  $(c_i/c_a)_{min}$  were from rainforest (*A. pantheri* grows in tropical cloud forest) (Table I). These qualitative observations are strongly supported by the highly significant correlation between  $(c_i/c_a)_{min}$  and  $\psi$  at  $(c_i/c_a)_{min}$  (Fig. 4), indicating that  $(c_i/c_a)_{min}$  provides a good index of drought tolerance (of the foliage at least) in plants not susceptible to patchy photosynthesis.

The dynamic response described for the narrow-leaved conifers was not found to occur in the broad-leaved angiosperm species used here. Both species exhibited relatively constant  $c_i/c_a$  during drought, as described in many other studies (Wong et al., 1985; Anderson et al., 1995). This type of response has classically been interpreted as showing a gradual increase in nonstomatal limitation of photosynthesis during drought. The data presented here do not, however, show any evidence for nonstomatal inhibition in *E. tenuiramis* until leaf conductance had declined to around 25% of the level found in undroughted plants (Fig. 6), indicating that the leaves had responded to drought well before nonreversible photoinhibition became significant.

The fact that both species are relatively drought tolerant, with *A. melanoxylon* foliage found to withstand  $\psi$  of around  $-3.5$  MPa (Brodrick and Hill, 1993) and *E. tenuiramis* capable of surviving soil water potentials in the field of around  $-2.9$  MPa without leaf damage (M. Battaglia and N. Davidson, personal communication), also suggests that it is unlikely that direct inhibition of photosynthesis should have occurred soon after water was withheld. Patchy stomatal closure provides a better explanation for the responses illustrated in Figures 5 and 6, and this is confirmed for *A. melanoxylon*, in which the number of stomata found to be fully closed increased rapidly with the onset of drought (Fig. 5). This type of preemptive stomatal closure may be explained by fluxes of ABA that are released from the roots and translocated in the xylem (Khalil and Grace,

1993; Jackson et al., 1995), since it has been shown that initial translocation of ABA occurs at  $\psi$  values equivalent to only mild drought (Correia and Pereira, 1994).

At some stage during drought it is inevitable that direct inhibition of photosynthesis will occur either by physical effects, such as turgor loss or cell damage, or by loss of chemical function in the chloroplasts. The important question is whether nonstomatal inhibition occurs early during drought, depressing photosynthesis while significant rates of gas exchange are taking place, or whether this effect occurs only in highly stressed plants as  $g_s$  approaches a minimum. All narrow-leaved conifers in this study exhibited the latter response to drought, resulting in substantial reductions in  $c_i/c_a$  prior to any expression of nonstomatal inhibition. This result supports  $^{13}C$  discrimination and fluorescence data, indicating a dynamic response of  $c_i/c_a$  to drought and a minimal nonstomatal influence on assimilation until plants are subject to a severe water stress.

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**Light response characteristics of a morphologically diverse group of Southern Hemisphere conifers as measured by chlorophyll fluorescence.**

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**Abstract** Unlike northern hemisphere conifer families, the southern family, Podocarpaceae, produces a great variety of foliage forms ranging from functionally broad, to needle-leaved. The production of broad photosynthetic surfaces in podocarps has been qualitatively linked to low light environments, and it was undertaken to assess the validity of this assumption by measuring the light response of a morphologically diverse group of podocarps. Light response of apparent photochemical electron transport rate (ETR) as measured by modulated fluorescence, was determined for ten species of this family, and six associated species (including five Cupressaceae and one functionally needle-leaved angiosperm) all grown under identical glasshouse conditions. In all species ETR was found to increase as light intensity increased, reaching a peak value ( $ETR_{max}$ ) at saturating quantum flux ( $PPFD_{sat}$ ), and decreasing thereafter.  $ETR_{max}$  ranged from  $217 \mu\text{mol.m}^{-2}.\text{s}^{-1}$  at a  $PPFD_{sat}$  of  $1725 \mu\text{mol.photons.m}^{-2}.\text{s}^{-1}$  in *Actinostrobus acuminatus*, to an ETR of  $60 \mu\text{mol.photons.m}^{-2}.\text{s}^{-1}$  occurring at a  $PPFD_{sat}$  of  $745 \mu\text{mol.photons.m}^{-2}.\text{s}^{-1}$  in *Podocarpus dispersmis*. Good correlations were observed between  $ETR_{max}$  and both  $PPFD_{sat}$  and maximum assimilation rate measured by gas-exchange analysis. Effective quantum yield at light saturation remained constant in all species with an average value of  $0.278 \pm 0.0035$  determined for all 16 species. Differences in the shapes of light response curves were related to differences in the response of non-photochemical quenching ( $q_n$ ), with  $q_n$  saturating faster in species with low  $PPFD_{sat}$ . Amongst the species of Podocarpaceae, the log of average shoot width was well correlated with  $PPFD_{sat}$ , wider leaves saturating at lower light

intensities. This suggests that broadly flattened shoots in the Podocarpaceae are an adaptation to low light intensity.

### *Introduction*

Conifer diversity in the Southern Hemisphere is dominated by the family Podocarpaceae, which has shown great flexibility in evolving a variety of foliage forms unrivalled amongst other conifer families. The genera of the Podocarpaceae exhibit foliar morphologies ranging from tightly imbricate shoots (analogous to needles), to bilaterally flattened foliage which takes the appearance and function of angiosperm broad-leaves (Fig. 1) The production of bilaterally flattened foliage in podocarps has occurred at least three ways (Hill 1995); in genera such as *Acmopyle*, *Dacrycarpus*, *Prumnopitys*, *Nageia*, *Retrophyllum* and *Afrocarpus*, the leaves on short shoots are oriented parallel to the shoot axis, and arranged in a manner typical of the leaflets on angiosperm compound leaves; in *Sundacarpus* and some species of *Podocarpus*, leaves have become very long, but still relatively narrow since they have only a single vein, and often arranged two dimensionally on the short shoot; and in *Phyllocladus* leaves have been replaced by a flattened phylloclade, which is multiveined and broad. *Nageia* has secondarily evolved multiple veins in the leaf, which enable leaf width to expand, a response otherwise difficult in univeined leaves. In all of these genera the evolution of flat photosynthetic foliage has been polyphyletic.

Podocarps are found in a wide variety of habitats from canopy dominant in dense rainforest, to understorey shrub in open woodland (Enright and Hill, 1995), and it has been suggested that it is the ability to produce functional 'broad-leaves' which has enabled them to compete with angiosperms in the low light environment under forest canopies (Hill, 1995). Relatively few Northern Hemisphere conifers produce two dimensionally flattened foliage, and those that do, generally inhabit dark, wet environments eg. *Metasequoia*, *Taxodium*, and *Abies* (Leverenz, 1995). The relationship between shoot architecture and light environment is best demonstrated in *Abies*, where shoots developed under high light are typically three dimensional, while those developed at lower irradiance show increasing bilateral compression (increased silhouette area/total leaf area) (Sorrensen-Cothorn et al., 1993).

Previous studies of Northern Hemisphere conifers have shown that maximum photosynthetic rate (per unit silhouette area) and

quantum yield of CO<sub>2</sub> fixation in several genera of Pinaceae (*Pinus*, *Abies*, *Tsuga*, and *Picea*) were related to the degree of shoot flattening (silhouette area/total leaf area), and that differences in physiology between flattened and non-flattened shoots were removed by illuminating stems from all directions during measurement (Carter and Smith, 1985; Leverenz, 1995). The variation in leaf and shoot morphology among species of the Podocarpaceae is far more profound than the differences observed in the Pinaceae, and the aim of this paper was to examine how these highly distinct morphologies were related to the light response characteristics of this unique group of conifers.

Instead of applying classical techniques whereby the potential of species to adapt to artificially imposed conditions of sun and shade are used to distinguish species better suited growth under high or low light environments (e.g. Jun and Taylor, 1986), plants here were all grown and measured under identical conditions of light, nutrient supply, temperature, etc. To ensure no species suffered from lethal extremes of light intensity, the light climate imposed was intermediate between sun and shade. The intention here was not to evaluate the short term adaptive potential (morphological or physiological) of shoots to imposed extremes of sun or shade, but to indicate the relative light climate preferences of different shoot morphologies grown under uniform conditions. From this information it was proposed to test the intuitive association between Podocarp "broad shoots" and low light environments. Several species of the conifer family, Cupressaceae, and a needle-leaved angiosperm (*Allocasuarina verticellata*) were also included to determine whether relationships between morphology and light response were common to more distantly related species.

The light response of conifers was measured here using a modulated fluorometer (Schreiber et al., 1986) to determine the quantum yield of photosystemII ( $\Phi_{PSII}$ ) of individual leaves, or of a small part of a compound shoot (<1.8cm<sup>2</sup>), avoiding the effects of self-shading. This allowed the response of leaves to be directly measured without the complicating effects of leaf arrangement. The use of chlorophyll a fluorescence as a tool for measuring light response has been limited to date (eg. McKiernan and Baker, 1992, Bilger et al., 1995), despite the considerable potential of this technique for assaying both the rate of energy transfer through PS II, and characteristics of the energy dissipating processes such as the xanthophyll cycle (Demmig-Adams et al., 1995) which modulate the response of photochemistry to light.



Rather than measuring the rate of CO<sub>2</sub> uptake at different light intensities, the response of apparent electron transport rate (ETR) to photosynthetic photon flux density (PPFD) was determined here. ETR expresses the relative rate of electron transport through PSII for reduction of NADP<sup>+</sup>, and is thus closely related to the rate of carboxylation (Edwards and Baker, 1993). The calculation of ETR involves multiplying the  $\Phi_{\text{PSII}}$  as determined by fluorescence (Genty et al., 1989) by the quantum flux absorbed by PSII. Thus, measurement of ETR provides a means of probing the primary photochemical response, and photochemical capacity of the leaf without contributing effects such as dark and light respiration rates.

### *Materials and Methods*

#### *Species*

Podocarps: *Acropyle pancheri* (Brongn. and Gris) Pilger, *Dacrycarpus dacrydioides* (Rich.) de Laubenfels, *Lagarostrobos franklinii* (Hook.) C.J.Quinn, *Microstrobos niphophilus* Garden and Johnson, *Phyllocladus aspleniifolius* (Labill.), *Podocarpus dispermis* White, *Podocarpus drouynianus* Mueller, *Podocarpus lawrencii* Hook.f., *Prumnopitys ferruginea* D.Don, and *Retrophyllum comptonii* (Buchh.) C.N.Page, and Southern Hemisphere Cupressaceae/Taxodiaceae: *Actinostrobus acuminatus* Parlatores, *Athrotaxis cupressoides* D.Don, *Athrotaxis selaginoides* D.Don, *Callitris rhomboidea* R.Br., and *Diselma archerii* Hook.f., and the needle leaf angiosperm *Allocasuarina verticellata* (Lam.) L. Johnson, were propagated in sand in Hobart. Upon establishment, all plants were transferred to a pine bark potting mix in 3 litre pots, and grown under ambient light conditions in a well irrigated, heated glasshouse near sea-level in Hobart.

All species were grown under identical light conditions, and only foliage which was directly exposed to the incident light was used in experimental work. Plants were grown in an opaque glasshouse where light intensity was uniform throughout, and in full sun, maximum light intensity was only 900  $\mu\text{mol.photons}^{-1}.\text{m}^{-2}.\text{s}^{-1}$  at the leaf surface. Thus leaves developed under conditions intermediate between full sun (approximately 1600  $\mu\text{mol.photons}^{-1}.\text{m}^{-2}.\text{s}^{-1}$ ) and shade, avoiding leaf damage due to excessive or insufficient PPFD. Measurements were carried out during a four week period in mid-summer, ensuring maximum stability of light intensity and photoperiod while experimental work was

being undertaken. All species were represented by at least 5 replicates (and in the case of the cuttings, from at least 3 parent trees), except for *A. pancheri*, *P. ferruginea* and *R. comptonii*, which could only be propagated from 2 cuttings each due to a high mortality rate during propagation, caused by extreme sensitivity to light and humidity conditions.

## Fluorescence

Chlorophyll a fluorescence was measured using a PAM 2000 fluorometer (Waltz, Effeltrich, Germany). Leaves, or portions of shoot, which had been dark adapted for approximately 30 minutes were clamped into a leaf clip (Bilger et al., 1995), ensuring that no self shading was occurring. Leaf temperature was maintained at approximately 20°C during fluorescence readings.

Initially  $F_o$  (minimum fluorescence with electron chain fully reduced) was measured in the dark, after which a saturating pulse of light was applied to the foliage, allowing measurement of  $F_m$  (maximum fluorescence with electron acceptors fully oxidised) and  $F_v$  ( $F_m - F_o$ ). An actinic light was then switched on, and measurements of light adapted  $F_m'$  carried out again by applying a saturating flash of light.  $F_o$  quenching was determined by illuminating the sample with far-red light for 3 seconds with actinic light switched off, facilitating reoxidation of the acceptor side of PS II while measuring  $F_o'$  (Schreiber et al., 1994). Actinic light intensities were stepped up gradually from 15 to around 2000  $\mu\text{mol.photons}^{-1}.\text{m}^{-2}.\text{s}^{-1}$ , allowing a period of 20 minutes for acclimation at each light intensity. An external halogen lamp, with fibre optic light delivery was used during the acclimation period at PPFDs greater than 400  $\mu\text{mol.photons}^{-1}.\text{m}^{-2}.\text{s}^{-1}$ , as continuous use of the PAM internal halogen caused excessive heating. The external light was switched off and internal halogen used, only briefly, during measurement, thus avoiding drift in measuring light intensity produced by heating of the apparatus. At light intensities above 1000  $\mu\text{mol.photons}^{-1}.\text{m}^{-2}.\text{s}^{-1}$  at least 30 minutes was allowed for acclimation.  $\Phi_{\text{PSII}}$ , photochemical quenching ( $q_p$ ), non-photochemical quenching ( $q_n$ ) of fluorescence were calculated at each light intensity using the equations of Schreiber and Bilger (1992). ETR was calculated using equation 1:

$$1. \quad \text{ETR} = \Phi_{\text{PSII}} \cdot I \cdot \alpha / 2$$

where :  $I$  = incident PPFD (in the waveband 400--700 nm);  $\alpha$  = leaf absorbance, taken here as 0.84 (Björkman and Demmig, 1987); and the factor of 2 accounts for the fact that 2 photons are required per electron passed through PSII, assuming linear electron flow, and even distribution of absorbed quanta between PSII and PSI. The average value of  $\alpha$  for green leaves of 0.84 (Björkman and Demmig, 1987) was used here, and it was assumed that excitation energy was evenly distributed between PSII and PSI (Bilger et al., 1995; Loreto et al., 1995). The units of ETR are  $\mu\text{mol.electrons.m}^{-2}.\text{s}^{-1}$ , although it should be noted that values of ETR will not be precise as leaf absorbance was not measured. The use of the average  $\alpha$  for leaves, of 0.84, as determined by Björkman and Demmig (1987) was considered a good approximation considering that all leaves were relatively thick and only healthy, green leaves were selected. The one podocarp measured in the survey by Björkman and Demmig (1987) was found to have an  $\alpha$  of 0.835, very close to the average value used here.

All fluorescence measurements were carried out at approximately 20°C, and response curves were measured from five replicates of each species, except for *A. pancheri*, *P. ferruginea* and *R. comptonii*, where two replicates were used. Polynomial curves were used to fit the pooled data for each species, and from these curves saturating PPFD ( $\text{PPFD}_{\text{sat}}$ ) and maximum ETR (at  $\text{PPFD}_{\text{sat}}$ ) were read.  $\text{PPFD}_{\text{sat}}$  was taken as the light intensity at the peak of the ETR vs PPFD response.

## Gas Exchange

Net  $\text{CO}_2$  uptake was measured using an open flow IRGA system (Hoddeson, England) as described in Brodribb (1996). In all plants the maximum rate of photosynthesis ( $A_{\text{max}}$ ) was determined under approximately optimal conditions, with leaves at 20°C, leaf-air vapour pressure deficit at 5--10  $\text{mmol.mol}^{-1}$ , ambient  $\text{CO}_2$  at  $350 \pm 5 \mu\text{mol.mol}^{-1}$ , and light intensity saturating (generally 1400  $\mu\text{mol.photons}^{-1}.\text{m}^{-2}.\text{s}^{-1}$ ).  $A_{\text{max}}$  for each species was averaged from all replicates, and a standard error calculated. Where possible, the same leaves were used for both fluorescence and gas-exchange analysis. In both cases, young, green foliage was selected.

The small volume of the cuvette (20mL) and careful selection of foliage allowed leaves to be arranged such that, no self shading occurred during measurement of gas-exchange. Following gas-exchange analysis,

the silhouette area of the shoot enclosed in the cuvette was determined using a digital camera (EDC-1000, Electrim Corp. Princeton, USA) after which leaves were detached, oven dried at 70°C for 72 hours, and leaf dry weight measured. Assimilation rate was expressed in terms of leaf silhouette area rather than total leaf area.

### Leaf Characters

Because leaves were arranged to prevent any self-shading, methods of describing shoot morphology such as silhouette leaf area/ projected leaf area, were not necessary. In all species, short shoots were functionally and morphologically analogous to leaves, in imbricate species, shoot width was less than 2mm, analogous to needle-leaves, and in non-imbricate species, short shoots were analogous to single broad-leaves (Fig. 1). For this reason, the width of the short shoot provided the easiest and best means of quantifying its morphological character in the various species.

The width of short shoots was measured at their widest point with a set of digital callipers (measuring to an accuracy of  $\pm 0.01\text{mm}$ ), and for each species, averages were taken from 30--40 shoots (depending on number of plants available).

The mass of foliage per unit area (LAI) was also calculated on a silhouette area basis, and values of LAI for each species represented averages from 5 shoots.

### Results

ETR increased in response to increasing light intensity, to a well defined maximum ETR ( $\text{ETR}_{\text{max}}$ ) occurring at  $\text{PPFD}_{\text{sat}}$ , and declined thereafter. Figure 2 shows average values of ETR at each light intensity for three species with different morphological and light saturating characteristics (*Actinostrobus acuminatus* and *Diselma archerii* both produce imbricate shoots, while *Retrophyllum comptonii* produces broad, flat short shoots). Fourth-order polynomials fitted the data reasonably well, and from these curves,  $\text{ETR}_{\text{max}}$  and  $\text{PPFD}_{\text{sat}}$  were measured. The initial slope of the PPFD vs ETR response was virtually identical in all species regardless of  $\text{ETR}_{\text{max}}$  or  $\text{PPFD}_{\text{sat}}$ .

Photochemical quenching of fluorescence ( $q_p$ ) decreased in a linear fashion as light intensity increased, with the rate of decrease inversely proportional to  $\text{ETR}_{\text{max}}$ ; slope  $q_p = 1.19 \times 10^{-6}(\text{ETR}_{\text{max}}) - 4.5 \times 10^{-4}$ ;  $r = 0.849$ .

Figure 3(a) illustrates the increasing decay rate of  $q_p$  in species with lower  $ETR_{max}$ , using the same three species as fig. 2. The relationship between non-photochemical quenching ( $q_n$ ) and PPFD was more complex (Fig. 3b), with a hyperbolic increase in  $q_n$  as light intensity increased. The rate of  $q_n$  saturation in each species was related to its photochemical capacity, with low  $ETR_{max}$  corresponding to rapid saturation.  $q_n$  saturation always occurred at light intensities higher than  $PPFD_{sat}$ , and in several species  $q_n$  did not saturate at the maximum light intensity (approximately 2500  $\mu\text{mol.photons.m}^{-2}.\text{s}^{-1}$ ).

A linear relationship was observed between  $ETR_{max}$  and  $PPFD_{sat}$  in the 16 species investigated (Fig. 4), with the maximum  $ETR_{max}$  of 217  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$  (*Actinostrobilus acuminatus*) occurring at a PPFD of 1725  $\mu\text{mol.photons.m}^{-2}.\text{s}^{-1}$ , and the minimum  $ETR_{max}$  of 60  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$  (*Podocarpus dispermis*) measured at a PPFD of 745  $\mu\text{mol.photons.m}^{-2}.\text{s}^{-1}$  (Fig. 3). This linear relationship indicates that  $\Phi_{PSII}$  at  $PPFD_{sat}$  was almost constant for each species. Measurement of average  $\Phi_{PSII}$  at  $PPFD_{sat}$  for each species, indicated that quantum yield was conserved, with an average value of  $\Phi_{PSII} = 0.278 \pm 0.0035$  found for all 16 species.

$ETR_{max}$  was also linearly related to the maximum rate of  $\text{CO}_2$  assimilation measured in each species by infra-red gas analysis (Fig. 5), the maximum rate  $ETR$  of 217  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$  corresponding to an assimilation rate of 37.8  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ . This regression was not expected to pass through the origin due to the fact that  $A_{max}$  was a measure of net  $\text{CO}_2$  flux, and hence would be negative (due to respiration) at an  $ETR$  of zero.

$PPFD_{sat}$  was significantly correlated ( $PPFD_{sat} = -241.\log(\text{shoot width}) + 1209$ ;  $p < 0.001$ ) with the log of width of short shoots in the 10 species of Podocarpaceae used here (Fig. 6). In narrow (imbricate) shoots,  $ETR$  was found to saturate at higher light intensities than in broader, flattened shoots (Table 1). Among the five species of Cupressaceae/Taxodiaceae and *Allocasuarina* included in the study there was no significant degree of shoot flattening, and thus there was no relationship between shoot width and light saturation characteristics in these non-podocarp species (Fig. 6).

A weak but significant positive linear relationship was present between LAI and  $PPFD_{sat}$  ( $r = 0.50$ ;  $p < 0.05$ ).

## Discussion

### Fluorescence technique

Analysis of chlorophyll a fluorescence provides a useful means for comparing light response characteristics among species. The utilisation of fibre optics to deliver light to a small portion of the leaf, means that high light intensities can be used without concomitant leaf heating problems. This allows the photochemical response curve to be extended above saturating light intensities, until a combination of high  $q_n$  and photoinhibition cause a decline in ETR. The advantage of this is that the maximum rate of ETR appears as a well defined peak, from which  $PPFD_{sat}$  can be determined precisely. In a previous field study (Bilger et al., 1995) high light intensities were associated with decreased accuracy in the measurement of ETR, this effect was not observed in the data here, and was probably associated with the use of ambient light in the field rather than artificial illumination.

The relationship between  $\Phi_{PSII}$  and  $\Phi_{CO_2}$  has been shown to be linear under a range of conditions in maize leaves (Edwards and Baker, 1993), however, the relationship for  $C_3$  plants has yet to be determined precisely, largely due to the complicating effects of photorespiration. Data presented here indicates a close correlation between  $ETR_{max}$  and  $A_{max}$  (Fig. 5) suggesting that photorespiration was not causing any significant variation in the relationship between  $\Phi_{PSII}$  and  $\Phi_{CO_2}$  under the standard measurement conditions for  $ETR_{max}$  and  $A_{max}$  applied here. The probable reason for this is that only a relatively small variation in the leaf internal  $CO_2$  concentration (between approximately 205 and 250  $\mu\text{mol.mol}^{-1}$ ) occurs in these species under the conditions which  $A_{max}$  was measured (Brodribb and Hill, submitted). This amount of variation in internal  $CO_2$  concentration would only be expected to produce a maximum variation of approximately 8% (using the stoichiometry of Farquhar et al., 1980) across the range of  $A_{max}$  due to the effects of differing Rubisco oxygenase/carboxylase activities.

Other potential problems such as uneven distribution of absorbed quanta between PSII and PSI and non-cyclic electron flow may have been present, but their effects did not alter the linear relationship observed between ETR and A.

### Light response characteristics

The photochemical capacity ( $ETR_{max}$ ) of foliage here was found to be proportional to the light intensity which just saturated photochemistry

(Fig. 4). This indicates that the light reactions in all plants were initially maximal, and saturated in a standard fashion (see Bordman, 1977) resulting in a constant  $\Phi_{PSII}$  at  $ETR_{max}$  of  $0.278 \pm 0.0035$  in all species investigated. This figure represents the quantum yield at light saturation prior to the saturation of  $q_n$  and the production of photoinhibitory effects which occur at super-saturating quantum fluxes. From this it is clear that all species were unstressed prior to measurement, and thus, that differences in the light response of the various species reflected functional characteristics of the photochemistry and were not an artifact of stressful light conditions during growth. Conventional techniques of quantifying the sun or shade adaptation potential generally involve subjecting foliage to extremes of sun or shade during the 'acclimation period' prior to measurement, and this has the potential to cause photochemical damage to the leaves, thus affecting the quantum yield at all light intensities.

The differences in light response observed between species here appear to be related to differences in the characteristics of  $q_n$  (Fig 3b). In species with low light requirement for  $PPFD_{sat}$ ,  $q_n$  was found to become saturated at light intensities around 1000--1500  $\mu\text{mol.photons.m}^{-2}.\text{s}^{-1}$ , whereas  $q_n$  in species with high  $PPFD_{sat}$  did not saturate even at the maximum light intensity used (2500  $\mu\text{mol.photons.m}^{-2}.\text{s}^{-1}$ ). This variation in the characteristics of  $q_n$  is most probably due to differences in the size and characteristics of the xanthophyll pool. The xanthophyll-cycle pigments are responsible for regulating the bulk of non-fluorescent energy dissipation from the photosystems, channelling excess excitation energy away from reaction centres so as to prevent the formation of toxic oxygen radicals. The size and composition of this xanthophyll pool has been correlated with the photosynthetic capacity of leaves of species occupying different light environments in tropical forest (Königer et al., 1995), with species adapted to growth in high light conditions found to possess larger, more dynamic xanthophyll pools (Demmig-Adams et al., 1995). A relatively large xanthophyll pool allows plants to rapidly respond to increasing light intensity (Demmig-Adams et al., 1995), while species adapted to low light environments have no need for efficient energy dissipation. This is consistent with the different saturating characteristics of  $q_n$  shown in figure 3(b), the rapid saturation of  $q_n$  in *R.comptonii* illustrating a high sensitivity to low to medium light intensity, but a low capacity for responding to light intensities over 1000  $\mu\text{mol.photons.m}^{-2}.\text{s}^{-1}$ . In contrast,  $q_n$  in *A.acuminatus* was able to respond to increasing light intensity even up to values of PPFD greater than full sun.

## Evolutionary implications of increased shoot width.

The relationship between shoot width and light response illustrated here (Fig. 6) has implications for the evolution of conifers in terms of their competitive interaction with angiosperms. One of the explanations used to account for the near complete replacement of conifers by angiosperms is that conifers only have slow rates of foliage production, especially during establishment, making them unable to compete for light with fast growing broad-leaved angiosperms, particularly in more productive temperate-tropical forest (Bond, 1989). The production of bilaterally compressed short shoots resembling broad-leaves, in several genera of the Podocarpaceae provides a mechanism for rapidly increasing foliar area for light capture, and the presence of these genera in temperate and tropical broad-leaf forest suggests that this morphological adaptation has contributed to the success of these genera.

The palaeohistory of conifers in the Southern Hemisphere suggests that the evolution of a broad, flat photosynthetic unit in the Podocarpaceae did not occur until diverse, broad-leaved angiosperm forests with a closed canopy had developed. In Australia, this had begun by the Late Paleocene, where a macroflora from southeastern Australia contains diverse broad-leaved angiosperms and conifers that include *Acmopyle* (Taylor et al. 1990, Hill & Carpenter 1991). Complex rainforest associations on the west coast of Tasmania in the Early Eocene contain *Dacrycarpus* and broad-leaved *Podocarpus*, and other extant and extinct genera of podocarps with similar adaptations are present by the Middle Eocene across southern Australia (Hill & Pole 1992, Hill 1995, unpublished data). However, Cretaceous forests in southern Australia and adjacent Antarctica were apparently more open, in response to low sun angles at the prevailing very high latitudes (Truswell 1991, Hill & Scriven 1995), and there is no evidence of podocarps with a broad photosynthetic unit.

It has been shown here that increasing shoot width is associated with a photosynthetic preference for lower light intensity, expressed as a low  $PPFD_{sat}$  and a low maximum photosynthetic capacity. This, in combination with ecological and palaeobotanical evidence, suggests that the production of broad short shoots in the Podocarpaceae is an adaptation to low light intensity.



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Figure 1. Various leaf arrangements on short shoots of 4 species of Podocarpaceae (a) *Podocarpus*- large single veined "broad-leaf"; (b) *Phyllocladus*- flattened shoot system; (c) *Acmopyle*- a single short shoot with leaves are arranged parallel to the shoot axis, each short shoot taking the functional role of a single photosynthetic unit; (d) *Microstrobos*- an imbricate leafed shoot, dimensionally and functionally acting as a needle-leaf; (e) shows the leaf arrangement of *Microstrobos* in detail. The 1cm scale bar applies to (a-d) while (e) is shown with a 1mm scale bar. Adapted from Hill (1995).

Figure 2. The response of apparent electron transport rate (ETR) to PPFD in the conifers *Actinostrobus acuminatus* - ▲ (imbricate shoot) , *Diselma archeri* - ◦ (imbricate shoot), and *Retrophyllum comptonii* - ■ (broad shoot). Values shown represent averages from 5 plants (except *Retrophyllum comptonii*, where only 2 plants were available). Curves fitted are fourth order polynomials. Values of saturating PPFD ( $PPFD_{sat}$ ) and maximum ETR ( $ETR_{max}$ ) were taken from these light response curves.

Figure 3. Changes in (a)  $q_n$ - photochemical and (b)  $q_p$ - non-photochemical fluorescence quenching in response to increasing PPFD in the same three species as Figure 2. Linear regressions were used to fit the  $q_p$  data and rectangular hyperbolae were fitted to the  $q_n$  data. Symbols are as in Fig. 2.

Figure 4. A linear relationship was observed between light saturation intensity ( $PPFD_{sat}$ ) and the maximum rate of electron transport through PSII as determined by fluorescence analysis. Among the 16 species shown are: 10 species of Podocarpaceae; 4- *P. lawrencei*, 8- *M. niphophilus*, 9- *P. aspleniifolius*, 10- *P. drouynianus*, 11- *A. pancheri*, 12- *D. dacrydioides*, 13- *L. franklinii*, 14- *R. comptonii*, 15- *P. ferruginea*, 16- *P. dispermis*. Five species of Cupressaceae; 1- *A. acuminatus*, 3- *A. cupressiodes*, 5- *C. rhomboidea*, 6- *D. archeri*, 7- *A. selaginoides*. And a "needle-leaved" angiosperm; 2- *A. verticellata*.

Figure 5. The relationship between electron transport rate as determined by fluorescence analysis, and the maximum rate of  $CO_2$  uptake, in all 16 species. A highly significant linear regression is shown ( $p < 0.001$ ). The relationship is not expected to go through the origin considering the fact that  $CO_2$  uptake at 0 ETR will be negative due to respiration. Values of  $CO_2$  uptake are average maximum rates from 5 individuals, except *P. ferruginea*, *R. comptonii*, and *P. dispermis*, which were all represented by 2 individuals.

Figure 6. The relationship between light saturation intensity and average width of short shoots in 10 species of Podocarpaceae - ■ . A highly significant log regression ( $p < 0.001$ ) is shown. The 6 species of non-podocarps also shown - ▲ did not fit on this regression. Species are as in fig. 4.

Figure 2

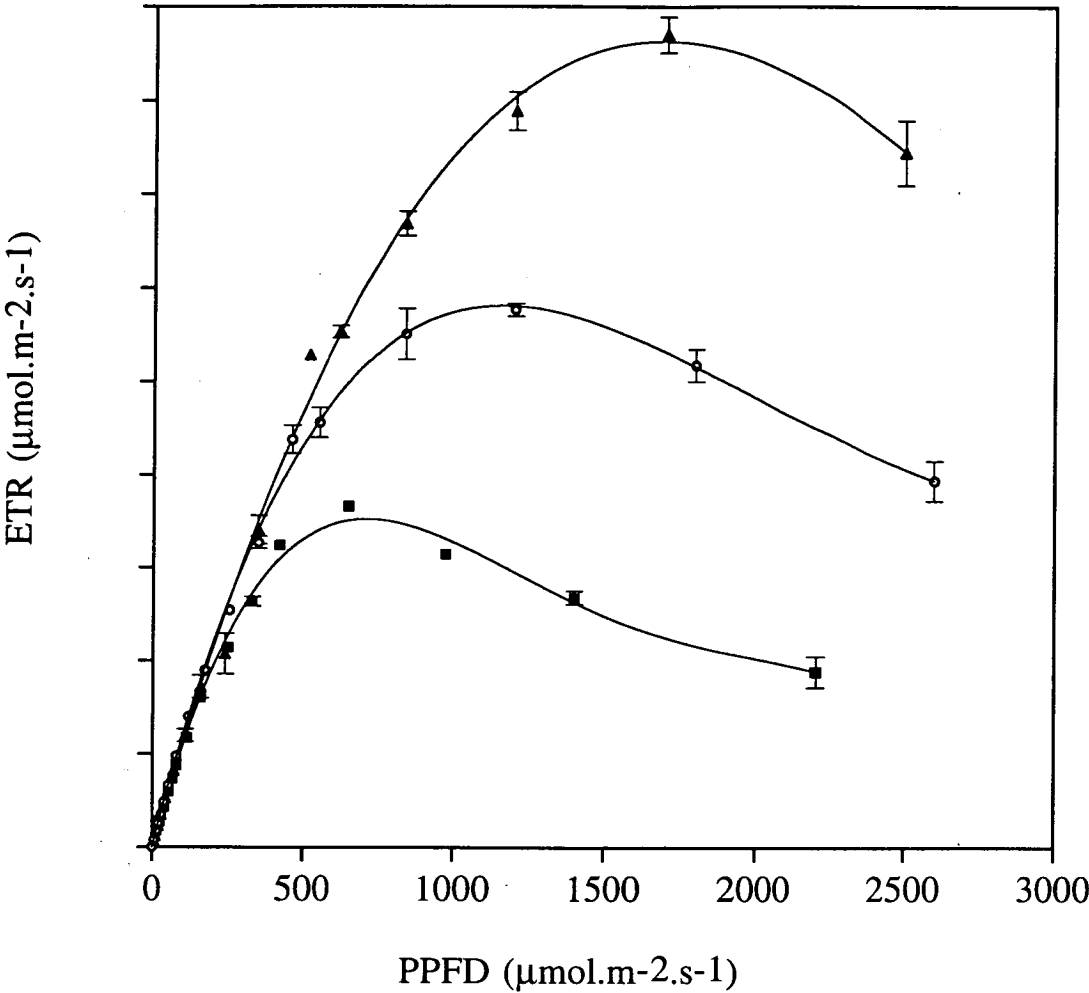


Figure 3(a)

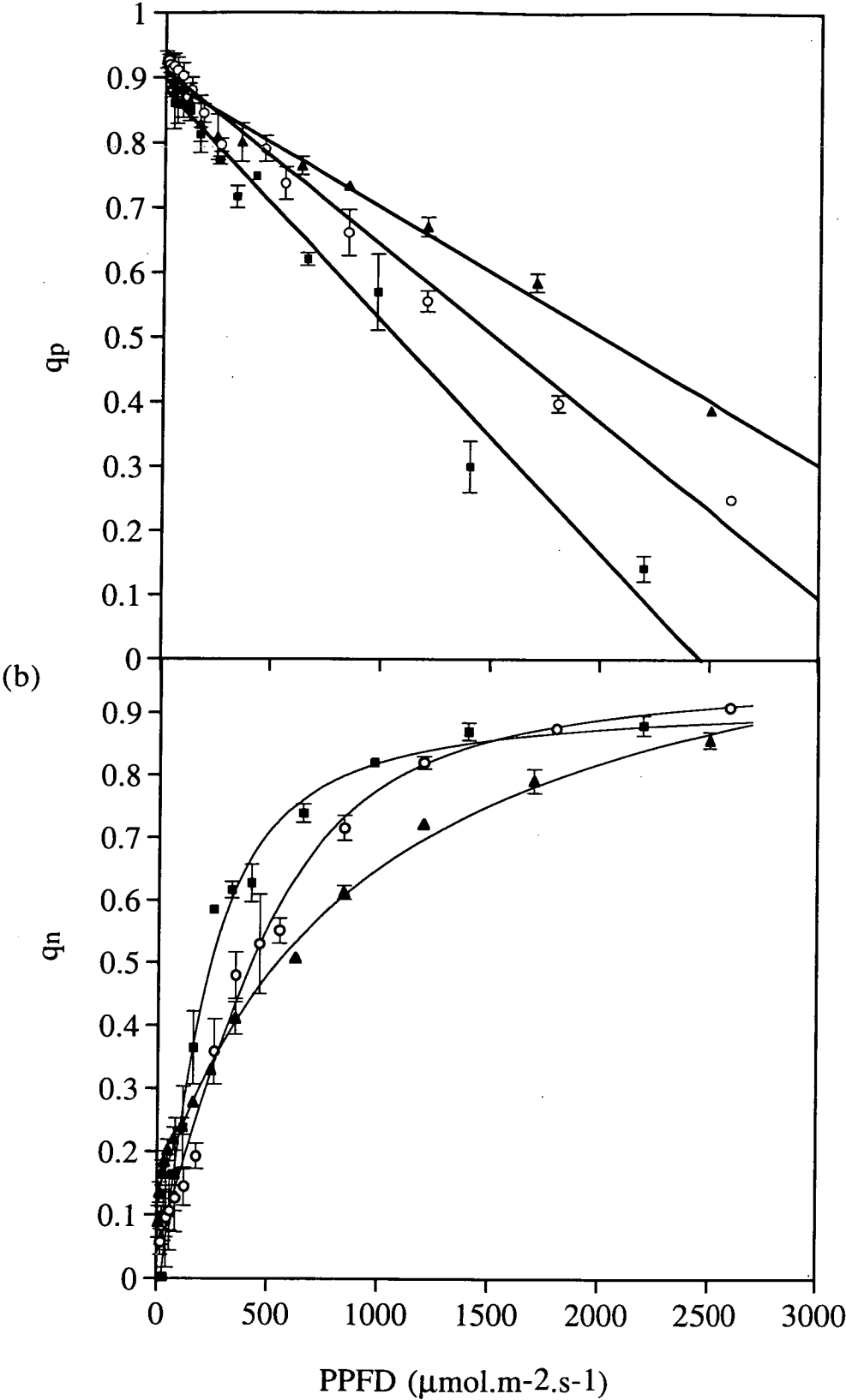


Figure 4

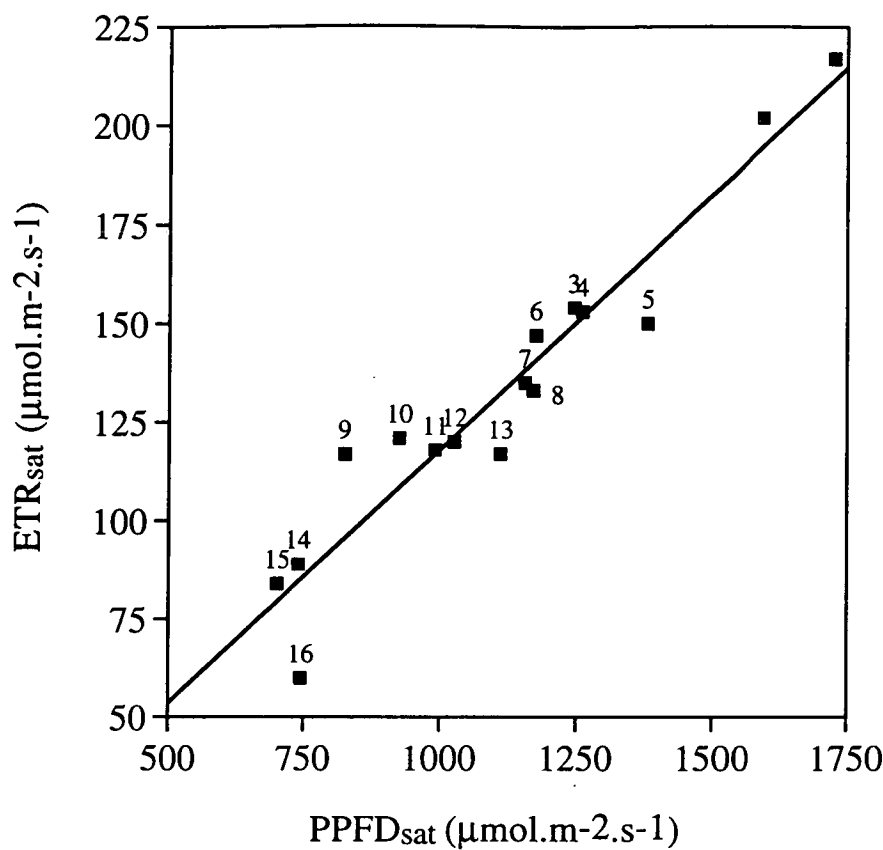


Figure 5

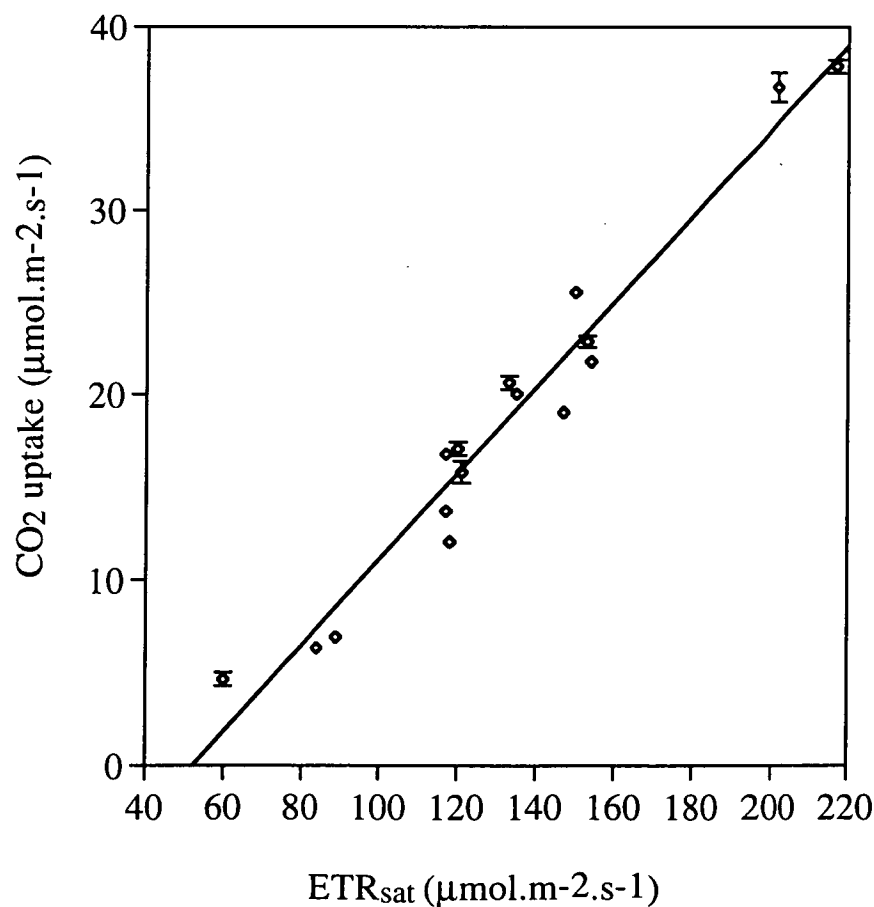
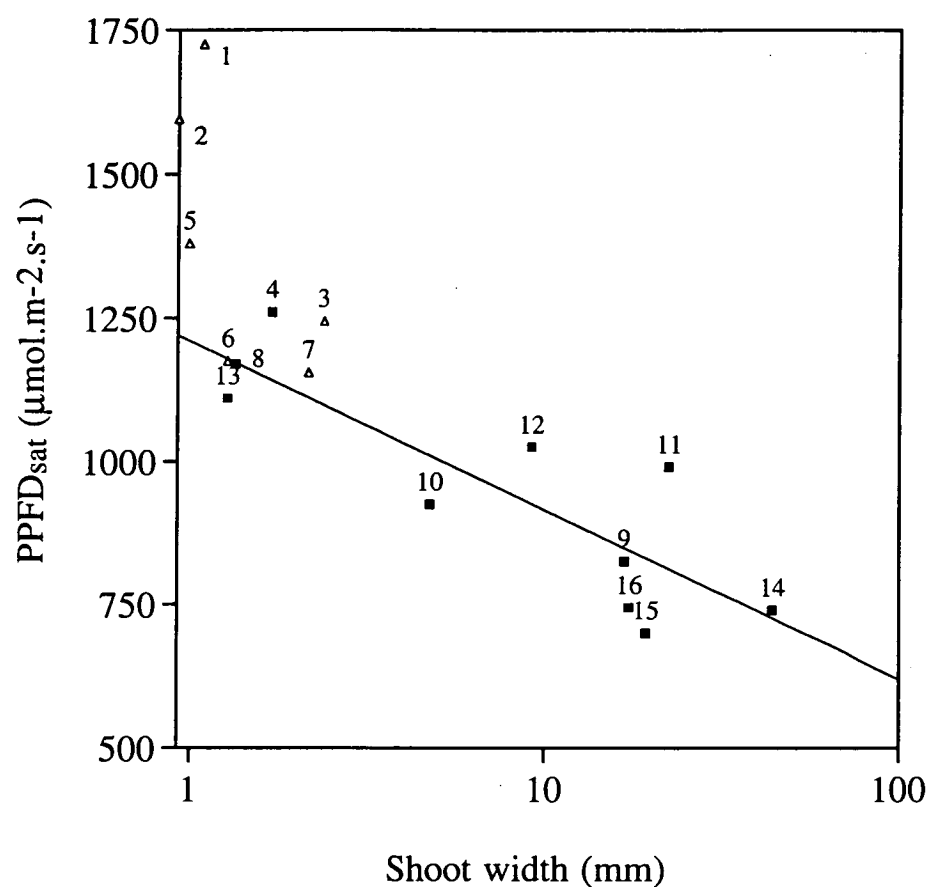




Figure 6



## **Imbricacy and Stomatal Wax Plugs Reduce Maximum Leaf Conductance in Southern Hemisphere Conifers.**

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### **Running head: Leaf Morphology and its Effects on Conductance**

#### *Abstract*

An examination of the relationship between theoretical maximum leaf conductance as calculated from stomatal dimensions, and measured maximum leaf conductance was undertaken in a group of Southern Hemisphere conifers. The relative effects of stomatal wax plugs, found in most species of conifers in the Southern Hemisphere, and imbricate leaf arrangement were expressed as a percentage inhibition of maximum leaf conductance ( $g_{\max}$ ) calculated from the ratio of measured  $g_{\max}$  to theoretical  $g_{\max}$ . Because of the similar stomatal dimensions of all species, measured  $g_{\max}$  was proportional to stomatal density in plugged and unplugged species, with species without wax plugs producing maximum leaf conductances on average 91% of calculated  $g_{\max}$ , while in species with plugged stomata measured  $g_{\max}$  was on average only 35% of theoretical  $g_{\max}$ . There was no effect produced by imbricacy in itself, but when combined with epistomy,  $g_{\max}$  was significantly reduced to about 17% of theoretical  $g_{\max}$ . This is clearly illustrated by juvenile-adult foliage comparisons, and closely related imbricate and non-imbricate species. The adaptational advantages of imbricacy and wax plugs, and the potential for inferring  $g_{\max}$  of fossil taxa are discussed.

#### **Introduction**

The photosynthetic characteristics of a leaf are intrinsically linked to the physical characteristics of its stomata. Being the only pathway for gas exchange, the size and density of stomata necessarily dictate the maximum leaf conductance ( $g_{\max}$ ) and hence the maximum

photosynthetic rate. A number of theoretical calculations of the physical resistance to diffusion imposed by the stomatal pore ( $r_s$ ) have been presented, most of which are based on the diffusion properties of gas through a cylinder (see Jones 1992). A more comprehensive derivation of  $r_s$  expressed in terms of stomatal pore geometry and density (Parlange and Waggoner 1970) has been found to correlate well with measured values of stomatal conductance ( $1/r_s$ ) in leaves which were subsequently cryo-fixed, and stomatal characters measured by electron microscopy (Van Gardingen *et al.* 1989). Despite this, no broad pattern has been described relating the physical characteristics of the stomata to either the physiological or ecological characteristics of species.

The most consistent trend observed between stomatal characters and environment is the relationship between the distribution of stomata on leaves and light intensity. Hypostomy (where stomata are confined to the abaxial leaf surface) has been qualitatively associated with leaves growing at low light intensities, and amphistomy (where stomata are evenly distributed on both leaf surfaces) with species from high light environments which produce high maximum photosynthetic rates and conductances (Mott *et al.* 1982; Körner *et al.* 1989; Peat and Fitter 1994). Stomatal density has also been reported to respond to short- and long-term changes in CO<sub>2</sub> concentration (Woodward 1987; Beerling and Chaloner 1993; Van der Water *et al.* 1994) although these results are still somewhat controversial (Körner 1988).

The difficulty with attempting to describe stomatal trends in the flora is that the size and shape of stomata vary considerably among genera (Meidner and Mansfield 1968), and differences in leaf morphology have the potential to substantially alter the relationship between pore area and leaf conductance. Common morphological features which must severely affect leaf conductance include the formation of stomatal crypts (common in the leaves of xeromorphic taxa), leaf pubescence, wax plugs (found in a very large proportion of conifers) and imbricacy (common in highland taxa). This paper focuses on the effects on gas exchange of two of these leaf characters, wax plugs and leaf imbricacy.

Wax plugs are occasionally found in angiosperms (e.g. Mihaich 1989), but are very common in conifers, being present in most Southern Hemisphere genera and a large number of northern taxa. Their function is not well understood, but antitranspirant (Jeffree *et al.* 1971), and antipathogen (Giles-Frankle *et al.* 1993) effects have been observed. The presence of large amounts of wax covering the stomatal pores clearly

affects the rate of leaf gas exchange by decreasing the cross-sectional area available for diffusion, and by decreasing the diffusion coefficient (depending on the size of the spaces between wax crystals). Whether wax plugs are a primary adaptation to reduce gas exchange, or the resultant inhibition of stomatal conductance is a secondary effect, is unknown. Imbricacy is also common amongst conifer genera, and in some species stomata are restricted to the adaxial surface of leaves which are tightly appressed to the stem, effectively shielding the stomata from the environment (Wells and Hill 1989a). Several of these species also possess a well-developed leaf marginal frill, which almost completely seals the remaining gap between leaves, and all have wax plugs in the stomatal pores. Again, this morphology must profoundly reduce the potential maximum leaf conductance, but the extent of this reduction, and the selective force which has resulted in such an unusual morphological response, are unknown.

An understanding of the relationship between stomatal characteristics such as density and morphology, and leaf physiology has considerable application for ecological and climatic interpretation of the fossil record. Temporal trends of decreasing stomatal density and increased imbricacy have been described for several genera from Tertiary macrofossils, and it has been suggested that these changes in leaf morphology represent adaptations to restrict water loss as a response to increasing aridity (Hill and Carpenter 1991). Such conclusions are not well founded without an understanding of the effects of these adaptations on the likely rate of gas exchange in living taxa.

In this study, the maximum leaf conductances of a group of Southern Hemisphere conifers with stomata of similar sizes, are compared in an attempt to quantify the effects of leaf morphology and stomatal arrangement on leaf gas exchange.

## Materials and Methods

### *Plant Material*

Cuttings and seed, were collected from 16 species of Podocarpaceae (all with stomatal wax plugs), and seven species of Cupressaceae (three of which possessed wax plugs). Podocarps used were; *Acmopyle pancheri* (Brongn. and Gris) Pilger, *Dacrycarpus compactus* (Wasscher) de Laubenfels, *D. dacrydioides* (Rich.) de Laubenfels, *D. imbricatus* (Blume) de Laubenfels, *Lagarostrobos franklinii* (Hook.) C.J.Quinn,

*Lepidothamnus laxifolius* (Hook.f.) C.J.Quinn, *Microstrobos niphophilus* Garden and Johnson, *Phyllocladus alpinus* Hook.f., *P. aspleniifolius* (Labill.) Hook.f., *P. hypophyllum* Hook.f., *Podocarpus dispermis* White, *Podocarpus drouynianus* Mueller, *Podocarpus lawrencii* Hook.f., *Prumnopitys ferruginea* D.Don, and *Retrophyllum comptonii* (Buchh.) C.N.Page. Cupressaceae used were; *Actinostrobus acuminatus* Parlatores, *Athrotaxis cupressoides* D.Don, *Athrotaxis selaginoides* D.Don, *Callitris columellaris* F.Muell., *C. rhomboidea* R.Br., *Diselma archeri* Marsh, and *Widdringtonia cedarbergensis* Marsh, were propagated in sand in Hobart. Upon establishment, all plants were transferred to a pine bark potting mix in 3L pots and grown under ambient light conditions in a well-irrigated, heated glasshouse near sea-level in Hobart.

In order to determine the combined effect of imbricacy and epistomy (the restriction of stomata to the adaxial leaf surface) on leaf conductance, the juvenile (non-imbricate) and adult foliage types of *Diselma archeri*, and *Microstrobos niphophilus* were compared. Also compared with the tightly imbricate species *Microstrobos niphophilus* was the closely related species *Microstrobos fitzgeraldii*, which possesses spreading, epistomatic leaves.

### Gas Exchange

Measurement of gas exchange was carried out on leaves or shoots enclosed in a cuvette attached to an open-flow infra-red gas analyser and dew point meter, as described by Brodribb (1996). Leaves and shoots were arranged carefully in the cuvette so as to minimise self-shading. Maximum values of leaf conductance were measured for each species in fully watered plants, photosynthesising under approximately optimal conditions, with leaves at 20°C, leaf-air vapour pressure deficit at 5-10 mmol.mol<sup>-1</sup>, light intensity at 1400  $\mu$ mol.photons.m<sup>-2</sup>.sec.<sup>-1</sup>, and an ambient CO<sub>2</sub> concentration of 350  $\pm$  5  $\mu$ mol.mol<sup>-1</sup>. Because the rate of stomatal opening in most species was very slow, leaves were exposed to full light and 100% relative humidity for a period of one to two hours prior to measurement of gas exchange to ensure maximum stomatal opening. For each species, an average of the maximum readings of leaf/shoot conductance from five individuals was calculated to give  $g_{max}$ .

Both stomatal density and  $g_{max}$  were expressed as a function of the silhouette area of foliage in the cuvette. This was measured with a digital camera (EDC-1000, Electrim Corp. Princeton, USA) after conductance readings had been taken. Thus, differences in leaf arrangement and shoot

morphology affected stomatal density and leaf/shoot conductance in parallel.

### *Stomatal Density*

After measuring the silhouette area of a small sample of leaf or shoot (approximately 1 cm<sup>2</sup>), the foliage was detached and stomata counted by one of three methods:

1. Stomata were counted directly using a light microscope with illumination of the sample from above, and a camera lucida attachment to enable counted stomata to be marked. This method was employed on small leaves with clearly visible stomata.

2. Epidermal impressions were made using cellulose acetate nail polish, and stomata counted using a light microscope. This method was used on larger leaves where whole-leaf impressions were made, and then subdivided for ease of counting.

3. Leaf cuticles were removed by dissolving leaf tissue in 5% aqueous chromium trioxide (CrO<sub>3</sub>) and stomata counted under a light microscope. This was employed for leaves with stomata restricted to grooves, making them impossible to count by methods 1 or 2.

In each case, all stomata were counted on the sample, or if leaves were perfectly symmetrical with an even number of stomatal bands, half the stomata were counted and the result doubled. At least one replicate was counted for each species, and results given as an average with standard error.

### *Theoretical Maximum Conductance*

The theoretical maximum shoot conductance was calculated for samples of imbricate, plugged, and unplugged foliage using equation 1 (Parlange and Waggoner 1970)

$$1. \ r_s = [(d/\pi.a.b) + \ln(4.a/b)/\pi.a]/D.n \quad \text{Equation (1)}$$

where:  $a$  = pore length/2;  $b$  = pore width/2;  $d$  = pore depth;  $D$  = diffusivity of water vapour in air;  $n$  = stomatal density.

Measurements of stomatal pore depth and width were carried out by sectioning leaves with a freeze microtome and staining sections with a cutin stain (Sudan Red) so as to visualise the stomatal pore and pit. A light microscope with graticule was then used to determine the distance from the inside of the pore to the cuticle surface, and the width of the pit (in all cases the stomatal pit was of very similar dimensions to the actual pore, and was thus considered as part of the pore (Fig. 1). Pore length was

determined by taking scanning electron micrographs of the inner cuticle surface and measuring the length of the stomatal slit.

To compare calculated conductance ( $g_{\max}$ ) and measured conductance ( $g_1$ ) it was necessary to add the boundary layer resistance to the calculated value of  $r_s$ , giving a theoretical leaf or shoot minimum resistance to diffusion, or  $1/g_{\max}$ . The boundary layer resistance was calculated by measuring the conductance of a wet, 2 mm-wide leaf replica made of blotting paper, as described in Jones (1992).

## Results

Stomatal density ranged widely from an average of 4280 cm<sup>-2</sup> in *Prumnopitys ferruginea* to 37,340 cm<sup>-2</sup> in adult *Microstrobos niphophilus* foliage (Table I). Variation in the size of the stomatal slit was minimal, with a range of pore lengths from 18 to 24.5 µm. The most obvious distinction between stomatal pores was the presence or absence of wax plugs occluding the opening of the stomatal antechamber (Figs 1,2). Plugs were present in all genera except *Actinostrobos*, *Callitris* and *Widdringtonia*. The relatively small variation in pore size emphasised the effects of leaf morphology, allowing a clear distinction to be seen between the stomatal density versus measured  $g_{\max}$  relationships for plugged, unplugged, and imbricate foliage (Fig. 3). For both plugged and unplugged species, positive linear regressions (significant at the  $p < 0.001$  level) described the relationship between number of stomata and measured  $g_{\max}$ .

The combined effects of imbricacy and epistomy are illustrated in figure 3 for the two species of *Microstrobos*. *Microstrobos fitzgeraldii* is epistomatic but the leaves are not imbricate, and hence its conductance was found to be consistent with other plugged species. Juvenile *M. niphophilus* has slightly spreading foliage and was found to be slightly displaced from the other plugged species, while adult *M. niphophilus* is entirely imbricate and epistomatic (Figs. 4, 5), and clearly possessed a much higher stomatal density relative to maximum conductance than the other plugged species (Fig. 3). Juvenile *Diselma archeri* has spreading, hypostomatic leaves, and produced a maximum conductance well within the expected range for plugged species. In adult foliage, however, leaves were imbricate, with 79% of stomata found to be restricted to the adaxial (enclosed) surface of the leaf, and in this foliage, stomatal density was also high relative to maximum conductance, although the magnitude of the

difference was not as large as for *M. niphophilus* (Fig. 3). Other (plugged) imbricate species such as *Lagarostrobos franklinii* and *Athrotaxis cupressoides*, which had stomata fully exposed to the atmosphere on the abaxial leaf surface, exhibited a similar association between stomatal density and maximum conductance to other plugged species.

In the three unplugged species, from three genera of Cupressaceae, maximum conductance varied linearly with stomatal density, with unplugged species producing maximum stomatal conductances more than twice that of plugged species with equivalent densities of stomata.

Theoretical values of  $g_{\max}$  using equation 1 were very close to the measured conductances for unplugged species (Fig. 6). The linear regression for measured versus theoretical  $g_{\max}$  indicates that measured values of leaf conductance were about 91% of the theoretical value calculated from the stomatal dimensions of these three species. *Callitris columellaris* was included to determine the effect of shoot morphology on  $g_{\max}$  in a species without stomatal plugs. Unlike the other three unplugged species, the stomata in *Callitris columellaris* were restricted to deep invaginations between leaves (Figs 7,8) and as a result of this measured  $g_{\max}$  was found to be reduced to 56% of the expected value (Fig. 6).

A linear relationship was also observed between measured and calculated theoretical maximum leaf conductance in the sub-sample of eight plugged species (Fig. 6). The slope of this regression illustrates that the measured  $g_{\max}$  in species with stomatal plugs was less than 35% of the value calculated from equation 1.

The maximum difference between measured and theoretical  $g_{\max}$  was found in the imbricate, epistomatic species *Microstrobos niphophilus*. Adult *Microstrobos niphophilus* foliage produced a maximum conductance of only 17% of the theoretical maximum expected from the density and size of stomata present (Table I). Leaf conductance in *Diselma archeri*, an incompletely epistomatic species, was slightly higher, with measured  $g_l$  being 25% of the maximum theoretical value. In contrast, juvenile *Diselma* foliage clearly fitted on the regression through the other plugged species (Fig. 6).

Because of the similar dimensions of the stomatal pores in all species the relationship between stomatal density and theoretical  $g_{\max}$  (calculated using equation 1) was approximately linear (Fig. 6). Again the effect of stomatal plugs was clearly evident when observed and expected



conductances were compared in species with a range of stomatal densities (Fig. 9).

## Discussion

The similarity between measured and theoretical  $g_{\max}$  in the three species of conifers used here with fully exposed, unobstructed stomata, further supports the application of the diffusion equation of Parlange and Waggoner (1970) as an indicator of the maximum potential for leaf gas exchange. This also provides evidence that the assumptions made in measuring the stomatal pores were valid, namely that the width of the stomatal antechamber was approximately equal to the maximum open diameter of the stomatal pore, and that the depth of the stomata included the depth of this pit. The close correlation between measured and calculated  $g_{\max}$  in these species also enables the inhibitory effects of stomatal morphology on maximum potential leaf conductance to be assessed with some confidence that the calculation of maximum theoretical conductance is accurate.

Maximum conductance in species with exposed stomata occluded by wax plugs was reduced to about 35% of that which would occur if the plugs were absent. This inhibition was approximately the same for all species sampled, including five genera of Podocarpaceae and two genera of Cupressaceae. Thus it is assumed that the wax crystals in the stomatal antechamber of each of these species are of similar size and density. A theoretical calculation of the effect on conductance of stomatal plugs in Sitka spruce suggested that plugs should reduce leaf conductance by only about 37% (Jeffree *et al.* 1971). The discrepancy between the 65% reduction shown here and the 37% reduction proposed for Sitka spruce may be due to differences in the nature of the stomatal wax, but may also be a function of inaccuracies in estimating the resistance of the stomatal plug, particularly in its effect on the coefficient of free diffusion.

The question remains as to why the majority of conifers in the Southern Hemisphere should produce leaves with a high potential for gas exchange, only to occlude all the stomata early during leaf development (Martin and Juniper 1970). Unlike most plants, where wax is distributed approximately uniformly on the leaf avoiding stomatal openings and guard cells, the wax in these conifers is most abundant within the stomatal antechamber (Figs 2, 4), and in some species wax is almost entirely confined to the stomatal openings. The most common

suggestion is that wax plugs serve an anti-transpirant function, a role which they clearly fulfil. However two lines of evidence suggest that wax plugs are not a primary adaptation to restrict water loss. Firstly, the conifer species which grow in some of the driest environments in the Australia, and hence have had the greatest need to reduce water loss, e.g. species of *Actinostrobus*, *Callitris* and *Widdringtonia* (Brodribb and Hill 1996) produce wax, but do not possess wax plugs. Second, amongst species with stomatal wax plugs, the frequency, size, and nature of wax plugs appears to be unrelated to the magnitude of the maximum leaf conductance. One might expect that if wax plugs were an adaptation to restrict water loss, some variation in the abundance of wax would be evident in species at opposite ends of the conductance spectrum ie. species with high photosynthetic rates and conductances might be expected to produce less wax than species with lower intrinsic photosynthetic capacity and maximum conductance. Such differences were not evident, and in *Diselma archeri* and *Microstrobus niphophilus*, where the conductance of seedling foliage is greatly enhanced relative to adult foliage (Fig. 6). In these species increased conductance accompanied large changes in leaf morphology, while the nature of the wax-plugs is believed to have remained unchanged. In fact, inhibition of photosynthesis, at least in the short term, is commonly accompanied by a decrease in wax deposition (Ylimartimo *et al.* 1994).

The presence of plugged stomata certainly pre-dates the spread of aridity during the Tertiary (Hill 1990), and it seems more likely that wax plugs may have evolved as an adaptation to ever-wet conditions. Leaf wetting inhibits photosynthesis both by blocking the stomatal pore and by directly lowering the capacity of the photosynthetic apparatus (Ishibashi and Terashima 1995). Decreased leaf wettability has been shown to allow increased conductance and assimilation under wet conditions (Smith and McClean 1989). The confinement of wax to the stomatal antechamber may facilitate gas exchange under ever-wet conditions by keeping the pore free of water, while avoiding the increased leaf reflectance associated with a uniform wax deposition on the leaf.

A second advantage associated with obstructing the antechamber is in the prevention of fungal invasion into the stomatal pore. Fungal spores accumulate in the stomatal pits of angiosperms and germinate there, presumably in response to the moist conditions (Figs 10, 11) This poses a particular threat to conifer leaves, due to their generally high longevity (Ewers and Schmidt 1981). Stomatal wax would prevent spore

and water accumulation around the stomatal pore, and provide a physical barrier to the invasion of fungal hyphae. This is supported by the fact that erosion of the stomatal wax plugs by airborne pollutants leaves foliage more susceptible to fungal attack (Meng *et al.* 1995).

Imbricacy in itself has no effect on the relationship between stomatal density and  $g_{\max}$ , but when combined with epistomy, leaf conductance was found to be substantially reduced. The effect is clearly illustrated in the comparison between *Microstrobos niphophilus* and *M. fitzgeraldii*, two closely related species with very similar leaf and stomatal morphology, but with the latter species possessing spreading rather than imbricate leaf arrangement (Fig. 6). In *M. fitzgeraldii*, leaf conductance was reduced to 32% of maximum theoretical conductance, whereas in *M. niphophilus*  $g_{\max}$  was reduced to 17% of theoretical  $g_{\max}$ . Assuming that 35% (average inhibition of all plugged species) of this effect is due to stomatal plugs, then the imbricate effect results in about a 48% reduction of  $g_{\max}$ . This reduction in conductance was less marked in *Diselma archeri* (Fig. 6) due to the fact that only 79% of stomata were on the adaxial surface of the leaf. No effect was observed in imbricate species such as *Lagarostrobos franklinii* or *Athrotaxis cupressoides* where leaves were not tightly imbricate, with less than 30% of stomata on the adaxial leaf surface.

Considering the large inhibition of conductance (and hence assimilation) affected by this morphology, there must be an equally large benefit conferred. The most obvious advantage is that of physical protection of the stomatal surface. *Microstrobos niphophilus* and *Diselma archeri* (and the only other imbricate epistomatic species, *Microcachrys tetragona*) are most common in sub-alpine shrubland in Tasmania; where mechanical abrasion of the leaf is likely to be high. Thus, dedication of the exposed (abaxial) leaf surface to structural support, by confining stomata to the adaxial leaf surface, would be advantageous in terms of minimising leaf damage from effects such as ice-abrasion. The fossil record provides some support for the protection hypothesis, with the description of another (extinct) epistomatic, imbricate species from a Tertiary high-altitude site in Tasmania (Hill and Carpenter 1991). However, the fossil record also indicates that this morphology has been far more widespread in the past, as fossil occurrences of these genera (*Microstrobos*, *Diselma*, and *Microcachrys*) are commonly associated with low-altitude, broad-leaved forest (Blackburn 1985; Wells and Hill 1989b;

Blackburn and Sluiter 1994; Jordan 1995), suggesting that the current restriction to highland areas may only be a recent phenomenon.

Another possibility is that this morphology functions primarily to restrict water loss, a purpose which it obviously serves extremely well. In the genus where this morphology is best developed (*Microstrobos*), the conductance of non-imbricate foliage (juvenile *M. niphophilus* and *M. fitzgeraldii*) is very high (Fig. 3), a factor no doubt largely responsible for the distributional restriction of *M. fitzgeraldii* to spray zones of waterfalls (Harden 1990). It seems plausible therefore that imbricacy may extend the range of *Microstrobos* by decreasing leaf conductance and allowing the genus to grow in drier habitats.

Restriction of water loss would be an explanation for the restriction of stomata to invaginations in the leaf of *Callitris columellaris*. Although not as effective as imbricacy in *Microstrobos*,  $g_{\max}$  was reduced to 56% of theoretical  $g_{\max}$ . The most common species with this morphology (*C. preissii*) extends into arid environments (Bowman and Harris 1995), and the effect of this morphology in increasing the resistance to diffusion from the stomatal pore to beyond the boundary layer would enhance water use efficiency during photosynthesis.

In conclusion, it can be said that maximum leaf conductance in Southern Hemisphere conifers is proportional to the stomatal density, although the nature of the proportionality is dependant on whether stomata are occluded by wax plugs, and whether leaves are partially or fully epistomatic and imbricate. With such information, maximum conductance can be predicted from extant or fossil leaves with some confidence.

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**Fig. 1.** Transverse section of a stomatal pore of *Actinostrobus acuminatus* showing the dimensions and unplugged nature of the stomatal antechamber.

**Fig. 2.** Transverse section of a stomatal pore of *Acmopyle pancheri*, illustrating stomatal antechamber filled with wax. In this section, the wax plug has become slightly dislodged during sectioning, showing how closely the plug conforms to the shape of the stomatal antechamber (scale bar for both = 50  $\mu\text{m}$ ).

**Fig. 3.** Stomatal density and maximum measured conductance in 23 species of conifers. Two separate regressions are shown through,  $\bullet$  - species with wax plugs ( $r = 0.88$ ) and  $\diamond$  - species without wax plugs ( $r = 0.97$ ). Two imbricate, epistomatic species, *Diselma archeri* ( $\square$ ) and *Microstrobos niphophilus* ( $\Delta$ ) are shown, but have not been included in the regression. Arrows indicate the juvenile to adult comparison for *Microstrobos* and *Diselma* and the non-imbricate *Microstrobos* species *M. fitzgeraldii* is labelled (*M.f*)

**Fig. 4.** Scanning electron micrograph of the adaxial (inner) leaf surface of *Microstrobos niphophilus*, showing large numbers of stomata with wax deposits in the stomatal pore (280x). **Fig. 5.** SEM of a shoot of *M. niphophilus* showing the imbricate leaf arrangement, and absence of abaxial stomata (12x).



**Fig. 6.** Measured versus theoretical maximum leaf conductance in a sample of eight species with stomatal plugs (- ◻ ), three species without plugs (- ◊ ), and two imbricate epistomatic species (- ■ ). Regressions through the plugged and unplugged data are both highly significant ( $r$  for both = 0.98). The dotted line represents equality between measured and theoretical values. The juvenile to adult comparison for *Diselma* is shown with an arrow, and the comparison between non-imbricate *Microstrobos fitzgeraldii* (M.f) and imbricate *M. niphophilus* is also arrowed. An unplugged species with stomata confined to leaf invaginations, *Callitris columellaris* (- ♦ ) is shown for comparison with other unplugged species, which presented fully exposed stomata.

**Fig. 7.** Transverse section of a whorl of three leaves of *Callitris columellaris*, where stomata are restricted to invaginations (arrowed) between leaves (scale bar = 500  $\mu\text{m}$ ). **Fig. 8.** A similar transverse-section for *Actinostrobus acuminatus* where stomata (arrowed) are fully exposed on the leaf surface (scale bar = 500  $\mu\text{m}$ ).

**Fig. 9.** The linear relationship between stomatal density, and measured (- ◊ ) and theoretical (- • ) maximum conductance in plugged species, and unplugged species ( ♦ ; measured   ♦ ; theoretical). Imbricate epistomatic species are not included in the plugged data. Regressions shown are theoretical  $g_{\text{max}}$  vs. stomatal density ( $r = 0.97$ ) and measured  $g_{\text{max}}$  vs. stomatal density in plugged species ( $r = 0.88$ ).

**Fig. 10.** Surface view of fungal hyphae in the stomatal pores of a typical broad-leaf angiosperm. Hyphae appear as dark blotches concentrated around the stomatal pores of *Olearia argophylla* (Labill.) Benth. (scale bar = 100  $\mu\text{m}$ ). Uninfected stomata appear as clear areas. **Fig. 11.** Detail of hyphae in three stomata. Fungii use the stomatal pore as a germination point, and to gain entry into the leaf (scale bar = 20  $\mu\text{m}$ ).

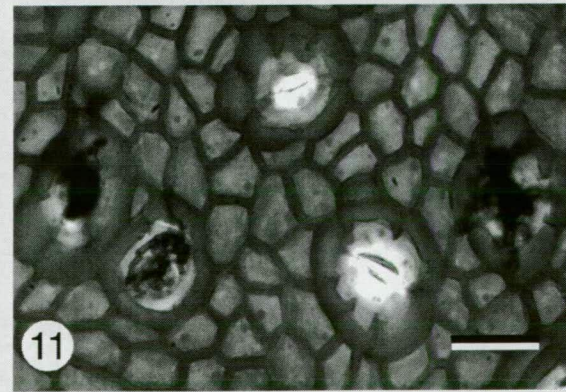
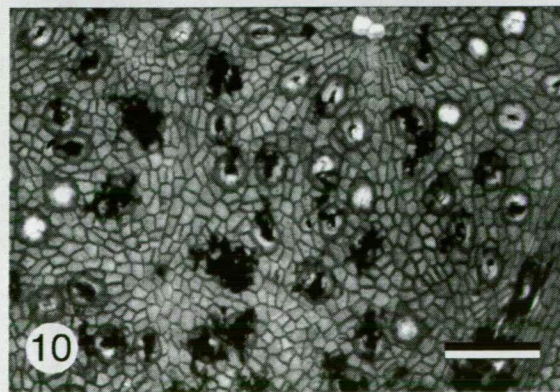
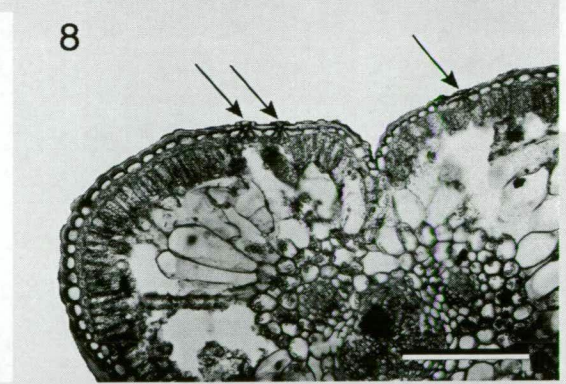
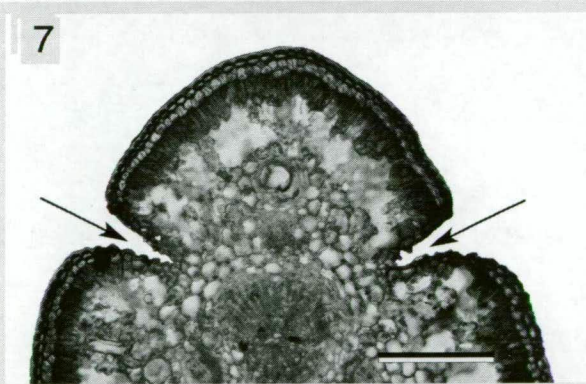
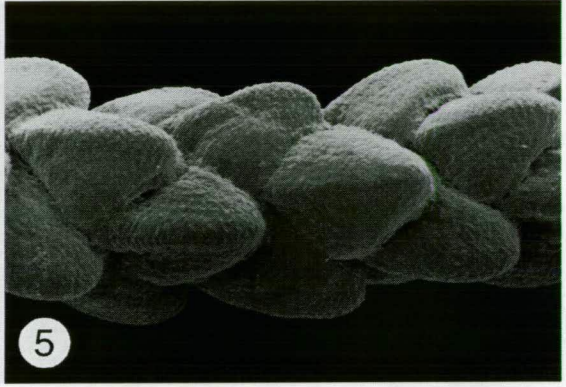
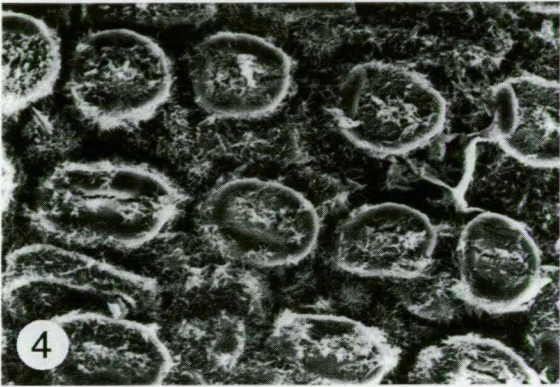
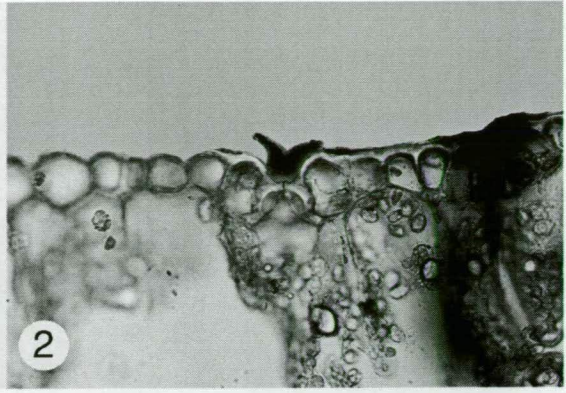
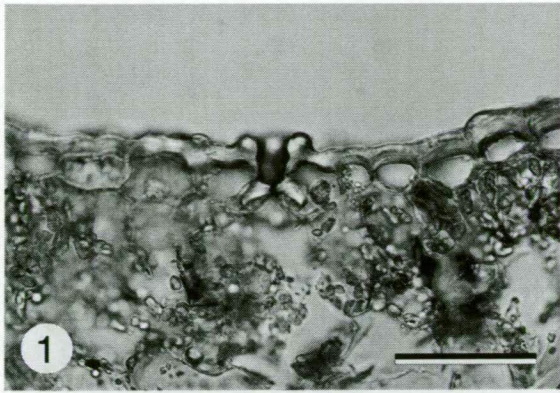


Figure 3

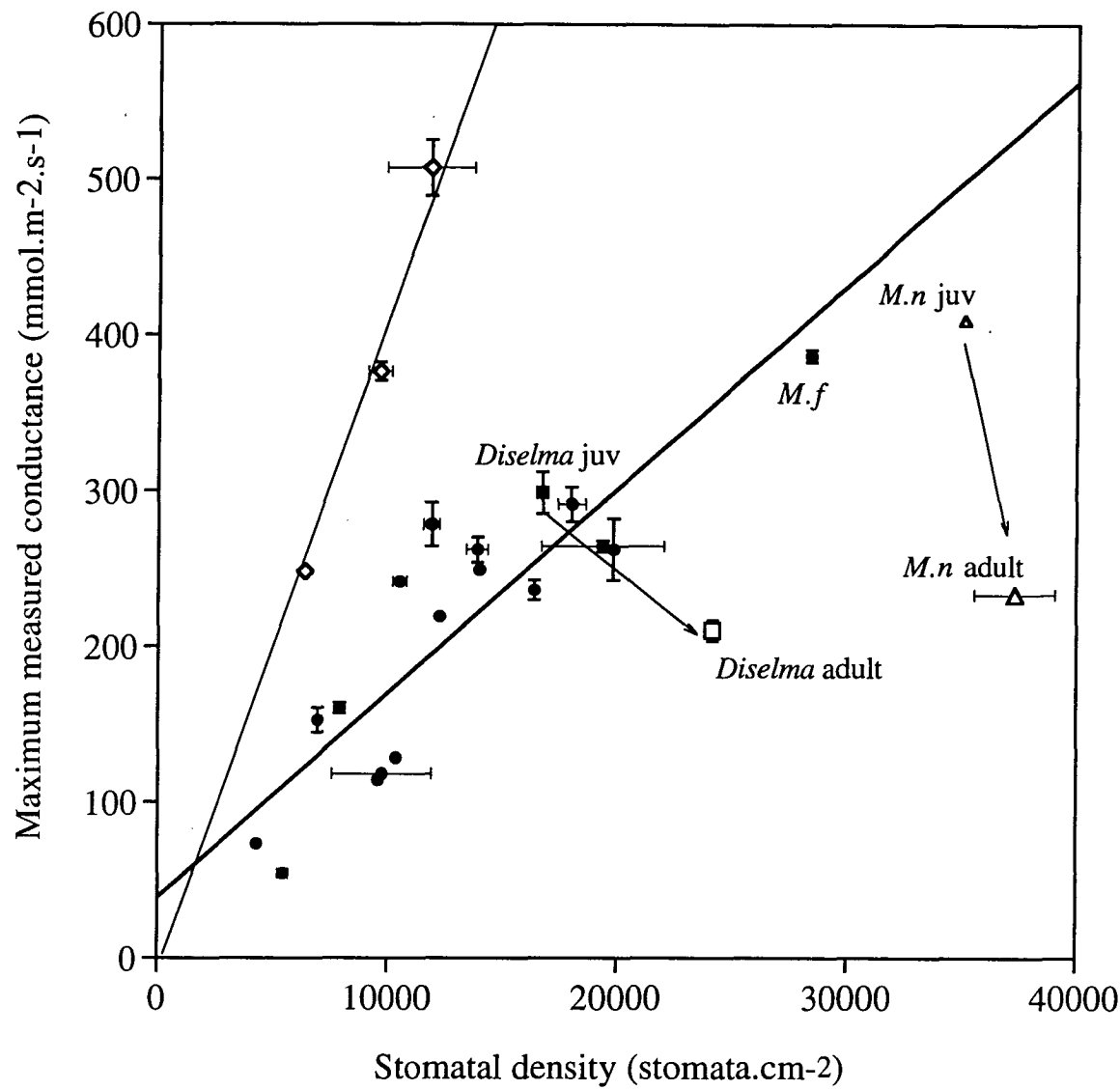


Figure 6

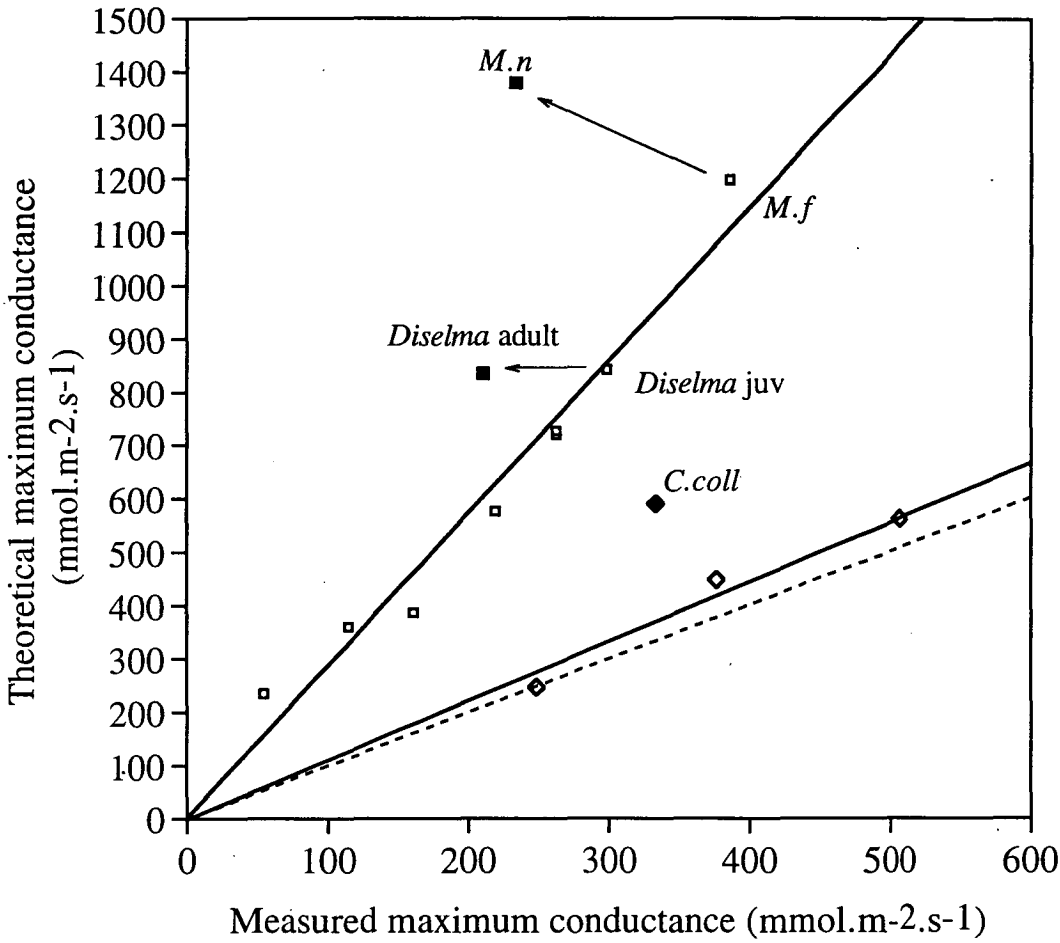


Figure 9

