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

Tim Brodribb[AB] *and Robert S. Hill*[A]

[A]Department of Plant Science, University of Tasmania, GPO Box 252–55, Tas. 7001, Australia.
[B]Corresponding author; email: brodribb@postoffice.utas.edu.au

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 comparisons of juvenile–adult foliage, 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_2 concentration (Woodward 1987; Beerling and Chaloner 1993; Van der Water *et al*. 1994), although these results are still somewhat controversial (Korner 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 anti-transpirant (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 apressed 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. & 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 & Johnson, *Phyllocladus alpinus* Hook.f., *P. aspleniifolius* (Labill.) Hook.f., *P. hypophyllus* Hook.f., *Podocarpus dispermis* White, *P. drouynianus* Mueller*, P. lawrencii* Hook.f., *Prumnopitys ferruginea* D.Don, and *Retrophyllum comptonii* (Buchh.) C.N.Page. Cupressaceae used were: *Actinostrobus acuminatus* Parlatore, *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 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^{-1}, light intensity at 1400 μmol photons m^{-2} sec^{-1}, and an ambient CO_2 concentration of 350 ± 5 μmol mol^{-1}. 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 1–2 hours prior to measurement of gas exchange to ensure maximum stomatal opening. For each species, an average of the maximum readings of leaf and 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 and shoot conductance in parallel.

Stomatal Density

After measuring the silhouette area of a small sample of leaf or shoot (approximately 1 cm^2), the foliage was detached and stomata were counted by one of the three following 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 were 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_3) and stomata were counted under a light microscope. This method 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 are 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):

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

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) 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_l), 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 by Jones (1992).

Results

Stomatal density ranged widely from an average of 4280 cm^{-2} in *Prumnoptiys ferruginea* to 37340 cm^{-2} in adult *Microstrobos niphophilus* foliage (Table I). Variation in the size of
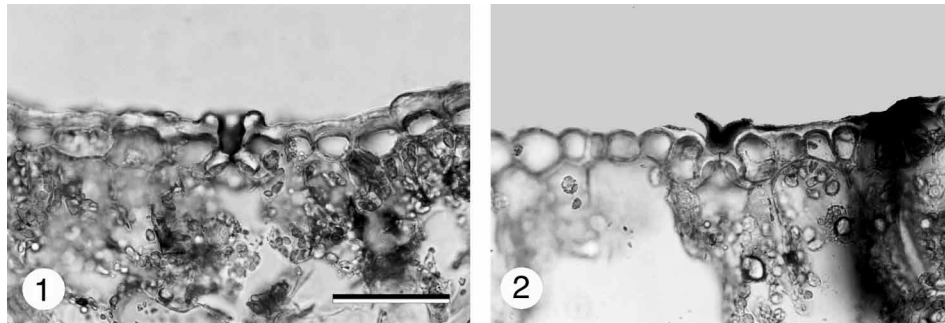
Fig. 1. Transverse section of a stomatal pore of *Actinostrobus acuminatus* showing the dimensions and unplugged nature of the stomatal antechamber.   **Fig. 2.** Transverse section of a stomatal pore of *Acmopyle pancheri*, illustrating stomatal antechamber filled with wax. In this section, the wax 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 µm).

the stomatal slit was minimal, with a range of pore lengths from 18 µm 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 *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_{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 Fig. 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} indicated 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

Table I. Morphological details of species investigated, and the ratio of measured, to theoretical conductance (expressed as a percentage)

Measured conductance (g_l) was the average maximum conductance from five plants, and theoretical conductance (g_{max}) came from measurement of the stomatal pore and application of Equation 1

Family and Species	Leaf Morphology	Stomatal density (cm^{-2})	gl/g_{max} (%)
CUPRESSACEAE			
Actinostrobus acuminatus	Unplugged stomata, imbricate leaves	11 803	90
Athrotaxis cupressoides	Plugged epistomatic	14 000	–
Athrotaxis selaginoides	Plugged, hypostomatic imbricate	13 897	36
Callitris rhomboidea	Unplugged imbricate	9643	84
Callitris columellaris	Unplugged (stomatal grooves) imbricate	14 773	56
Diselma archeri	Plugged, epistomatic, imbricate imbricate	24 137	24
Diselma archeri (juvenile)	Plugged hypostomatic	16 734	35
Widdringtonia cedarbergensis	Unplugged uneven amphistomatic	6360	100
PODOCARPACEAE			
Acmopyle pancheri	Plugged stomata, uneven amphistomatic	12 264	38
Dacrycarpus dacrydioides	Plugged amphistomatic	11 887	–
Dacrycarpus imbricatus	Plugged amphistomatic	6941	–
Lagarostrobos franklinii	Plugged imbricate uneven amphistomatic	10 535	–
Lepidothamnus laxifolius	Plugged uneven amphistomatic	9733	–
Microstrobos niphophilus	Plugged, epistomatic, imbricate	37 339	17
Microstrobos niphophilus (juvenile)	Plugged, epistomatic, losely imbricate	35 143	31
Microstrobos fitzgeraldii	Plugged, epistomatic, non- imbricate	28 410	32
Phyllocladus alpinus	Plugged uneven amphistomatic	19 352	–
Phyllocladus aspleniifolius	Plugged uneven amphistomatic	19 811	–
Phyllocladus hypophyllus	Plugged uneven amphistomatic	10 350	–
Podocarpus lawrencii	Plugged hypostomatic	18 025	–
Podocarpus drouynianus	Plugged hypostomatic	7909	41
Podocarpus dispermis	Plugged hypostomatic	5447	23
Prumnopitys ferruginea	Plugged approx. hypostomatic	4281	–
Retrophyllum comptonii	Plugged amphistomatic	9549	32

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 *M. niphophilus* foliage produced a maximum conductance of only 17% of the theoretical maximum expected from
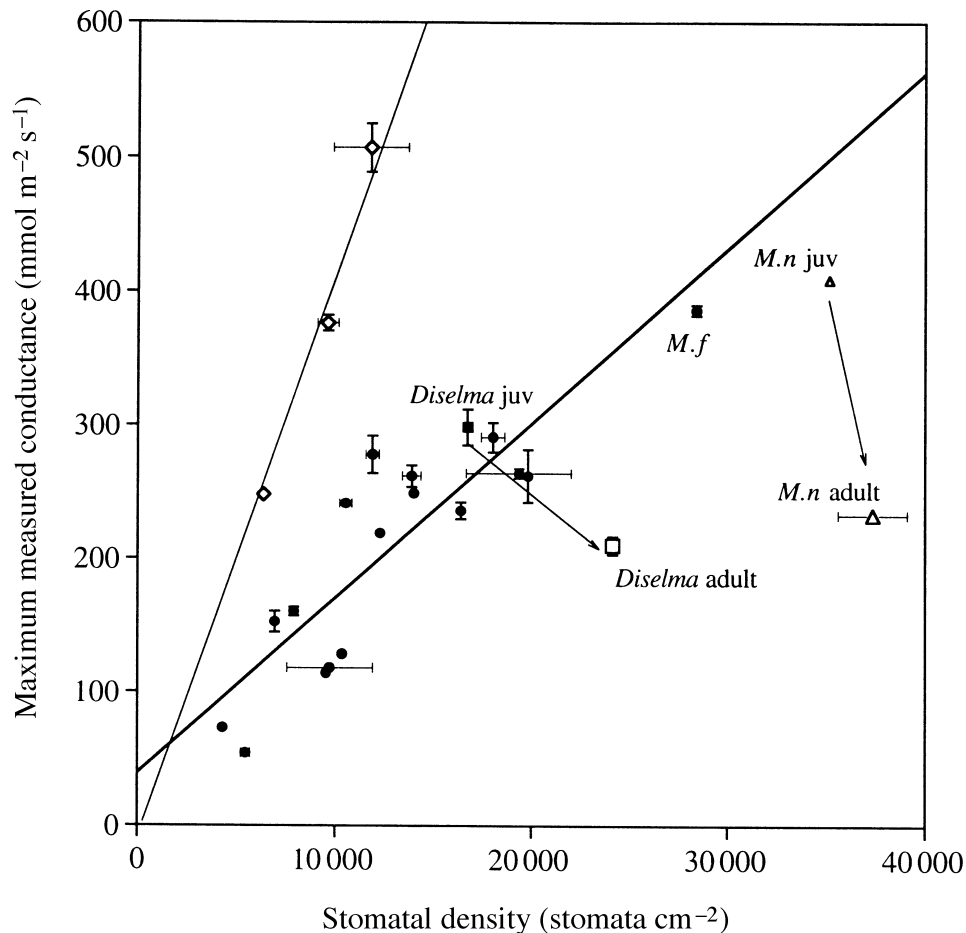
Fig. 3. Stomatal density and maximum measured conductance in 23 species of conifers. Two separate regressions are shown through, species with wax plugs (●) ($r = 0.88$) and species without wax plugs (◇) ($r = 0.97$). Two imbricate, epistomatic species, *Diselma archeri* (□) and *Microstrobos niphophilus* (*M.n*) (△) 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 *Microstrobus* species *M. fitzgeraldii* is labelled (*M.f*).

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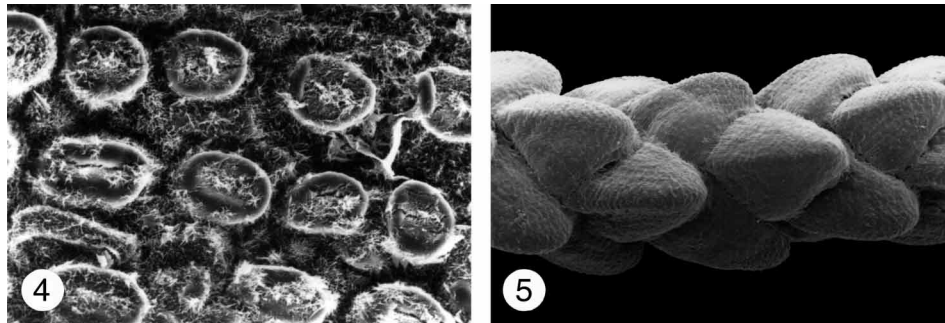
Fig. 4. Scanning electron micrograph of the adaxial (inner) leaf surface of *Microstrobus niphophilus*, showing large numbers of stomata with wax deposits in the stomatal pore (280×). **Fig. 5.** SEM of a shoot of *M. niphophilus* showing the imbricate leaf arrangement, and the absence of abaxial stomata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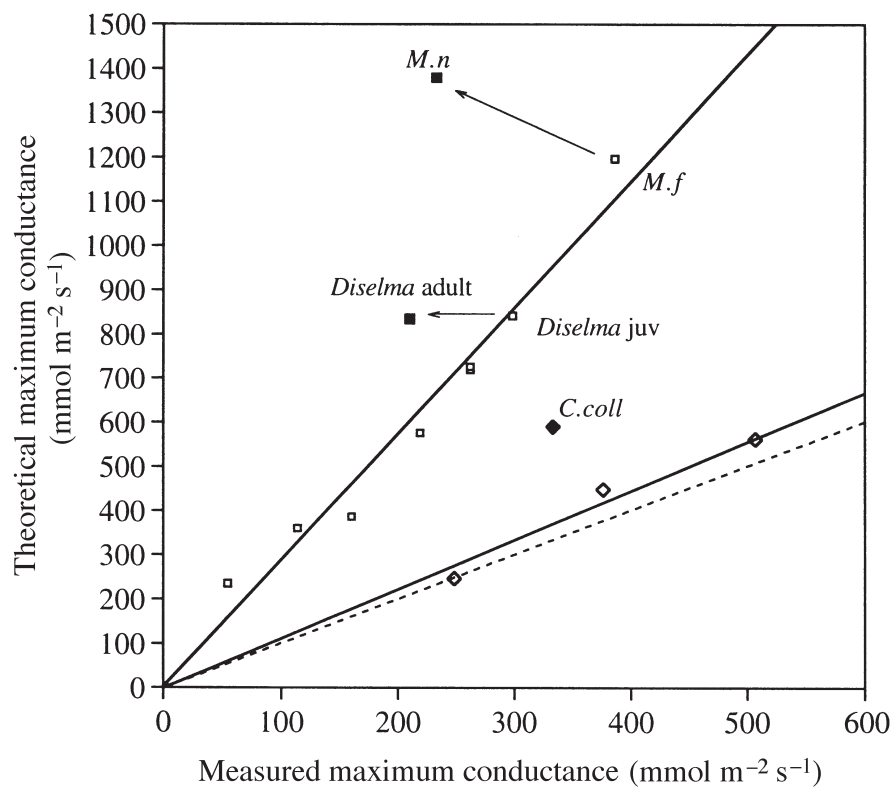

Fig. 6. Measured versus theoretical maximum leaf conductance in a sample of eight species with stomatal plugs (□), three species without plugs (◇), and two imbricate epistomatic species (■). Regressions through the plugged and unplugged data are both highly significant for both ($r = 0.98$). The dotted 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 *Microstrobus fitzgeraldii* (*M.f*) and imbricate *M. niphophilus* (*M.n*) is also arrowed. An unplugged species with stomata confined to leaf invaginations, *Callitris columellaris* (*C.coll*) (◆) is shown for comparison with other unplugged species, which presented fully exposed stomata.
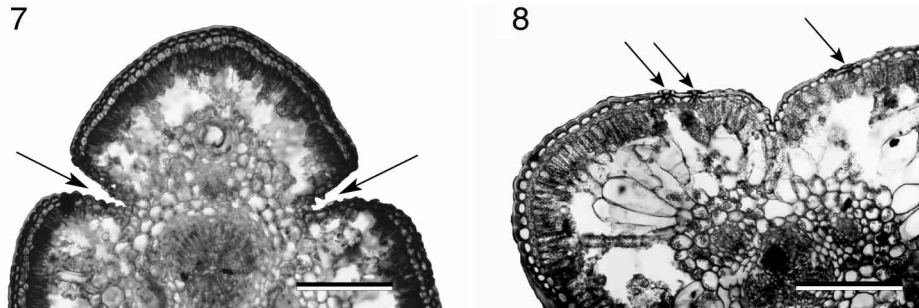
Fig. 7. Transverse section of a whorl of three leaves of *Callitris columellaris*, where stomata are restricted to invaginations (arrowed) between leaves (scale bar = 50 μm). **Fig. 8.** A similar transverse section of *Actinostrobus acuminatus* where stomata (arrowed) are fully exposed on the leaf surface (scale bar = 500 μm).
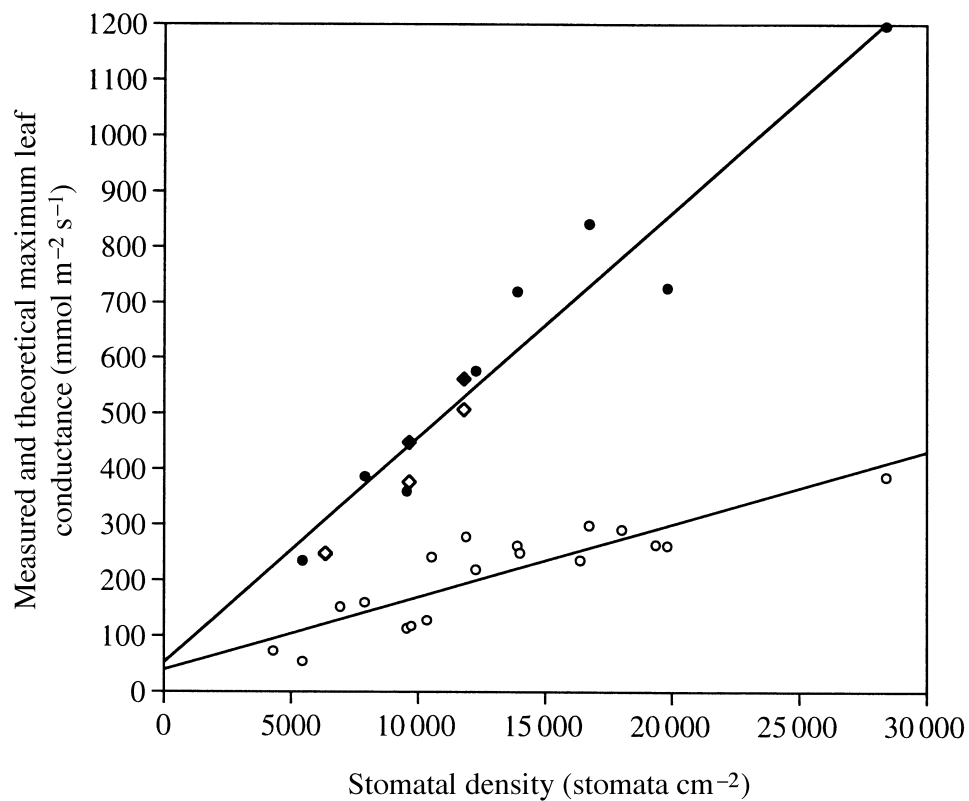

Fig. 9. The linear relationship between stomatal density, and measured (○) and theoretical (●) maximum conductance in plugged species, and unplugged species (◇, measured ◆, theoretical). Imbricate epistomatic species are not included in the plugged data. Regressions shown are theoretical g_{max} versus stomatal density ($r = 0.97$) and measured g_{max} versus stomatal density in plugged species ($r = 0.88$).

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 fulfill. 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 1998) produce wax, but do not possess wax plugs. Secondly, 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 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 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 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 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).
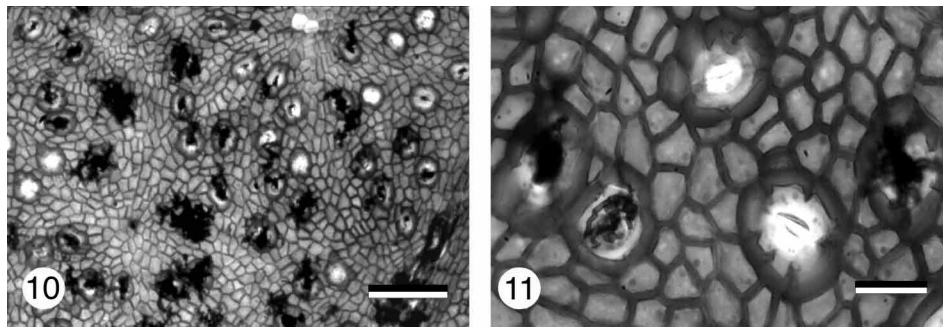

Fig. 10. Surface view of fungal hyphae in the stomatal pores of a typical broad-leaf angiosperm. Hyphae appear as dark blotches concentrated around the stomatal pores of *Olearia argophylla* (Labill.) Benth. (scale bar = 100 μm). Uninfected stomata appear as clear areas. **Fig. 11.** Detail of hyphae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

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 (*Microstrobus, Diselma,* and *Microcachrys*) are commonly associated with low-altitude, broad-leaved forest (Blackburn 1985; Wells and Hill 1989*b*; 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. fizgeraldii*) is very high (Fig. 3), a factor which is no doubt largely responsible for the distributional restriction of *M. fizgeraldii* 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.

Acknowledgments

References

Beerling, D. J., and Chaloner, W. G. (1993). Evolutionary response of stomatal density to global CO_2 change. *Biological Journal of the Linnean Society.* **48**, 343–353.

Blackburn, D. T. (1985). Palaeobotany of the Yallourn and Morwell coal seams. State Electricity Commission of Victoria, Palaeobotanical Project—Report No. 3, Melbourne.

Blackburn, D. T., and Sluiter, I. R. K. (1994). The Oligo–Miocene coal floras of southeastern Australia. In 'History of the Australian Vegetation'. (Ed. R. S. Hill.) pp. 328-367 (Cambridge University Press: Cambridge.)

Bowman, D. M. J. S., and Harris, S. (1995). Conifers of Australia's dry forests and open woodlands. In 'Ecology of the Southern Conifers'. (Eds N. J. Enright and R. S. Hill.) pp. 252–270. (Melbourne University Press: Melbourne.)

Brodribb, T. (1996). Dynamics of changing intercellular CO_2 concentration (c_i) during drought, and determination of minimum functional c_i. *Plant Physiology* **111**, (179–185).

Brodribb, T. J., and Hill, R. S. (1998). The drought physiology of a diverse group of Southern Hemisphere conifer species is correlated with minimal seasonal rainfall. *Functional Ecology* (in press).

Ewers, F. W., and Schmidt, R. (1981). Longevity of needle fascicles of *Pinus longaeva* (bristlecone pine) and other North American conifers. *Oecologia* **51**, 107–115.

Giles-Frankle, W., Hopkins, D. L., and Stall, R. E. (1993). Ingress of the watermelon fruit blotch bacterium into fruit. *Plant Disease* **77**, 1090–1092.

Harden, G. J. (1990). 'Flora of New South Wales.' Vol. 1. (New South Wales University Press: Sydney.)

Hill, R. S. (1990). *Araucaria* (Araucariaceae) species from Australian tertiary sediments—a micromorphological study. *Australian Systematic Botany* **3**, 203–220.

Hill, R. S., and Carpenter, R. J. (1991). Evolution of *Acmopyle* and *Dacrycarpus* (Podocarpaceae) foliage as inferred from macrofossils in south-eastern Australia. *Australian Systematic Botany* **4**, 449–479.

Ishibashi, M., and Terashima, I. (1995). Effects of continuous leaf wetness on photosynthesis: adverse aspects of rainfall. *Plant, Cell and Environment* **18**, 431–438

Jeffree, C. E., Johnson, R. P. C., and Jarvis, P. G. (1971) Epicuticular wax in the stomatal antechamber and its effects on the diffusion of water vapour and carbon dioxide. *Planta* **98**, 1–10.

Jones, H. G. (1992). 'Plants and Microclimate.' (Cambridge University Press: Cambridge.)

Jordan, G. (1995). Extinct conifers and conifer diversity in the Early Pleistocene of western Tasmania. *Review of Palaeobotany and Palynology* **84**, 375–387.

Körner, Ch. (1988). Does global increase of CO_2 alter stomatal density? *Flora* **181**, 253–257

Körner, C., Neumayer, M., Menendez-Riedl, S. P., and Scheel, A. (1989). Functional morphology of mountain plants. *Flora* **182**, 353–383

Martin, J. T., and Juniper, B. E. (1970). 'The Cuticles of Plants.' (Edward Arnold Publishers. Edinburgh: Great Britain.)

Meidner, H., and Mansfield, T. A. (1968). 'Physiology of Stomata.' (McGraw-Hill: London.)

Meng, F. R., Bourque, C. P. A., Belczewski, R. F., Whitney, N. J., and Arp, P. A. (1995). Foliage responses of spruce trees to long-term low-grade sulphur dioxide deposition. *Environmental Pollution* **90**, 143–152.

Mihaich, C. M. (1989) Leaf and epicuticular waxes in the taxonomy of the Epacridaceae. PhD Thesis, University of Tasmania.

Mott, K. A., Gibson, A. C., and O'Leary, J. W. (1982). The adaptive significance of amphistomatic leaves. *Plant Cell and Environment* **5**, 455–460.

Parlange, J., and Waggoner, P. E. (1970). Stomatal dimensions and resistance to diffusion. *Plant Physiology* **46**, 337–342.

Peat, H. J., and Fitter, A. H. (1994). A comparative study of the distribution and density of stomata in the British flora. *Biological Journal of the Linnean Society* **52**, 377–393.

Smith, W. K., and McClean, T. M. (1989). Adaptive relationship between leaf water repellency, stomatal distribution, and gas exchange. *American Journal of Botany* **76**, 465–469.

Van der Water, P. K., Leavitt, S. W., and Betancourt, J. L. (1994). Trends in stomatal density and 13C/12C ratios in *Pinus flexilis* needles during the last glacial-interglacial cycle. *Science* **264**, 239–243.

Van Gardingen, P. R., Jeffree, C. E., and Grace, J. (1989). Variation in stomatal aperture in leaves of *Avena fatua* L. observed by low-temperature scanning electron microscopy. *Plant Cell and Environment* **12**, 887–898.

Wells, P.M., and Hill, R.S. (1989*a*). Leaf morphology of the imbricate-leaved Podocarpaceae. *Australian Systematic Botany* **2**, 369–386.

Wells, P.M., and Hill, R.S. (1989*b*). Fossil imbricate-leaved Podocarpaceae from Tertiary sediments in Tasmania. *Australian Systematic Botany* **2**, 387–423.

Woodward, F. I. (1987) Stomatal numbers are sensitive to increases in CO_2 from pre-industrial levels. *Nature* **327**, 617–618.

Ylimartimo A., Paakkonen E., Halopainen T., and Rita H. (1994). Unbalanced nutrient status and epicuticular wax deposition of Scots pine. *Canadian Journal of Forest Research* **24**, 522–532.