Ecology and Ecophysiology of Subantarctic Campbell Island Megaherbs

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Abstract

The megaherb growth form is not common in the New Zealand flora; yet it is a distinctive feature of the flora of New Zealand's subantarctic islands, such as Campbell Island (52°33'S, 169°09'E). It occurs in four genera: *Pleurophyllum, Stilbocarpa, Bulbinella* and *Anisotome*. Their unusually large form and striking colourful flowers have long been commented as possible relics of a more widespread flora or possibly more recently dispersed and adapted to the present conditions. This research focussed on how well they are adapted to their environment and how adaptable they may be to novel conditions using ecophysiological methods. The breeding system of *Pleurophyllum* was also looked at as an example of the development of reproductive systems in an isolated island environment.

Biomass allocation patterns were investigated in two species, *A. latifolia* and *P. speciosum* in order to determine whether this growth form was extraordinary compared with other herbaceous perennials. Six shoots of each species were harvested on the island, and sorted into components (leaf, stem, rhizome, reproductive) and dried back on the mainland. Leaf:stem ratios were found to be higher in both species than other perennials. This may be a response to ensure adequate resource harvesting i.e. light, water, nutrients, in an exposed, competitive environment.

Gas exchange was studied in different light and temperature regimes using a growth cabinet in order to determine light and temperature tolerance and possible optima. Nine plants each of subantarctic *Pleurophyllum criniferum*, *Anisotome latifolia*, *Stilbocarpa polaris* (three plants only), New Zealand subalpine *Ranunculus lyallii*, and Chatham Island *Myosotidium hortensia* were exposed to three temperatures (7°C, 17°C, 25°C) and four light levels (0, 150, 380 and 950 μmol m^{-2} s^{-1}). *P. criniferum* had the fastest photosynthetic rate (of 8.6 μmol m^{-2} s^{-1}), followed by *R. lyallii* (7.8 μmol m^{-2} s^{-1}), *A. latifolia* and *M. hortensia* (both with 4.6 μmol m^{-2} s^{-1}) and *S. polaris* (2.1 μmol m^{-2} s^{-1}) (averaged over all light and temperature levels). All species had higher photosynthetic rates at the highest light level. *P. criniferum* did not appear to be inhibited by the high temperatures while *A. latifolia* did. Respiration rates increased with temperature with *A. latifolia* having the highest rate followed by *M. hortensia, P. criniferum, R. lyallii* and *S. polaris*. These results suggest that although the subantarctic megaherbs appear to be well adapted to the low light and cool temperature regime of their environment, they may be more plastic to environmental change at low ranges, especially temperature, than expected.

Experiments were carried out *in situ* on Campbell Island to ascertain the effect of environmental perturbations, using carbohydrate, chlorophyll, and growth analysis of leaves on *P. speciosum*, *P. hookeri* and *P. criniferum*, *Bulbinella rossii*, and *A. latifolia*. The microenvironment around each plant was altered by combinations of reducing light, increasing shelter, increasing nutrients, altering photoperiod and increasing temperature over the course of eight weeks. Most of the treatments had very little effect on the carbohydrate pools of the species suggesting that environments were not limiting growth on the island. However leaf growth in *P. criniferum* increased in reduced light as did leaf growth in *B. rossii*, suggesting etiolation (sensitivity to light). *A. latifolia* showed an increase in carbohydrate pools with increase in temperature, compared with *P. speciosum*. Contrary to expectations increased night length also had a small positive effect on growth. Chlorophyll content remained unaffected by treatment but differed significantly between species (ranging from 56 mg g^{-1} in *A. latifolia* to 149 mg g^{-1} in *B. rossii*). These results suggest that the megaherbs are quite plastic in their responses, relatively unaffected by environmental perturbations.

Megaherbs may be pollen limited in an environment that might be considered unfavourable to insects. 15 plants each of *P. criniferum* (discoid capitulum), *P. speciosum* (rayed capitulum) and *P. criniferum x speciosum* (rayed capitulum) were randomly chosen and four treatments - control, bagged and hand-outcrossed, bagged and hand-selfed, and bagged (no assisted pollination) - individually applied to four scapes on each plant. Capitula were collected at the end of the season and proportion of seed set analysed in the laboratory. *P. speciosum*, the most colourful of the genus, is an obligate out-crosser (*i.e.* self-incompatible). The less colourful *P. criniferum* is autonomously self-compatible, and the hybrid, while being able to set seed autonomously, sets more seed when outcrossed. These results indicate that these plants are not pollen or pollinator limited. Observations showed that small midges were the most active on these plants, although their efficacy was not examined. Their activity was significantly affected by wind. A selection of breeding systems and the existence of a hybrid suggests a capacity for recombination of genetic material and potential for adaptive radiation of species.

This study shows that the subantarctic megaherbs are well adapted to their environment. Their apparent plasticity in physiological responses to environmental, and their range of breeding systems, also indicates that they may be more adaptable to novel environments than previously considered. However, whether they are relicts of an ancient, more widespread flora, or whether they have evolved more recently *in situ* remains unresolved.

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CHAPTER ONE

Introduction

Megaherbs

'Megaherbs', 'pachycauls' (Mabberly, 1979) and 'giant rosettes' (Hedberg, 1964) all describe plants with little or no arborescent growth, and with a limited number of growing points. They tend to have a 'stocky' habit *i.e.* having short internodes, large leaves and large rhizome. Examples of these large plants occur in the Hawaiian islands as silverswords, *Argyroxiphium* (Goldstein *et al.,* 1989) in the slightly more dendroid forms in Kenya as *Lobelia* and *Dendrosenecio* (Schulze *et al.,* 1985) and as *Espeletia* (Monasterio and Sarmiento, 1991) in the Andes. The species mentioned inhabit environments where extremes of temperature occur on a diurnal basis and irradiances are high. Research into their physiology and ecology has shown a wide range of adaptations to cope with these conditions including pubescence (e.g. Meinzer and Goldstein, 1985), rosette form (e.g. Hedburg, 1965), leaf orientation (e.g. Melcher *et al.,* 1994) and large pith (Goldstein *et al.,* 1984). While diurnal temperatures are more extreme in the alpine tropics comparisons have been made with this climate and that of the New Zealand subalpine and subantarctic (Troll, 1960; Mark *et al.,* 2000), and with the plant life forms found there (e.g. Hedberg, 1964). One representative occurs on the Kerguelen Islands: the Kerguelen cabbage, *Pringlea antiscorbutica* (Aubert *et al.,* 1999). In the subantarctic islands of New Zealand, the growth form is a common element of the vegetation and is represented by four genera. Here it is a herbaceous growth form of typically large size (up to 1 m high), with a brightly coloured floral display and stout rhizome and occurs in the following species: *Pleurophyllum speciosum*, *P. criniferum*, *P. hookeri*, *P. criniferum x speciosum*, *Bulbinella rossii*, *Anisotome latifolia*, *A. antipoda*, *A. latifolia x antipoda*, *A. acutifolia* and *Stilbocarpa polaris,* all of which are endemic to the Southern Ocean.

New Zealand Subantarctic Islands and Flora

The subantarctic islands are a group of exposed islands lying in the Southern Ocean between 47°40'S and 52°38'S latitude and incorporating the Bounty Islands, The Snares, Antipodes Islands, Auckland Islands and Campbell Island (Figure 1). Their weather is characterised by cool but relatively stable temperatures, strong winds, a high degree of cloud cover and frequent precipitation giving a high degree of humidity (De Lisle, 1965).

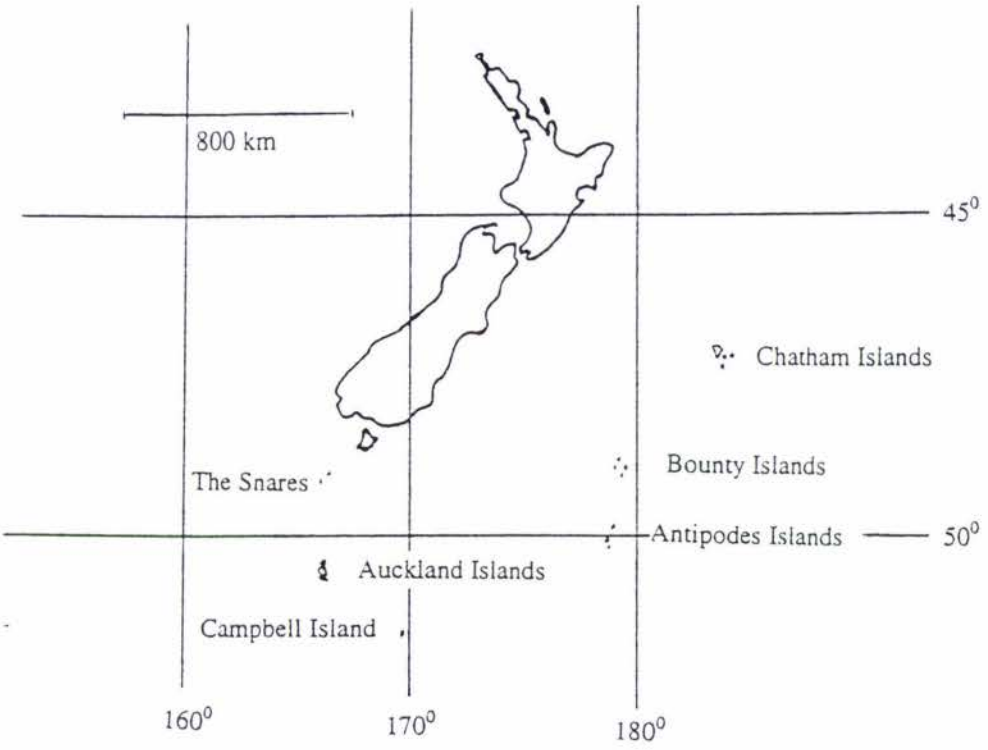

Figure 1: The subantarctic islands in relation to New Zealand. Campbell Island lies 700km south of New Zealand.

The vegetation of the subantarctic islands has been a source of great interest and centre of research since the expeditions of the early botanists (Hooker, 1844; Buchanan, 1883; Cockayne, 1903) and this continues to the present day (e.g. Godley, 1982; Meurk *et al.*, 1994). The reason for such interest lies in the contrasting features between these southern islands and the flora of the mainland, leading to much speculation and debate concerning their origin and development (e.g. Wardle, 1978; Godley, 1979; Lloyd, 1985). The megaherb growth form is considered unusual on the mainland, where the flora is characterised by diminutive flower form and pale colour and the herbs are generally small. Only a few examples of megaherbs occur outside the subantarctic: *Ranunculus lyallii, Anisotome lyallii* and *Aciphylla* species on the main islands and *Stilbocarpa* *robusta* and *S. lyallii* on Stewart Island, and the Chatham Island forget-me-not, *Myosotidium hortensia*.

There are several areas of intrigue surrounding the megaherb in the subantarctic context. Of interest is the reason for the abundance of this growth form in the subantarctic flora (and conversely its absence in the mainland flora), and how well suited it is to a subantarctic environment - *i.e.* one that is very windy, cool, often overcast and wet. Climate change, isolation and the shifting zone of the Antarctic Convergence (Walton, 1984) may all have influenced the evolution of the flora of these islands. This growth form may represent relicts of a more widespread pre-glacial flora (Godley, 1975), which disappeared from the mainland during less suitable conditions, the islands being possible remnants of a larger subcontinental landmass (Gressitt *et al.,* 1964). Alternatively, they may have dispersed there from neighbouring land masses and evolved *in situ* (Lloyd, 1982).

This study was based on Campbell Island, 52°33'S, 169°09'E, the southernmost of the New Zealand islands, situated 700 km south of Bluff, New Zealand (Figure 2). It receives a mean annual rainfall of 1360 mm distributed throughout the year (a mean of 265 annual raindays) and a mean wind speed of 32 km hour^{-1} (9.2 m s^{-1}). The mean annual temperature is 6.9°C while the mean monthly maximum temperature for the warmest month is 15.9°C and mean minimum for the coldest month is -2.7°C. Although sunny, bright days can occur the island is usually overcast receiving less than 1 hour of sunshine a day for 215 days of the year [De Lisle, 1965]) and only a mean of 659 hours of sunshine a year (NZ Meteorological Service, 1983).

The vegetation of Campbell Island, predominantly consists of *Dracophyllum* shrubland with mixture of *Myrsine* and *Coprosma*, and tussockland of *Chionochloa antarctica* and *Poa litorosa*. Megaherb meadows are scattered over various areas of the island consisting of *Pleurophyllum*, *Anisotome* and *Stilbocarpa* and other species (for a more detailed vegetation description of the island see Meurk and Given (1990) and Meurk *et al.,* [1994]). Megaherb species have quickly recolonised these areas from their previously restricted habitat of rocky ledges, with the removal of sheep (Meurk, 1982).

The development of the megaherb form in what is the equivalent of a subalpine/alpine environment (Bliss, 1979) is of interest. Altitude generally governs plant life forms by imposing increasingly harsh regimes of desiccation and temperature (Wardle, 1974). As temperatures decrease with altitude canopy height generally becomes lower allowing the prostrate plants to exploit the relatively warmer temperatures at ground level (Wardle, 1974). 'Phytomicroclimates' are created around plants and are influenced by the growth form - leaf arrangement, size, shape, pubescence - and may allow an increase in plant temperature more than surrounding air aiding metabolic processes (Billings, 1974). As alpine plants have relatively high respiration rates (Billings, 1974), carbon balance - net gain of photosynthesis over respiration - is also crucial at these altitudes. The gain during the growing season must outweigh both the structural and winter maintenance costs (Wardle, 1974) and so taller vegetation gives way to low woody shrubs which are in turn replaced by herbaceous plants as altitude increases.

Alpine environments are more extreme than subantarctic environments with below freezing temperatures, possibility of desiccation, and possibility of high temperatures during the day. The subantarctic climate in comparison is more equable and temperate. While low temperatures and strong winds may not favour woody vegetation, the high humidity might allow for the larger leaf form of the megaherb.

The Megaherb Growth Form

A study investigating the 'megaherb' growth form may require some justification for the term megaherb and what might typify a megaherb. Biomass allocation patterns such as shoot:root or shoot:stem ratios may indicate a characteristic megaherb feature. In such plants one might expect a significantly large proportion of biomass attributed to foliage compared with other herbaceous plants. A study of tissue allocation patterns of *P. speciosum* and *A. latifolia* (Chapter 2) explores the possibility of extraordinary allocation patterns and may give some indication into the advantages this growth form has in the subantarctic environment.

Floral Biology

In general, islands are noted for their lack of zygomorphic, tubular and brightly coloured flowers (Carlquist, 1966) *i.e.* flowers that attract specialist pollinators such as birds, butterflies, long-tongued bees. Instead the flowers are usually small, pale and simple, attracting a range of generalist pollinators, usually diptera, moths or beetles. This is especially so in the New Zealand environment (Lloyd, 1985). It is unusual then, given this trend, to find brightly coloured flowers on the subantarctic islands where, in accordance with the windy, cool, wet environment one would expect a high degree of autogamy, self-compatibility and wind pollination (Leppick, 1977). In Chapter 3 I investigate the pollination and breeding systems of the showiest genus, *Pleurophyllum: P. criniferum* and *P. speciosum* - and their hybrid - *P. criniferum x speciosum.*

The Plasticity and Adaptability of the Megaherb

Over time, after initial colonisation of islands, plants may undergo adaptive radiation (sometimes resulting from stochastic processes) (Barrett, 1996) to fit different niches offered by the new environment and climate. Alternatively, during the course of their evolution they may also suffer bottlenecks (Vitousek, 1988; Barrett, 1996) and consequently have smaller levels of genetic variation than mainland sister species (e.g. Barrett and Husband, 1990). This may actually reduce their ability to adapt to new environments.

If genetic variation is limited it might be expected that these species are restricted in the range of environmental conditions *i.e.* cool temperatures, low light levels and high winds. If exposed to light regimes above their optima, photoinhibition (damage to the photosynthetic apparatus) can result (Bjorkman, 1981a), while high temperatures might push respiration rates beyond economic levels (Bjorkman, 1981b). Under novel conditions such as controlled environments and environmental manipulation *in situ*, these possibilities may be examined using gas exchange measurements and carbohydrate analysis giving some insight into environmental optima and possible plasticity of the species (Chapters 4 and 5). Gas exchange studies have the advantage of providing a measure of a plant's immediate response to its environment (e.g. Korner and Diemer, 1987). Study of carbohydrate use on a daily basis, on the other hand, may give an indication of daily respiration loads on the plant, indicating the potential for growth. By using environmental manipulation *in situ* this type of study can be taken a step further to look at such factors as environmental optima and species plasticity. This may also aid the understanding of how they survived past climate change and may survive it in the future as well as suggesting how they might perform in cultivation.

Growing Interest in Megaherbs

Latterly, the subantarctic islands have become a popular attraction for visitors fascinated by these far-flung islands and the abundant wildlife. With the increasing profile of the islands, one area of attention has focussed on the megaherbs. Cultivation of these species on the mainland has been attempted with mixed results. *Anisotome*, *Stilbocarpa* and *Bulbinella* survive (pers. comm. Alison Evans) but *Pleurophyllum* has proven the hardest to grow. Yet it is *P. speciosum* in particular that has the most appeal with its broad, corrugated, hairy leaves and rose-purple daisy flowers on multiple stems. It is possible that while some species e.g. *Anisotome*, may be able to adapt to new conditions, *Pleurophyllum* is narrowly adapted to subantarctic conditions with little potential to change. This study investigates these possible limitations.

Aim

The aim of this study is to explore the phenomenon that is the subantarctic megaherb with a view to contribute towards the understanding of their evolution and development. It is hoped that the initial ecophysiological and biological investigations that follow may provide a background for further work in this little understood area.

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CHAPTER TWO

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SHORT COMMUNICATION

BIOMASS ALLOCATION IN SUBANTARCTIC ISLAND MEGAHERBS, *PLEUROPHYLLUM SPECIOSUM* (ASTERACEAE) AND *ANISOTOME LATIFOLIA* (APIACEAE)

Summary: We analysed biomass allocation of *Pleurophyllum speciosum* (Asteraceae) and *Anisotome latifolia* (Apiaceae) to explore the 'megaherb' phenomenon, the apparent importance of large-leaved, colourful forbs on southern oceanic offshore islands. The two species had similar shoot dry weights, with high leaf:stem ratios. Even within the megaherb form there are differences in shoot allocations, with *Pleurophyllum* investing more biomass in rhizome than foliage, compared with *Anisotome.* The megaherb form might be attributable to responses to the physical environment, involving the pre-emption of resources such as light, nutrients, water, or space; alternatively it may be related to the paucity of woody species at this latitude.

Keywords: Megaherb; subantarctic; Southern Ocean; island; allocation; phenology; strategy; leaf:stem ratio.

Introduction

Situated in the Southern Ocean south of New Zealand between 47°40' and 52°38'S latitude are a small and scattered group of volcanic and sedimentary islands, the current representatives of subantarctic landmasses present at these latitudes possibly since the end of the Miocene (Marshall and Browne, 1909). Their flora contains several so-called 'megaherb' species, members of the genera *Pleurophyllum* (Asteraceae), *Anisotome* (Apiaceae), *Bulbinella* (Liliaceae) and *Stilbocarpa* (Araliaceae). These herbaceous perennial forbs have large growth forms (often more than 1 metre high or wide), with large leaves and very colourful floral displays (Hooker, 1844). Their striking growth form (Fig. 1) appears extraordinary compared with other herbaceous perennials, and may be an adaptation to their southern oceanic island environment. Six randomly chosen plants each of *Pleurophyllum speciosum* and *Anisotome latifolia* from Campbell Island were destructively harvested and resource allocations studied. These species were then compared with other herbaceous perennials to identify biomass allocation patterns that might be characteristic of megaherbs.

Methods

Campbell Island lies 700 km south of Bluff, New Zealand at 52°33'S, 169°09'E. It is an 11 000 ha windswept island with moderate rainfall (mean of 1361 mm yr^{-1} distributed throughout the year) and low annual sunshine hours (659). Its oceanic climate results in a mean monthly maximum temperature for the warmest month of 15.9 C and minimum for the coldest month of -2.7 C (NZ Meteorological Service, 1983).

Pleurophyllum speciosum (Hook. f.) is a rosette herb (up to 50 cm high and 100 cm wide) with large (up to 75 cm or more wide), corrugated leaves. A single plant may produce up to 16 scapes containing 10 or more capitula with pink florets, which are pollinated by insects and possibly also wind (*pers. obs.*). While considered to be evergreen, the plant reduces in size over winter, with the outer leaves dying off, and spring regrowth is possibly supported by the large rhizome. *Anisotome latifolia* (Hook. f.) is a taller (70 cm), evergreen plant with long petioles supporting the pinnate laminae; one crown produces usually one dioecious scape. The habitat of these megaherbs varies from high altitude turf-meadow to maritime megaherb-tussock grassland (Meurk, Foggo and Wilson, 1994b).

The sampled habitat of *Anisotome* and *Pleurophyllum* was an open subalpine environment comprising tussocks (*Chionochloa* and *Poa*) and occasional shrubs of *Dracophyllum, Coprosma, Myrsine* and *Polystichum (*bordering *'Poa litorosa meadow and Chionochloa'*, and 'Tundra mosaic' as mapped by Meurk and Given (1990)). The site was at approximately 52° 33'S and 169° 09'E at approximately 140 m above sea level.

Six randomly chosen plants (with consideration to extraction logistics) of both species were excavated and destructively harvested in early February 1997.

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a)

b)

Figure 1: *(a) A group of* Pleurophyllum speciosum; *(b) a lone* Anisotome latifolia.

Roots proved difficult to harvest and were omitted from further analysis. Harvest of the megaherbs was after peak flowering time; the flowers had begun to dehisce in *Pleurophyllum* and the male scapes of *Anisotome* were beginning to rot; consequently some scape material could not be collected. The plants were sorted into foliage, reproductive matter (scapes and capitula), rhizome, and stem, and weighed fresh. They were then laid out to air-dry prior to transportation back to New Zealand.

The lamina:petiole ratio depicts allocations within the leaf to dedicated supporting *vs.* light harvesting tissue. While the lamina-petiole boundary is clear in *Anisotome latifolia*, the petiole in *Pleurophyllym speciosum* was arbitrarily defined as the basal part of the leaf below the expanded blade, which is considerably paler and more hairy than the lamina. In New Zealand each leaf of each plant was subdivided into lamina and petiole; reproductive material was divided into scapes, pedicels and capitula. All material was then dried for 72 hours at 65 C in a vacuum oven (-15atm.) and weighed. The quantity of tissue missing due to other sampling was visually assessed at time of weighing and values for the weighed portions adjusted.

Statistical analysis of tissue weights and allocations was by Analysis of variance (d.f. = 10) using SYSTAT (SYSTAT, 1992). Differences in allocations between large (assumed to be older) and small (assumed to be younger) leaves were examined by ranking leaves by size, with each plant standardised over the range 0 - 1. For mean lamina:petiole ratio, leaves of each plant were first grouped into quartiles by biomass.

Results

Pleurophyllum and *Anisotome* had contrasting fresh:dry weight ratios (*Pleurophyllum*: mean ± S.E. = 10.13 ± 0.43, *Anisotome*: 5.75 ± 0.66), indicating that *Pleurophyllum* is more succulent.

Table 1: *Mean tissue allocations (% of shoot dry mass), and leaf:stem and lamina:petiole mean ratios ± standard errors. Root material is omitted because of harvest difficulties, but subterranean rhizomes were excavated. Reproductive tissues include scapes and pedicels.*

	Proportion of shoot dry mass (%)				Dry mass ratio	
Species	Stem	Rhizome	Leaf	Reproductive	Leaf:stem	Lamina:petiole
Pleurophyllum speciosum	1	20	52	27	61.55 ± 9.29	5.45 ± 0.50
Anisotome latifolia	2	8	81	9	61.19 ± 10.42	1.80 ± 0.18

The two species of megaherb did not differ significantly in their total shoot dry weights (rhizome, stem, reproductive and foliage tissue), being 220 g per plant for *Pleurophyllum* and 180 g for *Anisotome* ($P = 0.18$, Error M.S. = 2287.7). There is very little variation in dry weight between the individual plants measured (*Pleurophyllum:* S.E. = 16.69; *Anisotome*: S.E. = 21.99).

Reproductive tissue (including scape) in *Pleurophyllum* makes up three times the proportion of shoot compared with *Anisotome* (Table 1). *Anisotome* is dioecious, and as collection occured late in the summer when male flowers were dying off not all reproductive material was sampled. The single female plant has a notably higher allocation to reproductive biomass (20%) compared with an average of 6% for the male plants.

Allocations to the non-reproductive shoot are comparable between the two species (mean ± S.E. for *Pleurophyllum* = 162g ± 15.96, and 163g ± 7.93 for *Anisotome;* $P = 0.9$, Error M.S. = 1728.9), though *Pleurophyllum* has a higher allocation to rhizome ($P = 0.001$, Error M.S. = 17.45), and *Anisotome* invests a high percentage of resources in foliage (Table 1; $P = 0.000$, Error M.S. = 43.85).

Pleurophyllum has similar leaf numbers to *Anisotome* (mean ± S.E. = 17.0 ± 1.5 and 20.5 ± 2.7 respectively, $P = 0.2$, Error M.S. = 28.65). There is a higher proportion of leaves in the lowest biomass class in *Pleurophyllum* compared with *Anisotome*, though the rest of the range of leaf sizes is similar (Fig. 2). A difference in leaf size patterns between the two species is suggested (Kolmogorov-Smirnov, using the combined data for all plants: $P = 0.07$).

Both species have similar leaf:stem biomass ratios (Table 1; $P = 0.98$, Error M.S. = 585.19). *Pleurophyllum* has a mean lamina:petiole ratio of 5.45, three times the ratio for *Anisotome.* In *Anisotome* the laminae of the smallest quartile of leaves contribute a large proportion of the leaf (Fig. 3; $P < 0.001$, Error d.f. = 38, Error M.S. = 1.58). This trend changes during foliage development with the largest leaves placing proportionately more resources into petioles. In *Pleurophyllum* these ratios have a reverse trend, with the laminae dominating the allocations of the largest leaves.

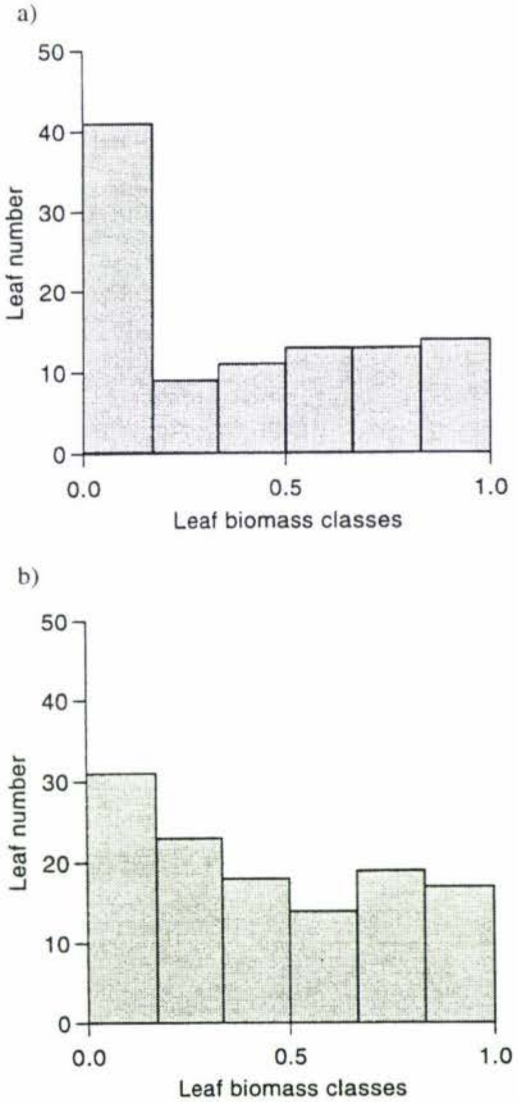

Figure 2: *Frequency distribution of leaf biomass in six classes for (a)* Pleurophyllum *and (b)* Anisotome, *standardised per plant from 0 - 1, and totalled over six plants of each species.*

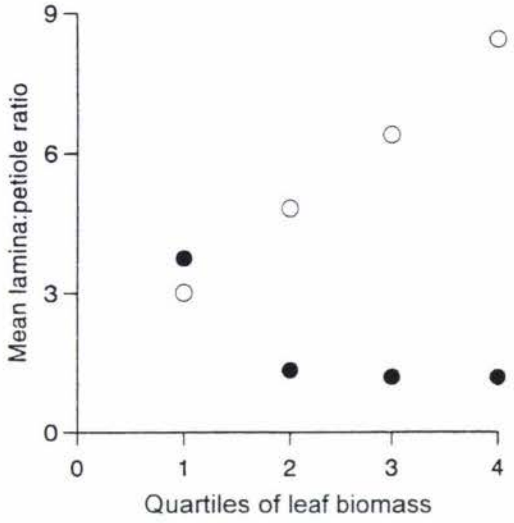

Figure 3: *Mean lamina:petiole ratios for each quartile of leaves when leaves are ranked by biomass from smallest (1) to largest (4) for* Pleurophyllum (○) *and* Anisotome (●).

Discussion

Herbaceous species with large leaves are a conspicuous feature of the vegetation on southern oceanic islands and have long been commented upon by botanists and naturalists (e.g., Hooker, 1844, Cockayne, 1903, Meurk *et al.*, 1994b). Do these species really have unique features that set them apart from other herbaceous species?

Strategies of *Anisotome* and *Pleurophyllum*

The two megaherb species studied have very similar standing crops at the time of harvest (early February, i.e., late summer). They show a higher leaf:stem ratio (61.2 for *Anisotome* and 61.5 for *Pleurophyllum*) relative to other herbaceous plants (2.5 ± 1.65, n = 10; Hickman, 1975; Hickman and Pitelka, 1975; Bostock and Benton, 1979; Abrahamson and Caswell, 1982; Gross, 1983; Jolls, 1984). Allocations to laminae within the leaf show considerable variation between the two species, with the petiole being small in the rosette of *Pleurophyllum*, and an important component of biomass in *Anisotome*.

Rhizome storage of carbohydrates is a key factor in maintenance and possible support of vegetative expansion, at least in alpines (Mooney and Billings, 1960; *c.f.* Hadley and Rosen, 1974). The shoot allocation (excluding reproductive biomass) to rhizome in *Anisotome* is considerably lower than in *Pleurophyllum*. *Anisotome* overwinters at a size comparable with the summer form (with "retained vegetative tissues" - Pugliese and Kozlowski, 1990), so that this species 'exerts much influence on the winter physiognomy of the meadow' (Cockayne, 1903). *Pleurophyllum* sheds its outer leaves and stays in "perpetual somatic youth" (Pugliese and Kozlowski, 1990), reducing from around 75 cm over summer to a winter rosette approximately 25 cm in diameter (Cockayne, 1903). *Pleurophyllum* may then require a rhizome-stored energy supply to support its initial regrowth in the spring. Thus the strategies of the two megaherbs do vary substantively.

A characteristic and competitively successful strategy of tall herbs in open grasslands is rapid vegetative growth during the growing season, often resulting in a large above-ground mass (Al-Mufti *et al.*, 1977); many of the leaves will be formed during that growing season (Yoshie, 1995). *Pleurophyllum* and particularly *Anisotome* may have such a competitive strategy with leaf size distribution, and thus possibly time of initiation, apparently being continuous throughout the growing season. This suggests that any winter cessation of leaf production may be controlled by temperature, rather than intrinsically, with growth of new foliage possibly being responsive at any time of the year to warmer than usual temperatures. In comparison, phenology is more rigidly controlled by the seasons in alpine areas (Mark, 1970), which are more constrained climatically, with shorter growing seasons and more extreme temperatures. The herbaceous form typical of mainland alpine regions is still adaptive in the less extreme southern oceanic island environments.

No autumn flower bud initiation was observed here. This is unlike some New Zealand alpine plants where floral initials are set during the previous growing season and sometimes even at the beginning of the season (Mark, 1970).

Evolution of the megaherb form

Large-leaved forbs are not rare. The mainland of New Zealand has a large orbicular-leaved buttercup, *Ranunculus lyallii* (Hook. f.), leaf diameter up to 30 cm, and an apiad, *Anisotome lyalli* (Hook. f.) with leaf length up to 60 cm (Allan, 1961). *Myosotidium hortensia* (Decne), a large forget-me-not, is endemic to the Chatham Islands (off New Zealand). The Chilean *Gunnera* (lamina diameter 100 cm with a petiole often over 150 cm long), Hawaiian silversword, *Argyroxiphium* (Goldstein and Meinzer, 1983), and Kenyan *Dendrosenecio* and *Lobelia* (Schulze *et al.*, 1985; Fetene *et al.*, 1998) are other examples. Yet the syndrome of the southern islands appears different with corrugations, stereom tissue, hairy and occasionally coriaceous laminae, a rosette form, fleshy root system, and colourful flowers.

Pleurophyllum is endemic to these southern ocean islands, while one species of *Stilbocarpa* extends to southern South Island, New Zealand. These genera contain only macrophyllous forbs. The other putative megaherb species have congeners throughout New Zealand; yet the megaphylls appear to be outside the normal size range of their genera.

While it is possible that the megaherb phenomena might be a chance evolutionary occurrence, megaphylls may convey several possible selective advantages in these subantarctic environments. Nutrient availability could be limiting as it varies with moisture holding capacity and acidity of peat, the main growing medium on the island (Meurk and Foggo, 1988). Large leaves may intercept nutrients from marine aerosols (Meurk *et al.*, 1994a), channelling resources directly to the stem base and onto roots (Enright, 1987; Agnew *et al.*, 1993). Alternatively, Wardle (1991) has suggested that a "greenhouse space" is set up between the large overlapping leaves of *Pleurophyllum,* with leaves acting as solar panels and focussing radiation towards the growing apex. Such temperature increases are as much as 25 C above ambient air in the Hawaiian montane silverswords, *Argyroxiphium* (Melcher *et al.*, 1994). Detrimental effects of cold are further reduced by decreases in wind speed and accompanying reductions in transpiration losses that are associated with the rosette growth form (Regehr and Bazzaz, 1976). Light is also often the limiting resource in open sites with tall herbaceous vegetation (Yoshie, 1995). In *Anisotome* the high allocation to petiole projects the leaves above the surrounding herb canopy, conferring a competitive advantage. By contrast, rosettes, such as *Pleurophyllum*, may suppress other competing herbs with their large rigid leaves.

The megaherb form might also be a response to the paucity of woody species. Yet the environment can sustain these as the tree daisy *Olearia lyalli* occurs on the more northerly of the islands and is invasive on the Auckland Islands (50° 45'S) (Lee *et al.*, 1991). The shrubby *Dracophyllum*, found on most subantarctic islands, including Campbell Island, tends to form thickets but mainly on the coastal fringes, while the Auckland Islands have a species of *Metrosideros*, also growing coastally. Elsewhere, megaherbs are aggressive, actively reoccupying their former habitat since the removal of exotic grazers (Meurk, 1982).

So the megaherb growth form must provide some adaptational advantages via pre-emption of resources and therefore be a derived syndrome. Certainly floral colour (other than white) is a derived element in subantarctic *Abrotanella* (Swenson and Bremer, 1997). Other aspects of the megaherb syndrome might also have evolved *in situ*.

Conclusions

This work is the first biomass allocation study of subantarctic island megaherbs. These species invest a large proportion (50-80%) of their shoot biomass in leaf; consequently, their proportionate rhizome and stem allocation is low. Even though strategies differ within the growth form, e.g., varying allocation within the leaf to lamina and petiole, these results support the suggestion that there is a 'megaherb' phenomenon, a growth strategy different to other herbaceous perennials, enabling these species to be a dominant and characteristic feature of subantarctic vegetation.

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CHAPTER THREE

Pollination in the subantarctic: *Pleurophyllum*

Abstract

The large, brightly coloured flowers of the megaherbs of the southern islands of New Zealand appear unusual in an environment supposedly unfavourable to pollinators. Studies of breeding systems of *Pleurophyllum* involving the out-crossing and selfing of flowers, and the exclusion of pollinators were carried out on Campbell Island, and observations made to determine the frequency of likely pollinators. While the insect life on Campbell Island is not diverse it was found that none of the *Pleurophyllum* species studied appeared to be pollen limited. *Pleurophyllum* shows an assortment of breeding systems. *P. speciosum*, the showiest, ray-flowered species, is an obligate out-crosser, showing no evidence of being self-compatible at all. The discoid *P. criniferum* is autonomously self-compatible, being able to set as much seed enclosed in a bag as with hand-selfing. The hybrid, *P. criniferum x speciosum*, shows partial self-compatibility and autonomous self-pollination, although sets more seed when out-crossed. It is suggested that a variety of breeding systems in *Pleurophyllum* provides a capacity for recombination of genetic material and potential for adaptive radiation.

Introduction

From as early as 1903 (Hutton, 1903; Cockayne, 1903) there has been considerable interest in the apparent disparity between the flowers of the mainland of New Zealand and those of the southern islands. The flora of the mainland is known for having generally 'unspecialised' floral characteristics (Lloyd, 1985). The flowers are usually small, pale and easily accessible and attract a wide range of relatively unspecialised groups of insects: Diptera, Lepidoptera and short-tongued bees. There are few butterflies and no long-tongued bees. The subantarctic flowers on the other hand, are known for their relatively large size, and large, colourful floral displays. These displays are normally associated with animal pollination (Godley, 1979), yet these species occur in a climate considered unfavourable to pollinators (Lloyd, 1985). On the Cape Expedition (1909) Hudson observed: "Campbell Island, in November at least, is not a good place for insects". Subjected to high winds and cool temperatures, the subantarctic islands are an environment in which one might expect more self-compatibility, autogamy and wind-pollination (Leppick, 1977).

The genus *Pleurophyllum* (Asteraceae) is endemic to the southern islands of New Zealand (the Auckland Islands, Campbell Islands) and Australia (Macquarie Island). It consists of three species - *P. criniferum*, *P. speciosum*, and *P. hookeri* and one hybrid *P. criniferum x speciosum*. All three species and the hybrid grow on Campbell and Auckland Islands (although the hybrid is unconfirmed on the latter) but only *P. hookeri* occurs on Macquarie. They are examples of the so-called "megaherb" flora of these islands, characterised by their large growth form (up to 1 m high), high foliage resource allocation (Nicholls and Rapson, 1999), brightly coloured flowers and stout rhizomes. These herbs are a distinctive feature of the subantarctic herbfields (Mitchell *et al.,* 1999).

Despite the apparent interest in flower colour, few pollinator observations have been carried out on New Zealand's subantarctic islands (Godley, 1982) and this is the first study carried out on pollination of *Pleurophyllum*. This study examines the breeding systems in *Pleurophyllum*, looking at self-compatibility, pollinator requirements and pollinator visitation rates. Two species of *Pleurophyllum* were studied - *P. criniferum*, *P.*

speciosum, and the hybrid *P. criniferum x speciosum* from December 1996 to February 1997.

Study Organisms

All species of *Pleurophyllum* reproduce sexually, but are also capable of limited asexual reproduction by increasing crown number, *i.e.* clumping, particularly in *P.hookeri* (this species was excluded from the study as it had already begun to set seed at the beginning of the study period). They all form rosettes, their leaves arising from the crown. *P. criniferum* is the tallest member of the genus, its leafy flowering scapes reaching 1 m high (Figure 1A). Its floral display consists of a tall scape of rayless, dark brown/maroon capitula suspended on long pedicels. The capitula have an indumentum when in bud. *P. speciosum* is the largest species - its corrugated, hairy leaves are capable of reaching 75 cm or more across. The flowers, on shorter scapes than *P. criniferum*, are the showiest of the genus (Figure 1B), with capitula 3-4 cm in diameter in varying shades of purple, and with the occasional white morph occurring (pers. obs.). The hybrid of these two species, *P. criniferum x speciosum* (Figure 1C), contains elements of both parents, the leaves being larger than *P. criniferum*, but not corrugated, and the capitula rayed, but not as prominently so as in *P. speciosum* and of a more intense maroon. The capitula also have indumentum similar to that of *P. criniferum* that is lacking in *P. speciosum*. In winter *P. speciosum* and *P. hookeri* reduce in size, retaining a small leaf rosette, while *P. criniferum* is completely deciduous.

Numerous capitula of both *P. speciosum* and *P. criniferum* contained Lepidoptera larvae burrowing at the base of the florets (Figure 2). This occurred in a couple of bagged capitulae which were tied on before the florets opened (and which were excluded from the analysis), so are almost certainly not pollinators, but may be seed predators (pers. comm. A. Robertson).

A) B) C)

Figure 1: Flowering inflorescences of A) *P. criniferum* showing the coloured ray florets ; B) *P. speciosum* showing the discoid, pendulous capitula; and C) *P. criniferum x speciosum* showing characteristics of both.

Study Area

The study took place on Campbell Island, one of the subantarctic islands 700km south of New Zealand in the Southern Ocean. It is 11 000 ha in size with a vegetation consisting largely of tussock, herbfields and shrubland with a littoral forest of tall *Dracophyllum* (Meurk and Given, 1990*)*. The atmosphere is damp with a moderate rainfall (mean 1361 mm yr^{-1}) distributed throughout the year, and the annual sunshine hours are low ($659yr^{-1}$). The temperatures reflect its oceanic climate - the mean monthly maximum temperature for the warmest month is 15.9°C and minimum for the coldest month is -2.7°C (NZ Meteorological Service, 1983).

The study populations were in two different sites. The *P. criniferum* population was coastal and associated with *Carex appressa* ('*Poa litorosa* maritime tall tussock grassland' as mapped by Meurk and Given [1990]). *P. speciosum* and *P. speciosum x criniferum* were studied at a higher altitude (120m above sea level) in a more open subalpine environment consisting of tussocks (*Chionochloa* and *Poa*) and occasional shrubs of *Dracophyllum*, *Coprosma* and *Myrsine* and the ferns *Polystichum* and *Blechnum* ("*Poa litorosa* meadow and *Chionochloa*" community as mapped by Meurk and Given [1990]).

Methods

Breeding Systems

The potential for self-compatibility and autogamy in each taxon was tested for experimentally. As the plants came into flower, 15 individuals of each species were chosen and four scapes on each plant were assigned to each of four treatments: hand-selfing ('bagged selfed'), hand-outcrossing ('bagged crossed'), pollinators excluded ('bagged'), and open pollination ('open'). For the hand-selfing and hand-outcrossing treatments, unopened capitula were enclosed in small mesh bags (Figure 3) and hand pollinated with a small paint brush with either the plant's own pollen or a mixture of pollen from other plants of the same species in the vicinity. To attempt to pollinate all florets open at the time, hand pollination was carried out at least twice on the same capitulum over the season as the capitula opened basipetally. Bags were made up of an open mesh of approximately 0.3 mm to allow the passage of wind but not insects. To determine the potential for autogamy, capitula were enclosed inside bags and left unpollinated. The open pollination capitula were left unbagged and untouched.

Capitula were collected in early February after 6 weeks from the start of the study and stored in 95% alcohol until analysis. While most flowers had been pollinated by this time, the achenes were not necessarily well developed. In the lab, the pickled capitulae were cut in half and both faces inspected using a dissecting microscope. Ovaries were recorded either as set, empty, aborted or immature, based on apparent development of the seed. Proportion of seed set was estimated as the number of filled seed/(unfilled seed + aborted seed + filled seed). There was some difficulty in assessing the difference between immature and unfertilised ovules in the centre of each capitula so these inner florets were omitted from analysis. In addition, some heads were considered too young to determine whether ovaries were empty or just too immature. These were also not included in the analysis.

Pollinator observations

Only *P. speciosum* was observed intensively as there was very little pollinator activity on *P. criniferum* or *P. criniferum x speciosum*. Groups of up to 6 capitula were observed for 25 periods of 10 minutes at a time and the number of visiting insects counted. Observations were made at varying times of the day between 9am and 4.30pm in varying overhead conditions (sunny or overcast) and wind conditions (light or moderate), and these conditions recorded. Visitation rates were corrected to visits per head per hour. A small sample of pollinators was collected, and later identified by Dr Ian Andrew, Massey University.

Data analysis

Seed set was analysed by means of a generalised linear model with a binomial error distribution and logit link function using S-PLUS version 4.5 (Mathsoft Inc. 1998). Data were treated as a randomised block design with the plants as blocks. *A posteriori* multiple comparisons of means using the Sidak (1967) method were used to compare the different treatments for each species. Visitation rate analysis was carried out on the log-transformed pollinator visits ($\log_{10}$ visits +1) using ANOVA (SYSTAT, 1992).

Figure 2: Capitula of *P. criniferum* showing burrowing by Lepidoptera larvae.

Figure 3: Pollination bags for hand-selfed, hand-outcrossed, and bagged treatments, attached to individual capitula of different scapes of *P. criniferum x speciosum.*

Results

Breeding Systems

The three taxa differed in their breeding systems and in pollination requirements. *P. criniferum* showed no significant difference between any of the treatments (Table 1; Figure 4A) suggesting full autonomous self-compatibility. In contrast, *P. speciosum*, shows a significant difference between the hand-selfed and the hand-crossed treatments (Figure 4B), indicating that it is strongly self-incompatible. This self-incompatibility is reinforced by its inability to self pollinate in the bagged treatment. The open treatment, where pollinators have free access to the heads, indicates that there is no pollen or pollinator limitation in this species. The hybrid, *P. criniferum x speciosum*, like *P. speciosum*, shows a significant difference between outcrossing and selfing (Figure 4C) but shows partial self-compatibility and is capable of autonomous self-pollination in bagged flowers. In none of the species does there appear to be any significant difference between the open-pollination treatment and hand-pollination suggesting there is no pollen limitation of seed production.

Of interest is the apparent higher (although more variable) seed set in the hybrid compared with the others. *P. criniferum x speciosum* has 43% achenes filled overall, followed by *P. criniferum* with 31% and *P. speciosum* has the lowest rate at 20%.

Pollinator observations

The visitation rate by all insects to *P. speciosum* was 6.2 visits per head per hour. Four types of insect were observed: a moth (unidentified), a hoverfly (*Melangyna novaezealandiae*, family Syrphidae), and 3 small midge-like flies (*Australimyza anisotomae*, family Australimyzidae or Carnidae; *Tetragoneura minima*, family Mycetophilidae; and Chironomidae species). The latter tended to sit on the corolla for long lengths of time, and make occasional movements onto the florets. The efficacy of these pollinators was difficult to determine although the resident pollinators clearly were efficient pollinators as the pollination experiment gave no evidence of pollen limitation in these species. Blowflies were very numerous in the vicinity, but on only one occasion was one observed on a flower.

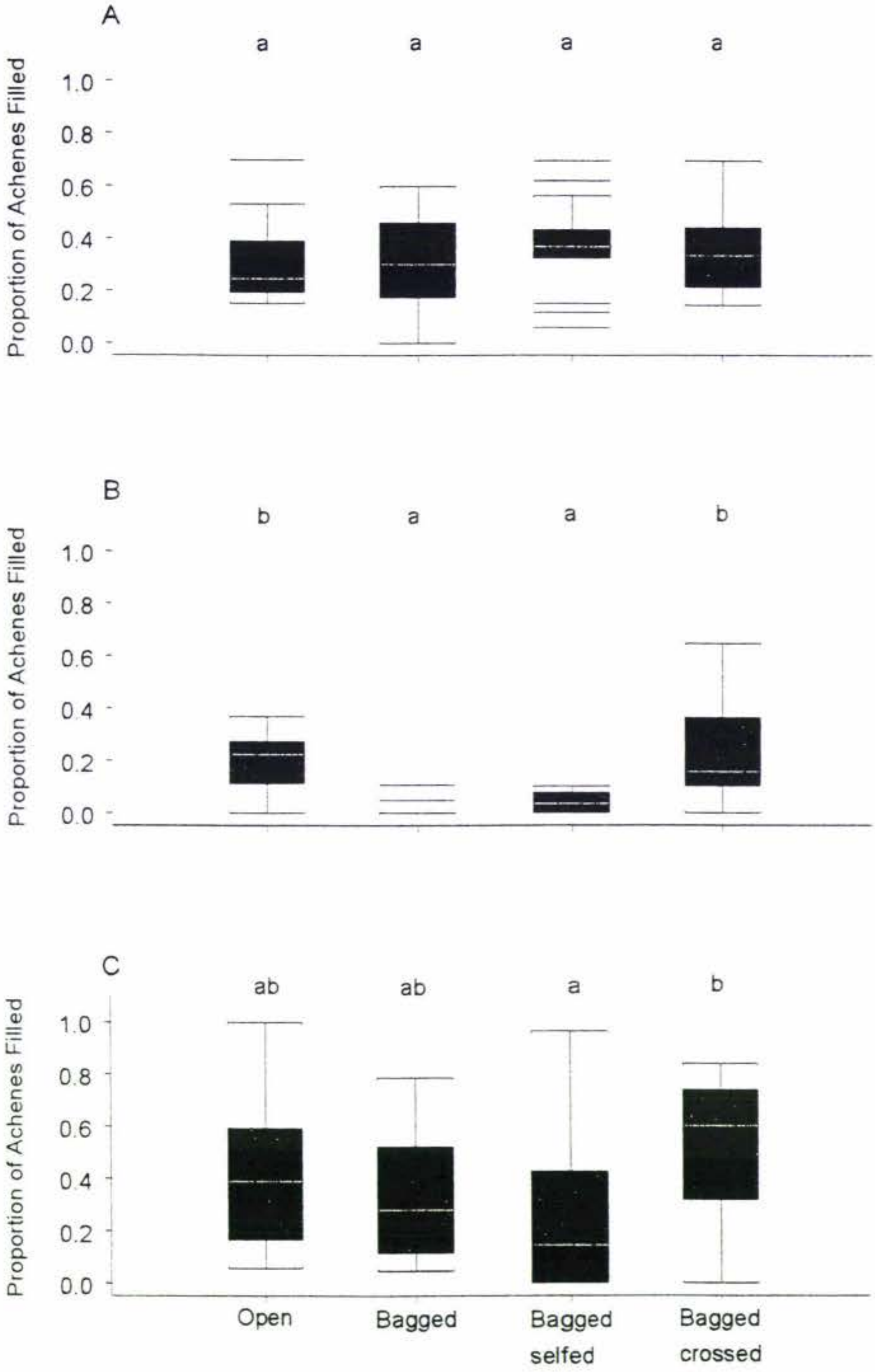

Figure 4: Boxplots showing proportion of achenes filled in each treatment for A) *P. criniferum*; B) *P. speciosum;* and C) *P. criniferum x speciosum.* Medians, upper and lower quartiles, the range and outliers are depicted. Within a species, shared letters indicate means that are not significantly different as shown by *a posteriori* tests.

Table 1: Binomial ANOVA of the treatments (open, bagged, bagged selfed, bagged crossed) on three *species P. speciosum, P. criniferum, P. criniferum x speciosum* (separately analysed).

Model	df	Deviance	Residual df	Residual deviance	% explained	P
P. criniferum						
Null			57	245.06		
Plants	14	74.06	43	171.00	30.2	<0.0001
Treatment	3	3.33	40	167.67	1.4	0.3436
P. speciosum						
Null			42	210.21		
Plants	13	91.75	29	118.46	43.7	<0.0001
Treatment	3	70.19	26	48.27	33.4	<0.0001
P. speciosum x criniferum						
Null			47	502.200		
Plants	13	162.32	34	339.87	32.3	<0.0001
Treatment	3	89.94	31	249.93	17.9	<0.0001

Wind conditions (light or moderate) significantly affected visitation rates while overhead conditions (sunny or overcast) did not quite (Table 2; Figure 5) The combination of sunny conditions with light wind however was the most favourable for pollinators, followed by overcast with light wind, sunny with moderate wind, and finally overcast with moderate wind (Figure 5).

Table 2: ANOVA on effect of overhead (sunny or overcast) conditions and wind (light or moderate) conditions on log-transformed pollinator visits ($\log_{10}$ visits + 1). $R^2 = 0609$ *i.e.* 61% of the data was explained by the analysis.

Source	Sum-of-squares	df	Mean-square	F-ratio	P
Overhead conditions	0.465	1	0.465	4.038	0.058
Wind conditions	2.930	1	2.930	25.458	0.000
Overhead*Wind conditions	0.165	1	0.165	1.431	0.245
Error	2.417	21	0.115		

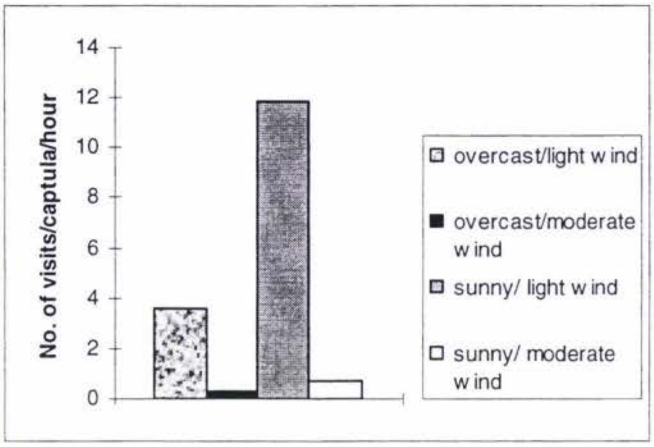

Figure 5: Least square adjusted means (back transformed) of visitation rates under different pollinating conditions.

Discussion

Self-incompatibility

Each of the subantarctic island megaherb species tested appears to have a different breeding system. *P. criniferum* is fully self-compatible and autonomously self-pollinating, in contrast to *P. speciosum* which is an obligate out-crosser, while the hybrid, *P. criniferum x speciosum*, sets more seed when out-crossed yet is also capable of some autonomous self-pollination. Of interest is the apparent higher rate of seed set in the hybrid than in the other species which may be a result of possible hybrid vigour. In general, self-fertile species have a higher seed set ratio than outcrossing species (Lloyd, 1987). However, it is not known whether the seeds from the hybrid are fertile.

The apparent relatively low level of self-incompatibility reported in the New Zealand flora may be connected with the generalised nature of New Zealand's pollinators (Webb and Kelly, 1993), although the true level of self-incompatibility is unknown, and almost certainly under reported (pers. comm. A. Robertson). It is therefore surprising to find self-incompatibility on an island where the pollinators are even less diverse. This may be ancestral as there is often a high degree of self-incompatibility in members of the Asteraceae (Burtt, 1977).

Self-compatibility may evolve from self-incompatibility in environments where selection pressure exists from lack of pollinator or compatible pollen (Wyatt, 1983) such as frontier habitats where population sizes are small, or where cross pollinator mechanisms may be under strain e.g. from 'excessive moisture in northern and oceanic regions' (Stebbins, 1970) such as might be found in these southern islands. An increase in altitude may also cause an increase in self-compatibility accompanying a decrease in pollinator activity (Billings, 1974; Arroyo *et al.,* 1982; Berry and Calvo, 1989). *P. criniferum*, may have evolved self-compatibility as a way of avoiding pollinator reliance in a climate seen as unfavourable to pollinators *i.e.* windy, often overcast, cool.

But while potential pollinators appear to be limited, they do play a significant role in the reproduction of *Pleurophyllum. P. speciosum* appears to be reliant on floral visitors, and pollinator observations confirm high visitation, the only species that requires a pollinator. Both *P. criniferum* and *P. criniferum x speciosum* show self-compatibility and both taxa show high degrees of self-pollination. Despite these differences in pollinator requirements there is no evidence of pollen limitation in any of the species, as hand-pollination did not increase the proportion of ovules that set in any of the taxa.

A wide range of seed set occurs in the Asteraceae with recordings up to 94% (Burd, 1994), but lower rates are not uncommon. Carr *et al.* (1986) found a range of 6-84% and 34-73% in assisted outcrossing pollination studies of the Hawaiian *Argyroxiphium sandwicense* subsp. *macrocephalum* and *Wilkesia gymnoxiphium* (both self-incompatible species). McMullen (1987) found 34% and 41% in the control (open-pollination) plants of the self-compatible Galapagos Island *Adenostemma platyphyllum* and *Bidens pilosa*. In a study of *Aster* species the majority of species examined had between 20-30% seed set, even with assisted pollination (Jones, 1978), and Andersson (1991) found 27% in *Achillea. Pleurophyllum* has similar low seed set rates, which may be due to resource limitation preventing development of more ovules as neither of the species were pollen limited.

Pollination

Despite the apparent paucity of pollinators on Campbell Island, there were a number of visits made to *P. speciosum*. The visitation rate of 6.2 visits per capitula per hour or 10.4 visits/capitula/100 minutes to *P. speciosum* is moderately high. A range of visitation rates has been recorded in Asteraceae. Berry and Calvo (1989) reported an average of 1.8 visits/capitula/100 minutes (over 13 species), ranging from 0.01-7.8 visits/capitulum/100 minutes in the Andean tropical alpine *Espeletia* while Andersson (1991) reported 100-1000 visits/capitulum/100 minutes for *Achillea ptarmica*.

Diptera and Hymenoptera generally account for a large proportion of pollinators of Asteraceae (Leppick, 1977). On Campbell Island, insect pollinators are likely to include Lepidoptera as well, as there are no native Hymenoptera (Godley, 1982). Gressitt *et al.*

(1964) found that the invertebrate fauna in general was not as limited as initial studies have suggested. But Godley (1982), studying pollination in *Gentiana* in the subantarctic, did not observe any insect visitation on *Gentiana*, but did observe one hoverfly, *Serphus novaezelandiae* (syn. *Melangyna novaezelandiae),* on *P. speciosum*. On the other hand, *Bulbinella rossii, Dracophyllum,* and *Anisotome latifolia* (pers. obs.) are usually well visited by numerous small flies (Hudson, 1909). While *P. criniferum* and *P. criniferum x speciosum* both presented pollen, very little activity was observed on them.

Solitary bees are uncommon at high altitudes (Billings, 1974; Arroyo *et al.,* 1982) and correspondingly high latitudes; as they are generally more energy demanding. They cannot undergo thermo-regulation as well as other insects where sun basking plays a large part in their daily activities (Arroyo *et al.,* 1982). Indeed, most of the insect visitors observed in this study spent most of their time positioned on the ray florets. Windy, cloudy and moist conditions also inhibit pollinator activity (Stebbins, 1970). In this experiment, pollinator activity was more affected by wind conditions while the combination of sunny conditions with light wind was the most favourable.

An increase in altitude has been shown to decrease pollinator activity (Billings, 1974; Arroyo *et al.*, 1982; Arroyo *et al.*, 1985). While Asteraceae is generally insect pollinated, wind pollination does occur in several genera e.g. *Artemisia* (Leppick, 1977), *Senecio* (Arroyo *et al.,* 1982) and *Espeletia* (Berry and Calvo, 1989). One characteristic of wind pollinated species - hanging capitula - is demonstrated in *P. criniferum.* However, the role of wind in the pollination of this species was not tested for in this study, and it is possible that wind may have contributed to the high apparent rate of autonomy.

The significant differences between the pollinator-excluded and the unmanipulated treatments in *P. speciosum* shows that this species requires a pollinator. There therefore appears to be a return on the relatively larger investment in colour and size associated with pollinator attraction in this species. Conspicuous ray flowers are usually associated with out-crossing in the Asteraceae as they are attractive to pollinators, often female, and the first florets to open (Burtt, 1977). In *Pleurophyllum* ray florets are also female and the disc florets are perfect. Removal of ray petals from *Achillea,* resulted in less visits to the capitula compared with the rayed capitula (Andersson, 1991). Their removal however, had only a small negative effect on seed set indicating their removal had a greater effect on the male component in pollination (Andersson, 1991). This suggests that ray petals are efficient attractants for pollinators and are structures worthwhile investing in. Conversely, in the species lacking the bright ray florets, *P. criniferum*, self-compatibility is evident and may be a comparatively recently evolved characteristic, avoiding pollinator reliance in the subantarctic environment. A combination of the two species, as embodied in *P. criniferum x speciosum*, may enable maintenance of a wider gene pool increasing potential for adaptive radiation.

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CHAPTER FOUR

Impact of *in situ* environmental manipulations on megaherb carbohydrate, chlorophyll and growth levels

Abstract

Megaherbs are a characteristic feature of New Zealand's subantarctic island flora. They are large herbaceous perennials with large leaves, and bright, colourful floral displays making a striking contrast amongst the tussocks. The growth form is not common in mainland New Zealand and some megaherb species such as *Pleurophyllum*, fail to survive when cultivated. This study looked at possible limitations to megaherb growth by investigating ecophysiological responses of several megaherb species (*Pleurophyllum criniferum*, *P. hookeri*, *P. speciosum*, *Bulbinella rossii*, and *Anisotome latifolia*) to micro-environmental manipulation in the field: decreased light, increased foliar nutrients, increased shelter, increased temperature, and increased night length were studied. Carbohydrate concentration and leaf growth were used as an indicator of physiological tolerance.

B. rossii had the highest carbohydrate pools of all species. *A. latifolia* maintained a higher lamina carbohydrate concentration than *P. speciosum* in all treatments and responded to increased temperature, while *P. speciosum* was relatively unresponsive to increase in temperature. *P. criniferum* showed an increase in the rate of leaf growth in decreased light conditions, while *B. rossii* responded to added nutrients. Increased night length and increased shelter had no apparent effect on any of the species. Seasonal changes in carbohydrate pools within the plants were detected which may be related to phenological developments within the plant, or alternatively, to weather. Carbohydrate pools were rarely consistent with gas exchange work, suggesting more investigation is required. Chlorophyll levels were extremely high in all species compared with the literature although *a:b* ratios

were within the range of temperate species. Overall, the low sensitivity to most of the treatments applied suggest a higher degree of plasticity than expected.

These mixed responses suggest the difficulty in isolating any one factor responsible for growth inhibition on the mainland, and that a combination of environmental factors such as warmer temperatures, different rainfall regime and higher irradiance levels relative to the subantarctic is more likely to affect megaherb growth in cultivation.

Introduction

The flora of New Zealand's subantarctic islands has attracted a great deal of interest since their initial discovery on the early botanical expeditions, to those expeditions of the present day. Botanists of all descriptions, from scientists to gardeners have become fascinated with the large herbs or 'megaherbs': large-leaved species with large and colourful floral displays that are a characteristic feature of the vegetation and not commonly found in mainland New Zealand.

Several attempts to grow megaherbs on the mainland have had mixed successes (pers. comm. Carol West). Buchanan noted on one of the earlier expeditions in 1883 that 'it is doubtful if in the absence of these moist conditions ['moist vegetable soil' and 'fog-shaded atmosphere'] much success will attend the cultivation in New Zealand of plants removed there' (Buchanan, 1883). Three subantarctic species do, however, grow well on the mainland, *Anisotome latifolia*, *Stilbocarpa polaris*, and *Bulbinella rossii*; but the most alluring one - *Pleurophyllum* - has yet to survive New Zealand conditions.

Plants are not necessarily perfectly adapted to their environment (Parkhurst and Loucks, 1972). Their existing environment does not necessarily represent the limits to their potential environmental range as the limited distribution of a species may be purely historical, e.g. an artefact of dispersal (a possibility in island populations) (Moore, 1979), rather than be the result of physiological limitation. Experimentally, modifying micro-environmental conditions around megaherbs and measuring the effects of extending the plants' range may provide a key to understanding physiological limits to performance in island conditions with a view to understanding the evolution of the megaherb syndrome. This could also be extrapolated to explain responses to mainland environments, especially failure to thrive.

This study was carried out on Campbell Island over a period of 10 weeks during the summer months of December to February of 1997/1998. The species studied were *P. speciosum* (Figure 1A), *P. criniferum* (Figure 1B) and *P. hookeri* (Figure 1C), all of which fail to thrive on mainland New Zealand, along with *B. rossii* (Figure 2) and *A. latifolia* (Figure 3) which were chosen as comparative species as these species are able to grow in cultivation on the mainland. They also have natural congeners on the mainland, e.g. *B. hookeri* and *A. lyallii*, which might imply a potential genetic capacity for adaptability at the generic level.

The primary aim of this study was to determine how physiologically sensitive these species are to an altered environment by experimentally altering nutrient levels, light levels, wind, temperature and photoperiod (day length). Carbohydrate (CHO) concentration was chosen as an indicator of plant physiological status - *i.e.* whether sufficient resources for growth are being supplied by a healthy plant, or whether the plant is in resource deficit as a result of stresses imposed by treatments. This method of measuring a plant's response to its environment was preferred to gas exchange measurements for logistic reasons (*i.e.* repeated non-destructive samples could be obtained, easily stored and transported, and no electricity was required). A decrease in light may test the plant's capacity for growth in low light conditions where stress would be indicated by a decline in CHO resources to promote the plant's growth. Additionally, this may suggest a response to high light conditions such as experienced in cultivation on the mainland. An increase in (foliar) nutrients would test whether the plant's physiology is limited by nutrient availability (e.g. Parsons *et al.,* 1994) or whether growth would be unaffected or even inhibited by extra nutrients. A reduction in wind may increase the size of the boundary layer of the leaves, increasing resistance to convective heat transfer and reducing heat loss. Similarly an increase in temperature using a cloche may increase metabolic processes (Bjorkman, 1981). An increase in night length might reduce carbohydrate resources consumed by nocturnal respiration to a level that cannot be replaced during the day.

A) (photo M. Joy)

B) (photo M. Joy)

C)

Figure 1: A) *P. speciosum* (M. Joy photo); B) *P. criniferum* (M. Joy photo); and C) *P. hookeri* (in seeding stage).

A)

B)

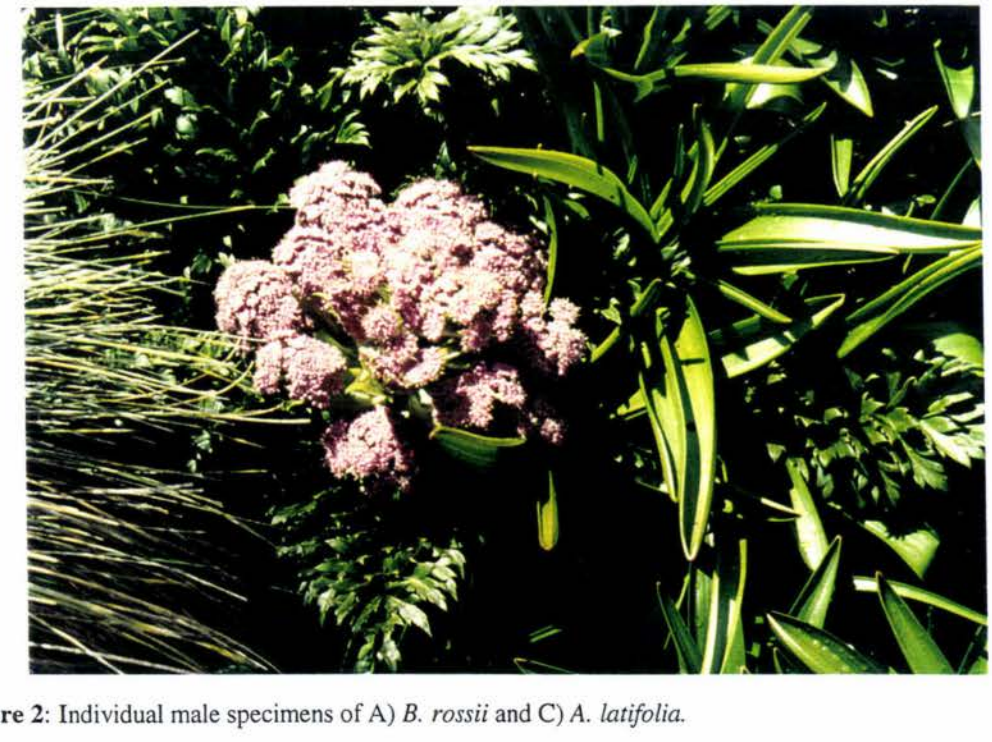


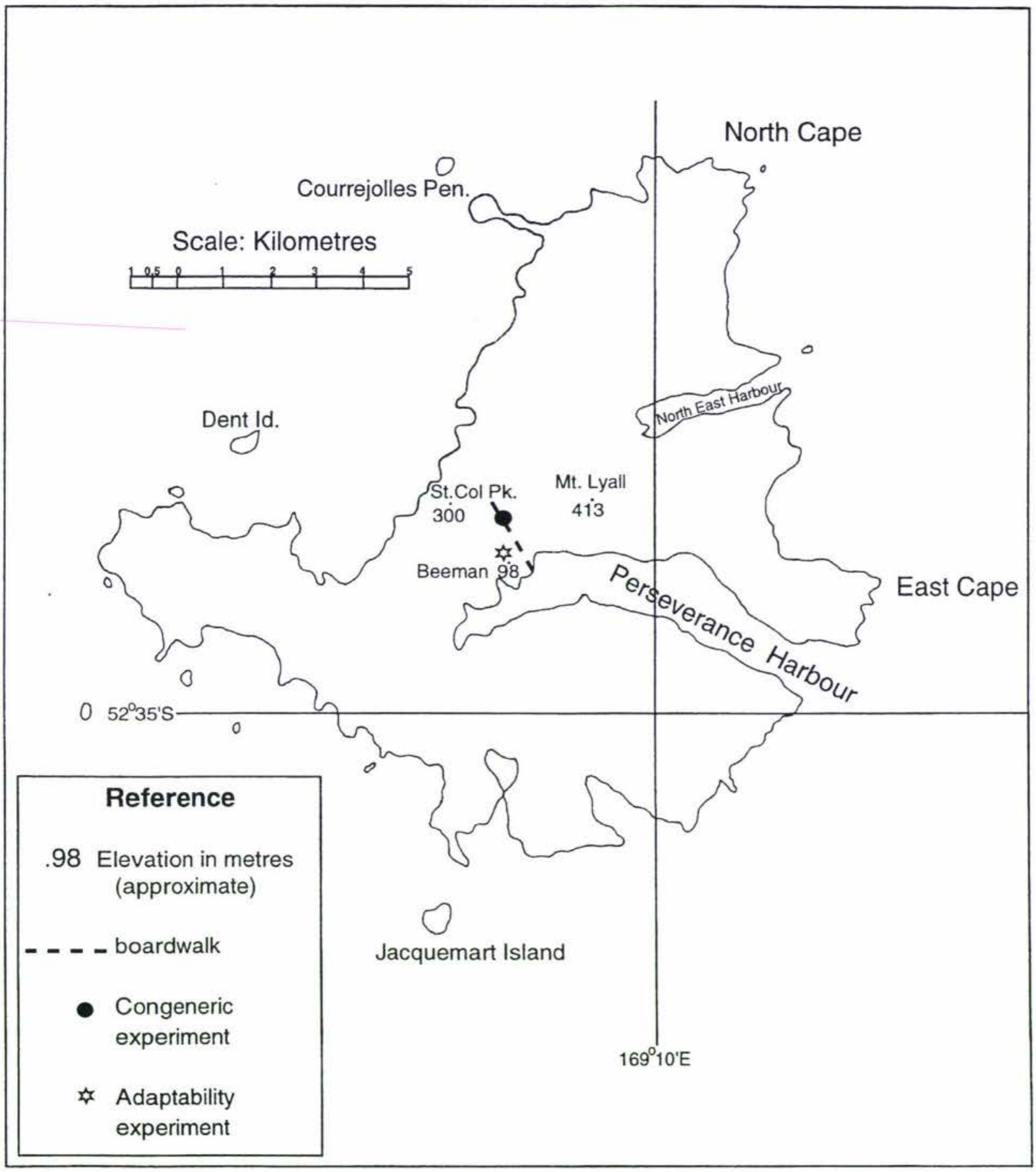
Figure 2: Individual male specimens of A) *B. rossii* and C) *A. latifolia.*

Methods

The study was carried out on 11 000 ha Campbell Island (Figure 4) which lies 700 km south of New Zealand in the Southern Ocean. It has a damp atmosphere with a moderate rainfall (mean 1361 mm yr^{-1}) distributed evenly throughout the year, and low annual sunshine hours (659). The temperatures reflect its oceanic climate - the mean annual temperature is 6.9°C, the mean monthly maximum temperature for the warmest month is 15.9°C and minimum for the coldest month is -2.7°C (NZ Meteorological Service, 1983). The vegetation consists largely of tussock, cushion bogs, herbfields and shrubland with a littoral forest/shrubland of *Dracophyllum* (for a more detailed vegetation description of the island see Meurk *et al.*, 1994a, and Meurk and Given, 1990).

Two experiments, each at a different site (Figure 4), were set up applying different treatments and sampling regimes to various species. Sampling was carried out in the morning, measuring each plant's CHO pool remaining after the night's respiration and representing capital gain for growth. Some plants were also sampled in the evening to measure their CHO gain during the day. The Congeneric Experiment studied the effects of shade (= low light), reduced wind, and increased foliar nutrients on *P. speciosum*, *P. criniferum*, *P. hookeri* and *B. rossii*, while the Adaptability Experiment studied the effects of increased temperature and increased night length on *P. speciosum* compared with the apparently more adaptable *A. latifolia.*

The three *Pleurophyllum* species all have a similar rosette growth form but two types of life cycles. *P. criniferum*, the tallest species, reaching over 1 m, is completely deciduous over winter. *P. hookeri*, the smallest of the genus, and *P. speciosum*, the most colourful, remain evergreen, although reduce in size. *B. rossii*, a dioecious monocotyledon, has a bulb and the above ground potion dies away completely over winter. *A. latifolia* is a dioecious perennial herb with large pink inflorescences, and unlike the other species studied it maintains its size throughout the year.



Campbell Island - Study Sites

Figure 4: Campbell Island showing study sites situated near Beeman Hill.

Congeneric Experiment

P. speciosum, *P. criniferum, P. hookeri* and *B. rossii* were sampled in a community of herbs, tussocks (*Chionochloa* and *Poa*) and occasional shrubs of *Dracophyllum*, *Coprosma* and *Myrsine*, and the ferns *Polystichum* and *Blechnum* (mapped as '*Coprosma* - *Myrsine*-fern swamp shrubland to scrub' by Meurk and Given [1990]). The site was a southwest facing slope, approximately 120 m at 52°33' S and 169°09' E with a 200° aspect (Figure 5). Five plots were chosen within the area. Because of the clonal nature of *P. hookeri* and *B. rossii* some effort was made to sample individual plants by selecting isolated specimens of *B. rossii*, and keeping crown numbers of *P. hookeri* to fewer than six per plant. Plants were selected and randomly allocated to each of four treatments. Where a plant had more than one crown, sampling and measurements were applied only to the largest crown, although the treatment was applied to the whole plant.

"Shade"

Individual shade structures - 80 cm x 80 cm x 80 cm - enclosed each plant with 66% neutral light reduction cloth, avoiding contact with leaves wherever possible (Figure 5B).

"Wind"

To increase shelter a single-sided shield of doubled-over windcloth - 80 cm x 80 cm - was placed between the plant and the prevailing wind. While the wind often changed direction during the course of a day, the direction that sculptured the vegetation was considered to be the prevailing wind, *i.e.* from the north-west.

"Nutrients"

In the nutrient treatment a foliar fertiliser, Phostrogen, (15 g in 4.5 l water) was applied every 4 days - approximately 8-9 ml of solution for *B. rossii* while *P. hookeri, P. speciosum* and *P. criniferum* each received 13-18 ml as they were larger plants.

A)

B)

Figure 5: Congeneric Experiment site on both sides of the board walk; A) study site showing open tussockland with occasional shrubs; megaherbs are interspersed throughout; and B) erecting a "shade" structures over an individual *P. speciosum*.

Initially the plants' leaf growth were measured at 12 day intervals but this was later shortened to 6 day intervals. Four expanding but not juvenile leaves on each plant were randomly chosen, and measured along their abaxial length from the base of the petiole to the leaf tip. To calculate leaf growth rate only measurements showing a change in size were used. A line was then fitted to individual plant's measurements over time. The growth rate (slope) for all the replicates was averaged and used to compare growth rates between treatments and species. The data were analysed with ANOVA.

Brief phenological observations were also made throughout the season.

Leaf samples for CHO analysis were taken three times (termed 'Periods') throughout the season. This was done over four consecutive mornings between 8am and 10.30am to represent the overnight minimum of CHO in storage. 3 cm x 3 cm portions of leaf tissue were taken from the perimeter of healthy leaves, finely chopped up with secateurs and placed in vials of 10 ml of methanol, water and formic acid (12:2:1 by volume). These vials were then stored in a dark, cool place. In some cases sampling of individual plants had to be discontinued as they had either blown over (*P. criniferum*), or begun to die off (*B. rossii*).

Adaptability Experiment

The study site was an open subalpine environment comprising tussocks (*Chionochloa* and *Poa*) and occasional shrubs of *Dracophyllum, Coprosma,* and *Myrsine*, and *Polystichum* (bordering '*Poa litorosa meadow and Chionochloa*', and 'Tundra mosaic' as mapped by Meurk and Given (1990)). The site was at 52° 33' S and 169° 09' E, at 140 m with 270° aspect (Figure 6A). The experiment consisted of three replicates exploring the effect of increased temperature or increased night length.

"Cloche"

Individual plastic cloches with 80 cm x 80 cm x 80 cm frames covered with reinforced plastic were fitted around randomly selected plants with one side only clipped shut to allow access (Figure 6B).

“Night”

Night length was extended using cloths made out of black weedmatting on similar frames placed over the plants (and guyed down) every night at approximately 7.30pm, and removed at 7am to give 11.5 hours of night. As the hours of daylight are approximately 5am to 10pm at this latitude (*i.e.* a photoperiod of 17 hours averaged over the course of the experiment), this increased the time of darkness by four hours (Figure 6B).

Plant growth was measured and analysed similarly to the Congeneric Experiment and brief phenological observations were made. For CHO sampling 3 cm x 3 cm pieces of leaf tissue were removed from each plant, finely dissected and placed in vials as for Congeneric Experiment. Sampling was done under a different regime however - at night (*i.e.* from 7pm to approximately 10pm), and in the morning (*i.e.* 7am to approximately 10am (‘PMAM’). This was repeated two days in a row: *i.e.* night and next morning, followed by the night of the next day and the subsequent morning. Duplicates were taken of each sample and sampling occurred four times (termed ‘Periods’) throughout the season.

A)

B)

Figure 6: Adaptability Experiment on the NW flanks of Beeman Hill; A) study site showing mixed tussockland with occasional shrubland; and B) cloche and night treatment covers in place.

Environmental data

Meteorological data were obtained during the course of the season at both sites using meteorological stations consisting of pairs of rain gauges, Stevenson screens at ground level measuring air temperatures (min/max), and soil temperatures recorded at a depth of 15 cm. These were read every four days. Occasionally throughout the season air temperatures were recorded daily with maximum/minimum thermometers at approximately 75 cm above ground in different treatments over a weekly period. Electronic recorders, 'Tinytalks', were also used on several occasions to record temperature every 15 minutes inside the treatments in the Adaptability Experiment. Spot readings were taken on several occasions of leaf IR temperature and light intensity in the separate treatments. IR readings were taken 5 cm above the leaf surface with a Barnes infrared gun. Light measurements were taken at plant height (*i.e.* 5 cm above plant surface) and expressed as % of ambient light, recorded at 1.3 m height. Occasional diurnal light readings were recorded at hourly intervals. Light readings were taken in the Increased Night treatments to confirm plants were receiving no light during the artificial darkness period. Wind speeds were recorded with a hand-held spinning cup anemometer, at half hourly intervals on four different days at each study site. Average wind speed was noted over a 5 second interval and maximum gust speed recorded. Spot readings were also taken comparing the ambient conditions and the wind shelter treatment.

2 cm x 2 cm x 10 cm deep plugs of peat were extracted from below the root mat from both study sites. These were stored in a cool place until they were analysed for nutrients (K, Ca, Mg, Na, and Cation Exchange Capacity (CEC) by Fertiliser and Lime Research Centre, Institute of Natural Resources, Massey University), and carbon content (obtained by burning off the carbon at 500°C for 5 hours).

Test for sampling effect

Because of potential impacts on an individual plant's CHO status from repeated sampling (32 samples taken in total from each plant), a test for sampling effect was carried out on *A. latifolia* and *P. speciosum.* Six randomly chosen individuals of both species underwent a similar sampling regime to that of the Adaptability Experiment but plant material was simply discarded. At the end of the study period (6 weeks after the initial sampling), samples were taken from these six plants along with samples from six other plants that had been selected but not sampled previously. These samples were all retained and stored for CHO analysis.

CHO analysis methods

The samples were kept cool for up to 50 days on Campbell Island prior to being transported back by boat to New Zealand. Unfortunately some vials leaked during travel, but this was taken into consideration during laboratory and mathematical analysis (see below). Back on the mainland the samples were stored at 5°C in the dark until analysis.

The technique for sugar analysis was adapted from Haslemore and Roughan (1976), omitting the chloroform scrub (only necessary when extracting lipids in grasses (pers. comm. Haslemore)) from the process. The vials were placed in a waterbath at 55°C for 1 hour. Supernatant was then added to tubes containing 0.1 ml of saturated lead acetate to precipitate phenolic materials. A 4 ml sample of supernatant was used for *Pleurophyllum*. However, the *B. rossii* and *A. latifolia* samples were found in preliminary tests to be too concentrated (outside the range of the standard CHO solutions), so 2 ml and 1 ml were used respectively. Their volume was then made up to the standard 4 ml using more methanol solution. This modification was allowed for in later analysis. After 10 minutes with occasional agitation the teflon-capped tubes were sealed and centrifuged. From each tube a 0.5 μl aliquot was extracted and added to testtubes containing 1 ml of 5% phenol solution (5 g phenol in 100 ml water). 4 ml of concentrated H_2SO_4 was then rapidly added releasing heat and developing a pink-orange colour in the presence of CHO (Figure 7). The solution absorbances were then read with a Jenway 6105 U.V./Vis. spectrophotometer at 490 λ wavelength in conjunction with a set of standards (see below). The shredded plant material was then removed from the vials, dried and weighed for three days at 90°C to determine the concentration of CHO per unit dry weight. For those samples that had been spilt, the volume lost was estimated to within 1 ml and the absorbances of the remaining liquid were read. The remaining solutions were then topped up to 10 ml, extracted and read again, the two absorbances being added together allowing for the volumes in each portion in later analysis.

For *A. latifolia* and *P. speciosum* plants where biomass data are known (Chapter 2/ Nicholls and Rapson, 1999) the total CHO content of the foliage of individual plants was calculated by multiplying the CHO concentration by the biomass.

Figure 7: Analysis procedure showing vials of samples of CHO solution from the island on the left; in the centre are the centrifuged tubes from which the aliquot is extracted (note the hard pellet at base of tubes); and on the right are the coloured solutions containing H_2SO_4 depicting varying concentrations of CHO.

For standards, a sucrose solution of 10 mg cm^{-3} in 62.5% methanol was prepared daily, and standards of concentrations 0.25%, 0.5%, 0.75% and 1% CHO were analysed concurrently with each batch of samples (Figure 8). The standard curves of the 61 batches analysed were very similar. They were averaged, graphed and a standard curve fitted (equation: CHO content = 0.6158*(absorbance), $r^2 = 0.9999$). This equation was then used for all batches. Where less supernatant was used the absorbances were scaled appropriately. The CHO contents were converted to concentrations per unit plant dry matter prior to statistical analysis.

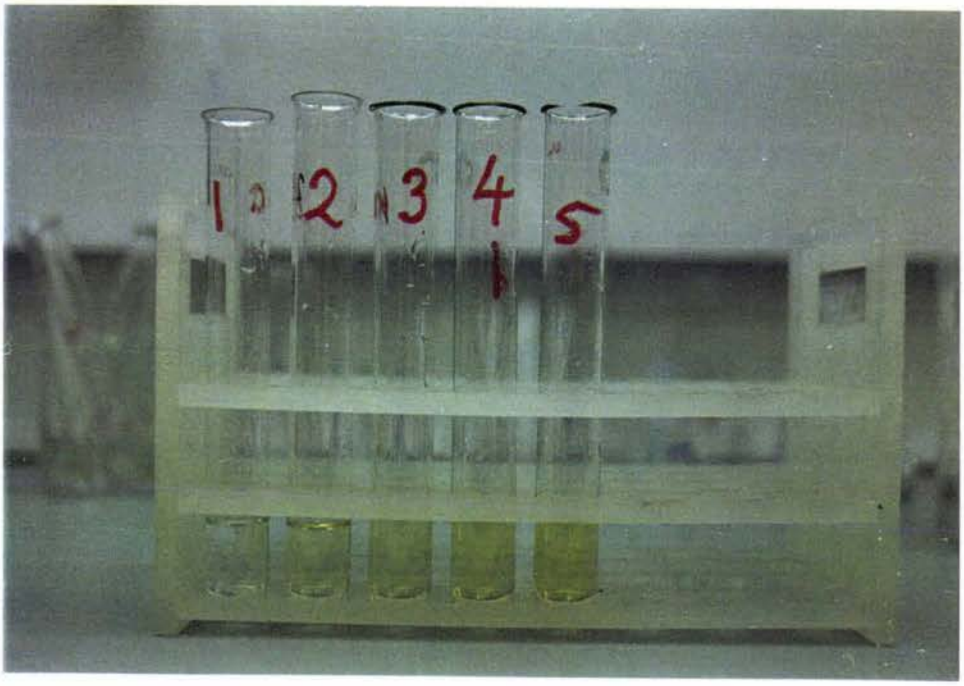


Figure 8: Sucrose solution standards 1 = 0%, 2 = 0.25%, 3 = 0.5%, 4 = 0.75% and 5 = 1% CHO.

Chlorophyll

Leaf samples (3 cm x 3 cm) were taken from all plants in both experiments in early January for chlorophyll analysis. The Adaptability Experiment was sampled again (with duplicates) in early February. The samples were cut up and placed in vials of 5 ml of Dimethylsulphoxide (DMSO) and stored until return to New Zealand. Chlorophyll was extracted by heating the vials for 1 hour in a water bath (65°C) before centrifuging for ten minutes. The liquid was decanted into crystal cuvettes and absorbances read with a spectrophotometer (as above) at 645, 652, and 663 nm wavelengths to obtain measurements of chlorophyll *a*, *b* and *a*:*b* ratio following Bruinsma, 1963. The plant tissue was then dried for 72 hours at 92°C and reweighed to generate chlorophyll content in mg g^{-1}.

Statistical Analysis

ANOVA (SYSTAT, 1992) was used to analyse the data. As the samples were taken from the same plants 3-4 times over the season (Periods), the morning and evening data (in the Adaptability experiment) and the repeated sampling data (*i.e.* the measurements on consecutive days) were treated as nested with repeated measures. Where duplicate samples were taken they were chemically analysed separately and averaged prior to statistical analysis. Replicates were treated as blocks.

Results

Environmental conditions

Rainfall

Environmental conditions were cool and oceanic with a mean maximum air temperature of 16-18°C for the two sites during the period December - March (*i.e.* mid summer) (Table 1A). Campbell Island is routinely wetter and cooler than the mainland station at Invercargill (Table 1C). In particular, maximum air temperatures are 11°C cooler than Invercargill. The 1997-1998 summer on the island appears to have been warmer and drier, particularly in February, than the normal New Zealand Meteorological Society recordings of 1941-1980 (Table 1B and C) for Tucker Cove, Campbell Island. There were two rainfall peaks over the sampling period (Figure 9A) with corresponding falls in air temperatures (Figure 9B and C). The Congeneric Experiment site had a slightly higher rainfall than the Adaptability Experiment (Table 1B). Overall soil temperatures fluctuated little, demonstrating the buffering effect of soil on temperature (Figure 9B and C), as the air temperatures fluctuate more, and show cooler periods.

Temperature

In the applied treatments, wind protection and shade, the air temperature was raised slightly above the control (Figure 9D). The amelioration is 2°C in mean maximum temperature and 0.1°C in minimum temperature. No differences were apparent between the temperatures in wind and shade treatments. However, these are not reflected in higher leaf (IR radiation measurement) temperatures (Table 2A). IR leaf temperatures in shade treatments are lower than the control, though variable in the wind treatment (Table 1A). Differences between species (Table 2B) are minimal. While the cloche raises leaf temperature in *A. latifolia* (Table 2C) compared with the control it does not do so in *Pleurophyllum*. The wind treatment raises the IR temperature of *P. speciosum* but not that of *P. criniferum*.

Dataloggers showed the cloche treatment had a higher daytime temperature than the control treatment (Table 3), of over 3°C in January lessening to 2°C in February. At night all three treatments have similar temperatures, while during the day the increased night treatment is slightly warmer than the control but 1.3°C cooler on average than the cloche.

Table 1: A) Mean maximum and minimum air and soil temperatures for each four day period measured (n = 14) over the duration of the study (30 December - 18 February, 1997/1998), and total rainfall over the sampling period for each experiment site; B) mean monthly maximum and minimum temperatures and rainfall (mm) from both experiment sites, and raindays/month compared with NZ Met. Service (1980) long-term data from Tucker Cove. The February results for the experiment are only for half the month and so the New Zealand Met. data for rainfall and raindays are also halved; and C) long term mean meteorological data taken at Invercargill Airport and Tucker Cove, Campbell Island (NZ Met. Service, 1980).

A) Experiment site climate over study period.

	Adaptability	**Congeneric**
Maximum air temperature (°C)	18.6	16.6
Minimum air temperature (°C)	5.6	4.8
Maximum soil temperature (°C)	10.4	9.7
Minimum soil temperature (°C)	7.3	6.8
Rainfall (mm)	63.5	95

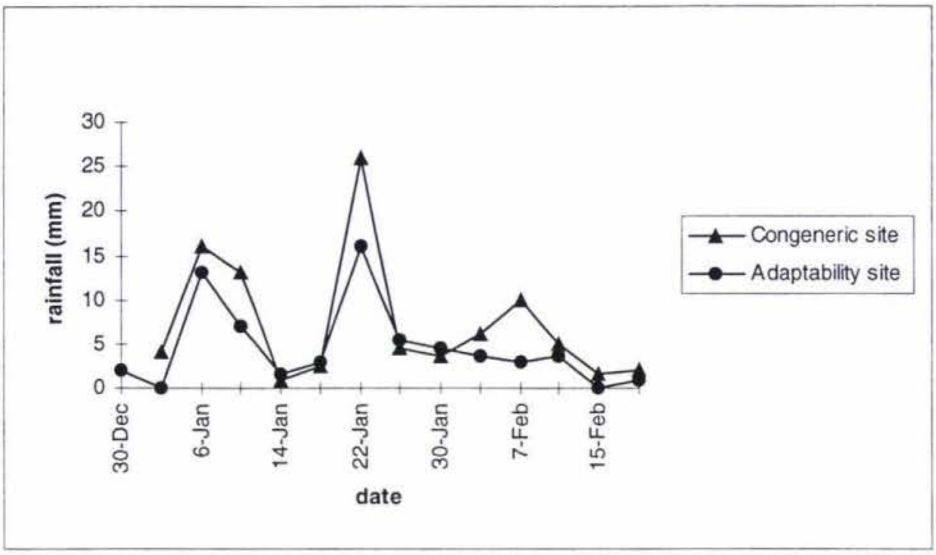
B) Experiment site climate for January and half of February compared with long term climate.

	January			**February**		
	NZ Met.	**Adaptability**	**Congeneric**	**NZ Met.**	**Adaptability**	**Congeneric**
Max. mean temperature (°C)	15.9	18.9	16.8	15.3	18.4	16.5
Min. mean temperature. (°C)	2.1	5.3	4.4	2.2	6.1	5.4
Rainfall (mm)	118	50.5	70.5	53.5	11	24.5
Rain days	19	12	12	9	8	8

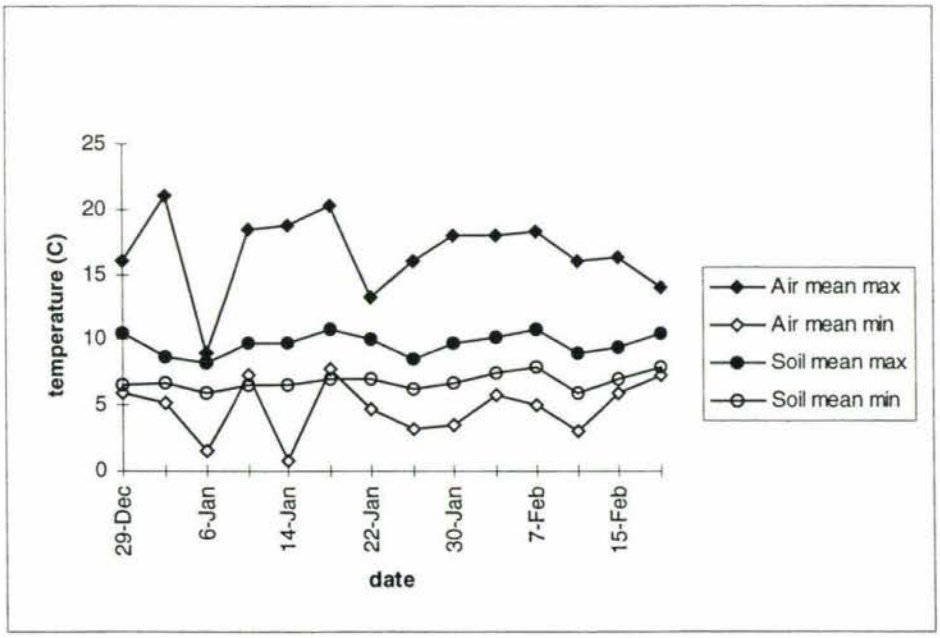
C) Comparison between mainland climate (Invercargill Airport) and Campbell Island (Tucker Cove)

	January		**February**		**July**	
	Invercargill	**Campbell**	**Invercargill**	**Campbell**	**Invercargill**	**Campbell**
Rainfall (mm)	92 (1939-80)	118 (1951-80)	75	107	70	107
Rain days	13 "	19 "	11	18	13	22
Monthly max. temp. (°C)	26.9(1905-80)	15.9 (1941-80)	26.5	15.3	14.2	9.7
Monthly min. temp. (°C)	3.0 "	2.1 "	2.4	2.2	-4.3	-2.7
Soil temp.10cm depth (°C)	14.1 (1951-80)	10 (1969-80)	13.6	9.4	3.5	4.0
Total sunshine (hrs/year)	187 (1935-80)	99 "	163	81	92	13

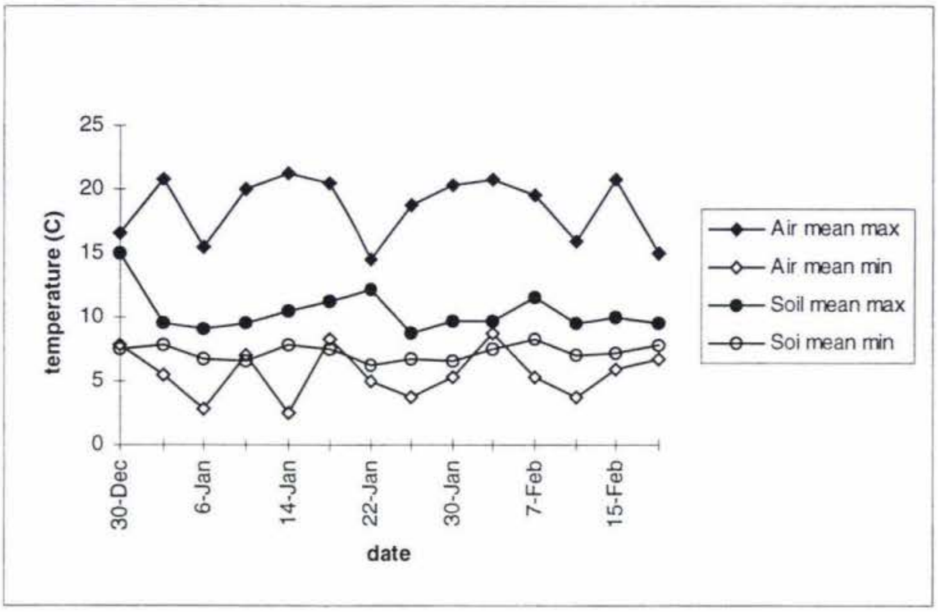
A) Rainfall (mm).



B) Congeneric Experiment temperatures



C) Adaptability Experiment temperatures



D) Treatment effects (Congeneric Experiment)

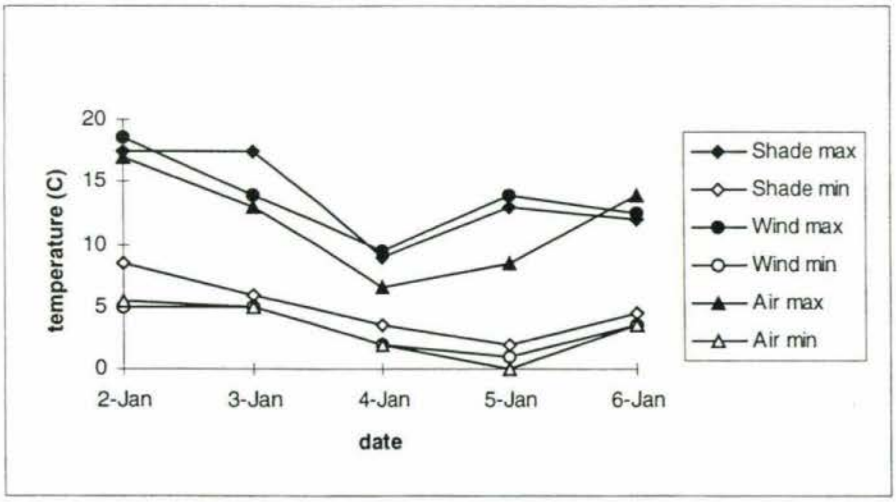


Figure 9: A) Rainfall (mm) at both study sites measured at 4 day intervals between the 30 December and 18 February; maximum and minimum air and soil temperatures at B) Congeneric Experiment and C) Adaptability Experiment sites measured at four day intervals; and D) daily air temperature (maximum and minimum) recorded in three different treatments (shade, wind, control) in Congeneric Experiment over 6 days.

Table 2: Mean light (% of ambient) and leaf IR daytime temperatures for A) two species (n = 3) in three treatments (Congeneric Experiment); B) three species in one treatment (n = 6) (Congeneric Experiment), on 8 February; and C) two species in two treatments on 1-2 days, 15 January and 2 February (Adaptability Experiment).

A) Congeneric Experiment

	P. speciosum (11 Jan)		*P. criniferum* (31 Jan)	
treatment	**light (%)**	**IR (°C)**	**light (%)**	**IR (°C)**
Control	59.1	8.5	84.5	9.1
Shade	37.7	8.1	24.7	8.5
Wind	70.1	10.7	75.8	8.5

B) Control treatment, Congeneric Experiment

species	**light (%)**	**IR (°C)**
P. criniferum	92.1	6.4
P. hookeri	84.1	6.3
P. speciosum	81.7	6.4

B) Adaptability Experiment

	A. latifolia (15 Jan) n = 12		*A. latifolia* (2 Feb) n = 9		*Pleurophyllum* (2 Feb) n = 9	
Treatment	**light (%)**	**IR (°C)**	**light (%)**	**IR (°C)**	**light (%)**	**IR (°C)**
Cloche	64.6	9.0	67. 1	13.8	61.53	12.2
Control	65.1	7.7	68.4	9.4	67.6	11.5

Table 3: Mean day/night temperatures using dataloggers for two treatments for *A. latifolia* 12 - 18 January (n = 705) and three treatments on 11 - 13 February (n = 295).

	12 - 18 Jan		**11 - 13 Feb**		
	Control	Cloche	Control	Extended Night	Cloche
Mean night temperature (°C)	7.7	7.6	9.3	9.5	9.4
Mean day temperature (°C)	9.7	12.7	9.7	10.3	11.6

Light

Overhead light conditions vary markedly on the island. The island receives less than 700 sunshine hours on average a year giving predominantly overcast conditions; a mean light level of 600 μmol $m^{-2} s^{-1}$ was recorded over three days at half hourly intervals. However, bright, sunny days did occur (up to 3000 μmol $m^{-2} s^{-1}$) where the irradiation fluctuated considerably due to cloud and wind (Figure 10).

Light at plant level is less than ambient due to the influence of other plants nearby (Table 2A) and the treatments all reduced available light with respect to ambient conditions. The shade treatment reduced available light by about 50%-60% of the control while the wind treatment had values within the range of the control (Table 2A). The Night treatment covers blocked out 99.99% (n = 6, data not reported) of ambient light during the morning and evening.

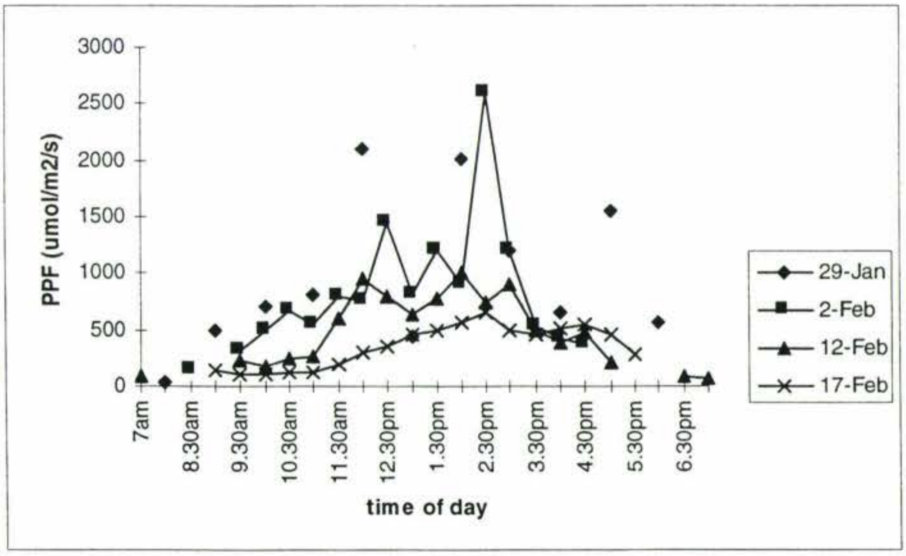


Figure 10: Daily light tracking over the course of four non-consecutive days: 29 January - sunny day with clouds, readings intermittent, so points not joined by line; 2 February - bright day, but overcast in the morning with sun at mid day and overcast after 3pm; 12 February - grey morning with sunny patches midday followed by a grey afternoon; 17 February - overcast conditions.

Wind

Wind speed was measured several times over three representative days at both study sites. The mean wind speed above the plants used in the Congeneric Experiment was 9.4 m s^{-1} with mean gusting up to 16.6 m s^{-1} (n = 34) and for plants used in the Adaptability Experiment, 4.8 m s^{-1} and 12.8 m s^{-1} (n = 38). This compares with wind speed recordings on average of 7.4 in Meurk *et al.* (1994a, Table 4) ,and a mean of 9.2 m s^{-1} in NZ Met. Service records for January and February. The mean maximum gusting speed over both study sites was 20.7 m s^{-1}. The wind cloth reduced wind speed (Table 4) but not significantly (mean wind speed: p = 0.158, n = 5, df = 4; and for gusting wind speed: p = 0.305, n = 4, df = 3).

Table 4: Wind reduction (% of wind measurements taken at 2m above ground) of both mean non-gusting speed and gusting speed at plant height behind wind cloth, compared with control.

Treatment	Mean non-gusting %	Mean gusting %
Control	27.5	37.1
Wind cloth	79	30

Soils

There was very little seasonal variation in nutrient levels between replicate sites in the Adaptability Experiment and the Congeneric Experiment, so data were averaged over the sites and season within each experiment (Table 5A). The low quantity of cations present indicate that the soils have a low nutrient content. The cation contents are slightly lower than those found in other analyses of Campbell Island soils (Foggo and Meurk, 1983), but match with the shallow peat soils of Campbell's analysis (1981) There is also very little variation over time and space in organic content between sites with high readings in both areas (Table 5B). Cation Exchange Capacity (CEC) indicates the potential fertility of a soil, giving a measure of the available sites for chemical reactions to take place. The CEC is high in these soils due to their high organic matter. In comparison, a typical productive silt-loam under pasture provides 0.6 K, 15 Ca, 1.5 Mg and 0.2 Na (units cmol (+) kg^{-1})* with a CEC of 20-30 (pers. comm. Lance Currie) having a much higher Ca content but lower Mg, Na and CEC values.

*(+) = anion

Table 5: A) Soil nutrient levels and Cation Exchange Capacity (CEC) (mean ± s.d.) from the five replicate samples in the Congeneric Experiment (sampled once during the middle of the season), and three replicate samples in the Adaptability (sampled three times) (units: cmol(+) kg^{-1}); and B) % carbon content of soils (mean ± s.d.) (n = 47).

A) Soil nutrients

Experiment	K	Ca	Mg	Na	CEC
Congeneric	0.98 ± 0.85	3.79 ± 1.88	8.48 ± 4.11	3.29 ± 0.99	69.78 ± 12.54
Adaptability	1.28 ± 0.77	5.82 ± 2.50	14.16 ± 3.19	5.74 ± 1.88	87.22 ± 9.19

B) Carbon content over three sampling periods

Experiment	Period 1	Period 2	Period 3
Congeneric	83.88 ± 2.34	86.82 ± 3.55	82.05 ± 7.29
Adaptability	86.48 ± 6.49	85.84 ± 5.35	76.71 ± 26.61

Repeated sampling test

While the two species studied demonstrated significantly different CHO concentrations (*A. latifolia* 72.2 ± 10.3 mg g^{-1}, and *Pleurophyllum* 12.8 ± 1.6 mg g^{-1}), repeated tissue removal had no effect (Table 6). Also the species did not respond in different ways to the treatments. Repeated sampling therefore was discounted as having any effect on the results.

Table 6: ANOVA of sampling effect. Significant effects are in bold.

Source	SS	df	MS	P
Species	10566.027	1	10566.027	**<0.001**
Treatment (repeated sampling)	6.965	1	6.965	0.695
Species*Treatment	33.333	8	33.333	0.400
Error	337.162	8	42.145	

Congeneric Experiment

Phenology

At the beginning of the sampling period (30 December), *P. hookeri* was the most phenologically advanced species being in full flower, with some plants possibly setting seed. *B. rossii* was in flower as was *P. speciosum* which also had buds developing. *P. criniferum* was in bud. By Period 2 (29 January) the flowers of *P. speciosum* were losing perianths and possibly setting seed, although some plants still contained buds; *P. criniferum* was in flower and inflorescences were beginning to be forced over in their shade structures as they grew taller; *P. hookeri* was dispersing seed; *B. rossii* was beginning to set seed, and change colour, the leaves becoming tinged with orange. By the last sampling period (8 February) *P. speciosum, P. criniferum* and *B. rossii* were all at different stages of seed setting.

Growth rates

The species differ in their growth rates (Table 7; Figure 3A) with *P. criniferum* having the fastest growth rate, followed by *B. rossii*, *P. speciosum* and *P. hookeri*. The treatments also have a significant effect on plant growth rates (Table 7) with shade and increased nutrients causing the fastest leaf growth in plants (Figure 3B). The significant species and treatment interaction is strongly influenced by the increased growth of *P. criniferum* in the shade treatment, and *B. rossii* in the nutrients treatment (Figure 3C). *P. speciosum* shows a slightly higher rate of growth in the shade treatment compared with the other treatments and *P. hookeri* shows little response to any of the treatments.

Table 7: A) ANOVA for growth measurements for plants form the Congeneric Experiment.

	SS	df	MS	P
Species	1.784	3	0.595	**<0.001**
Treatment	0.100	3	0.033	**0.010**
Species*Treatment	0.389	9	0.043	**<0.001**
Error	2.309	265	0.009	

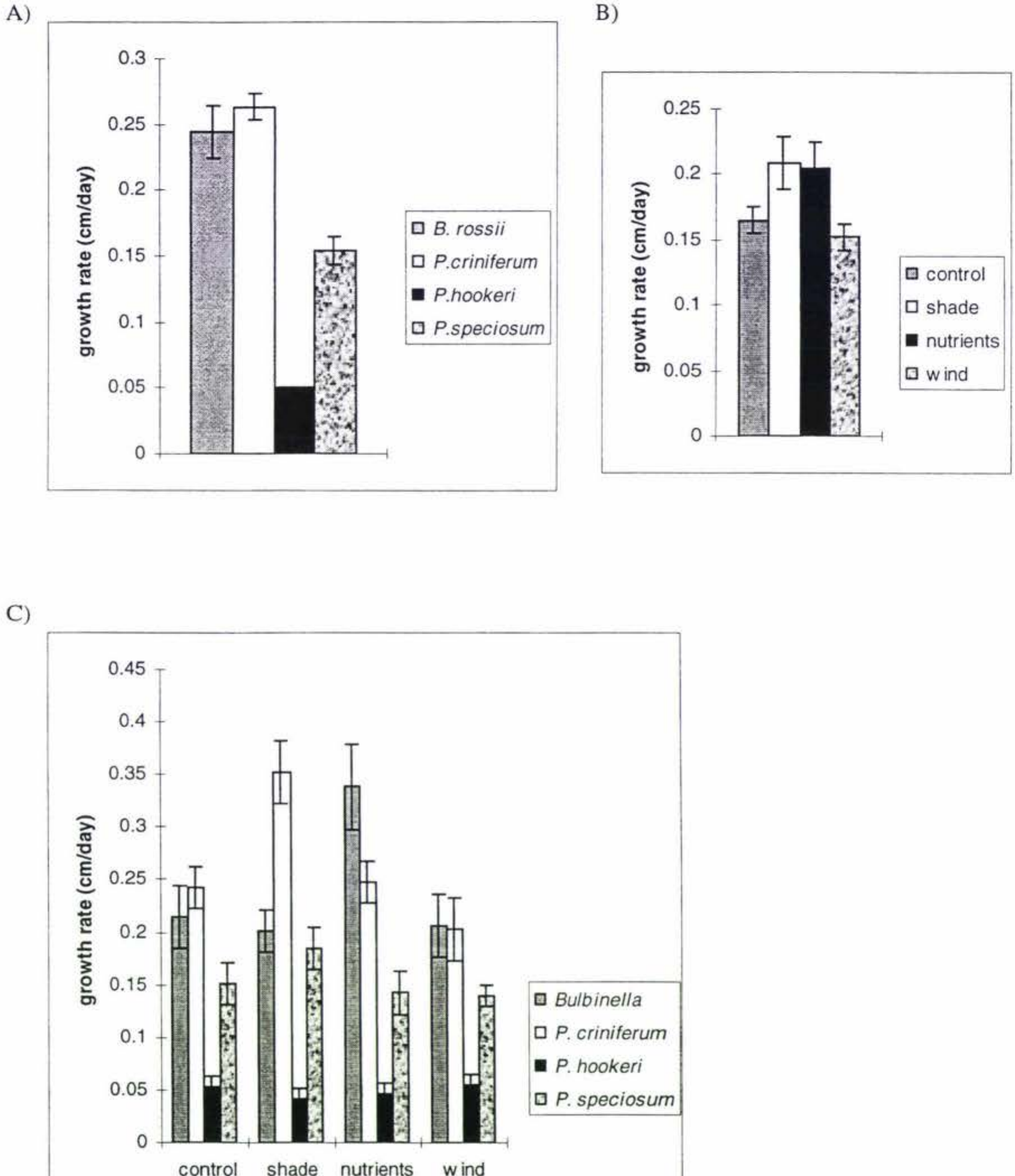


Figure 11: Mean growth rates in the Congeneric Experiment (n = 281). A) growth rate of each of four species averaged over all treatments and replicates; B) effect of each treatment on growth rate averaged over all species and replicates; C) growth rate of each species in each treatment averaged over all replicates. Standard error bars shown.

Chlorophyll

The measured absorbances at 663 nm (nanometers) were highly correlated ($r^2 = 0.9754$; n = 76) with the expected absorbances at 663 nm (when calculated using the absorbances of 645 nm and 652 nm in the equation: A663 = (27.80/8 x A652) - (20.29/8 x A645) [Bruinsma, 1963]) confirming the reliability of the absorbance records. The treatments had no effect on chlorophyll *a*, *b*, or *a*:*b* ratio, but there was a significant difference between the species with *P. hookeri* having the highest chlorophyll *a* content and *a*:*b* ratio, while *B. rossii* had the highest chlorophyll *b* content (Table 8).

Table 8: A) P-values for ANOVAs for chlorophyll content in plants from the Congeneric Experiment (significant values in bold);and B) mean values of chlorophyll content (mg g^{-1}), and chlorophyll *a:b* ratio;

A) ANOVA

Source	**Species**	**Treatment**	**Species* Treatment**	**df**	**Error MS**
Chlorophyll *a*	**< 0.001**	0.732	0.925	60	678.826
Chlorophyll *b*	**< 0.001**	0.367	0.890	60	122.586
Chlorophyll *a:b*	**< 0.001**	0.964	0.756	60	0.231

B) Chlorophyll values

Species	**Chlorophyll *a***	**Chlorophyll *b***	**Chlorophyll *a:b***
B. rossii	85.0	64.1	1.3
P. criniferum	69.7	40.8	1.7
P. hookeri	101.6	42.5	2.4
P. speciosum	45.3	22.7	2.0

Carbohydrates

The species showed significant differences in their CHO concentrations (Table 9A) with *B. rossii* having over four times the average carbohydrate concentration of the *Pleurophyllum* species (Table 9B). The three *Pleurophyllum* species have similar levels, with *P. hookeri*, having the lowest CHO concentration. These CHO levels also changed over the three sampling periods (Table 9C) with levels peaking in the middle sampling period (29 January - 1 February) and declining in early February (mainly due to the response of *B. rossii*; Table 9B). Over the sampling periods, the species responded in different ways (Figure 12) with *B. rossii* increasing then declining over the season, *P. speciosum* gradually declining and the other species maintaining similar levels. The treatments had no significant effect on CHO levels, with mean values (± s.d.) for control of 35.3 ± 30.4, nutrient addition of 34.9 ± 39.3, shade of 33.0 ± 34.4, and wind of 34.6 ± 34.8 (mg g^{-1}). Nor was there any significant interaction between treatments and species, or a species by treatment by time interaction.

Table 9: A) ANOVA for plants from the Congeneric Experiment; B) CHO (mg g^{-1}) levels (mean ± s.d.) of the four species averaged over all sampling periods; and C) CHO means for each sampling period averaged over all species (n = 72-78).

A) ANOVA

Source	SS	df	MS	P
Replicate	2565.985	4	641.496	0.283
Species	126773.962	3	42257.987	**<0.001**
Treatment	567.555	3	189.185	0.765
Species*Treatment	552.182	9	61.354	0.999
Error	22639.363	46	492.160	
Within subjects				
Period	5547.844	2	2773.922	**<0.001**
Period * Replicate	2325.563	8	290.695	0.142
Period*Species	14385.901	6	2397.65	**<0.001**
Period*Treatment	708.300	6	118.050	0.697
Period*Species*Treatment	3283.163	18	182.398	0.478
Error	16948.018	92	184.218	

B) Species differences in CHO (mg g^{-1}).

B. rossii	*P. criniferum*	*P. hookeri*	*P. speciosum*
84.1 ± 35.2	25.6 ±13.4	13.0 ± 3.6	18.5 ± 9.6

C) Period differences in CHO (mg g^{-1}).

30 Dec - 3 Jan	29 Jan - 1 Feb	8 Feb - 11 Feb
35.8 ± 30.6	39.3 ± 44.7	28.5 ± 22.51

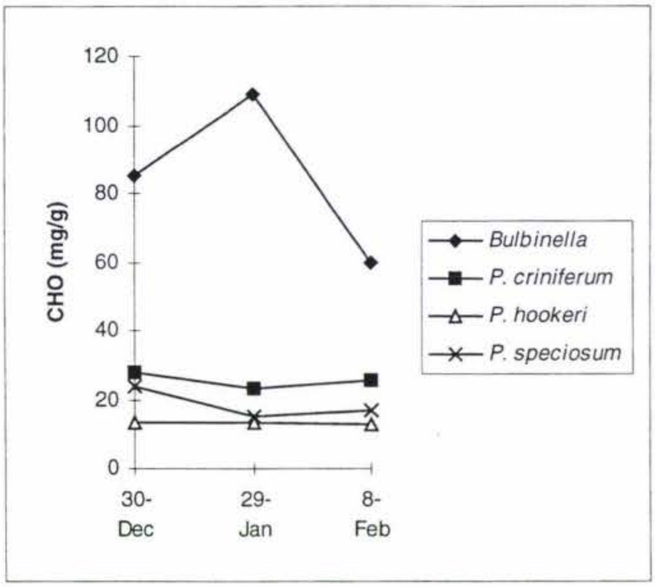


Figure 12: Changes in CHO levels in four species over the three sampling periods.

Adaptability Experiment

Phenology

A. latifolia and *Pleurophyllum* were at different stages of flowering during the sampling period (6 January - 15 February). At the start of sampling *Pleurophyllum* was in bud and flower, while *A. latifolia* female flowers were beginning to set seed and the male inflorescences were almost finished. By the 20 January the male inflorescences of *A. latifolia* were beginning to collapse and the seeds were swelling; *Pleurophyllum* was still producing buds. By early February *Pleurophyllum* flowers were dying off, with some possibly setting seed, and by mid-February, while the occasional flower was still out, most plants were probably setting seed.

Growth Rates

The species do not differ significantly in their growth rates (Table 10), the mean ± s.d. for *P. speciosum* being 0.163 ± 0.07 cm day^{-1}, n = 31, and for *A. latifolia* being 0.106 ± 0.11cm day^{-1}, n = 15, (although the data set was limited). Neither species responded to the treatments (growth rates in control, night and cloche treatments being 0.124 ± 0.005, n = 16, 0.154 ± 0.001, n = 12, and 0.127 ± 0.004, n = 18 respectively).

Table 10: ANOVA for growth measurements for plant from the Adaptability Experiment. Significant effects are in bold.

	SS	df	MS	P
Species	0.019	1	0.019	0.076
Treatment	0.003	2	0.001	0.788
Species*Treatment	0.013	2	0.007	0.311
Error	0.224	40	0.006	

Chlorophyll

A. latifolia and *Pleurophyllum* do not differ significantly in their chlorophyll content; nor is there a treatment response (Table 11).

Table 11: A) P-values for ANOVA of chlorophyll content for plants in the Adaptability Experiment; and B) mean values for chlorophyll contents (mg g^{-1}) for species averaged over all treatments, and by treatment averaged over all species.

A) ANOVA

Source	Species	Treatment	Species* Treatment	df	Error MS
Chlorophyll *a*	0.191	0.985	0.236	29	257.137
Chlorophyll *b*	0.805	0.123	0.182	29	44.038
Chlorophyll *a:b*	0.153	0.490	0.727	29	0.489

B) Chlorophyll contents

	Chlorophyll *a*	**Chlorophyll *b***	**Chlorophyll *a:b***
A. latifolia (n = 17)	33.8	22.2	1.6
Pleurophyllum (n = 18)	41.1	21.6	1.9
Control (n = 12)	36.8	20.7	1.8
night (n = 11)	37.7	25.3	1.6
cloche (n = 12)	37.9	19.7	1.9

Carbohydrates

A. latifolia has a significantly (Table 12A) higher CHO content than *Pleurophyllum* in all circumstances (Table 12B). The treatments *per se* do not significantly affect CHO levels (Table 12A) (the mean ± s.d. for control, extended night and cloche are 34.2 ± 24.4, 37.6 ± 26.3, and 40.4 ± 29.6 mg g^{-1} respectively).

Overall CHO levels declined significantly with time (Table 12A) until the last period (Table 12B). The species responded to this time change differently (Table 12B) with *Pleurophyllum* showing an increase in CHO from Period 1 to Period 2 (from 4% above the mean of all periods to 6%), dropping considerably (18% below the mean) in Period 3 and rising again in Period 4 (to 7% above the mean) (Table 12B). *A. latifolia* does not have such marked responses: CHO levels drop slightly between Period 1 and Period 2 (1.5% to 0.9% above the mean), followed by an 11% drop below the mean in Period 3, and ending in a 10% rise above the mean in Period 4 (Table 12B).

The three treatment effects on overall CHO changed over time (Figure 5A) with the night treatment fluctuating the most and the control treatment having a much reduced CHO level by Period 4. The cloche treatment increased CHO levels, relative to the control by up to 24% (in Period 3) while the night treatment increased them by up to 20% (in Period 4).

The species also responded differently to the treatments over time (Table 12B), most notably in the varied responses to treatments of *A. latifolia*, while *P. speciosum* is relatively unresponsive (Figure 5B and C). In *A. latifolia* the concentration in the control is considerably lower than the other treatments and decreases sharply relative to the other treatments until Period 4, whereas in *P. speciosum* the Period 3 value is the lowest for the control and increases to Period 4. The night treatment produces fluctuating results with regard to the other treatments.

CHO levels are significantly different depending on the time of day ('PMAM') (Table 12A) - the evening levels are almost 10% higher than the morning levels: 39.1 ± 27.3 mg g^{-1}, and 35.6 ± 26.3mg g^{-1} respectively. This 3.5 mg g^{-1} difference represents night time use of CHO for respiration. Although there was some difference between replicates none of the treatments showed signs of affecting this pattern, even the extended night treatment which was hypothesised to use more CHO during the longer dark period.

CHO content

The total CHO content in foliage of 6 plants used in a biomass allocation study (Chapter1/Nicholls and Rapson, 1999) corresponds to mean (± s.d.) values for *Pleurophyllum* 1358 ± 319 mg and *A. latifolia* of 9095 ± 2211 mg.

Table 12: A) ANOVA for Adaptability Experiment; and B) CHO (mg g^{-1}) concentration (mean ± s.d.) for both species over the four sampling periods.

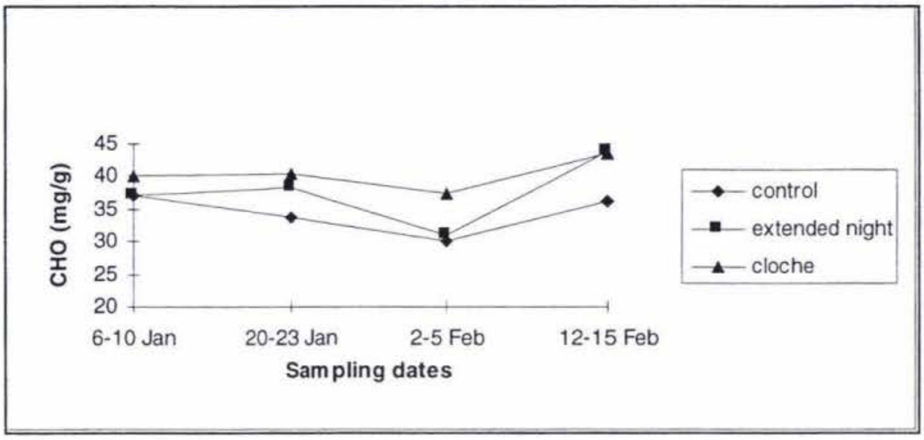
A) ANOVA

Source	**SS**	**df**	**MS**	**P**
Replicate	351.600	2	175.800	0.489
Species	93830.174	1	93830.174	**<0.001**
Treatment	903.523	2	451.761	0.189
Species*Treatment	653.525	2	326.762	0.284
Error	2284.478	10	228.448	
Within subjects				
Period	1281.395	3	427.132	**<0.001**
Period*Replicate	93.487	6	15.581	0.457
Period*Species	516.461	3	172.154	**<0.001**
Period*Treatment	308.487	6	51.415	**0.014**
Period*Species*Treatment	246.399	6	41.067	**0.039**
Error	478.076	30	15.936	
PMAM	440.491	1	440.491	**<0.001**
PMAM*Replicate	77.281	2	38.640	**0.038**
PMAM*Species	23.174	1	23.174	0.127
PMAM*Treatment	0.253	2	0.127	0.985
PMAM*Species*Treatment	10.703	2	5.352	0.548
Error	83.755	10	8.375	

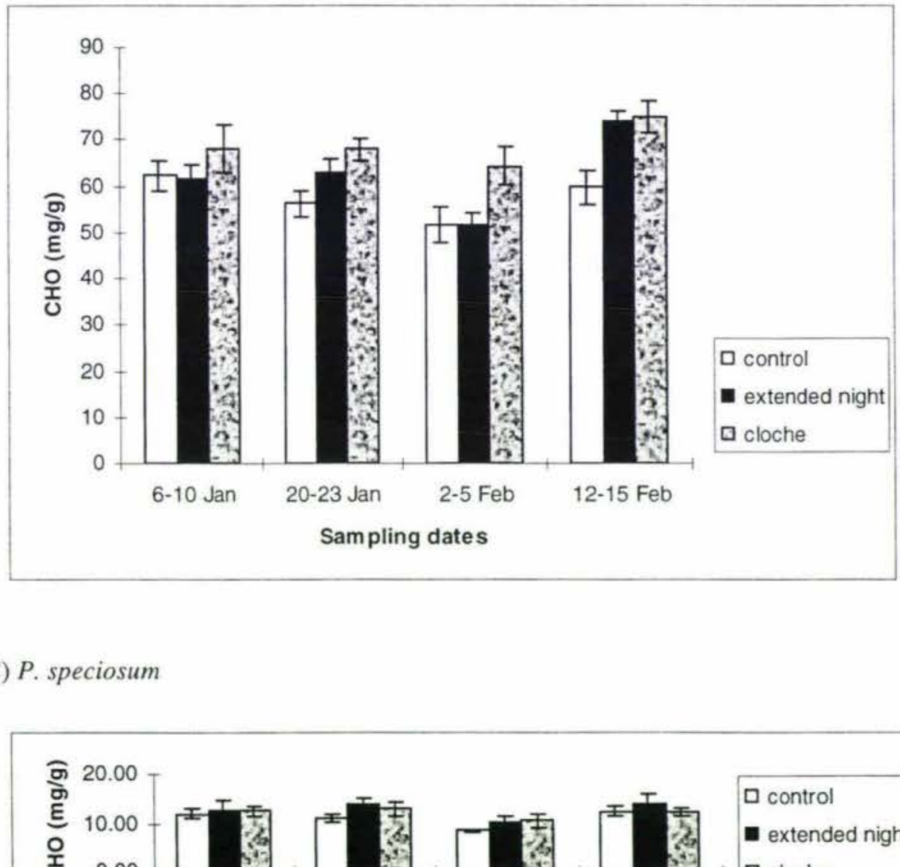
B) CHO concentrations

Species	**Period 1**	**Period 2**	**Period 3**	**Period 4**	**Mean**
	6-10 Jan	**20-23 Jan**	**2-5 Feb**	**12-15 Feb**	
A. latifolia	63.9 ± 9.7	62.4 ± 8.1	55.9 ± 10.5	69.5 ± 10.3	62.9 ± 10.7
Pleurophyllum	12.3 ± 3.4	12.6 ± 3.0	9.7 ± 2.5	12.7 ± 3.3	11.9 ± 3.3
Mean	38.1 ± 27.0	36.5 ± 25.9	32.8 ± 24.6	41.1 ± 29.7	37.4 ± 26.8

A) *A. latifolia* and *P. speciosum* CHO combined over four periods.



B) *A. latifolia*



C) *P. speciosum*

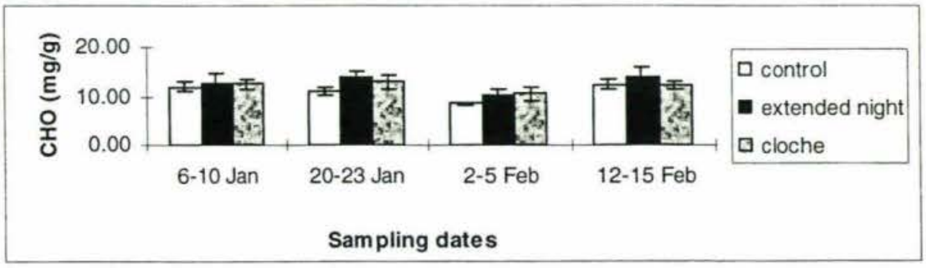


Figure 13: A) CHO concentrations over the four sampling periods in three treatments; and for both species in the three treatments over time for B) *A. latifolia* and C) *P. speciosum.* Standard error bars shown.

Discussion

Megaherbs with their large growth form and bountiful floral display appear extraordinary, seemingly at odds with their windy, wet and cool environment analogous to that of the mainland subalpine zone. And just as Wardle (1965) noted that subalpine flora shows xeromorphic features in the humid subalpine zones, Cockayne (1903) had commented earlier on the possible xerophytic nature of the Campbell Island flora due to a combination of low aeration and waterlogging of the peat. The plants however are apparently relatively well adapted to island conditions, as attempts at growing species (mainly *Pleurophyllum*) on the mainland have failed; this raises questions about limits to their ecophysiological tolerances. It is possible that they are physiologically so well adapted to the subantarctic islands environment that they have no capacity (plastic or genetic) to grow elsewhere. This has implications for understanding their evolutionary history.

Light effects on megaherbs

As sunshine hours over the growing period on Campbell Island appear to be approximately half that of the mainland (Invercargill), the plants are subjected to a naturally low Photosynthetic Photon Flux (PPF). As plants from low light situations cannot easily adapt to high light conditions (Salisbury and Ross, 1991), higher irradiance levels on the mainland may cause stomatal closure, limiting CO_2 uptake, and making the plants more susceptible to photoinhibition (Bjorkman, 1981). Conversely, light might be limiting on the island. Being unable to experimentally increase light in the field, we decreased it by applying neutral shadecloth which reduced available light to 1/3 of ambient conditions, testing the megaherbs' tolerance of low light conditions instead. While the plants showed no significant decline in their CHO concentrations under decreased light conditions, there appears to be a significant increase in leaf growth in *P. criniferum*, and to a lesser extent in *P. speciosum*. Although the increased growth may be an etiolation response, it is not reflected in increased chlorophyll *b*, the 'low light' chlorophyll' . This result suggests that low light levels on the island are not limiting for the species tested and they may have the capacity to photosynthesise in even lower light situations.

'Shade' plants usually have a higher chlorophyll content (mg g^{-1} of leaf) and a lower chlorophyll *a*:*b* ratio than sun plants (Boardman, 1977). A reduction in the *a*:*b* ratio can maximise chloroplasts' light harvesting capabilities [Tognetti, 1993, Chow *et al.*, 1991]). Total chlorophyll content in plants appears to be quite variable; e.g. mean of 5 woodland species was 3.1 mg g^{-1} (from Table 1 in Boardman, 1977), and up to 19 mg g^{-1} in a study on shade plants of *Solanum dulcamara* (Clough *et al.,* 1979). Values for other subantarctic species vary between 3.7 and 6.7 mg g^{-1} (Smith, 1984), and the Macquarie Island *P. hookeri* had a fresh weight of 0.43 mg g^{-1} (Jenkin and Ashton, 1970). The plants in this study had an extremely high weight of chlorophyll that is difficult to explain (ranging from 56 mg g^{-1} in *A. latifolia* to 149 mg g^{-1} in *B. rossii*). In contrast, the chlorophyll ratios of the species in this study are relatively low when compared with other plants e.g. (mean ± s.d.) 2.8 ± 0.4 for a selection of 'sun' plants (Black, 1973), 5.06 in high light for *Agrostis capillaris* (Rapson, 1985), >3 in peas (increasing with increasing light [Lee and Whitmarsh, 1989]), 1.79-1.85 in 4 forest shade species (Chow *et al.,* 1991), and 2.4 ± 0.4 and 2.2 ± 0.4 for two *Agrostis* species on the subantarctic Marion Island (Pammenter *et al.,* 1986). Several subantarctic grass species have been found with concentrations between 3 and 4 (Smith, 1984). Those species tested here had ratios from 1.3 to 2.4. The significant difference between the species in the Congeneric Experiment, with *B. rossii* having the lowest *a*:*b* ratio (of 1.3) and *P. hookeri* the highest (2.4), might indicate a tolerance in *Pleurophyllum* to high light conditions.

Given that these megaherbs species seem uninhibited by low light levels, they may also be uninhibited by high light, such as experienced on the mainland. If so, the plants on Campbell Island may be utilising available sunshine on a 'sunfleck' basis. Forest floor species utilise the short periods of irradiance known as sunflecks to photosynthesise sufficiently (e.g. Chazdon and Pearcy, 1986; Pearcy, 1988). In a low irradiance situation such as Campbell Island, where days are often completely overcast, or sunny days have fast moving clouds (Figure 2), perhaps the plants do most of their photosynthesis in the sunny periods, the 'sunfleck' being a matter of a few moments to several minutes or hours. A study of high *vs.* low altitude species by Korner and Diemer (1987) found that plants in both altitudes utilised the brightest, warmest times for maximum photosynthetic activity, even though this was only 25-30% of the time. While Campbell Island has many overcast days (less than 700 hours sunshine per year compared with 1621 at Invercargill), a maximum PPF of 3000 μmol m^{-2} s^{-1} was recorded in this study, so sunflecks are available. Light fluctuates constantly creating a very erratic resource which megaherbs are possibly adapted to; Wardle (1991) suggests the large leaves act as solar panels, harvesting as much light as possible when available. Gas exchange measurements *in situ* in response to light fluctuations are necessary to investigate these possibilities.

Photoperiod effects

As photosynthetic rates are probably low during the day due to a low mean PPF on the island, imposing a longer night than usual might have been expected to decrease the megaherb's net photosynthesis to a level unsustainable for growth. Black-out covers that extended the night to nearly 12 hours (as compared with their normal 7 hours) altered the photoperiod, increasing dark respiration while possibly reducing total net photosynthesis for the day. However, if anything, both *P. speciosum* and *A. latifolia* showed signs of actually benefiting (as reflected in the increased CHO) from the treatment at different times over the season. The shorter daylengths of the mainland then may not be limiting to growth. This also indicates that high respiration costs are not limiting productivity of megaherbs such as appears to happen in *Chionochloa* species transplanted to low altitudes (pers. comm. J. Rapson).

Nutrient effects

Peat provides little in the way of mineral nutrients for plants, being very low in calcium, a primary element. The high Na, Mg and Cation Exchange Capacity values are an indication of the maritime influence and the high organic content: Meurk *et al.*, (1994b), in a study on nutrients on Campbell Island found that airborne nutrients (aerosols) contain a high proportion of ions such as Na, Mg, Ca, and contribute significantly to nutrient supply. The addition of foliar fertiliser in this study tested whether or not the plants might be nutrient limited. CHO concentrations showed no response, but leaf growth in *B. rossii* appeared to increase with increased nutrients. This is not reflected in CHO concentrations, as the mean for *B. rossii* in this treatment is no higher than the mean for the other treatments. This suggests any increase in CHO is immediately consumed in increased growth so nutrients on the island do not appear to be limiting for *Pleurophyllum*, but may be for *B. rossii*. As the usual uptake of nutrients appears to be through the foliage, perhaps on the mainland, where aerosols, if they occur, are not as ion rich (Meurk *et al.*, 1994b), nutrients may be in short supply and necessary trace elements may be lacking.

Temperature effects

The winter temperature regime of Campbell Island demonstrates the buffering effect of the ocean: the mean minimum soil and air temperatures are warmer than those of the most similar latitude and altitude on the mainland and the winter mean maximum air temperatures of the two places are closer than in they are in the summer. This illustrates the large range in the temperature regime of the mainland compared with Campbell Island. Summer temperatures on the mainland are considerably warmer than on Campbell Island, with a mean monthly maximum in January and February at Invercargill of 26°C, compared with approximately 15°C on Campbell Island. An 11°C increase in maximum temperature, along with the larger range on the mainland, may prove detrimental to growth depending on the species' optima for gas exchange. The plastic cloches applied to the plants raised the daytime ambient temperature by up to 3°C, and this increased the CHO concentration relative to the control by 9% for *P. speciosum* and 20% for *A. latifolia*. This contrasts with work on *Pringlea antiscorbutica* from the Kerguelen archipelago (Aubert *et al.,* 1999) where it was found that temperature appeared to have little influence on CHO production.

As temperature does seem to affect CHO levels of *A. latifolia*, low temperature may be limiting on the island for this species, and that it may in fact do just as well, or even better, with higher daytime temperatures. These responses correspond with cultivation on the mainland: *A. latifolia* is able to grow but *P. speciosum* does not survive. *P. speciosum* could have a lower temperature optimum for photosynthesis than *A. latifolia,* proving to be less adaptable, but the reverse was found to be the case in gas exchange studies (Chapter 5). These are inconsistencies that may be resolved with longer-term studies. Alternatively, *P. speciosum* may be able to produce more CHO during the mainland day, but, as night temperatures are greater than occurred in this experiment (where there was no difference between ambient and the cloche treatments at night; Table 3A), the increased production may be offset by increased nocturnal respiratory demand.

Large leaves maintain a higher temperature than small leaves. Wardle (1991) has suggested that a "greenhouse space" is set up between the large overlapping leaves of *Pleurophyllum,* with leaves acting as solar panels and raising leaf temperature up to 15°C above the air temperature. With radiation focussed towards the growing apex, temperatures of up to 25°C above ambient air have been recorded in the apex of Hawaiian montane silverswords, *Argyroxiphium* (Melcher *et al.,* 1994). Leaf pubescence also allows for thermoregulation by increasing boundary layer thickness: *Espeletia* leaf temperature has been found to be over 5°C higher than ambient temperature (Meinzer and Goldstein 1985). An increase in air temperature may increase plant temperature above the optimum and may result in decreased photosynthetic rate, possibly solarisation (decreased starch production), bleaching (chlorophyll destruction) and denaturing of enzymes (Nobel 1976). No IR differences in leaf temperature were detected in this experiment however. More measurements and more sensitive testing may be required to confirm that this 'solar panel' idea does apply in subantarctic environments.

Below ground temperatures might also influence plant growth. Soil temperatures on the mainland are approximately 4°C warmer in the summer than on Campbell. Studies on CHO storage and use in alpine perennials (e.g. Mooney and Billings, 1960) have found that respiration over the dormant period is a major sink, in some cases accounting for over 50% of stored CHOs (Wyka, 1999). An increase in soil temperature, such as occurs in cultivation, might further increase respiration of underground plant parts, reducing CHO production more, and affecting long-term fitness. *In situ* soil warming might be informative (refer to Buxton Climate Change Impacts Laboratory, Sheffield).

Shelter effects

Large leaves have large boundary layers enhanced by fine hairs which reduce heat loss. Because transpiration occurs at a similar rate per unit area in a large leaf as in a small leaf, large leaves get hotter and are so are less adaptive for hot conditions (Nobel, 1976). When incoming radiation and leaf resistances are low, wind can increase transpiration and reduce plant temperature by removing the boundary layer (Salisbury and Ross, 1991). However, when compared with glaucous leaves, leaf pubescence has been shown to buffer temperature change by reducing temperature drop in increased wind speeds (Meinzer and Goldstein, 1985). Providing shelter might preserve a larger boundary layer possibly raising the plant's temperature while decreasing water loss due to the complex ratio of boundary layer resistance to stomatal resistance, and leaf pubescence (Meinzer and Goldstein, 1985). On the mainland, where conditions are less windy, megaherb temperatures, particularly in the pubescent *Pleurophyllum* (Figure 14A and B), may be raised above their optima. Conversely, if temperature is limiting on the island growth might actually be enhanced by shelter, and reflected in increased CHO production during the day.

While the sheltering treatment appeared to reduce ambient and gusting wind speed, it did so insignificantly ($p = 0.158$ and 0.305 respectively) and did not have any effect on CHO concentrations. Wind speed is markedly reduced at plant level anyway (from 12.6 m s^{-1} at 2 m height to 3.1 m s^{-1} at <1 m, $p < 0.001$, $n = 14$, *i.e.* approximately 37%). This suggests that perhaps the plants are subjected to less extreme wind conditions than is initially apparent, perhaps as a consequence of the compact megaherbs rosette form (although *P. criniferum* can be 'emergent' above the surrounding vegetation). However, wind speed on the island is still greater than that of the mainland where boundary layers are likely to be thicker, keeping transpiration low, but increasing leaf temperature.

Seasonal effects

Using CHO concentration as a measure of response, *A. latifolia* and *P. speciosum* reacted differently to the increased temperature and increased night length as the season went on, although this was not apparent in leaf growth. The significant response over time, as manifested in the decline up to the third sampling period (2-5 February) followed by a rapid increase, may indicate an increased use of CHO for growth and reproduction. Flowering was virtually over by this stage and seeds were beginning to set. Alternatively it could be weather related - records show temperatures were high prior to this sampling period and rainfall was low; the plants may have been suffering moisture stress (the effect of which was not investigated in this study). The marked increase in CHO in the final sampling period over both species is quite a rapid change in only a week. Without testing CHO levels in other parts of the plants, e.g. rhizome, roots and inflorescences, one can only speculate that CHO use for growth, perhaps in seed setting, is concluding, and is now being accumulated for overwintering. This is a general pattern found in CHO studies of alpine plants (e.g. Mooney and Billings, 1961; Payton and Brasch, 1978).

A)

B)

Figure 14: Pubescence in *P. speciosum* A) on leaves showing entrapment of moisture; and B) surrounding reduced stem.

CHO levels in *B. rossii* also showed a difference over time and this is reflected in growth rates, particularly after the addition of nutrients, although CHO of the other three species in the Congeneric Experiment remained fairly constant. The change over time could be attributed to seasonal changes in CHO within *B. rossii* as the plant translocates its CHO. The leaves of this species were showing signs of advanced senescence by the end of the study period. 20-30% of dried shoot and rhizome biomass is devoted to reproductive tissue (scape and inflorescences) in megaherbs (Nicholls and Rapson, 1999), comparable to 34% in *Celmisia haastii* (Hadley and Rosen, 1974), showing a large mobilisation of energy to reproduction.

Plant CHO pool size changes over the season depending on vegetative or reproductive growth or senescence. A study on *Celmisia* (Hadley and Rosen, 1974) found that the evergreen species of *Celmisia haastii* relied on ongoing photosynthesis for growth and reproduction and not on CHO storage; in fact, above and below ground parts of the species studied did not act as overwintering storage sites. This is contrary to perennials in arctic conditions, where a short growing season of a few weeks constricts perennials to the use of stored CHO in underground plant parts at least for the initial spring growth (e.g. Mooney and Billings, 1960; Wyka, 1999). In megaherbs growth may be from both ongoing photosynthesis and storage. This requires further investigation to understand CHO utilisation throughout the whole plant and how the environment might influence this.

Phenology

Mark (1970) found that floral primordia initiation in alpine plants occurs in the autumn and a bad winter can deplete the next flowering season's display. However, this only occurs in a few subantarctic species (mostly grasses), possibly because of the longer growing season and longer day length (Smith, 1984). Even so, megaherbs can be erratic in both quantity of flowering (Meurk, 1977) and timing of display (see below) and the study year (1996-1997) was a particularly good summer in terms of flowering (pers. comm. Peter Moore). The buds were developing at the end of December, and flowering was almost over by the 6 February, although in 1959 one *P. speciosum* was seen flowering in October, some in early December, and some in May (Bailey and Sorensen, 1962). In 1998 buds of *P. speciosum* were appearing in early January and still being produced mid February (pers. comm. Ian Flux).

The life history of *B. rossii* differs from the other species in that it is a bulbous monocotyledon, although. *P. criniferum* also has a deciduous lifestyle. There are both advantages and disadvantages to having such a lifestyle. While it may allow survival over a possibly hostile time it also involves a lot of energy to start afresh every year (Billings and Mooney, 1968). Here I have evidence of increased production of CHO pools, perhaps for storage, as winter approaches.

Leaf growth in *P. criniferum* and *B. rossii* appeared to be enhanced by low light and increased nutrient treatments (respectively). Leaf growth is likely to be more marked in these two species as they renew their growth every year compared with the other species which retain some foliage over the winter. Data collected by Ian Flux from 7 January 1998 to 15 February suggest that *P. criniferum* appears to have a finite number of leaves while in *P. speciosum* and *P. hookeri* new leaves are continually being formed and still growing. This is consistent with findings in a study of biomass allocation for *P. speciosum* in Chapter 2 (Nicholls and Rapson, 1999) where leaf production appeared to be continuous.

Conclusion

This study, the first exploration of megaherb physiology *in situ*, indicates that these species have specific responses which appear adaptive to their island environments. That megaherb growth is not detrimentally affected by all treatments suggests that the species are not restricted to the subantarctic environment, and that they may be able to exploit a wide range of conditions. Some species have the capacity to adapt to new or different environments. *A. latifolia* is capable of this but *Pleurophyllum* is not. It is interesting to note that *B. rossii* and *A. latifolia*, the most responsive species in the two experiments, have congeners on the mainland which might imply these genera have some genetic flexibility in their environmental requirements. Yet because they often do not grow well on the mainland, this suggests some other process must be limiting. The temperature results here suggest gas exchange rates may be crucial (examined in Chapter 5). Additionally, longer-term studies (e.g. Haag, 1974; Parsons *et al.*, 1994; Shevtsova *et al.*, 1997)) of environmental manipulation *in situ* would be productive, as would more rigorous treatment, and more detailed, though destructive, carbohydrate analysis of all portions of the plant.

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CHAPTER FIVE

Photosynthesis and Respiration of Five Megaherb Species in a Range of Light and Temperature Regimes

Abstract

Gas exchange of a selection of subantarctic and other megaherbs was studied in controlled environments to determine physiological limitations to their environmental range and to test their potential to adapt to other environments, *i.e.* their plasticity. Three subantarctic species were used (*Pleurophyllum criniferum*, *Anisotome latifolia*, *Stilbocarpa polaris*) and compared to the New Zealand subalpine *Ranunculus lyallii*, and the Chatham Island *Myosotidium hortensia*. Three levels of light and three temperature regimes were applied to the plants and their photosynthesis and respiration was measured using an infra-red gas analyser. The species' mean net photosynthetic rates varied with *Pleurophyllum* (8.6 μmol m^{-2} s^{-1}) and *Ranunculus* (7.8 μmol m^{-2} s^{-1}) having the highest overall rates, *Anisotome* and *Myosotidium* (both 4.6 μmol m^{-2} s^{-1}) the next highest rates, and *Stilbocarpa* (2.1 μmol m^{-2} s^{-1}) the lowest. Light optima for the species were not detected although the highest light level (950 μmol m^{-2} s^{-1} PPF) generated the highest photosynthetic rates, except in *Stilbocarpa* which had very little difference in photosynthetic rate between 380 μmol m^{-2} s^{-1} PPF and 950 μmol m^{-2} s^{-1} PPF). Respiration rates increased with temperature, and while the species had different mean rates there was no species by temperature interaction. These results imply that, contrary to expectation, these species have wide light and temperature ranges. Although the subantarctic megaherbs are physiologically well adapted to the subantarctic climate, they show a plasticity that suggests a greater ability than expected to adapt to novel conditions and possibly environmental change.

Introduction

Large, colourful, herbaceous perennials - 'megaherbs' - are a common growth form in the subantarctic islands of New Zealand, where several species are endemic. In order to survive in the subantarctic environment they must be adapted to usually overcast conditions, strong winds, high humidity and cool temperatures. Studies on these plants have mainly been confined to some early anatomical descriptions (Herriott, 1905), ecology (e.g. Cockayne, 1903; Oliver and Sorensen, 1951; Meurk, 1980), vegetation monitoring (e.g. Meurk *et al.*, 1994), and some phylogenetics (Mitchell *et al.*, 1999), but there has been very little physiological studies to date. Because of their size and beauty, several attempts have been made at growing them on the mainland of New Zealand (pers. comm. Carol West). While some species appear to adapt to mainland conditions, others do not thrive in cultivation (pers. comm. Alison Evans).

It is possible that megaherbs are narrowly adapted to their subantarctic environment and have low plasticity which restricts their ability to adjust to other environments. While field investigations of photosynthesis (e.g. Korner and Diemer, 1987; Goldstein *et al.*, 1989; Shevtsova *et al.*, 1997; Fetene, *et al.*, 1997) give an understanding of a plant's response to its current environment, investigation under a controlled environment can give an insight into the plant's potential for adaptation and limitations, *i.e.* its plasticity. Such environmental manipulations are a common method of investigating plant physiology and photosynthetic capacity (e.g. Scott *et al.*, 1970; Schulze *et al.*, 1985; Landhausser *et al.*, 1997). Here, gas exchange of selected megaherbs species was measured using an infra-red gas analyser to obtain an indication of their environmental requirements and optima. Living in such an environment these plants might be expected to have low light and temperature optima, possibly becoming photoinhibited at high irradiances (Bjorkman, 1981a) and suffering heat injury at higher temperatures (Bjorkman, 1981b).

Available vegetative species were tested on the mainland of New Zealand in controlled conditions (growth cabinet) at Crop and Food Research, Invermay. Three subantarctic species, *Anisotome latifolia*, *Stilbocarpa polaris* and *Pleurophyllum criniferum* were studied. *Anisotome latifolia* (Apiaceae) is a dioecious evergreen with rigid, pinnate leaves and large, pink inflorescences (see Chapter 4, Figure 2A). *Stilbocarpa polaris* (Araliaceae) is an evergreen with thick petioles and large pleated leaves covered in bristles. The large massed flower heads are green. *Pleurophyllum criniferum* (Asteraceae) is a deciduous herb with flowering scapes reaching 1m supporting pendulous, discoid capitula (see Chapter 4, Figure 1B). Its leaves have a dense layer of indumentum on the underside (Figure 1). These were compared with two other 'megaherb-like' species, *Myosotidium hortensia* and *Ranunculus lyallii*. *Myosotidium hortensia* (Boraginaceae) is an evergreen with large, glabrous leaves and large heads of blue flowers in the spring. It is endemic to Chatham Islands where it grows in peat on the coast. *Ranunculus lyallii* (Ranunculaceae) is a subalpine herb with large glabrous orbicular leaves and large white flowers in the summer. It grows in the subalpine zone of the North and South Island mountains of New Zealand.

Figure 1: The lower leaf of a young *P. criniferum* plant showing a fine covering of indumentum.

Methods

All plants except *M. hortensia* were supplied by Crop and Food Research, Invermay. *A. latifolia* and *S. polaris* were grown from seed collected on Campbell Island in 1994; *P. criniferum* plants were raised from tissue culture from seed collected on Campbell Island; and *R. lyallii* raised from seed collected from the Ben Ohau Range (inland Canterbury) in 1994. Plants ranged from 1 and 4 years old. *M. hortensia* was purchased commercially and taken to Invermay several months earlier to acclimatise to the local conditions. *A. latifolia* and *M. hortensia* plants were grown in bags (150 mm x 150 mm) while *S. polaris*, *P. criniferum* and *R. lyallii* were small plants in 80 mm x 80 mm pots.

Nine plants of each species were used except for *Stilbocarpa* which, due to limited supply, were only three individuals in total. Plants were exposed to three temperatures: 7°C, 17°C and 25°C using a growth cabinet. Temperature was set the previous night to allow the growth cabinet to stabilise. Three different individuals of each species (one for *Stilbocarpa*) were used for each temperature level as it was considered accumulated handling might damage the plants. All 13 plants were placed at random within the growth cabinet.

Within each temperature, the plants were exposed sequentially to four different light levels (PPF) of 950 μmol m^{-2} s^{-1}, 380 μmol m^{-2} s^{-1}, 150 μmol m^{-2} s^{-1} and 0 μmol m^{-2} s^{-1} to record respiration rates. Light was supplied by 6 x 375 W Philips HPI/T mercury iodide high pressure lamps and 2 x 1000 W Philips tungsten halogen lamps giving a maximum of 900-1000 μmol m^{-2} s^{-1} at leaf height (Parmenter and Littlejohn, 1998). CO_2 levels were ambient. Light was controlled using muslin screens placed under the overhead light source, and plants were left to acclimatise for 10 minutes at each light level before being measured. Gas exchange was measured using an infrared gas analyser Li6200 under a closed system with change in CO_2 recorded over a 30 second period (Figure 2).

Figure 2: Measuring photosynthesis in the growth cabinet.

Two leaves on each plant were used for gas exchange measurements while still attached to the plant providing six recordings for each light level (and two for *Stilbocarpa*). Two different cuvettes were used depending on leaf size: a small cuvette (305 cm^3) was used for *Pleurophyllum* and *Stilbocarpa* and a larger cuvette (1800 cm^3) was used for *Anisotome, Ranunculus* and *Myosotidium*. To seal the leaf chamber, foam rubber (draught stopper) was used on both chambers while "play dough" was used to seal the petiole aperture in the larger cuvette. At the conclusion of the measurements, leaf area was obtained non-destructively by photographing the measured leaves against a grid, cutting out the leaf image and weighing it, and comparing the weights to the weight of a known area on the photograph.

Photosynthesis was calculated from the expression

$$A = -K*V/S*\delta C/\delta t$$ (Jarvis and Catsky, 1971 and Li-Cor, 1986)

where A is the photosynthetic rate ($\mu mol\ m^{-2}\ s^{-1}$); V is the volume of the total system (m^3) (0.001954 for the large leaf chamber, and 0.000459 for the smaller chamber); S is the leaf area (m^2); and $\delta C/\delta t$ is the rate of change per unit volume of CO_2 over time (µmol). K is a correction from STP (Standard Temperature and Pressure): 40 at 25°C, 42 at 17°C and 43 at 7°C. As each plant was measured at four light levels within each temperature, and the same two leaves were measured on each plant each time, the data were analysed as a nested ANOVA (SYSTAT, 1992) with repeated measures.

Results

Photosynthesis

The species had significantly different overall photosynthetic rates (Table 1A) which varied fourfold (Figure 3). *Pleurophyllum* and *Ranunculus* had similarly high rates, *Anisotome* and *Myosotidium* had equal rates and *Stilbocarpa* had the lowest. Light had a strong effect on photosynthesis, with photosynthesis increasing at each light level (Figure 4) though the response was not linear, the rates of increase declining over the two highest light levels. This suggests the species may be approaching their light optima although this was not verified. Light also generated significantly different responses in the species (Table 1A). *Stilbocarpa* showed little response to light levels over 380 μmol m^{-2} s^{-1} (Figure 5). *Pleurophyllum, Ranunculus* and *Anisotome* appear to have light optima just beyond 950 μmol m^{-2} s^{-1} as their photosynthetic rates are decelerating. *Myosotidium* appears to have a slower rate of increase and is likely to have a lower optimum than the other species.

While there is no temperature effect *per se* (mean values for all species combined being 6.3 μmol m^{-2} s^{-1}, 6.1 μmol m^{-2} s^{-1} and 5.8 μmol m^{-2} s^{-1} respectively at each temperature) there is a third order interaction between temperature, light and species (Figure 6). *Stilbocarpa* appears unresponsive to all light and temperature treatments. Similarly *Myosotidium* is very unresponsive to temperature differences though it does respond to light increase. *Anisotome* becomes increasingly sensitive to temperature at higher light levels, while *Ranunculus* responds with increasing photosynthetic rates to both light and temperature. *Pleurophyllum* results indicate variable responses to temperature with light increase.

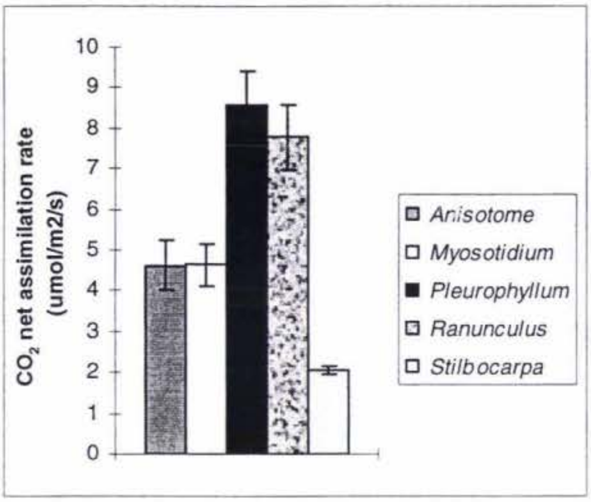


Figure 3: Net assimilation rates for five megaherb species averaged over three replicates (one for *Stilbocarpa*), three temperatures and three light levels. Standard error bars shown.

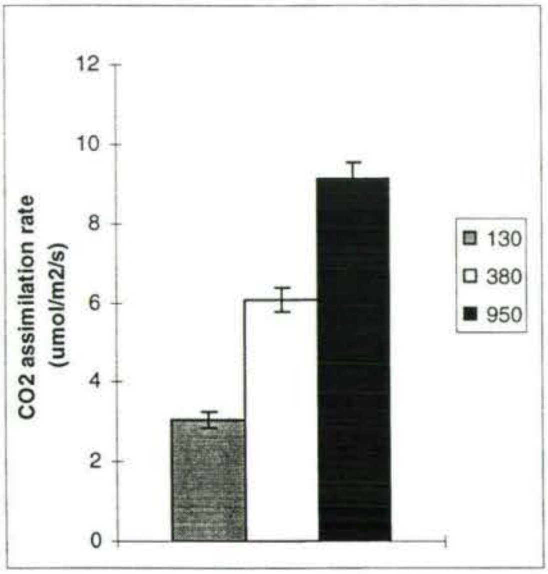


Figure 4: Net assimilation rates at three light levels (µmol m^{-2} s^{-1}), averaged over three replicates, five species and three temperatures. Standard error bars shown.

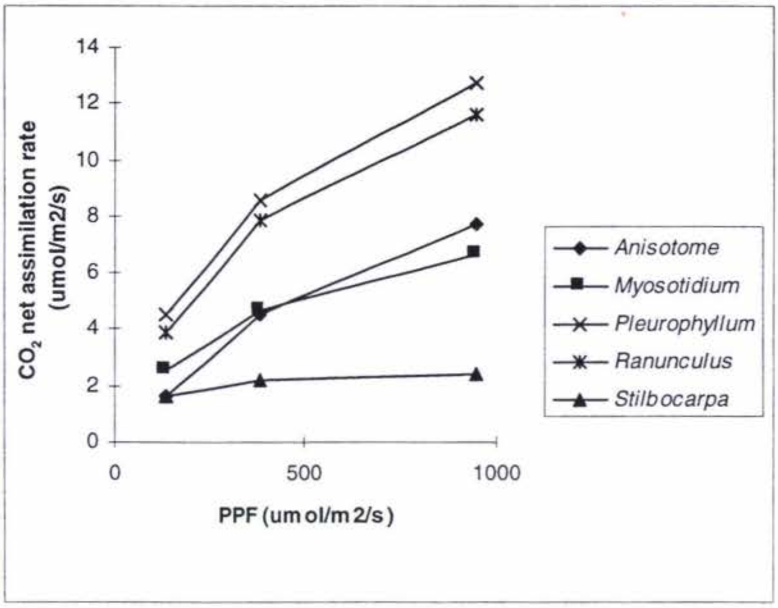
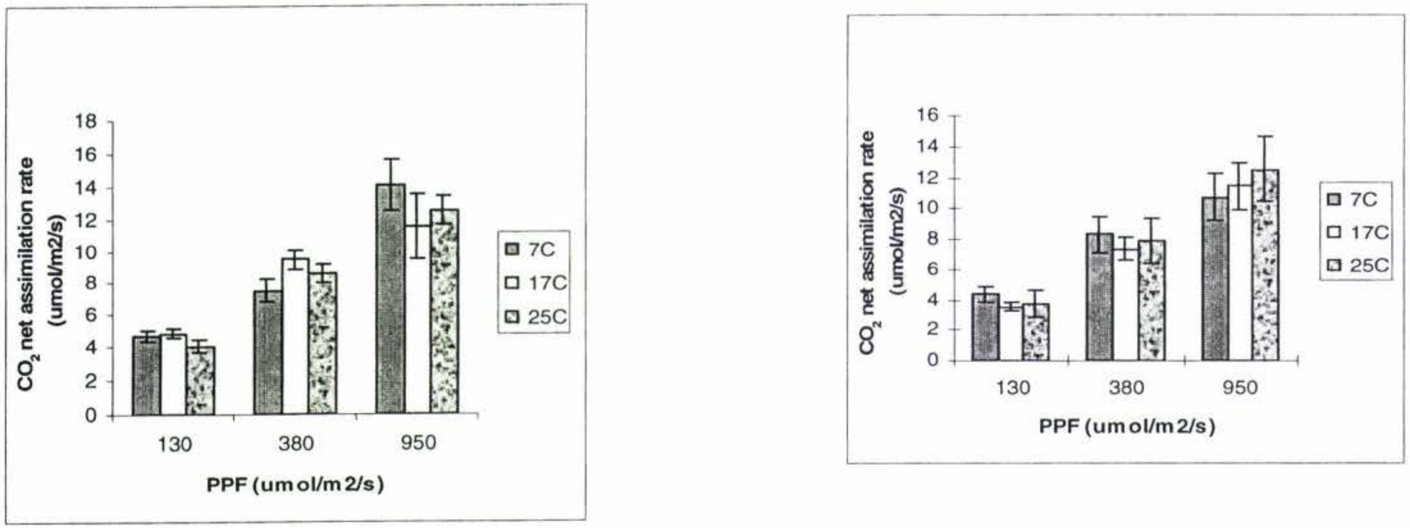


Figure 5: Net assimilation rates for individual species (mean of three replicates and three temperatures) with increasing light from 130 μmol m^{-2} s^{-1} to 950 μmol m^{-2} s^{-1}.

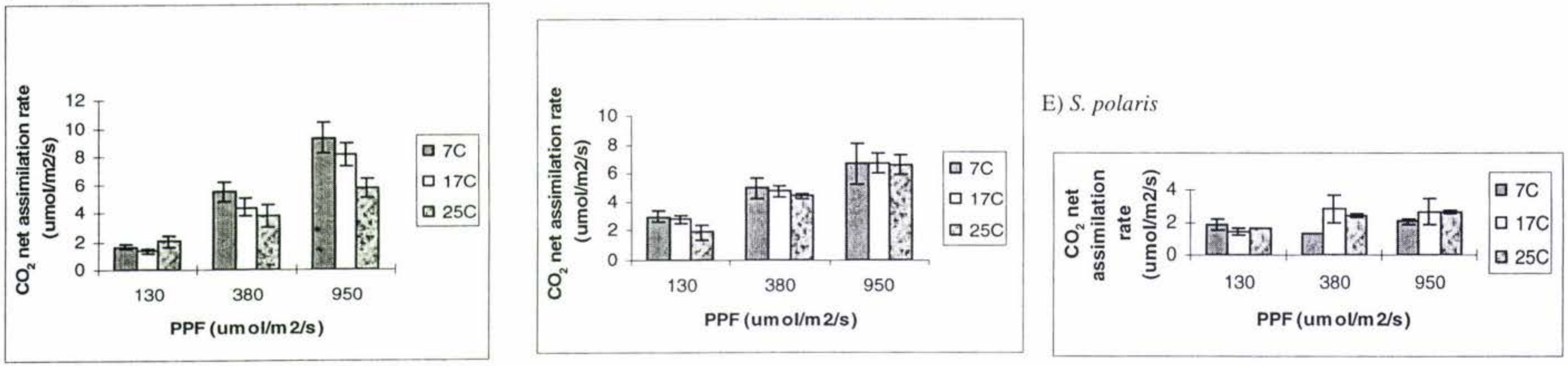
Table 1: A) ANOVA for photosynthesis of five species (*Anisotome, Myosotidium, Pleurophyllum, Ranunculus* and *Stilbocarpa*) over three light levels (130 μmol m^{-2} s^{-1}, 380μmol m^{-2} s^{-1} and 950μmol $m^{-2}s^{-1}$) and three temperatures (7°C, 17°C, and 25°C). (Bolding indicates significance at the 5% significance level.)

Source	**SS**	**df**	**MS**	**P**
Species	997.513	4	249.378	**<0.001**
Temperature	4.557	2	2.279	0.587
Species*Temperature	22.984	8	2.873	0.699
Error	96.157	23	4.181	
Within subjects				
Light level	897.458	2	448.729	**<0.001**
Light level *Species	180.595	8	22.574	**<0.001**
Light level*Temperature	3.104	4	0.776	0.694
Light level*Species*Temperature	68.651	16	4.291	**0.001**
Error	63.912	46	1.389	



A) *P. speciosum*

B) *R. lyallii*



C) *A. latifolia*

D) *M. hortensia*

E) *S. polaris*

Figure 6: Net assimilation rates of CO_2 for individual species at each light and temperature level for A) *A. latifolia*; B) *M. hortensia*; C) *P. criniferum*; D) *R. lyallii*; E) *S. polaris*. Standard error bars shown.

Respiration

Overall respiration significantly increased with increase in temperature (Figure 7; Table 2). All species had significantly different respiration rates especially high in *Ranunculus* and *Anisotome* (Figure 8), though these rates did not necessarily correspond to their photosynthetic rates. Averaged over three replicates, three light levels, and three temperatures, respiration in *Anisotome* accounted for 19% of gross photosynthesis, *Ranunculus* 10%, *Pleurophyllum* 5%, *Myosotidium* 12%, and *Stilbocarpa* 21%.

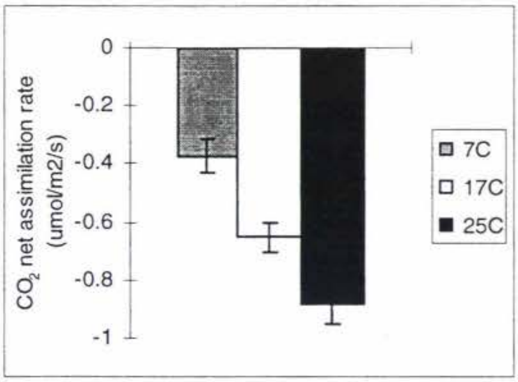


Figure 7: Overall respiration rates with increasing temperature, averaged over three species and three replicates (one for *Stilbocarpa*). Standard error bars shown.

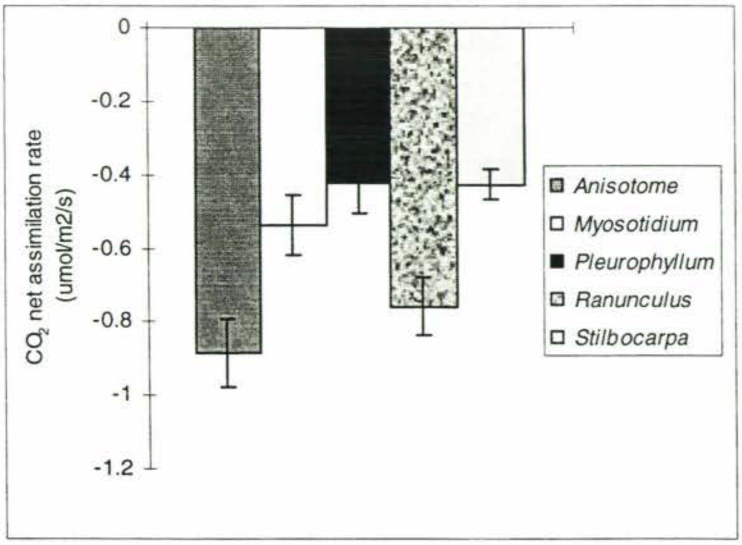


Figure 8: Individual respiration rates of five species averaged over three temperatures and three replicates (one for *Stilbocarpa*). Standard error bars shown.

Table 2: ANOVA for dark respiration of five species (*Anisotome, Myosotidium, Pleurophyllum, Ranunculus* and *Stilbocarpa*) over three temperatures (7°C, 17°C, and 25°C). (Bolding indicates significance at the 5% significance level.)

Source	**SS**	**df**	**MS**	**P**
Species	2.562	4	0.640	**<0.001**
Temperature	2.413	2	1.207	**<0.001**
Species*Temperature	0.426	8	0.053	0.699
Error	1.784	23	0.078	

Discussion

Subantarctic megaherbs which live in an environment characterised by a high degree of cloud cover and cool temperatures, might be expected to have low light and temperature optima, possibly becoming photoinhibited at relatively low irradiances and undergoing heat injury at higher temperatures (Bjorkman, 1981a; Bjorkman, 1981b). In contrast to such expectations, this study showed that, while these megaherbs all have individualistic light and temperature requirements, at least two of the subantarctic species have the potential to photosynthesise at higher irradiances and temperatures than they probably commonly receive on the island.

Light

Photosynthetic Photon Flux (PPF) in the habitat of the subantarctic megaherb is commonly low, the island receiving only 16% of possible sunshine hours (NZ Meteorological Service, 1980). While on a daily basis this can rapidly change (Chapter 4, light intensity is usually lower than 950 μmol m^{-2} s^{-1} (Chapter 4, Figure 10). *Pleurophyllum*, *Ranunculus* and *Anisotome* have different photosynthetic rates which appear to approach an optimum beyond 950 μmol m^{-2} s^{-1}. Light optima for species from similar subantarctic environments vary. *Agrostis* on Marion Island reaches saturation at 300 - 600μmol m^{-2} s^{-1} (Pammenter *et al.*, 1986), while *Pringlea* on Kerguelen saturates at 1500 μmol m^{-2} s^{-1} (Aubert *et al.*, 1999), and *Poa cookii*, from Marion Island, saturates at over 2000 μmol m^{-2} s^{-1} (Bate and Smith, 1983), demonstrating a wide range of photosynthetic capacities.

Ranunculus has the second highest average photosynthetic rate in this study with a light optimum of over 950 μmol m^{-2} s^{-1}. Similar photosynthetic rates and response to light was found by Parmenter and Littlejohn (1998). In a subalpine environment this species is likely to experience a variety of light conditions, from prolonged cloud cover to intense sunlight. Korner and Diemer (1987) found that *Ranunculus* species in the Austrian alps are always light-limited, despite light levels of up to 3000 μmol m^{-2} s^{-1}. While the light optimum for *Ranunculus* was not reached, it was decelerating at the highest level tested and is likely to approach an optimum of less than 2000 μmol m^{-2} s^{-1}, suggesting different photosynthetic capacities between European and New Zealand *Ranunculus* species.

The lower light optimum for *Myosotidium* in comparison with the others is unusual given that its natural habitat is the open coast of the Chatham Islands where it is likely to receive higher irradiance conditions than Campbell Island (a more northerly island receiving 38% of possible sunshine hours [NZ Meteorological Service, 1980]). However, in cultivation on the mainland it appears to prefer shade conditions (although this might be compensation for its native humid conditions).

Temperature

The lack of an overall temperature effect on photosynthesis is surprising, as temperatures tested ranged from those normally found on the island (mean summer temperature of 9°C) to a temperature expected to be outside the optimal range for subantarctic island-adapted species (25°C). Clearly photosynthesis is not limited by either cooler or warmer temperatures than normally experienced on the island (mean summer maximum of 15°C). This contrasts with recent gas exchange work carried out on *Ranunculus* (Parmenter and Littlejohn, 1998) which indicates a cooler optimum temperature of 17°C. High temperature optima are not uncommon in the subalpine/alpine habitat of *Ranunculus*. *Celmisia* was found to have an optimum of 30°C, somewhat higher than expected (Scott, 1970). At high temperatures however, respiration is also high. The species tested here had low respiration rates compared with other subalpine grasses (Mark, 1975) but similar rates to those found by Parmenter and Littlejohn (1998). It is doubtful that respiration rates are limiting adaptive responses to increased temperature in megaherbs.

Anisotome shows a stronger response to the cooler temperatures at all light levels. This compares with work on subalpine species *Chionochloa rubra* and *Celmisia spectabilis* which shows maximum photosynthetic rates at high light with low temperatures (Scott and Menalda, 1970). The subalpine species tested here, *Ranunculus*, shows a positive response to simultaneously increased light and temperature while *Myosotidium* shows very little response to temperature. *Stilbocarpa* also shows very little response to any treatment, though possibly this is an artefact of the small number of individuals tested.

In *Pringlea*, another subantarctic species from Kerguelen Islands, the photosynthetic rate varied less than 25% over a temperature range of 2.5-25°C (the optimum being 15°C) so that *Pringlea* is also influenced more by light than temperature (Aubert *et al*., 1999). Moreover, *Pringlea* continues to grow throughout the winter. It has been suggested that this low influence of temperature on photosynthesis might be a result of its historical distribution (Aubert *et al*, 1999). Considered to be a relict of the Tertiary flora (Cheeseman, 1909), *Pringlea* would have to be plastic enough to tolerate wide fluctuations of temperature over this time (Aubert *et al*., 1999). On Marion Island where the mean temperature of the coldest month is 3.2°C, *Poa cookii* is able to photosynthesise at 0°C (Bate and Smith, 1983). Campbell Island megaherbs might be expected to behave similarly. Similar investigations into the winter growth of the New Zealand species would be of interest although *Pleurophyllum* species tend to either be deciduous or reduce in size over this time (Cockayne, 1903), suggesting that they do not have a positive photosynthetic rate at low temperatures.

Conclusion

Plants are not necessarily optimally designed for their environment. They may merely be adapted to it (Parkhurst and Loucks, 1972), possibly possessing a plasticity enabling them to adapt to other environments. The species tested here (except for *Stilbocarpa*) show no immediate signs of stress to their photosynthetic apparatus in conditions warmer and lighter than their native habitat suggesting that they have a genetic ability to adapt to novel environments.

This study of gas exchange was restricted to investigating the effect of light and temperature on CO_2 assimilation rate and as such should be considered as a preliminary indication of their photosynthesis. For a more comprehensive survey of their physiological adaptiveness further work should include extending the light and temperature range tested, defining optima and determining light compensation points. It would also be beneficial to measure the gas exchange of megaherb species on the island, exploring possible sun-fleck responses and determining how they behave in their natural environment.

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CHAPTER SIX

Discussion

The origin and evolution of the megaherb in the windy, wet, cool and overcast subantarctic environment is a conundrum that has intrigued many over the years. The megaherbs may be old phylogenetically, as Cheeseman (1909) and Godley (1975) suggest, in which case they would show many ancestral characters, and would persist as relicts of a now largely extinct flora *i.e.* palaeoendemics. Or they may be of more recent and derived origin, having undergone *in situ* speciation and adaptive radiation in these subantarctic habitats from a small number of colonists. Various methods have been used to investigate this issue, such as plant ecology (e.g. Meurk *et al.,*1994a), biogeography (e.g. Lloyd, 1982), palynology (e.g. Moar, 1958) and phylogenetics (Mitchell *et al.,*1999). In this thesis, the approach has been to use ecophysiological methods in the first comprehensive study of the responses of these subantarctic megaherb species. The underlying assumptions are that relict species on islands have become narrowly adapted to their habitats due to accumulation of recessive or fixed genes during inbreeding in small or isolated populations (Barrett, 1996), and they are non-plastic due to these accumulated costs and not able to extend their range (Rapson and Maze, 1994; Barrett, 1996). Alternatively, more recently dispersed species, or those derived from recent radiations, should be closely adapted to their habitats, but may have evolved a high level of plasticity as a response mechanism, and may therefore be capable of expanding their gene pool and range (Stebbins and Major, 1965).

Biogeographically, Cheeseman (1909) identified three main elements in the subantarctic flora. One element is of South American origin and is both early and recent; this connection between vegetation types of Tierra del Fuego and the subantarctic islands has been well documented (e.g. Godley, 1960). The next element is made up of species still extant on the mainland of New Zealand. These species have arrived relatively recently, probably via trans-oceanic routes such as birds, sea or wind currents. A final element comprises endemism and contains two parts. The first part includes those endemic taxa with close relatives still in New Zealand. They have possibly dispersed from New Zealand either trans-oceanically, or by ancient land bridges (Cheeseman, 1909) and have newly evolved *in situ* (*i.e.* neoendemics). The second part of this endemic element are possible relicts of a much more widespread, ancient flora (Cheeseman, 1909; Godley, 1975), *i.e.* palaeoendemics. It is to this last group that the subantarctic megaherbs possibly belong, since none of these megaherb species occur on mainland New Zealand (though several have congeneric relatives in southern New Zealand, and in the alpine zone).

Phylogenetic Considerations

The subantarctic islands of New Zealand are of mixed age and origin. The Bounty Islands and the Snares are outcrops of ancient metamorphic rock (over 100 million years), while the Auckland Islands and Campbell Island are Tertiary volcanoes and the Antipodes much younger (12-23 million years old, 6-11 million years old, and 1-5 million years old respectively). Campbell Island volcanic activity occurred in the late Miocene period during times of great uplift on the mainland of New Zealand. During the colder Quaternary period (approximately 2 - 2.5 million years ago to present day) when continual glaciation and associated climate changes encouraged adaptive radiation in New Zealand (Raven, 1973), the oceanic climate of the subantarctic islands may have allowed for preservation of these existing species, despite some glaciation.

During the Quarternary other subantarctic islands also underwent glaciation (e.g. Kerguelen Islands 49°30'S, Marion Island 46°54'S, Heard Islands 53°S, South Georgia 54°25'S) leaving only a few islands as refugia. As a result, most of their vascular plants are considered to be ‘postglacial’ (Smith, 1984) with the possible exception of two species, *Pringlea* and *Lyallia* which survived on the Kerguelen Islands (Cheeseman, 1909; Young and Schofield, 1973; Aubert *et al.,* 1999). While *Lyallia* is a dwarf cushion plant, *Pringlea* is a large-leaved perennial similar in form to the New Zealand subantarctic megaherb that may share a similar survival history.

If a relict species, the large-leaved “megaherb” species would already have been in existence in refuges and re-colonised these islands as they became available. One way of assessing this possibility is to examine aspects of ecology and ecophysiological responses as a key to adaptiveness and plasticity outside of their range.

The Megaherb Form

Subantarctic megaherbs are herbaceous perennials of typically large size (up to 1 m high) with brightly coloured massed floral displays. Their leaves are either coriaceous or pubescent (see Chapter 4, Figure 14A), and they have a stout rhizome (Figure 1). Their similar morphologies suggest that they may be examples of convergent evolution in response to the present environment conditions (Mitchell *et al.*, 1999), evolving, possibly *in situ,* from diverse, possibly smaller ancestors, as demonstrated in phylogenetic work on *Stilbocarpa.*

Figure 1: The rhizome of *P. speciosum* (approx. 3 cm diameter).

Vegetation types and associations between the tropical alpine zone and the subantarctic have been compared (Troll, 1960), and the occurrence of a similar growth form, the giant rosette has been commented on (Hedberg, 1964): *Pringlea* in the Kerguelen Islands, *Argyroxiphium* in Hawaii, and in the tropical alpine zone of the Andes and Africa, the 'pachycauls' *Lobelia, Dendrosenecio* and *Espeletia.* While not considered by Troll or Hedberg, I would add to this assemblage the New Zealand subantarctic megaherb (although it lacks the extreme development of the stem; see Chapter 2; Chapter 4, Figure 14B).

The megaherb growth forms in the alpine tropics are mainly a result of extreme diurnal, rather than seasonal, fluctuations, frequent frosts, high irradiances, and occasional dry periods (Hedburg, 1964). Comparisons of this tropical alpine zone with the subantarctic islands (Troll, 1960) and the New Zealand subalpine zone (Mark *et al.,* 2000), have often been made, rather than with the temperate alpine zone, on the basis of a shared lack of marked seasons, or at least an extended, but variably favourable growing season. The growing season for *P. hookeri* and *S. polaris* on Macquarie Island is approximately eight months (Jenkin and Ashton, 1970). While not being as 'isothermic' as the more southern Macquarie Island, the New Zealand subantarctic islands do not suffer extreme temperature variability during the growing season either; the mean annual temperatures over the New Zealand subantarctic range (47°S to 53°S) is 11°C to 6°C while Macquarie at 54°S is approximately 5°C. Temperatures for summer and winter on Campbell Island are 9°C and 5°C respectively. It is this environment which appears conducive to the development of the megaherb form.

From personal observation there appears to be variable populations of some of these species. The populations of *P. hookeri* growing on Auckland Islands, Campbell Island and Macquarie Island appear to be slightly different in form. Campbell Island has a slightly more slender leaf (Figure 2A), the other island forms having wide leaves (Figure 2B and C). Also, the Macquarie Island form seems to have a larger growth habit than the other islands. This may be a result of plasticity in the different environments, or may be due to genetic variation illustrating adaptive radiation. These forms are worth investigating at both the molecular level and ecophysiological levels.

A) B) C)

Figure 2: *P. hookeri* occurs on Campbell, Auckland and Macquarie Islands. Variation in form may suggest genetic variation between the islands; A) Campbell Island form; B) Auckland Island form; and C) Macquarie Island.

Phenological Responses

Due to the absence of a marked growing season, continuous growth is commonly found in the tropical alpine species and in *Pringlea* from Kerguelen Island (Aubert *et al.*, 1999). Campbell Island's *A. latifolia* also remains evergreen during the local 'winter'. In contrast, however, *Bulbinella* and *Pleurophyllum* species are either deciduous or reduce in size during the winter period, showing the variety of megaherb strategies.

While Kerguelen Island archipelago is at a lower latitude than Campbell Island, it lies closer to the Antarctic Convergence, with mean temperatures of 7.6°C and 2 .0°C for its warmest and coldest months respectively, with a mean annual temperature of 4 - 5°C. These means are slightly cooler than those for Campbell Island at 9.3°C and 4.7°C and 6.9°C respectively. As *Pleurophyllum* dies down in the winter it may not be as adapted to the island environment as its apparent ability to cope with a range of temperatures (Chapter 5) suggests. This response could be a relict of a colder climate, from which adaptive variation has been slow, although *Bulbinella rossii,* in the Liliaceae, has many low altitude mainland relatives that are routinely only summer-green. But as Hutton (1903) points out, not every character is of necessity adaptive, and this may merely be an artefact of other effects.

Analysis of resource allocation implies an almost continuous leaf production in both *A. latifolia* and *P. speciosum*, at least over the summer period (Nicholls and Rapson, 1999/ Chapter 2). New leaf production over most of the year has also been found in productivity studies on Macquarie Island (Jenkin and Ashton, 1970). This suggests that growth is indeterminate and therefore not as rigidly controlled as in alpines (Mark, 1970). Study of carbohydrate movement and storage over the winter period of these species would be beneficial to ascertain the level of growth or dormancy maintained during these months.

While the different species of megaherbs examined here have different strategies e.g. *P. speciosum* having a higher lamina:petiole ratio and allocation to rhizome than *A. latifolia,* they both have a high allocation to foliage (proportionate to the rest of the plant) (Chapter 2). Note only does this define the megaherb form (in the subantarctic), but it demonstrates the adaptive importance of resource harvesting as suggested by other workers (see Foliar Panels below).

The evolution of another characteristic of this growth form - colour - has ignited debate as to whether pigmentation such as found in the subantarctic flora is ancestral or derived (Wardle, 1978, cf. Lloyd, 1985). In a phylogenetic study on *Abrotenella*, Swenson and Bremer (1997) found floral pigmentation arose twice in the lineage supporting Lloyd's view of colour as being derived. A possible advantage for evolving colour in the cool subantarctic is that the pigmentation increases the heat inside the flower making it more attractive to the pollinator. Another explanation is that the possible increase in temperature might aid early seed development but as yet this is unfounded. Alternatively, colour may simply be an accidental byproduct of other adaptive changes (Frias *et al.*, 1975; Horowitz, 1976). Unfortunately, no conclusion can be drawn about the adaptive advantages of colour from my study. Though the *Pleurophyllum* hybrid is more intensely purple than *P. speciosum* (pers. obs.) (Chapter 3, Figure 1C), any influence this might have had on the greater quantity of seed set is difficult to determine. However, white morphs in *P. speciosum* do exist (pers. obs.; Figure 3), albeit in small numbers, and so there may be potential to do some comparative work on colour in this species. The existence of adaptive flower colour would indicate, as Swenson and Bremer (1997) suggests for *Abrotenella*, that megaherbs are derived, rather than relictual species.

Contrary to what one might expect in a windswept, damp, overcast environment *Pleurophyllum* showed no pollinator, or pollen limitation (Chapter 3). The main pollinators are very small but numerous and effective. Pollination (*i.e.* by biotic vectors) has been credited with the restriction of some species to their habitat, limiting expansion to other areas (Stebbins and Major, 1965). *Pleurophyllum* showed a combination of breeding systems - obligate out-crossing in the rayed *P. speciosum*, autonomous self-compatibility in the discoid *P. criniferum*, and a combination of autonomous selfing but higher seed set while out-crossing in *P. criniferum x speciosum*. Self-compatibility and autonomy may evolve in an environment where selection pressure exists from lack of pollinators or limited compatible pollen (Wyatt, 1983). In the Andean *Espeletia*, Berry and Calvo (1994) found that hybrids readily occurred due to the high outcrossing from generalist pollination allowing for adaptive radiation in the high Andes. That the New

Zealand subantarctic megaherb species belong to families with compound inflorescences that allow for generalist pollination shows that pollination may not be a limiting factor in their distribution, and that, combined with a variety of breeding systems, these species are likely to have the potential for adaptive radiation.

Figure 3: *P. speciosum* is usually purple but occasionally occurs in the white form.

Ecophysiological Responses

Ecophysiological work on Campbell Island megaherb species (Chapters 4 and 5) shows that they are more plastic in their behaviour than expected for a relict species (e.g. Stebbins and Major, 1965) and that they may be adaptable enough to survive changes to their temperature regime (Chapters 4 and 5). This is demonstrated in *A. latifolia* by increased carbohydrate stores in leaf lamina as a result of increased temperature effects on CO_2 assimilation (Chapter 4), and in *P. speciosum* by a photosynthetic capacity for a wide temperature range (Chapter 5). Their carbohydrate levels are unaffected by other environmental perturbations such as increased shelter, increased night length, reduced light and increased nutrients suggesting that the species are quite flexible to novel conditions. However, responses to reduced light and increased nutrients were apparent in leaf growth of *P. criniferum* and *B. rossii* respectively, which suggests these factors might be limiting and may need further investigation.

P. criniferum and *A. latifolia* both photosynthesise at rates within the range of other subantarctic species (Bate and Smith, 1983; Pammenter *et al.,* 1986) and the tropical Andean *Espeletia* (Goldstein *et al.,* 1989). Positive net photosynthesis in a temperature range between 7°C and 25°C suggests they have the potential to grow at both moderate and low temperatures. Physiological work on other subantarctic island species - on *Pringlea* in the Kerguelens (Aubert *et al.,* 1999) and on grasses on Marion Island (Pammenter *et al.,* 1986; Bate and Smith, 1984) also shows they are well adapted to the low light, and cool and windy conditions, but are capable of photosynthesising at higher light and/or temperature regimes. This demonstrates that these subantarctic species are all quite plastic in their behaviour, as is confirmed by many of my own results.

Pubescence is a feature of megaherbs, both in the tropical alpine zone and the subantarctic: *S. polaris* and *Pleurophyllum* (see Chapter 4, Figure 14; Chapter 5, Figure 1). Not only may pubescence contribute towards humidity control by trapping moisture from the atmosphere, but it also plays an important thermoregulation role in photosynthesis. Pubescence can enhance metabolic processes raising leaf temperature up to 7-8°C above the cooler surrounding air (by increasing leaf boundary layer) (Meinzer and Goldstein, 1985). But it may also reduce CO_2 assimilation by intercepting incoming radiation (Goldstein *et al.*, 1989), and when exposed to the irradiance and temperature levels of the mainland the effect of pubescence on plant temperature may be detrimental. Gas exchange studies of these plants did not give consistent results regarding pubescence (*S. polaris* had the lowest photosynthetic rate and *P. criniferum* had the highest rate; see Chapter 5, Figure 3). However, a difference in carbohydrate levels was found in the cloche treatment with the glabrous species (*A. latifolia*) responding more positively to temperature increase than the pubescent species (*P. speciosum*) (see Chapter 4, Figure 13

B and C). Whether pubescence had any influence on this result is not certain, but its effect on megaherb gas exchange requires investigation.

Respiration rates of those species tested (see Chapter 5, Figures 7 and 8) are well below those of New Zealand alpine tussock species (Mark, 1975), below that for New Zealand subalpine *Celmisia spectabilis* (Scott and Menalda, 1970) and subantarctic *Poa* (Bate and Smith, 1983), but similar to those found for *Ranunculus lyallii* (Parmenter and Littlejohn, 1998). This suggests that megaherbs have a high net photosynthetic rate and may not be sensitive to increased respiration rates. The tolerance to a wide temperature range was unexpected, particularly in *Pleurophyllum* as this genus, especially *P. speciosum*, has proven to be the more difficult of the megaherbs to cultivate on the mainland; it was hypothesised that this may be due to mainland temperature conditions being supra-optimal for respiration for the species. From these results it appears that this is not necessarily so as *P. criniferum* at least appears tolerant of a wide temperature range.

This apparent low sensitivity to temperature may indicate a plasticity to past climate changes, and possibly future changes. Indeed, *Pringlea*'s survival as relicts of a Tertiary flora is attributed to its low sensitivity to temperature (Aubert *et al.*, 1999).

The light saturation point for photosynthesis could not be tested, though *Pringlea's* saturation point is around 1500 µmol $m^{-2} s^{-1}$ (Aubert *et al.*, 1999), *i.e.*, about half peak summer sunshine at mainland latitudes. However, there are indications from the light/photosynthesis curves that the megaherbs could be approaching light saturation points at irradiances just higher than the tested high of 950 µmol $m^{-2} s^{-1}$ (Chapter 5). That *P. criniferum* also showed a positive response in leaf growth with decreased light *in situ*, suggests this species has a particularly wide tolerance to the lower light levels (Chapter 4) and may have a low light compensation point. While these megaherbs are well adapted then to the low light environment of the subantarctic, a detailed study of their tolerance of higher light levels is still required.

Foliar Panels

Wardle (1991) suggests that the large leaves of species such as *P. speciosum* act as 'solar panels' harvesting as much heat as possible in the normally cool and overcast environment. But my IR measurements indicate that leaf temperature was not significantly different than the ambient temperature (see Chapter 4, Table 2). In the cloche treatment, plant temperature was raised from between 6% and 50% above the control, with no apparent decline in lamina carbohydrate pool indicating that megaherbs are able to maintain their carbon balance in higher temperature regimes.

As the local peat substrate can be nutrient poor (Campbell, 1981), nutrient input may come from the sea, commonly transported as ion-rich aerosols; the large leaves may then act as nutrient harvesting panels (Meurk *et al.,* 1994b). Indeed, airborne cations play a considerable part in the plant life on the island and have been found to be an influential determinant of vegetation distribution on Campbell Island (Meurk and Foggo, 1988; Meurk *et al.,* 1994b). Yet in my experiment (see Chapter 4, Table 9A), when additional nutrients were applied to the foliage of several species, mimicking supplementation of aerosols, no apparent increase in carbohydrate level was attained, indicating that nutrients do not appear to be limiting the carbon balance for any of the species tested, although leaf growth of *B. rossii* appeared to be affected (Chapter 4, Figure 11C). This suggests that most plants are receiving all the nutrients they need (either from the air or the substrate). If there is a reliance on nutrient input from the air then this might be a limiting factor for establishment of megaherbs on the mainland by cultivation unless near the sea; manually applying aerosols is an obvious way to satisfy this possible requirement.

Humidity, another environmental variable, was not tested for here and possibly has an influential effect on photosynthesis given the humid nature of the subantarctic environment. As noted above, several species are markedly pubescent (*P. speciosum*, *P. hookeri*, *Stilbocarpa*) which may enhance entrapment of nutrients and moisture, maintaining high humidity around the leaf. Harvesting atmospheric moisture may also supplement water uptake from the soil if water requirements are high. Certainly the rosette form is an efficient arrangement for channelling nutrients and water to the root zone (Monasterio and Sarmiento, 1991; Agnew *et al.,* 1993).

Further, perhaps the large leaves may confer a competitive advantage by suppressing nearby smaller plants as has been found in *P. hookeri* (Jenkin and Ashton, 1979). The rapid reinvasion of megaherbs into suitable habitats following removal of introduced herbivores (Meurk, 1982) suggests a high competitive ability.

Attempting to isolate any one factor responsible for growth limitation is difficult, however, due to the complex and often synergistic interactions between environmental factors and physiological requirements. A unique combination of these may be responsible for restricting megaherb growth.

Cultivation

Cultivating plants outside their natural range is not always easy; for example many alpine species do not do well in cultivation. The subantarctic species, if narrowly adapted to their specific environment, may reasonably be expected to be 'difficult' plants to establish. *P. criniferum* is currently being grown in containers under shade and frequent misting regime at Invermay, Dunedin, and has survived two summers (pers. comm. G. Parmenter), but long-term survival is not necessarily guaranteed. In addition to limits discussed above, possible reasons for difficulty in adapting to mainland conditions may include the following: if nutrient aerosols are a significant component in a plant's life, or they require a micronutrient from the marine environment then growth might be difficult inland; diseases such as botrytis, grey mould, root rots, or 'sudden death syndrome' caused by latent fungi (Cartman, 1985), may all attack plants, often resulting in death; mycorrhizae associations occur in many plant species, playing a significant role in the assimilation of nutrients (Allen, 1991) and if this applies to the megaherb species it is possible that their chances of coming into contact with appropriate mycorrhizae may be limited outside of their native peat environment. This also requires further examination.

Future Work

Due to logistical constraints the *in situ* study reported here was only of a short duration. Longer term studies, ideally from one growing season to the next, particularly during the winter, of the impacts of environmental manipulations on carbohydrate movement and mobilisation within the plant would be beneficial to track any seasonal changes in carbon economy. Gas exchange measurements of the species *in situ,* and more controlled environment work with more extreme conditions would add to the preliminary data gathered here, identifying potential limitations on productivity. Studying the effect of humidity on plant performance would also be necessary as humidity is a significant component of the subantarctic environment and could well be a limiting factor in cultivation on the mainland.

Further pollination studies of megaherb species, including testing for wind pollination and the effect of colour, may demonstrate how species have adapted to the island conditions and would assist the understanding of evolution of breeding systems in isolated, island environments.

Molecular work comparing these species with their nearest New Zealand relatives could also be helpful in assisting with approximate times of dispersal, or evolution, shedding light on whether they are ancient relicts, or recent adaptations. Studying populations of the same species from different latitudes may also indicate how similar the species are throughout the Southern Ocean, *i.e.* if the species are maintaining their convergence, or if they are diverging into different forms.

Conclusion

There is a distinction between endemics that, as a result of reduced genetic composition, are limited by their narrow tolerances to their environment (possibly relictual), and those that are limited by the narrow confines (or uniformity) of their habitat (Stebbins and Major, 1965). Palaeoendemics, or relict species, would fall into the former group, and would possibly be approaching extinction (Stebbins and Major, 1965). The physiological tolerances of species then play a large part in the maintenance of their range and duration as a species.

These studies show that the megaherb growth form is well adapted to the present environment of the New Zealand subantarctic islands. However, given that this ecophysiological work also demonstrates that they are less dependent on temperature and light than expected, it is possible that they may have been adaptable enough to have survived long term environmental change. And, as in the case of the Kerguelen *Pringlea,* they may well be ancient relicts of a past, more widespread flora. But it is equally possible that they have evolved more recently *i.e.* since the last ice age, and adapted to the present climate. Their apparent plasticity to light and temperature in such a uniform environment and climate as the subantarctic, suggests that they do not have as reduced genetic variation as would be typical of a relict species. Megaherbs remain a conundrum.

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