The physiological importance of small leaf sizes in the Mediterranean-type ecosystem vegetation of the Cape Floristic Region

Megan Yates

Submitted in partial fulfilment of the degree of Bachelor of Science with Honours at The University of Cape Town

KD YATE
2007

This thesis was supervised by Michael Cramer.

Plagiarism Declaration:

1. I know that plagiarism is wrong. Plagiarism is to use another's work and pretend that it is one's own.
2. I have used the Harvard convention for citation and referencing. Each contribution to, and quotation in this report from the work(s) of other people has been attributed, and has been cited and referenced.
3. This report is my own work.
4. I have not allowed, and will not allow, anyone to copy my work with the intention of passing it off as his or her own work.
5. I acknowledge that copying someone else's assignment, or part of it, is wrong, and declare that this is my own work.

Signed by candidate

Signature Removed

Megan Yates

The physiological importance of small leaf sizes in the Mediterranean-type ecosystem vegetation of the Cape Floristic Region

Megan Yates

Botany Department
The University of Cape Town

Abstract 1

Introduction 2

Materials and Methods 5

Results 7

Discussion 9

Conclusions 13

Acknowledgements 14

References 14

Tables 18

Figure Legends 20

Figures 23

The physiological importance of small leaf sizes in the Mediterranean-type ecosystem vegetation of the Cape Floristic Region

Megan Yates

Corresponding author: M. Yates, email: megyates@gmail.com

Address: Department of Botany, the University of Cape Town

Abstract

Numerous "Fynbos" species of the Cape Floristic Region (CFR) have particularly fine, narrow leaves. The rates of transpiration and heat loss are partially dependent on boundary layer conductance, which is determined by leaf shape and size, surface modifications and wind speed. We expected fine-leaved species with higher boundary layer conductance to transpire faster than broad–leaved species at low temperatures whereas at higher temperatures we expected transpiration to be limited by stomatal conductance. In contrast, the rate of heat loss may be constrained by thick boundary layers in larger leaves at high temperatures. Leaf gas exchange characteristics at various temperatures were correlated with boundary layer thickness, leaf area and specific leaf area for 14 Proteaceae species using phylogenetically independent contrast species. When the temperatures of individual leaves were altered, while ambient temperature was kept at 18ºC, water loss decreased significantly at both 12ºC and 30°C with increased leaf size and thus boundary layer thickness. At 30°C, small leaves with thin boundary layers resulted in leaf temperatures below ambient, while larger leaves with thicker boundary layers had leaf temperatures closer to ambient. However, at 30ºC the variation in leaf temperature between the smallest and largest leaves was only 3.4ºC. Such a small variation in leaf temperature is unlikely to alter temperature-dependent physiological processes. We conclude that the small boundary layer associated with small leaves enables fine-leaved species to transpire at faster rates when water is plentiful. This may be a particularly important strategy for plants that take up most of their nutrients in the wet winter months from nutrient-poor highly leached soils of the CFR region.

We suggest that fine leaves are an adaptation for nutrient uptake during winter, although they may also have the benefit of improved coupling of leaf to ambient temperature during the summer drought period.

Keywords: Fynbos, transpiration, boundary layer, leaf size, Proteaceae

Abbreviations:

Photosynthetic rate, A; stomatal conductance, g_s; transpiration rate, E, water use efficiency, WUE, specific leaf area, SLA.

Introduction

Dutch settlers to the Cape of South Africa referred to the vegetation of the south-western region as 'fijnbosch' (Cowling 1992). This term refers to the predominant fine- or small-leaved component of the vegetation (Taylor 1978). Fynbos is a fire-prone shrubland occurring on nutrient-poor Table Mountain Group Sandstone-derived soils in the Mediterranean climate region of South Africa (Cowling 1992, Deacon *et al.* 1992). During winter, the fynbos biome receives large amounts of precipitation and the high run-off causes excessive nutrient leaching from the soil (Cowling & Holmes 1992). The summer drought presents a period of severe water limitation coupled with high temperatures.

Sclerophyllous leaves are a conspicuous feature of fynbos (Stock & Allsopp 1992), as they are in the other Mediterranean-type ecosystems of the world where sclerophyllous plants are dominant. Sclerophylly has advantages for nutrient use efficiency and drought-tolerance in nutrient-poor environments (Stock *et al.* 1992). Sclerophylly is correlated with other leaf characteristics, such as leaf longevity, and is thought to enhance carbon return per unit of nutrient invested (Orians & Solbrig 1977). The specific leaf area (SLA), which is a measure of sclerophylly, to leaf longevity relationship represents a trade-off, with species with lower SLA having a slower relative growth rate but a greater leaf life span and enhanced nutrient conservation (Westoby 1998, Aerts & Chapin 2000). Species with a higher SLA tend to have a greater photosynthetic capacity per unit leaf area due to the larger area for light capture per unit mass (Field & Mooney 1986). In Australian shrubland species for example, there is a positive correlation between independent contrasts between leaf area and SLA; i.e. fine, small leaves tend to be thicker and more dense (Ackerly & Reich 1999).

Correlations between leaf size and various environmental factors, such as temperature (Gates *et al.* 1968) and water relations (Taylor 1975) have been investigated. Species growing in arid and nutrient-poor environments tend to converge towards a lower SLA. This allows species from low-nutrient habitats to reach longer leaf life spans (Reich *et al.* 1997, Wright *et al.* 2002). Leaf size tends to decline with increasing elevation (Cordell *et al.* 1988), decreasing mean annual temperature, mean annual rainfall (Cowling & Campbell 1980) and lower soil fertility (Givnish 1987). For example, leaf size in several taxa in southeast Australia declined with both lower soil P and rainfall, although there was no correlation between soil P and rainfall (McDonald *et al.* 2003).

Are fine-leaves an adaptation to summer drought in the fynbos? The size of the still air boundary layer at the surface of the leaf depends on leaf size and wind speed and influences movement of air around the leaf and conductance of gases in and out of the leaf (Nobel 1999). The thickness of this layer is reduced in small leaves and results in higher boundary layer conductance and increased convective cooling. Fine-leaves enable maintenance of leaf temperature close to ambient temperature, which is particularly important in extremely arid environments (Gates *et al.* 1968). Several small-leaved desert plants were compared with *Opuntia* in the same locale, and had leaf temperatures within 3°C of ambient, while those of *Opuntia* were 10 to 16 °C higher than air temperature (Gates *et al.* 1968). Bigger leaves absorb larger amounts of radiant energy and have large boundary layers resulting in leaf temperatures higher than air temperature and corresponding higher rates of transpiration due to the effect of temperature on the water vapour saturation content of air (Gates *et al.* 1968, Taylor & Sexton 1972, Taylor 1975, Geller & Smith 1982). Thus at high temperatures, small leaves may act to conserve water (Geller & Smith 1982).

The thickness of the boundary layer of air at the surface of the leaf through which water vapour must diffuse after leaving the stomata governs the conductance of the boundary layer to water vapour, which in turn affects the transpiration rate (Geller & Smith 1982, Martin *et al.* 1999, Nobel 1999). Fine leaves have a smaller boundary layer, which reduces boundary layer resistance to water vapour enabling faster movement of water vapour out of the leaf (Noble 1999). Therefore fine-leaves may lose more water, independent of the effects of boundary layer thickness on leaf temperature, which may be disadvantageous in arid areas. Givnish (1979) argued that although fine leaves, with smaller boundary layers, would have high transpiration rates, high boundary layer conductance would also allow greater convective cooling with a possible net reduction in water loss through evapotranspiration.

The effect of high boundary layer conductance on water loss may be especially significant at low air temperatures corresponding to reduced water potential gradients between air and leaf. Under these circumstances fine leaves should lose more water than larger leaves as a result of their reduced boundary layer size and higher boundary layer conductance to water vapour (Nobel 1999). Could the predominance of fine-leaved species in the fynbos be an adaptation to nutrient uptake in plants growing on nutrient-poor soils and receiving high winter rainfall? Nutrient uptake is restricted to the wet winter months for many members of the Proteaceae family with a Meditteranean distribution (Shane & Lambers 2005). Mass-flow of dissolved nutrients and water through the soil to the roots is driven by transpiration. The significance of mass-flow for delivery of nutrients to the roots depends on water flow and nutrient concentration (Barber 1995). Transpiration may be partially up-regulated in plants grown under limited nutrient availability enabling greater mas-flow of nutrients. For example, a perennial grass, *Ehrharta calycina*, grown with nutrient pellets not directly accessible to the roots had transpiration rates 1.68 fold higher than plants grown with nutrient pellets in the rhizosphere (Cramer, Hoffmann and Verboom, unpublished).

We suggest that in fynbos, fine-leaves are an adaptation for nutrient uptake during winter, when water is available for nutrient uptake. Fine-leaves could also be an adaptation to summer drought, based on their lower absorption of radiant energy, higher convective cooling and corresponding lower rates of water loss. Thus, at high temperatures, fine-leaves may act to conserve water. To test these hypotheses, we selected 14 species in the Proteaceae family with varying leaf size and measured gas exchange parameters over a range of temperatures.

Materials and Methods

Characterisation of the variation in water and temperature loss with size

Pieces of filter paper of equal length (4 cm) and widths ranging from 0.1 to 1.5 cm were saturated with water and weighed at time intervals during drying in a phytotron chamber set to three different temperatures (8, 20 and 30°C). The rate of water loss per area (mmol m^{-2} s^{-1}) was plotted against boundary layer thickness (mm) to determine the relationship between boundary layer thickness and water flux. The same paper strips were allowed to equilibrate with the surrounding air for 2 min before surface temperatures of the papers were measured using an LS infrared thermometer (Optris, Berlin, Germany). Measurements were taken at minimal irradiance (*ca.* 5 μmol m^{-2} s^{-1}) to eliminate the effect of absorbance of radiation. The effect, therefore, represents only boundary layer conductance to heat transfer and convection. The difference between filter paper temperature and ambient temperature (ΔT) was plotted against boundary layer thickness to determine the physical effect of boundary layer thickness on leaf cooling.

Plant cultivation

14 Proteaceae species with varying leaf sizes were obtained from Kirstenbosch Nursery, Cape Town. The species were grown in a 1:1 mixture of sand and compost in 2 L plastic bags. All species selected are perennial and occur in the winter-rainfall region of the Western Cape, South Africa. Species from the *Leucadendron*, *Leucospermum* and *Protea* genera were chosen. To minimise the effects of phylogenetic history on the results, fine- and large-leaved species were selected from each genus. Species ranged from the very fine-leaved *Leucadendron laxum* to the broad-leaved *Protea eximia* and averaged 20 cm in height. Plants were grown in a temperature controlled phytotron set to 20°C with a photoperiod of LD 10:14 (irradiance of 500 μmol m^{-2} s^{-1}) and were watered every 2 days and immediately prior to shoot gas exchange measurements.

Shoot gas exchange measurements

Photosynthetic rate, transpiration rate, stomatal conductance and leaf temperature of the youngest fully expanded leaves of plants growing in the phytotron were measured using an LI-6400 Portable Photosynthesis System (LICOR, Lincoln, NE, USA). An LI-6400-05 conifer chamber, in which whole leaves could be placed, was used to include the effect of boundary layer on gas exchange measurements. To assess the plant response to changes in ambient temperature, the phytotron chamber was set to four different temperatures (8°C, 15°C, 22°C and 30°C) prior to gas exchange measurements. The response of individual leaves to temperature changes was assessed by altering the LI-6400-05 conifer chamber temperature to 12°C and 30 °C while maintaining the phytotron chamber temperature at 18°C. Chamber irradiance was set to 1000 µmol m^{-2} s^{-1} at plant height during the gas exchange measurements.

Specific leaf area and boundary layer measurements

Leaf area was measured using an LI-3000 Area Meter (LICOR, Lincoln, NE, USA). Leaves were dried in an oven at 80°C for 24 h to determine leaf dry weight. SLA was calculated as the leaf area (m^2) per dry weight (kg). The diameter of the largest circle area that could be accommodated in the area of the leaves was used as a leaf dimension to estimate boundary layer thickness (mm) according to the equation $\delta = 4.0\sqrt{\frac{l}{v}}$, where l = leaf dimension (m) and v = windspeed (m s^{-1}) (Nobel 1999). Wind speed was assigned an arbitrary value of 1 m s^{-1} in boundary layer calculations and thus the reported *d* values are an index of boundary layer size.

Mass spectrometer determination

Leaves were oven-dried, ground and weighed into 8 by 5 mm tin capsules (Elemental Microanalysis Ltd., Devon, U.K.) on a Sartorius microbalance (Goettingen, Germany). The samples were combusted in a Thermo Flash EA 1112 series elemental analyzer (Thermo Electron Corporation, Milan, Italy). The gases released were fed into a Delta Plus XP isotope ratio mass spectrometer (Thermo Electron Corporation, Milan, Italy) via a Thermo Finnigan Conflo III control unit (Thermo Electron Corporation, Milan, Italy), where their $d^{13}C$ values were determined. This was done at the University of Cape Town, where our own internal standards were run to calibrate our results relative to the international standard, CO_2 in PeeDee belemnite. The deviation of the sample from the international standard is expressed as $d^{13}C = (R_{sample}/R_{standard}-1)*10^3$, where R_{sample} represents the ratio between the heavy and lighter isotopes of the sample and $R_{standard}$ the ratio of the international standard.

Phylogenetic Contrast Analysis

Known Proteaceae phylogenies (Barker *et al.* 2002, Barker *et al.* 2004, Barraclough & Reeves 2005) were used to generate a tree showing the relationships between the 14 species used in the experiment. Trait relationships were evaluated based on correlation analyses using phylogenetically independent contrasts (Felsenstein 1985) based on the generated phylogeny. Branch lengths were assumed to be equal and assigned a value of 1.0. Comparative analyses were conducted using CACTUS (Version 1.13, Build 9, Prickly Software), which calculates independent contrasts and their correlations over the phylogenetic tree. Independent contrasts signify differences between the trait values of two sister taxa. This analysis was used to determine the significance of correlations based on phylogenetic history (CC) as well as independent of phylogenetic history (AC).

Results

Leaf characteristics

Boundary layer thickness of the 14 Proteaceae species ranged between 0.12 and 0.56 mm and was strongly correlated with leaf area (CC $p < 0.001$, Fig. 1b). SLA showed no relationship with boundary layer (CC $p = 0.288$), although thin, needle like leaves, such as those of *P. aristata*, *Ld. laxum*, *Ld. linifolium* and thin leaves of *Ld. platyspermum,* tended to have low SLA (Fig. 1a).

Leaf gas exchange and temperature response to changes in ambient temperature

When the leaf temperature was altered, fine leaves were cooler than broad leaves at all four temperatures (8°C, 15°C, 22°C and 30°C); however the correlation between boundary layer thickness and temperature were not significant (Fig. 2, Table 1). On average, the rate of transpiration was 4.5-fold higher at 30°C than at 8°C. However, transpiration and stomatal conductance showed no correlation with boundary layer at any temperature (Fig. 3, Table 1). Photosynthesis reached at a maximum at 15°C and was low at 8°C and 30°C for all species (Fig. 4). There were, however, no significant correlations between photosynthetic rate and boundary layer size at any temperature (Table 1). WUE (A/E) showed no relationship with boundary layer thickness at any temperature (Table 1). $d^{13}C$, which is a proxy for WUE integrated over the duration of leaf development, was not correlated with leaf dimension but was negatively correlated with SLA (p = 0.001). Thus thicker leaves, with low SLA, fixed more CO_2 than they lost H_2O.

Leaf temperature response to changes in leaf temperature

Cooling of filter paper was, on average, 5-fold greater at 28°C than at 10°C and 20°C due to the steeper vapour pressure gradient. The physical relationship between boundary layer size and leaf cooling indicates that small boundary layer size allows maintenance of temperatures closer to ambient (Fig. 5a). Thus when, boundary layer size was low, the difference between ambient temperature and filter paper temperature (ΔT) was low. Values were negative because only the effects of boundary layer conductance on heat transfer and convection were included, while the effect of absorbance of radiation was eliminated. When the temperature of individual leaves was altered, finer-leaves were cooler (Fig. 5b). At 12°C, fine-leaves were closer to ambient temperature, while larger leaves had temperatures significantly higher than ambient (CC p = 0.015). At this temperature, there was a 1.9°C difference in ΔT between small leaves with thin boundary layers and large leaves with thick boundary layers. At 30°C, fine-leaves had temperatures lower than ambient, while larger leaves had temperatures closer to ambient (CC p = 0.001). There was a 3.4°C difference in ΔT between leaves with thick boundary layers and leaves with thin boundary layers.

Gas exchange response to changes in leaf temperature

The physical relationship between water flux and boundary layer thickness obtained from the rate of water loss from different sizes of filter paper at different temperatures followed a reciprocal logarithmic relationship (Fig. 6a). Water flux increased substantially for low boundary layer sizes and the rate of increase was steeper at higher temperatures. When the plants were maintained at 18°C and the temperature of individual leaves altered to 12°C or 30°C, water flux (E) from the leaves was strongly correlated with boundary layer (CC p = 0.016 and 0.020, respectively) and leaf area (CC p = 0.029 and 0.019, respectively) (Figs. 6b and 7). The decline in water flux with increasing boundary layer followed the physical relationship predicted by water flux from filter paper. At 12°C and 30°C, leaves with small boundary layers (<0.2 mm) had rates of water flux substantially higher than that of leaves with larger boundary layers (>0.2 mm). On average, water flux was 2-fold higher at 30°C than at 12°C. The decrease in water flux with increasing boundary layer was steeper at 30°C than at 8°C. When boundary layers exceeded 0.2 mm, the variation in water flux between leaves with boundary layers of 0.2 mm and those of 0.6 mm at both temperatures was low.

At 12°C and 30°C, stomatal conductance declined with increasing boundary layer thickness (CC $p = 0.014$ and 0.038, respectively) (Fig. 8). The rate of decline of stomatal conductance with increasing boundary layer was similar at both 12°C and 30°C (Student's T-test, $p > 0.05$). Thus, although stomatal conductance declined at the same rate at both temperatures, transpiration declined at a faster rate at 30°C. The rate of photosynthesis was not correlated with boundary layer at both 12°C and 30°C (CC = 0.436 and 0.449, respectively). Water use efficiency was significantly correlated with boundary layer at 12°C, however, the correlation was weak ($r = 0.11$, CC $p = 0.008$) (Table 2).

Discussion

The predominance of fine-leaved species is a striking feature of the sclerophyllous vegetation of the Cape Floristic Region. Species of *Erica* (Ericaceae), which is the largest constituent genus in the Cape Floristic Region (Schumann *et al.* 1992), are predominantly fine-leaved. The fine-leaved component of Fynbos vegetation also includes members of the daisy (Asteraceae), legume (Fabaceae), jujube (Rhamnaceae), fibre-bark (Thyme-laeaceae) and blacktip (Bruniaceae) families, certain species of which have leaves similar in structure to those of the Ericaceae (van Rooyen & Steyn 1999). The Restionaceae and Cyperaceae which are also highly represented in the CFR have needle like culms with no leaves or very reduced leaves. High variation in leaf form is evident in the Proteaceae family, ranging from the broad leaves of species like *P. cynaroides* and *P. eximia* to the needle-like leaves of *P. aristata* and *Ld. laxum* and some *Sorocephalus* spp. Some Proteaceae species also have dissected leaves, e.g. *Serruria* and *Paranomus* spp. (Rebelo 2001). So, what are the ecophysiological advantages of fine-leaves in this Mediterranean-type ecosystem?

The relationship between water flux and boundary layer was described by a reciprocal logarithmic curve, with fine leaves, with smaller boundary layers, transpiring at significantly higher rates than larger leaves (Fig. 6b). However, boundary layer thickness influenced water flux substantially only when boundary layer thickness was less than 0.2 mm. The effect of boundary layer size on water flux was far greater at 30°C than at 8°C. Boundary layer conductance and stomatal conductance operate in series and their relative magnitudes dictate which conductance is the main controller of transpiration (Martin *et al.* 1999). The Omega factor (O) is a dimensionless coefficient ranging between 0 and 1, which indicates the extent of stomatal control on transpiration (McNaughton & Jarvis 1983). When stomatal conductance is less than boundary layer conductance, as is the case for fine leaves, O is close to 0 and stomata are the overriding regulator of transpiration (McNaughton & Jarvis 1983). For larger leaves, O is closer to 1 and any change in stomatal conductance will have a small effect on the rate of transpiration due to the low boundary layer conductance (McNaughton & Jarvis 1983). Thus when boundary layers are small, plants may be able to exert more control over transpiration by regulating stomatal conductance. This may be important at high temperatures, where the effect of boundary layer on water flux was higher (Fig. 6b). Why would maximisation of the stomatal control of transpiration in fine leaves be an advantage?

Plants with narrow leaves are found on nutrient-poor podsols, in montane rainforest and cloudforest with highly leached soils and in bogs (Givnish 1987). Mass-flow of dissolved nutrients through the soil is driven by transpiration (Barber, 1995). The importance of mass flow is dependent on nutrient concentration and solubility and water flux (Barber 1995). Transpiration driven water flux has been shown to be upregulated in plants grown under limited nutrient availability (Cramer, Hoffmann and Verboom, unpublished). Several environmental cues regulate stomatal conductance, one of which is nutrient availability. Recent research has revealed that this cue, in particular, may be highly important in regulating the degree of stomatal aperture. Stomatal closure has been shown to be induced in N-deprived plants due to changes in xylem sap composition (Dodd *et al.* 2003). Nitric oxide is a key signal molecule mediating ABA-induced stomatal closure and is synthesised by nitrate reductase in the guard cells (Desikan *et al.* 2002). Nutrient availability therefore plays an important role in the regulation of transpiration. We suggest that fine leaves and the related ability to transpire rapidly may be particularly important for nutrient-acquisition, especially in fynbos species growing on nutrient-poor, highly leached sandstone-derived soils. However, very high transpiration rates are rather risky and can be highly costly, possibly leading to reduced leaf water potential and growth impairment (Lambers *et al.* 1998). Fine-leaves, which had high rates of transpiration, had no additional cost in terms of WUE (A/E) at both 8°C and 30°C, as might have been expected. The nutrient-acquisition advantages of high transpiration would only be beneficial when water is plentiful. The fynbos region experiences high precipitation during winter, which leads to leaching of soil nutrients, such as phosphorous and inorganic nitrogen. Plants of Mediterranean ecosystems are thought to take up nutrients in winter and to store them for growth in spring and summer (Shane *et al.* 2004). In the CFR, phosphorous and nitrogen are the main limiting nutrients but since phosphorous is sparingly soluble, mass flow most likely enhances uptake of inorganic nitrogen. Thus during winter, high transpiration rates may be particularly advantageous for nutrient uptake.

At low temperature (12°C), fine leaves with smaller boundary layers had temperatures closer to ambient while the temperatures of larger leaves were higher than ambient (Fig. 6b). At high temperature (30°C), fine leaves had temperatures lower than ambient, while leaves with larger boundary layers were closer to ambient. The low boundary layer size of fine leaves allowed increased cooling due to increased boundary layer conduction to heat transfer and convection. At 30°C, the difference between leaf temperature and ambient temperature was only 3.4°C higher in leaves with large boundary layers than in leaves with small boundary layers. The effect of evapotranspirational cooling was included in these temperature measures. Thus if evapotranspirational cooling, which would be higher in fine leaves, was excluded the differences in leaf temperature and ambient between fine- and large-leaved species would be even less. This consideration is important as evapotranspirational cooling may be low during summer. Is the observed difference of 3.4°C enough to substantially alter temperature-dependent physiological processes and to be a selective pressure driving the evolution of fine leaves? In *Abies amabilis*, a subalpine forest tree, elevated leaf temperature (6°C higher than ambient) had no significant impact on carbon gain during the growing season (Martin *et al.* 1999). We suggest that this difference of 3.4°C is not substantial enough to significantly alter temperature-dependent physiological plant processes and while the increased cooling of fine-leaves may confer some advantage at high temperatures, they are not an adaptation for summer drought.

Givnish (1979) proposed that for fine leaves, with smaller boundary layers, transpiration could be high, however, high boundary layer conductance allowed greater convective cooling with a net reduction in water loss through evapotranspiration. Cooling of leaves can occur in several ways, including convective cooling, conductive cooling and evapotranspirational cooling. The effect of leaf cooling is, however, complicated by the effect of the boundary layer on water loss from leaves. In large-leaved species, transpiration is expected to be higher due to the increased leaf temperature as a result of higher absorbance of solar radiation and reduced convective cooling; however, the thick boundary layer also impedes loss of water from the leaves. Thus, when stomatal conductance is high, larger-leaved species transpire at slower rates and lose less water than fine leaves. In arid areas, small leaves are favoured because high evapotranspirational cooling of leaves cannot be supported and cooling of leaves is reliant on convective cooling to dissipate absorbed radiation (Lambers *et al.* 1998). Evapotranspirational cooling would only have leaf cooling advantages at high temperatures and when water is plentiful (Lambers *et al.* 1998). The low boundary layer size may enhance the extent to which transpiration can be controlled by stomatal conductance (McNaughton & Jarvis 1983) and may enable desert plants to exert greater control over the rate of transpiration. A complication to these single leaf predictions regarding leaf cooling is the difference in the number of leaves per plant. Several fynbos species have numerous, fine leaves while narrow-leaved desert plants have only a small number of narrow leaves. In desert-adapted plants, the fewer number of leaves present per plant may substantially reduce the amount of radiation absorbed per plant whereas in the fynbos the very high number of leaves of fine-leaved species would confer no advantage for reduced absorption of radiation as the total leaf area per plant may be similar to large leaved species (Yates and Cramer, unpublished).

The plant response to changes in ambient temperature varied from the responses of individual leaves to changes in temperature when growth temperature was maintained at 18°C. When the ambient temperature of the whole plants was reduced to 8°C, the transpiration rates of all species were low and showed no correlation with boundary layer (Fig. 3). At low and high temperatures, the rate of photosynthesis was low (Fig. 4). Thus at low temperatures, when carbon assimilation was low, the rate of transpiration was low and transpiration and photosynthesis were consequently coupled. At high temperature (30°C), the vapour pressure difference between air and leaf increased and water was lost at faster rates. Carbon assimilation was low while transpiration was high, which implies an uncoupling of photosynthesis and transpiration at high temperatures. Kramer and Boyer (1995) suggest that transpiration is the "unavoidable evil" of photosynthesis. This negative view of transpiration can be misleading, as transpiration plays an important role in several plant functions, such as nutrient uptake and evapotranspirational cooling. Stomatal conductance, which is regulated by a cascade of endogenous controls in response to various environmental cues, may have influenced the plant response of gas exchange measures and resulted in the low correlations between boundary layer and gas exchange measures. We postulate that the effects of boundary layer size on gas exchange-dependent processes may only become apparent when both photosynthesis and transpiration are at their respective environmentally-determined maxima.

Conclusions

Leaves tend to reflect adaptations to various ecological conditions, although there is usually considerable divergence in the expression of leaf traits within habitats (Givnish 1987). Water flux was significantly greater in plants with boundary layers smaller than 0.2 mm in thickness. Thus, fine-leaved species, which have numerous leaves, have the potential to transpire at high rates at relatively low temperatures. This may be beneficial on the nutrient-impoverished, leached soils of the Cape Floristic Region, where high transpiration rates when water is plentiful, may increase mass-flow of nutrients to the roots. The high boundary layer conductance and convective cooling of fine-leaved species also allowed maintenance of leaf temperatures cooler than those of large leaves with increased boundary layer size. However, we suggest that the 3.4°C difference between leaf and ambient temperatures, at an ambient temperature of 30°C, between large- and fine-leaved species was not high enough to provide a major adaptive advantage, although it would certainly confer some advantage. We conclude that fine-leaves in the CFR enable high transpiration rates in order to promote nutrient uptake, particularly of N, during winter.

Acknowledgements:

Funding assistance was provided by the University of Cape Town and the National Research Foundation (NRF). I would like to thank Michael Cramer for his valuable advice, constant patience and original, exciting suggestions on the topics of boundary layers, transpiration and leaf adaptation. Tony Verboom provided assistance with Proteaceae tree building and phylogenetically independent contrast analysis. Timothy Moore helped with using the program Cactus. Vera Hoffman was a constant source of positive energy throughout this project. I am grateful to Paul, from Kirstenbosch Botanical Gardens, Cape Town for assisting in species selection.

References

Ackerly, D.D. and Reich, P.B. (1999) Convergence and correlations among leaf size and function in seed plants: A comparative test using independent contrasts. *American Journal of Botany* **86** (9): 1272-1281

Aerts, R. & Chapin, F.S. (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research* **30**: 1–67

Barber, S.A. (1995) Soil Nutrient Bioavailability: A Mechanistic Approach, 2nd edition. John Wiley & Sons, New York

Barker, N.P., Weston, P.H., Rourke, J.P. and Reeves, G. (2002) The relationships of the southern African Proteaceae as elucidated by internal transcribed spacer (ITS) DNA sequence data. *Kew Bulletin* **57**: 867-883

Barker, N.P., Vanderpoorten, A., Morton, C.M. and Rourke, J.P. (2004) Phylogeny, biogeography, and the evolution of life-history traits in *Leucadendron* (Proteaceae). *Molecular Phylogenetics and Evolution* **33**: 845-860

Barraclough, T.G. and Reeves, G. (2005) The causes of speciation in plant lineages: species-level DNA trees in the African genus *Protea*. In: Plant Species-Level Systematics: New perspectives on pattern and process. Eds: Bakker, F.T., Chatrou, L.W., Gravendeel, B., and Pelser, P.B. Gantner Verlag, Ruggell, Liechtenstein

Cordell, S., Goldstein, G., Muellerdombois, D., Webb, D. and Vitousek, P.M. (1998) Physiological and morphological variation in *Meterosideros polymorpha,* a dominant Hawaiian tree species, along an altitudinal gradient - the role of phenotypic plasticity. *Oecologia* **113**: 188-196.

Cowling, R.M. and Campbell, B.M. (1980) Convergence in vegetation structure in the Mediterranean communities of California, Chile and South Africa. *Vegetatio* **43**: 191-197

Cowling, R.M. and Holmes, P.M. (1992) Flora and vegetation. In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK

Cowling, R.M. (1992) Preface. In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK

Deacon, H.J., Jury, R.M. and Ellis, F. (1992) Selective regime and time. In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK

Desikan, R., Griffiths, R., Hancock, J. and Neill, S. (2002) A new role for an old enzyme: Nitric-reductase mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana. PNAS* **99** (25)

Dodd, I.C., Tan, L.P. and He, J. (2003) Do increases in xylem sap pH and/or ABA concentration mediate stomatal closure following N deprivation. Journal of *Experimental Botany* **54** (385): 1281-1288

Felsenstein, J. (1985) Phylogenies and the comparative method. *American Naturalist* **125**: 1-15

Field, C. and Mooney, H.A. (1986) The photosynthesis-nitrogen relationship in wild plants. On the Economy of Plant Form and Function. Ed: Givnish, T.J. pp. 25–55. Cambridge University Press, Cambridge.

Gates, D.M., Alderfer, R. and Taylor, S.E. (1968) Leaf temperatures of desert plants. *Science* **159**: 994-995

Geller, G.N. and Smith, W.K. (1982) Influence of Leaf Size, Orientation and Arrangement on Temperature and Transpiration in Three High-Elevation, Large-Leaved Herbs. *Oecologia* **53**: 227-234

Givnish, T. J. (1979) On the adaptive significance of leaf form. Pp. 375-407 In: Topics in plant population biology. Eds: Solbrig, O.T., Jain, S., Johnson, G.B. and Raven, P.H. Columbia University Press, New York, New York, USA

Givnish, T.J. (1987) Comparative structures of leaf form: assessing relative roles of selective pressures and phylogenetic constraints. *New Phytologist* **106**: 131-160

Kramer P.J. and Boyer J.S. (1995) *Water Relations of Plants and Soils*. Academic Press, San Diego

Lambers, H., Chapin, F.S., and Pons, T.L. (1998) Plant Physiological Ecology. Springer-Verlag

Martin, T.A., Hinckley, T.M., Meinzer, F.C. and Sprugel, D.G. (1999) Boundary layer conductance, leaf temperature and transpiration of *Abies amabilis* branches. *Tree Physiology* **19**: 435-443

McDonald, P.G., Fonseca, C.R., Overton, J.McC and Westoby, M. (2003) Leaf-size divergence along rainfall and soil-nutrient gradients: is the method of size reduction common among clades? *Functional Ecology* **17**: 50-57

McNaughton, K.G. and Jarvis, P.G. (1983) Predicting effects of vegetation changes on transpiration and evaporation. In: Water Deficits and Plant Growth, Vol III. Ed. Kozlowski, T.T. Academic Press, Inc., San Diego, pp. 1-47

Nobel, P.S. (1999) Physicochemical and environmental plant physiology. Academic Press, San Diego, California

Orians, G.H. and Solbrig, O.T. (1977) A cost-income model of leaves and roots with special reference to arid and semi-arid areas. *American Naturalist* **111**: 677-690

Rebelo, T. (2001) Proteas: A Field Guide to the Proteas of southern Africa. Fernwood Press, Cape Town

Reich, P.B., Walters, M.B. & Ellsworth, D.S. (1997) From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences*, USA, **94**: 13730–13734

Schumann, D., Kirsten, G. and Oliver, E.G.H. (1992) Ericas of South Africa. Fernwood Press, Cape Town

Shane, M., McCully, M. and Lambers, H. (2004) Tissue and cellular phosphate storage during development of phosphorous toxicity in *Hakea prostrata* (Proteaceae). *Journal of Experimental Botany* **55** (399): 1033-1044

Shane, M. and Lambers, H. (2005) Cluster roots: A curiosity in context. *Plant and Soil* **274**: 101-125

Stock, W.D. and Allsopp, N. (1992) Functional Perspectives of Ecosystems. In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK

Stock, W.D., van der Heyden, F. and Lewis, O.A.M. (1992) Plant structure and function. In: The Ecology of Fynbos: Nutrients, Fire and Diversity. Ed: Cowling, R.M. Oxford University Press, Oxford, UK

Taylor, S.E. (1975) Optimal leaf form. In: Perspectives in Biophysical Ecology. Eds D.M. Gates & R.B. Schmerl. Springer, Berlin, Germany. pp. 73–86

Taylor, H.C. (1978) Capensis. In: Biogeography and ecology of southern Africa. Ed: Werger, M.J.A. The Hague. pp. 171-230

Taylor S.E. and Sexton O.J. (1972) Some implications of leaf tearing in Musaceae. Ecology **53**: 143-149 van Rooyen, G. and Steyn, H. (1999) Cederberg, Clanwilliam and Biedouw Valley: South African Wild Flower Guide 10. Botanical Society of South Africa, Cape Town.

Westoby, M. (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil* **199**: 213–227.

Wright, I.J., Westoby, M. and Reich, P.B. (2002) Convergence towards higher leaf mass per area in dry and nutrient-poor habitats has different consequences for leaf life span. *Journal of Ecology* **90**: 534-543

Table 1. Trait correlations between boundary layer/leaf area and gas exchange measures across 14 Proteaceae species using phylogenetically independent contrasts (CC) as well as excluding the influence of phylogenetic history (AC). Growth temperature was altered prior to gas exchange measurements.

Trait 1	Trait 2	Temperature (°C)	AC P-value	CC P-value
Boundary Layer (mm)	E (mmol m^{-2} s^{-1})	8	0.606	0.980
		15	0.307	0.456
		22	0.454	0.559
		30	0.848	0.920
	g_s (mol m^{-2} s^{-1})	8	0.059	0.266
		15	0.307	0.456
		22	0.539	0.679
		30	0.508	0.385
	WUE (mol)	8	0.292	0.269
		15	0.495	0.227
		22	0.291	0.246
		30	0.755	0.755
	A (µmol m^{-2} s^{-1})	8	0.905	0.572
		15	0.560	0.786
		22	0.702	0.819
		30	0.514	0.417
	Δ T (°C)	8	0.150	0.185
		15	0.108	0.204
		22	0.166	0.193
		30	0.261	0.147
	$d^{13}C$		0.253	0.410
Leaf Area (cm^2)	E (mmol m^{-2} s^{-1})	8	0.608	0.899
		15	0.413	0.740
		22	0.464	0.649
		30	0.996	0.738

Table 2. Trait correlations between boundary layer/leaf area and gas exchange measures across 14 Proteaceae species using phylogenetically independent contrasts (CC) as well as excluding the influence of phylogenetic history (AC). The temperature of individual leaves was altered while plants were maintained at 18°C.

Trait 1	**Trait 2**	**Temperature (°C)**	**AC P-value**	**CC P-value**
Boundary Layer (mm)	E (mmol m^{-2} s^{-1})	12	**0.007**	**0.016**
		30	**0.003**	**0.020**
	gs (mol m^{-2} s^{-1})	12	**0.039**	**0.014**
	0	30	**0.005**	**0.038**
	WUE (mol)	12	0.497	0.156
		30	**0.018**	**0.008**
	A (μmol m-2 s^{-1})	12	0.106	0.436
		30	0.095	0.449
	ΔT (°C)	12	**0.014**	**0.015**
		30	**0.005**	**0.001**
Leaf Area (cm^2)	E (mmol m^{-2} s^{-1})	12	**0.002**	**0.029**
		30	**0.002**	**0.019**

Figure Legends

Figure 1. Correlations of SLA (a) and leaf area (b) with boundary layer (n = 14). The symbols and bars represent means ± S.E. The correlation coefficient (r) and regression equation are indicated on the graph. SLA showed no correlation with boundary layer (CC p = 0.288), while leaf area and calculated boundary layer thickness were strongly correlated (CC $p = 8.12 \times 10^{-7}$).

Figure 2. Correlation between boundary layer and ΔT, the difference between leaf temperature and ambient temperature, at four different temperatures; 8°C (a), 15°C (b), 22°C (c) and 30°C (d). The ambient temperature was altered immediately prior to gas exchange measurements. Symbols and bars represent means ± S.E. Correlations between ΔT and boundary layer were not significant at any temperature (CC p = 0.185, 0.204, 0.193 and 0.147, respectively).

Figure 3. Correlations between boundary layer and E at four different temperatures; 8°C (a), 15°C (b), 22°C (c) and 30°C (d). The growth temperature was altered immediately prior to gas exchange measurements. Symbols and bars represent means ± S.E. Correlations between E and boundary layer were not significant at any temperature (CC p = 0.980, 0.456, 0.559 and 0.920, respectively).

Figure 4. Correlation between boundary layer and A at four different temperatures; 8°C (a), 15°C (b), 22°C (c) and 30°C (d). The ambient temperature was set immediately prior to gas exchange measurements. Symbols and bars represent means ± S.E. The correlations between boundary layer and A were not significant any temperature (CC p = 0.572, 0.786, 0.819 and 0.417, respectively). Across all species, maximum A was reached at 15°C (inset).

Figure 5. Relationship between cooling and boundary layer. The physical relationship between cooling and boundary layer size was determined by measuring the temperatures of pieces of filter paper in a temperature-controlled chamber set to three different temperatures; 10°C, 20°C and 28°C (a). The degree of heating was assessed as the difference between paper temperature and ambient temperature (ΔT). Pieces of filter paper with smaller boundary layers were more coupled to the environment and had temperatures closer to ambient temperature. ΔT values were

temperatures did not differ significantly (Student's T-test, $p > 0.05$). g_s declined with increasing boundary layer at 12 and 30°C (CC $p = 0.014$ and 0.038, respectively).

negative due to the exclusion of the effect of radiation. The relationship between leaf temperature and leaf boundary layer size was assessed by altering the temperature of single leaves, while maintaining plants at an ambient temperature of 18°C (b). The correlation coefficients (r) and regression equations are shown. At 12°C, leaves with smaller boundary layer had temperatures closer to ambient, while leaves with larger boundary layers were significantly higher than ambient (CC p = 0.015). At 30°C, leaves with smaller boundary layers were significantly lower than ambient (CC p = 0.001). Thus fine-leaves, with higher boundary layer conductance were cooler.

Figure 6. Relationship between water flux and boundary layer at different temperatures. The physical relationship between water flux and boundary layer was determined by the rate of water loss from pieces of filter paper in a temperature-controlled chamber (a). The relationship between water flux and leaf boundary layer thickness was assessed by altering the temperature of single leaves while maintaining plants at an ambient temperature of 18 °C (b). The curves obtained from the physical experiment were fitted to the data of water flux for single leaves at the different temperatures (b). Symbols and bars represent means ± S.E. The correlation coefficients (r) and equations of the curves are shown. For single leaves, the correlation between water flux and boundary layer was significant at 12°C and 30°C (CC p = 0.016 and 0.020, respectively).

Figure 7. Correlation of water flux with leaf area. The phytotron temperature was maintained at 18°C, while the cuvette temperature, in which single leaves were placed and gas exchange measurements taken, was altered. Symbols and bars represent means ± S.E. The correlation coefficients (r) and regression equations are shown. E significantly declines with leaf area at 12°C and 30°C (CC p = 0.029 and 0.018 respectively), however the rate of decline was higher at 30°C.

Figure 8. Correlation between g_s and boundary layer. The cuvette temperature, in which individual leaves were placed and gas exchange measurements taken, was altered while the ambient temperature was maintained at 18°C. Symbols and bars represent means ± S.E. The correlation coefficients (r) and regression equations are shown. The slope and intercepts of the regression lines at the two different

Figure 1

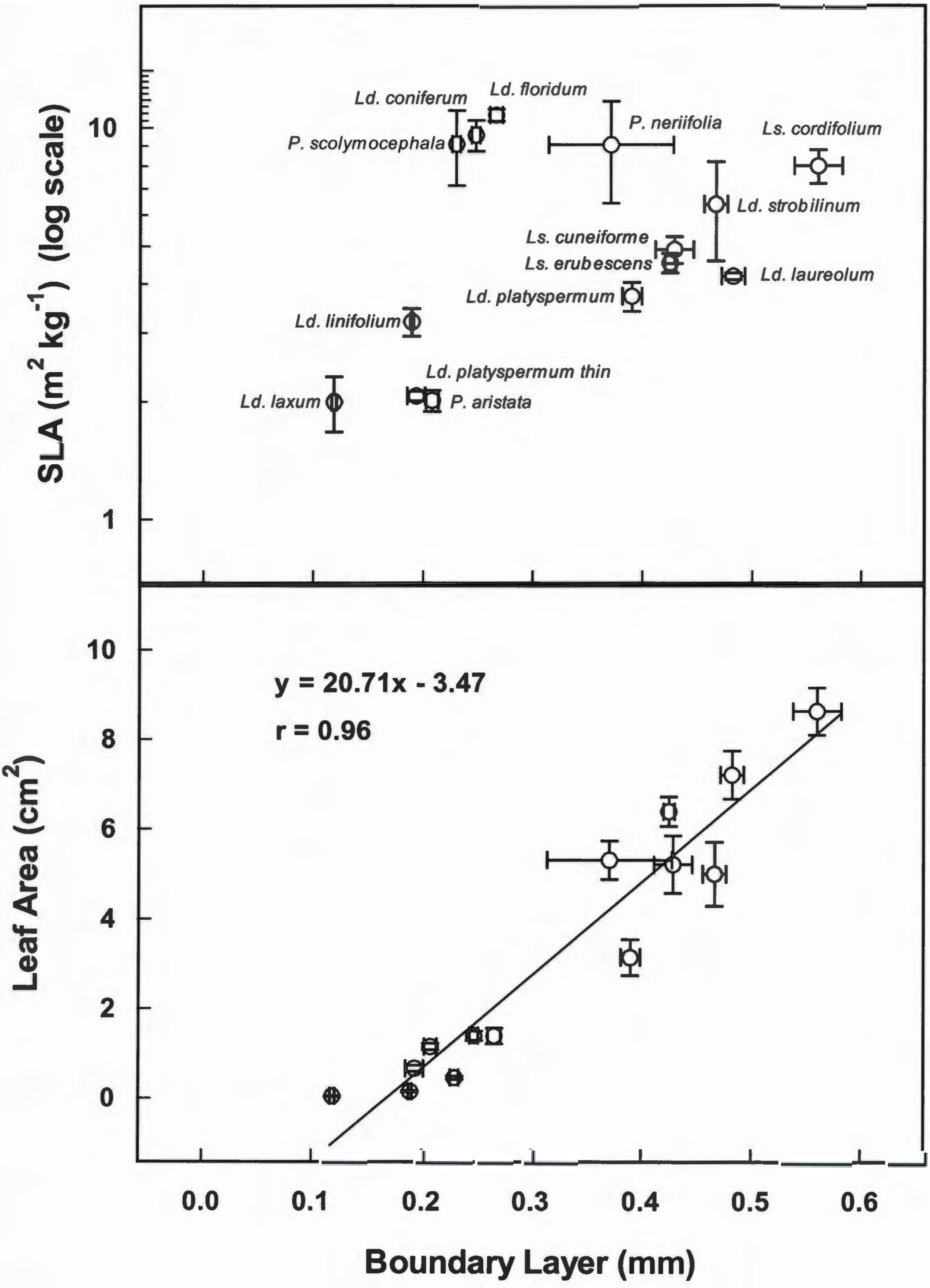

SLA (m^2 kg^{-1}) (log scale)
10
1
Ld. coniferum
Ld. floridum
P. scolymocephala
P. neriifolia
Ls. cordifolium
Ld. strobilinum
Ls. cuneiforme
Ls. erubescens
Ld. laureolum
Ld. platyspermum
Ld. linifolium
Ld. platyspermum thin
Ld. laxum
P. aristata
Leaf Area (cm^2)
10
8
6
4
2
0
y = 20.71x - 3.47
r = 0.96
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Boundary Layer (mm)

Figure 2

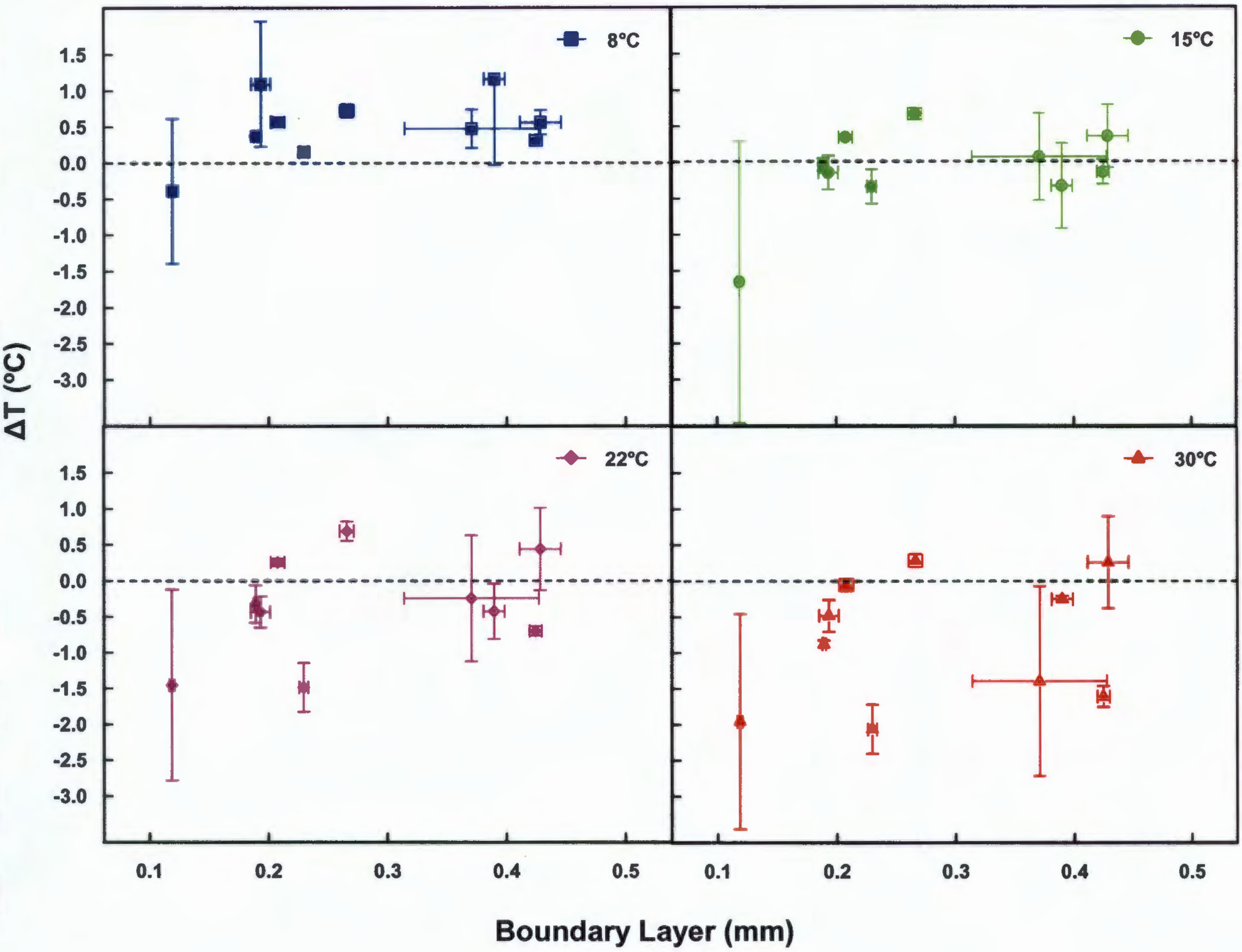

8°C
15°C
22°C
30°C
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
ΔT (°C)
0.1
0.2
0.3
0.4
0.5
Boundary Layer (mm)

Figure 3

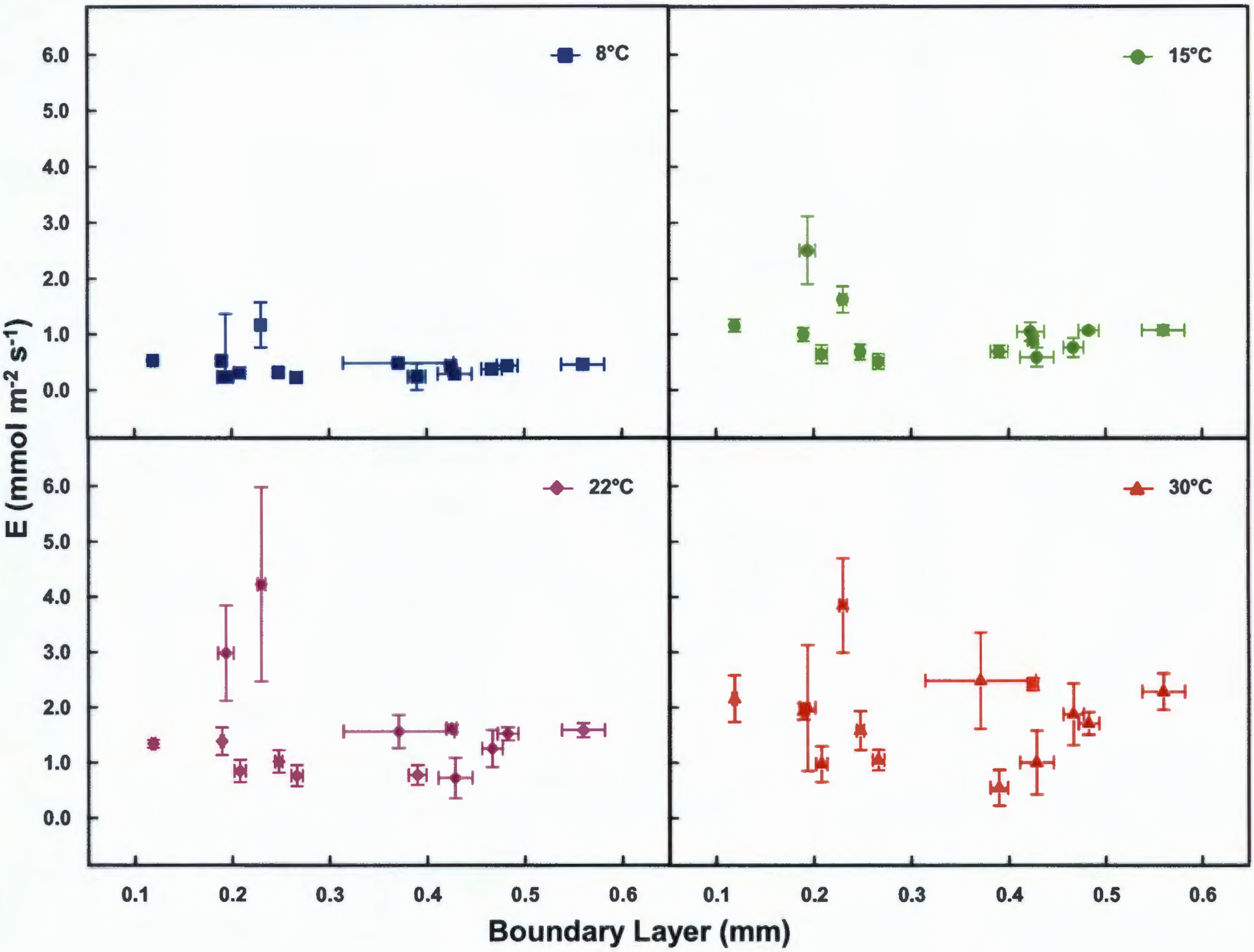

8°C
15°C
22°C
30°C
E (mmol m-2 s-1)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Boundary Layer (mm)

Figure 4

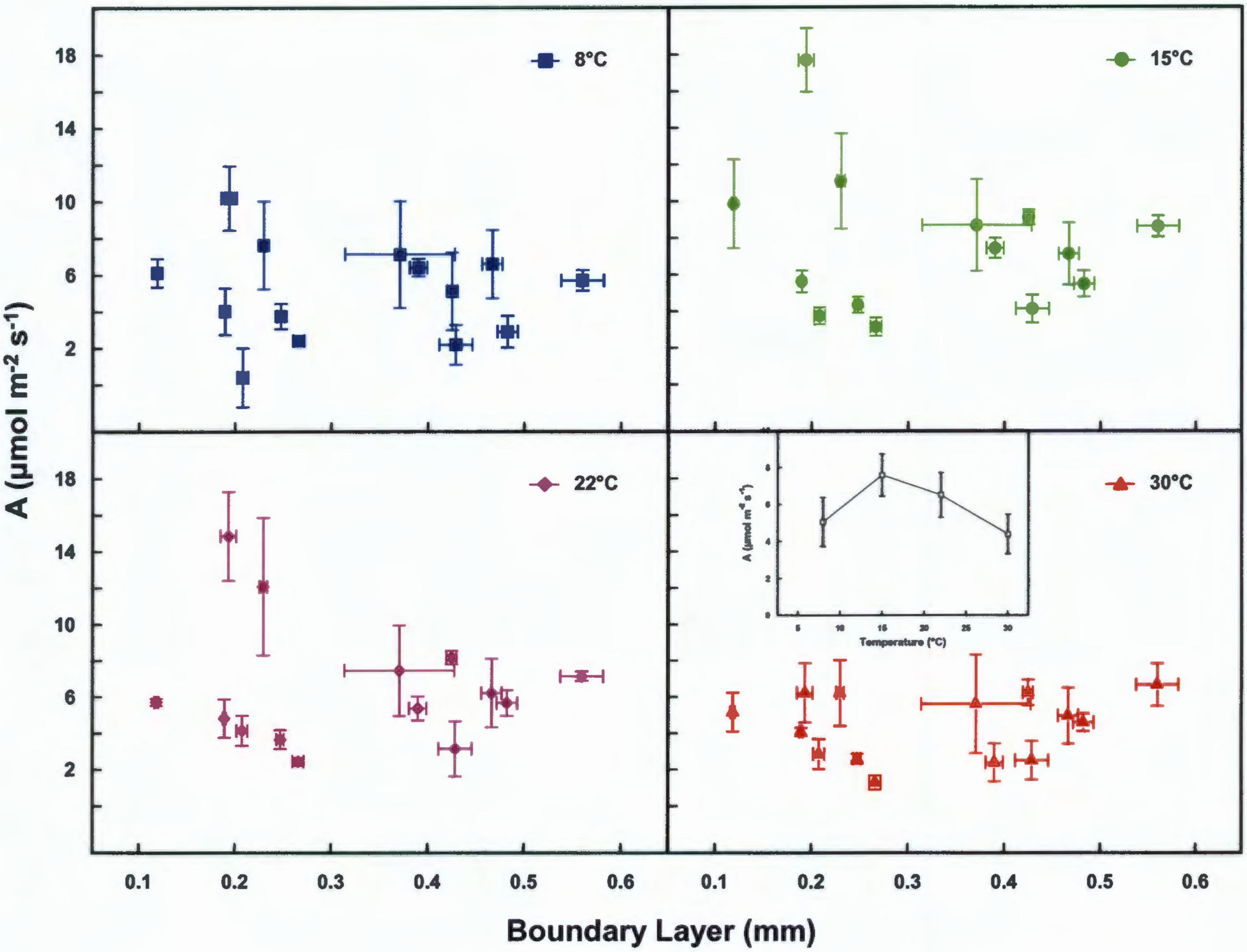
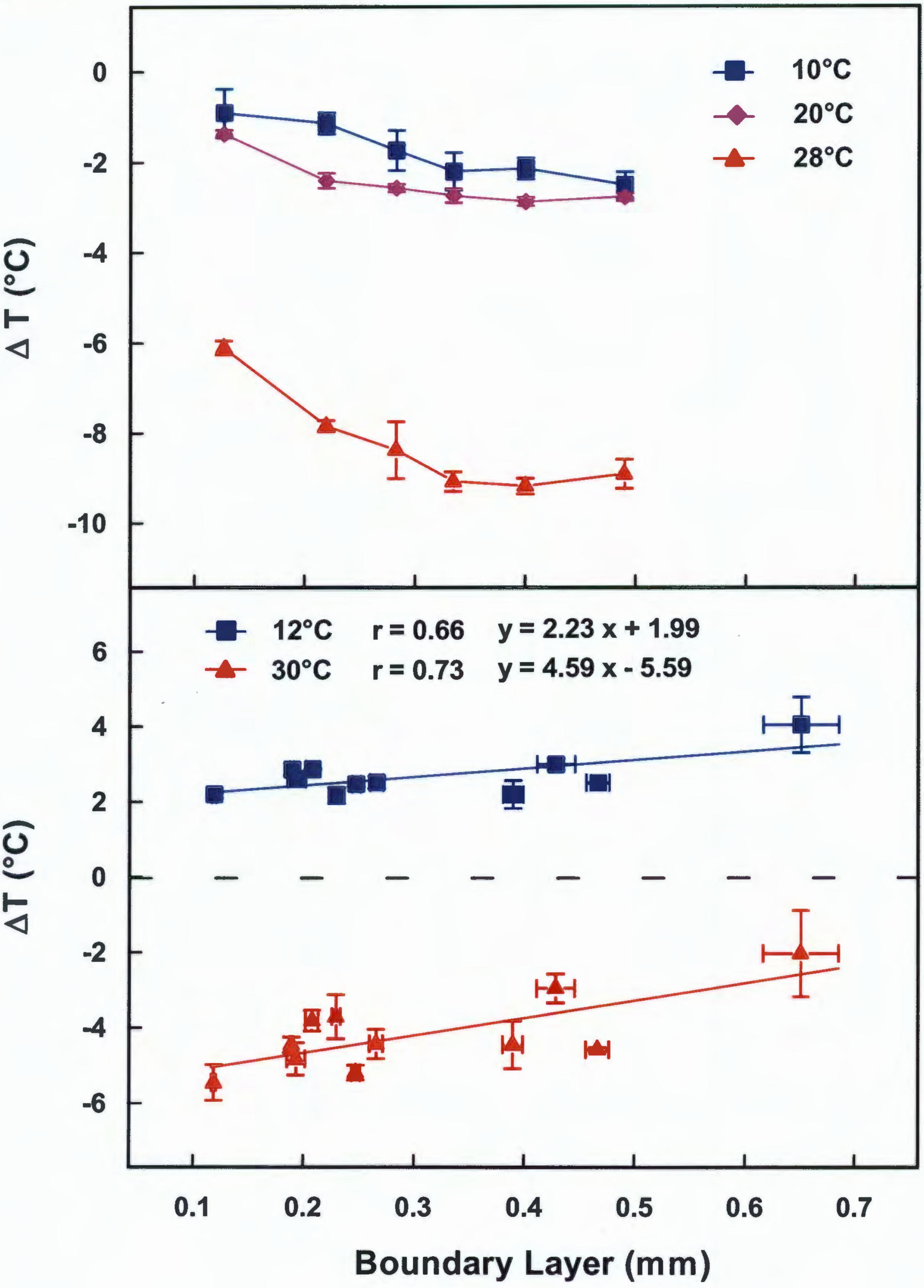

8°C
15°C
22°C
30°C
A (μmol m⁻² s⁻¹)
18
14
10
6
2
Boundary Layer (mm)
0.1
0.2
0.3
0.4
0.5
0.6
A (μmol m⁻² s⁻¹)
0
2
4
6
8
5
10
15
20
25
30
Temperature (°C)

Figure 5



0
-2
-4
-6
-8
-10
Δ T (°C)
10°C
20°C
28°C
6
4
2
0
-2
-4
-6
ΔT (°C)
12°C r = 0.66 y = 2.23 x + 1.99
30°C r = 0.73 y = 4.59 x - 5.59
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Boundary Layer (mm)

Figure 6

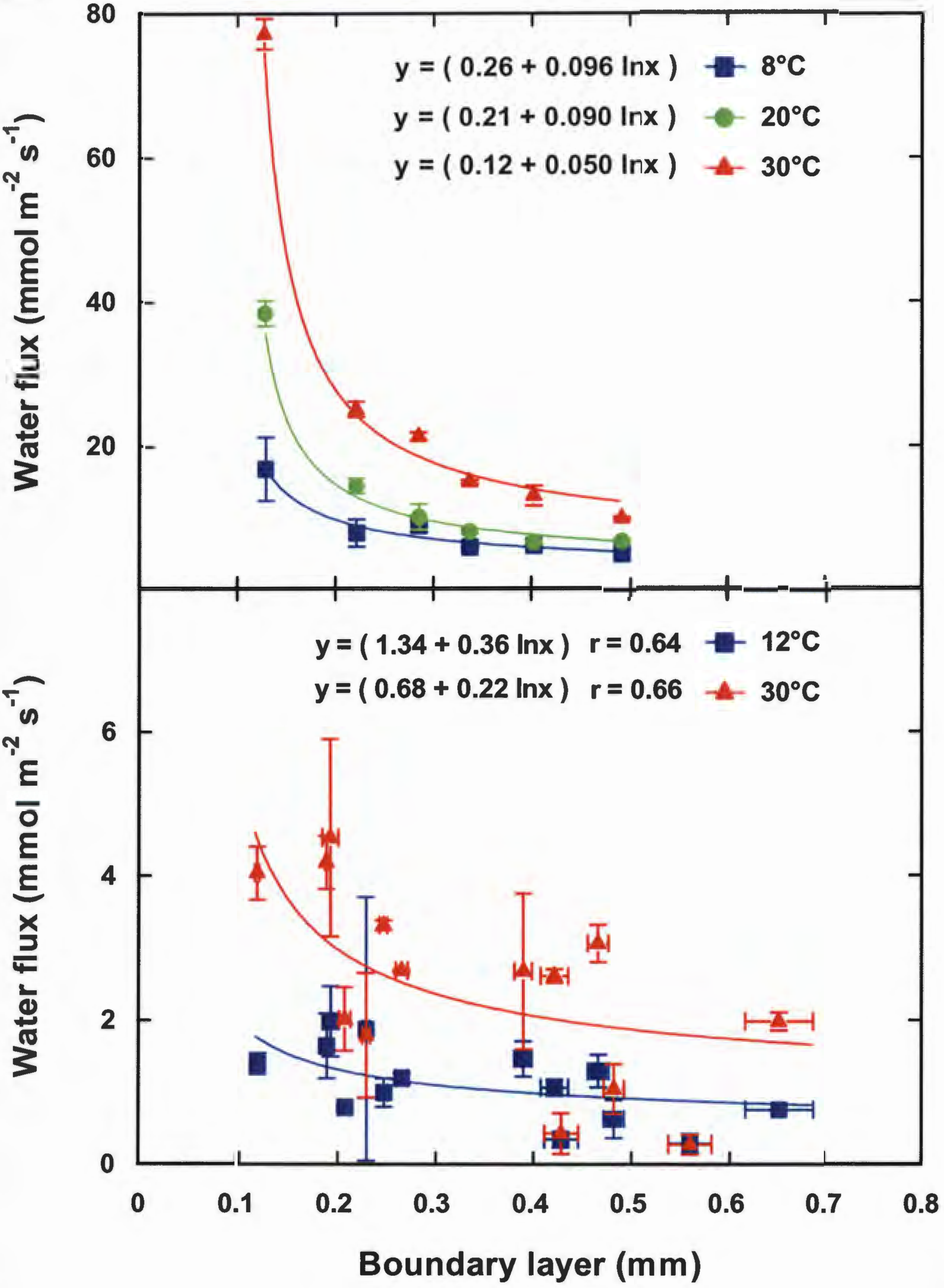

80
60
40
20
Water flux (mmol m^{-2} s^{-1})
y = (0.26 + 0.096 lnx)
8°C
y = (0.21 + 0.090 lnx)
20°C
y = (0.12 + 0.050 lnx)
30°C
6
4
2
0
Water flux (mmol m^{-2} s^{-1})
y = (1.34 + 0.36 lnx) r = 0.64
12°C
y = (0.68 + 0.22 lnx) r = 0.66
30°C
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Boundary layer (mm)

Figure 7

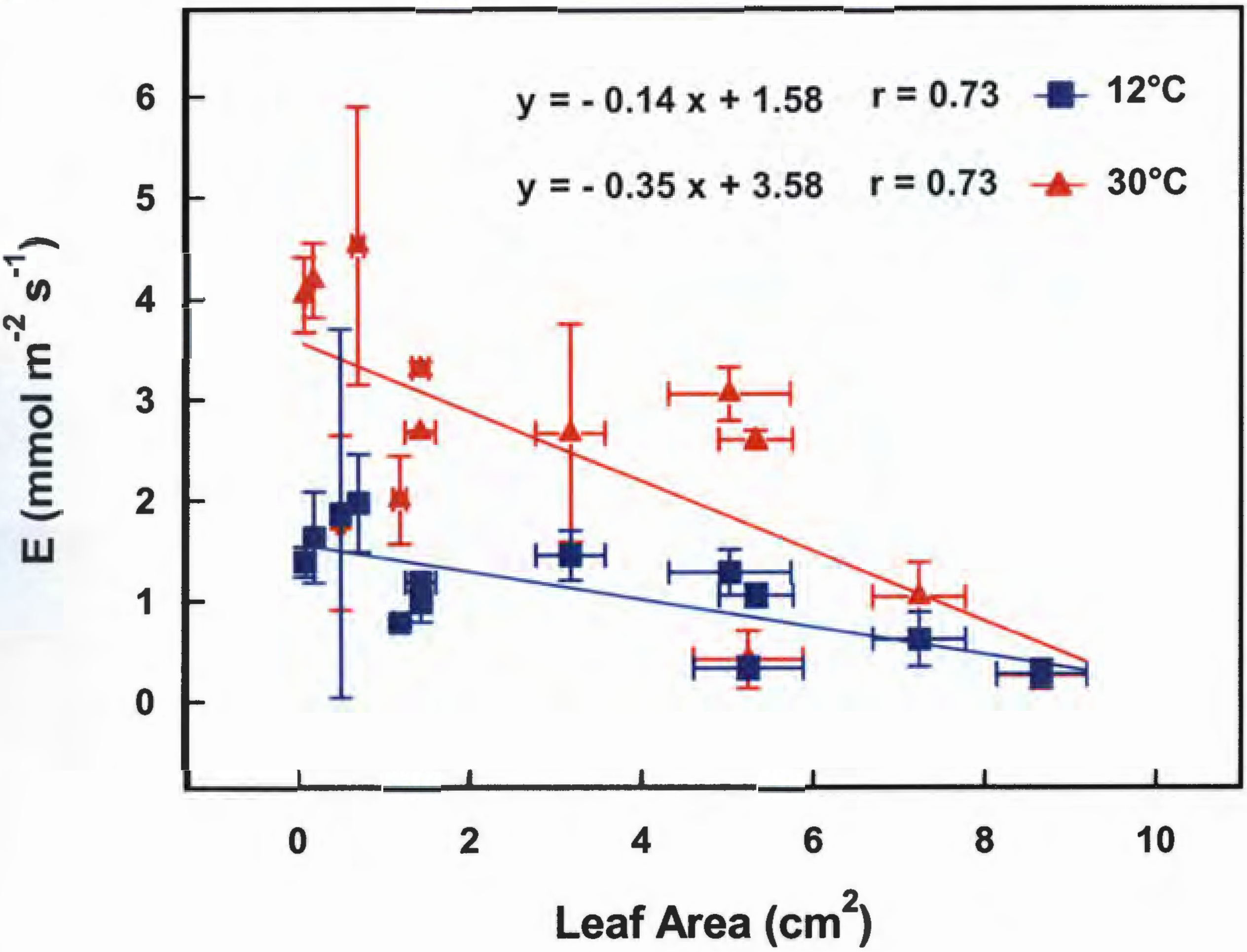
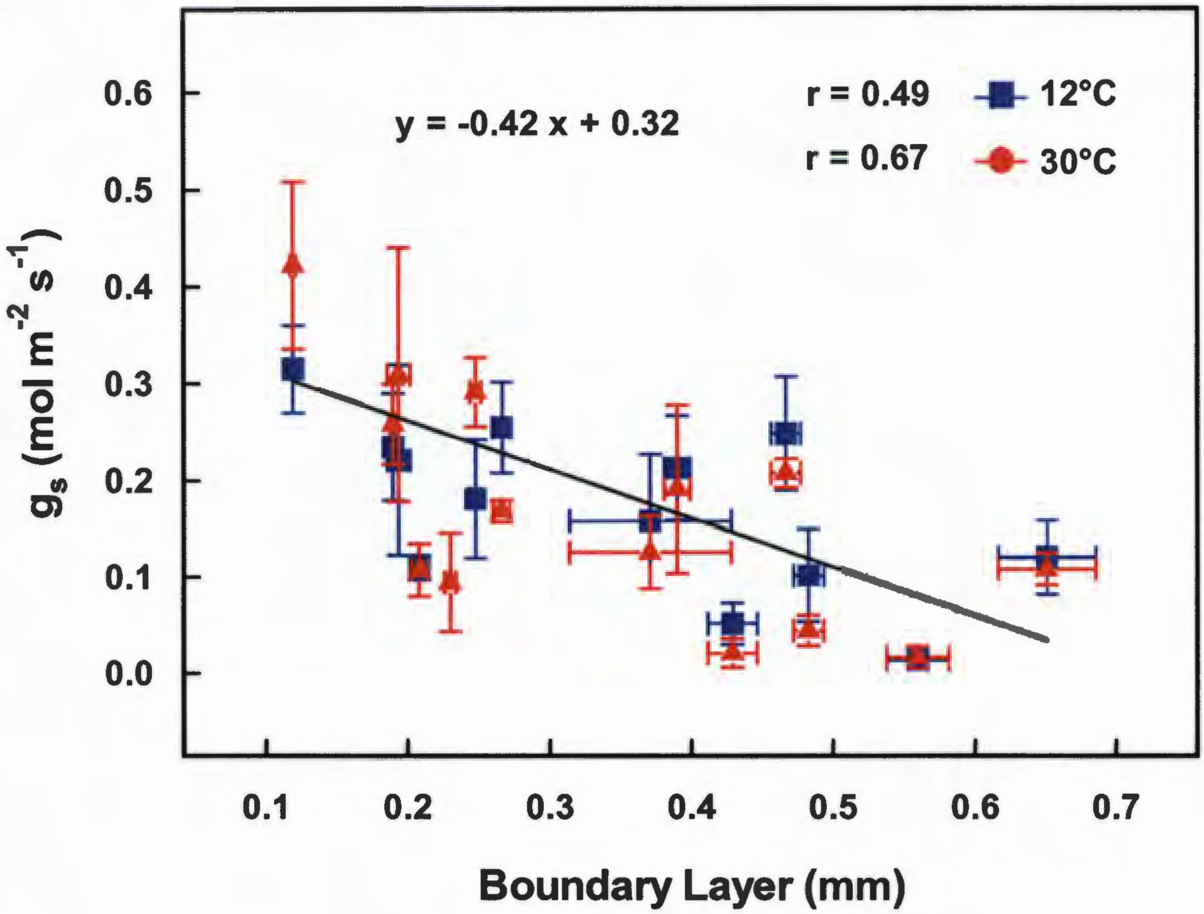

y = - 0.14 x + 1.58
r = 0.73
12°C
y = - 0.35 x + 3.58
r = 0.73
30°C
E (mmol m^{-2} s^{-1})
0
1
2
3
4
5
6
0
2
4
6
8
10
Leaf Area (cm^2)

Figure 8


y = -0.42 x + 0.32
r = 0.49
12°C
r = 0.67
30°C
g_s (mol m^{-2} s^{-1})
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Boundary Layer (mm)