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Frost and leaf-size gradients in forests: global patterns and experimental evidence

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Two tables and four figures (two colour)

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Summary

- Explanations of leaf size variation commonly focus on water availability, yet leaf size also varies with latitude and elevation in environments where water is not strongly limiting. We provide the first conclusive test of a prediction of leaf energy balance theory that may explain this pattern: large leaves are more vulnerable to night-time chilling, because their thick boundary layers impede convective exchange with the surrounding air.
- Seedlings of 15 New Zealand evergreens spanning 12-fold variation in leaf width were exposed to clear night skies, and leaf temperatures measured with thermocouples. We then used a global dataset to assess several climate variables as predictors of leaf size in forest assemblages.
- Leaf-minus-air temperature was strongly correlated with leaf width, ranging from -0.9 to -3.2 °C in the smallest- and largest-leaved species, respectively. MAT and frost-free period were good predictors of evergreen angiosperm leaf size in forest assemblages, but no climate variable predicted deciduous leaf size.
- Although winter deciduousness makes large leaves possible in strongly seasonal climates, large-leaved evergreens are largely confined to frost-free climates because of their susceptibility to radiative cooling. Evergreen leaf size data can therefore be used to enhance vegetation models, and to infer palaeotemperatures from fossil leaf assemblages.

Introduction

The organs that give most terrestrial ecosystems their characteristic colour all perform essentially the same functions, harnessing solar energy to power plant metabolism and the synthesis of construction materials, and to drive a transpiration stream that supplies the plant's tissues with water and minerals. Yet the range of sizes they show is bewildering, from the scale-like leaves of some conifers and heathland shrubs to the megaphylls of some tropical rainforest plants (Raunkiaer, 1934). The form and function of leaves has been a major focus for scientists researching plant, ecosystem and biosphere processes (Parkhurst & Loucks, 1972; Wright *et al.*, 2004; Wright *et al.*, 2017), reflecting the influence of leaf traits on plant fitness and ecosystem properties, and the importance of leaves as the prime interface between the atmosphere and terrestrial ecosystems.

The marked geographic patterns in leaf size noted by early biogeographers (Schimper *et al.*, 1903) suggest this trait must play some critical role in adapting plants to their environments. However progress towards a comprehensive theory of its functional significance has been fitful, and leaf size is usually omitted from models that use physiological principles to predict vegetation pattern and process (Dong *et al.*, 2017). Leaf energy balance theory (Parkhurst & Loucks, 1972) provides the most promising basis for a theory of leaf size. Large leaves should be less closely coupled to ambient air temperatures than small leaves, as their thicker boundary layers impede convective heat exchange. Recent experiments have confirmed that leaf width (the main determinant of boundary layer thickness: Parkhurst & Loucks, 1972) is strongly positively correlated with temperatures of leaves exposed to radiative heating at low wind speeds (Yates *et al.*, 2010; Leigh *et al.*, 2017). The prevalence of small leaves in open, arid environments therefore probably reflects the vulnerability of large leaves to overheating when water shortage prevents leaves being cooled by transpiration. Leaf energy balance theory could also explain latitudinal and elevational variation in leaf size of woody plants in environments where water is not strongly limiting: large leaves exposed to radiative cooling on clear nights should also chill more than small leaves (Parkhurst & Loucks, 1972; Leuning, 1988; Jordan & Smith, 1995), again because of the effect of leaf width on boundary layer thickness—and hence on convective exchange with the surrounding air. Assuming that transpiration can be neglected, energy balance theory predicts leaf minus air temperature (ΔT, in K) as:

$$\Delta T = R_n / (c_p \, g_b) \tag{1}$$

where R_n is the (negative) net radiation at the leaf surface (W m^{-2}), c_p is the heat capacity of air (29.3 J mol^{-1} K^{-1}), and g_b, the leaf boundary-layer conductance to heat (mol m^{-2} s^{-1}), is proportional to $\sqrt{(u/d)}$

where *u* is wind speed (m s^{-1}) and *d* is the characteristic dimension (about ¾ of the width) of the leaf (m). Low-growing alpine plants with closely-packed leaves can have much larger leaf - air temperature differentials than would be predicted by this equation (Körner & Cochrane, 1983), reflecting substantial interaction between the boundary layers of individual leaves (Monteith & Unsworth, 2007). The predicted effects of leaf size on daytime and night-time energy balance have recently been used to model global patterns in leaf size (Wright *et al.*, 2017). However, the predicted effect on night-time chilling has never been tested experimentally across a wide range of leaf sizes.

Here we use two lines of evidence to assess the role of frost in shaping global patterns of leaf size in environments where water is not strongly limiting. First, we experimentally tested the effect of leaf size on vulnerability to frost, comparing night-time radiative chilling of the leaves of 16 New Zealand evergreen angiosperms that spanned a 12-fold range in width (Table 1). We then tested frost-free period and several other climate variables as predictors of average leaf size of angiosperm trees and shrubs across 165 forest assemblages (comprising 3,024 species) from throughout Asia, Oceania and the Americas (Table S1). Our focus on forest vegetation was intended to isolate the effect of leaf size on night-time leaf - air temperature differences, and to minimize the influence of daytime effects that are dominant in arid and semi-arid regions (Wright *et al.*, 2017). We treated evergreen and deciduous species separately; we expected leaf sizes of deciduous species to be less responsive to frost regimes, as their leaves are less likely to be exposed to sub-zero temperatures, especially in continental temperate climates with strong temperature seasonality.

Methods

Effect of leaf width on leaf - air temperature differences

Seedlings of 15 New Zealand angiosperm trees and shrubs were obtained from commercial nurseries (Table 1). These were chosen as representative of the range of leaf size present in the New Zealand woody angiosperm flora, and the range of forest formation types found on the three main islands of the archipelago. This forest region encompasses ~7 ˚C variation in MAT (8-15 ˚C), and wide range of frost-free periods (< 90 days at high elevations and on some inland South Island sites, to 365 days on coastal northern sites). Average seedling leaf width of these 15 species was strongly correlated with the 5th percentile of July minimum temperatures experienced throughout species' ranges (r = 0.82), and less strongly with other temperature parameters (Table 1). Four seedlings of each species were grown under 50% shade-cloth for at least three months before the experiment. Foliage disposition of all seedlings was approximately planophile, and all species have simple leaves.

Thermocouples were used to record leaf temperatures of seedlings fully exposed to the sky on two successive clear winter nights on 8-9 August 2016. The experiment was carried out on a lawn on level ground at Plant and Food Research Ruakura campus on the outskirts of Hamilton (37.7757˚S, 175.3128˚ E), measuring leaf temperatures of two replicate plants of each species on each of the two nights (Fig. S1). Thermocouples were prepared from TT-T-40-500 insulated wire (Omega Engineering, Stamford CT), and attached with porous surgical tape (3M™ Micropore™ Surgical Tape, 3M, Maplewood, MN) to the centre of the upper surface of the uppermost fully-expanded leaf on the central axis of each plant, or on a major branch. This criterion ensured that the chosen leaves were well exposed to radiative cooling, with minimal shading by other leaves. As significant temperature gradients occur close to the ground during radiation frosts (Leuning & Cremer, 1988), the height of each thermocouple above ground was recorded (Table 1), enabling this variable to be incorporated in statistical analysis of the results. Temperatures were recorded every 10 minutes using a data-logger and thermocouple multiplexor (CR10X and AM25T, Campbell Scientific, Logan, UT). Two additional thermocouples and an anemometer (A100LM, Vector Instruments, Rhyl, UK) were used to measure air temperatures and wind speeds at the same height above the lawn. Plants were deployed in a circle c. 4.5 m in diameter around the datalogger (Fig. S1), and their order randomized. Minimum air temperatures recorded on the two nights were -2.9 and -0.3°C, respectively. Wind speeds ranged from 0.00 to 1.03 m s^{-1} on the first night, and from 0.00 to 0.58 m s^{-1} on the second. We did not measure relative humidity at our experimental site, but the meteorological station on the Ruakura campus recorded figures of 78-96% on the first night, and 66-93% on the second night; these measurements were taken higher above the ground (1.2m) than our experimental measurements.

Electronic calipers were used to measure effective width of leaves, the main determinant of boundary layer thickness (Parkhurst & Loucks, 1972). As leaf shapes ranged from lanceolate and oblanceolate to orbicular, with entire or weakly serrated margins, this dimension was simply the lamina width at the widest point, which was measured on each leaf used for temperature measurements.

We used the lme4 package of R (Bates *et al.*, 2015) to fit a Generalized Linear Mixed Model (GLMM) to analyse determinants of leaf - air temperature differentials. As well as fixed effects of (log) leaf width and (log) leaf height above ground, we incorporated species and measurement night (7 or 8 August) as random effects.

Climatic modelling of leaf size in forest assemblages

Leaf size (area) data were obtained only from eudicot and magnoliid trees and shrubs because only these plant groups were consistently sampled in all the source datasets (See Table S1 in Supporting Information). As there is no universally accepted definition of forest vegetation, we used an *ad hoc* climatic criterion (MAP/PET ≥ 0.5) that separated most closed-canopy tree-dominated vegetation from savannas, Mediterranean shrublands and other vegetation types typical of semi-arid climates. Leaflet area of compound-leaved species was recorded (i.e. the minimum photosynthetic unit: Kraft *et al.*, 2008), as this variable was considered likely to have more influence on boundary layer thickness than total area of a compound leaf. A total of 3,024 species were sampled, the number of species per site ranging from six to 268.

Published studies reporting leaf size data accounted for accounted for 157 of our 165 sites (Table S1). Leaf area measurements were made on fresh material collected from these 157 sites, usually from sun leaves of adult plants—exceptions are identified in Table S1. Gaps in subtropical and cool-temperate South America were filled by obtaining tree and shrub species lists from floristic studies carried out in those regions (Dollenz, 1981; Aidar *et al.*, 2001; Ruschel *et al.*, 2005; 2014), and matching leaf size data at nearby sites from the Herbario Virtual Austral Americano (Herbario Virtual Austral Americano, 2016); we added four sites using this approach. The online herbarium specimens which were measured were obtained from well-lit foliage of adult plants, as shown by the presence of fruit or flowers on specimens. Leaf(let) areas of herbarium specimens were measured using ImageJ (Schneider *et al.*, 2012), correcting deciduous and evergreen species for shrinkage by 27% and 15 %, respectively (Blonder *et al.*, 2012). Species' arithmetic mean leaf areas at each site were used for the analyses described below.

We used GLMMs with Gaussian error distributions to compare several temperature variables as predictors of log-transformed average leaf size of angiosperm trees and shrubs in forest assemblages. Models were fitted using the lme4 package of R (Bates *et al.*, 2015). Climate variables considered potentially relevant to leaf size were obtained from GIS surfaces (Hijmans *et al.*, 2005; Fischer *et al.*, 2008). Some tree species occurred at multiple sites so we computed site-specific species averages of leaf size, and conducted the analysis at this level of observation. To account for this data structure in every mixed model, we modelled random species intercepts and random site intercepts nested within regions. Our incorporation of these random effects recognizes that (a) different regions have historical peculiarities not accounted for by present climate; (b) leaf size differences between sites are influenced by factors other than macroclimate (e.g. topography, aspect, soil fertility, drainage); and (c) species sorting on temperature gradients is influenced by functional and ecological differences other than leaf size, such as leaf angle (Falster & Westoby, 2003) and differential exposure to climatic stresses because of differences in regeneration niche (Lusk & Laughlin, 2017). We computed both marginal (R^2_m) and conditional R^2 values (R^2_c) using the MuMIn package of R (Barton, 2015) to estimate variation that is explained by the fixed effects (R^2_m) and the variation that is explained by both fixed and random effects (i.e., R^2_c).

Because of the predicted effect of leaf size on nocturnal chilling, we hypothesized the average frost-free period would be the strongest climatic correlate of leaf size variation in forest assemblages. We fitted separate sets of models for evergreen and deciduous species, including frost-free period and seven other climatic variables in turn as a predictor of leaf area (Table 2). We used the Akaike Information Criterion (AIC) to determine how well each of the eight models was supported by the data (Anderson, 2008). The model with the lowest AIC is the most supported. We computed AIC differences as $\Delta_i = AIC_i - AIC_{min}$, where AIC_i is the AIC for the *i*th model and AIC_{min} is the minimum AIC among all the models. We did not consider models with multiple predictors because of the high collinearity ($r > 0.8$) among those climate variables that were strongly correlated with leaf size.

Results

Effect of leaf width on leaf - air temperature differences

As species differences in leaf temperatures were largest during the first half of both nights, we analyzed data from that period only (5:40 pm till 12:20 am). Leaves became colder than the surrounding air almost immediately after sunset on the first night, and before sunset on the second night; the effect of leaf width on leaf temperatures was also apparent by then (Fig. 1; Table S2). Although these patterns persisted until sunrise, they became weaker after midnight on both dates. This attenuation probably had different causes on the two nights. During the first night, the range of leaf - air temperature differentials was positively correlated with wind speed ($r = 0.71$), which fell to near zero after midnight; the reduced rates of cooling and dampening of the effect of leaf width thereafter cannot therefore be attributed to wind. At Hamilton airport (10km south of our experimental site), laser ceilometer readings detected no cloud cover on either night, but a small amount of fog was reported in the early morning hours of the first night (Meteorological Service of New Zealand, unpublished data); fog is therefore a possible cause of the dampening of the effect of leaf width after midnight (Fig. 1). In contrast, the range of leaf - air differentials was negatively correlated with wind speed on the second night ($r = -0.59$), suggesting that attenuation of the effect of leaf width during the second half of the night was caused mostly by the stronger and more variable winds occurring after ~11pm on that date (Table S2).

Leaf - air temperature differentials during the first halves of the two nights were tightly negatively correlated with leaf width (Fig. 2; $R^2 = 0.95$). Phylogenetically independent contrasts (Harvey & Pagel, 1991) gave a very similar result ($R^2 = 0.96$). Average leaf - air differentials of the 15 species spanned 2.3 °C, ranging from -0.9 °C in the smallest-leaved species (*Coprosma rhamnoides*) to 3.2 °C in the largest-leaved (*Beilschmiedia tarairi*). A linear mixed model showed a highly significant effect of leaf width on leaf - air differentials ($P < .0001$) and a marginal effect of leaf height above ground ($P = 0.0535$).

Climatic modelling of leaf size in forest assemblages

Mean annual temperature (MAT) and frost-free period were good predictors of mean evergreen leaf size in forest assemblages (Table 2), as shown by strong positive correlations (Fig. 3). Evergreen leaf size in forests approximately doubles for every 100-day increase in frost-free period (Fig. 3a), and nearly quadruples for every 10˚C increase in MAT (Fig. 3b). The model with MAT provided the most parsimonious explanation of variation in evergreen leaf size, as shown by an Akaike Information Criterion > 10 units lower than any other model (Table 2). MAT explained 33% of the variation in evergreen leaf size, after accounting for random species, site, and region effects (Table 2). Random species, site, and region-level effects together accounted for 70 - 80% of variation in leaf size within most models (Table 2). Quantile regression gave similar results: the best predictor of the 0.9 quantile of evergreen leaf size was MAT, closely followed by frost-free period (Table S3). At any given MAT or frost-free period, the 0.1 to 0.9 quantile range included up to two orders of magnitude variation in leaf size of individual evergreen species (Fig. 3). Neither frost-free period nor MAT was strongly related to deciduous leaf area (Figure 3c-d); indeed, none of the chosen climatic variables could explain much variation in deciduous leaf area (all $R^2_m < 0.03$).

Similarly, latitudinal gradients in leaf size are more evident in evergreen assemblages than in deciduous assemblages (Fig. 4). Only in the tropics are evergreen assemblages dominated by mesophylls (sensu Webb, 1968), and only at high latitudes or high elevations are they dominated by nanophylls. In contrast, large-leaved species can dominate deciduous assemblages at a wide range of latitudes in the northern hemisphere (Fig. 4).

Discussion

Our results are consistent with frost playing an important role in shaping evergreen leaf size in environments where water is not strongly limiting. Although leaf shapes of our 15 study species ranged from lanceolate to near-orbicular, lamina width alone explained 95% of variation in leaf - air temperature differentials (Fig. 2). The effect of leaf width on nocturnal chilling was found to be smaller than its effect on daytime radiative heating (Yates *et al.*, 2010; Leigh *et al.*, 2017). However the 2.3 °C range of leaf - air temperature differentials we report here (Fig. 2) is particularly significant for plants in temperate oceanic climates and in (sub)tropical uplands, where the annual range in mean monthly minimum temperatures is invariably <10 °C, and sometimes as little as 6 °C (di Castri & Hajek, 1976; New Zealand Meteorological Service, 1983; Australian Bureau of Meteorology, 2017). In such climates, the range of leaf - air temperature differentials in our dataset equates to 2-3 months' difference in the frost-free period effectively experienced by plant leaves at a given air temperature.

Although the best predictor of evergreen leaf size was MAT, rather than frost-free period (Table 2), the sum of the evidence presented here points to frost as the main limiting factor. MAT and frost-free period were highly correlated across our 165 sites (r = 0.90), making them difficult to tease apart. The inability of temperature variables to predict deciduous leaf size is consistent with limitation of evergreen leaf size in forests mainly by low temperatures (which deciduous species largely avoid), rather than by annual averages. Furthermore, although many warm-temperate and subtropical sites are predicted to be frost-free year round (Fischer *et al.*, 2008), this probably does not accurately represent the threat of chilling damage to large leaves at such sites; as large leaves can be several degrees colder than ambient air on clear nights (Fig. 2), they may suffer frost damage even when air temperatures are above zero.

Some (but by no means all) of the interspecific variation in leaf size in evergreen assemblages is associated with differential exposure to night-time chilling, as a result of differences in regeneration niche and life history. At an inland lowland site in New Zealand, (Lusk & Laughlin, 2017) found that small leaves were common among species that colonize open sites, where seedlings are most exposed to frost and to water deficits; species that establish in more sheltered microenvironments tended to have larger leaves. Shade-tolerant shrubs and small trees may spend most of their lives sheltered from night-time chilling, although tree falls will inevitably expose some individuals to the night sky (e.g. Cheng *et al.*, 2017).

Our analysis assumes that leaf-size related susceptibility to frost damage significantly affects plant growth and survival. The first night of our own experiment provided some support for this assumption: both seedlings of two large-leaved species (*Corynocarpus laevigatus*, *Melicytus ramiflorus*) were killed by the -2.9° C air frost they were exposed to that night (Table S4). Most other species suffered some degree of damage, but no other seedlings died. After a series of hard frosts at an inland site in the North Island of New Zealand, many *Beilschmiedia tawa* and *Melicytus ramiflorus* trees suffered heavy defoliation, whereas some of their associates appeared unaffected (Kelly, 1987). Although few trees died, many *B. tawa* lost the upper third of their crowns, later resprouting lower down. Radial growth of frost-damaged *B. tawa* trees was greatly reduced for the following three years. Kelly (1987) concluded that occasional extreme frost events may mediate the competitive balance between species, and so influence forest composition.

What are the relative roles of leaf size and tissue-level frost resistance in sorting evergreens along temperature gradients? The range of leaf - air differentials across our study species (2.3 °C) is dwarfed by the reported range of maximum frost resistance of their leaf tissues: -4 °C in *Beilschmiedia tarairi* to -12.4 °C in *Lophozonia menziesii* (Bannister, 2007). However, maximum frost resistance is only developed during winter, and species from different climate zones tend to differ less in frost resistance during the growing season than during the coldest months (Bannister, 2007). The main value of small leaves at high latitudes and high elevations might therefore be in minimizing chilling on clear nights in spring and autumn (Parkhurst & Loucks, 1972; Wright *et al.*, 2017), with evergreens relying more on tissue-level frost resistance during winter; although conifers were not included in our dataset, this may be especially true of needle-leaved conifers at high latitudes in the northern hemisphere. Boreal winter temperatures fall to levels that render boundary layer effects trivial, but having very narrow leaves may enable evergreen conifers to photosynthesize and grow during frost-prone weeks of spring and autumn (Parkhurst & Loucks, 1972), outside the shorter growth period of their deciduous angiosperm associates (Griffis *et al.*, 2003).

Although our main focus is on global and regional patterns, frost regimes are also likely to influence local variation in leaf size in hilly and mountainous regions. Topography has strong effects on minimum air temperatures close to the ground, with cold air drainage often causing temperature inversions on floodplains and low-lying terraces, and in hollows (Bootsma, 1976; Kalma *et al.*, 2012). The effect of leaf size on night-time chilling may thus help explain the prevalence of small-leaved species on inland terraces and in frost hollows in New Zealand (Wardle, 1977; Lusk, 2014) and in south-eastern Australia (Hobbs *et al.*, 2018).

The optimal leaf size of evergreens in forests may depend on a trade-off between avoidance of damagingly low temperatures, and efficiency of light interception. Large leaves enable seedlings to display large foliage areas without excessive self-shading or support costs (Falster & Westoby, 2003; Lusk *et al.*, 2012), and larger trees to suppress competitors by casting long dark shadows (Valladares, 1999). In tropical rainforests, large leaf size may also confer a net carbon gain advantage by minimizing respiration, which (unlike photosynthesis) increases exponentially with temperature (Berry & Bjorkman, 1980; Drake *et al.*, 2016): leaf energy balance theory predicts that evapotranspiration will cool large leaves to several degrees below air temperatures in such environments, once again because of the insulating effect of their thick boundary layers (Gates, 1968). All else being equal, in hot wet environments small leaves should respire at faster rates than large leaves, as they will stay closer to air temperature when wind speeds are low.

Our findings suggest it would be timely to reconsider the traits that enabled angiosperm radiation into freezing environments. The innovations thought to be key to this development are the evolution of small-diameter conduits, deciduousness, and (in herbaceous plants) senescence of aboveground tissues during winter (Zanne *et al.*, 2014). However, the latter two of these phenomena are of most relevance to the continental climates of the North Temperate Zone—they are less common in oceanic temperate climates, and rare on tropical mountains. At inland sites in oceanic temperate regions such as New Zealand, southern South America and south-eastern Australia, carbon gain opportunities are interspersed with frost events throughout much of the year; small evergreen leaves enable plants to exploit these unpredictable opportunities while minimizing vulnerability to frost damage (Lusk *et al.*, 2016; McGlone *et al.*, 2016), and possibly reducing the need for investment in specialised frost protection mechanisms. Similarly, small evergreen leaves predominate at high elevations on tropical mountains (Grubb, 1977), where frost can occur at any time of year.

This study advances understanding of leaf size by showing the differential sensitivity of evergreens and deciduous species to temperature gradients, and by confirming experimentally that this may reflect the vulnerability of large leaves to frost—as predicted by leaf energy balance theory (Parkhurst & Loucks, 1972; Leuning, 1988; Wright *et al.*, 2017). Modellers are focusing increasingly on the value of plant traits as potential predictors of how biodiversity and ecosystem function vary along climatic gradients and in response to global environmental change. To date, these efforts have focused on character syndromes related to the carbon economics of leaves and stems (Sitch *et al.*, 2003; Scheiter *et al.*, 2013; Sakschewski *et al.*, 2015). The potential role of leaf size as a determinant of plant susceptibility to damage in different types of environments, and the costs and trade-offs this may imply in terms of survivorship or competitive success, is not considered by current global ecosystem models (Dong *et al.*, 2017; Wright *et al.*, 2017). Our experimental results support the predicted effect of leaf size on nocturnal chilling, recently invoked as a partial explanation for global patterns of leaf size (Wright *et al.*, 2017). Our empirical confirmation of this effect puts modellers in a position to incorporate leaf size into the next generation of vegetation models, enhancing the ability of these models to predict species distributions, forest composition, and ecosystem dynamics. Our findings also confirm the utility of leaf size for inferring paleotemperatures from fossil assemblages (Peppe *et al*., 2011; Reichgelt *et al*., 2017), assuming that deciduous and evergreen fossil leaves can be reliably distinguished.

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Author Contributions

CHL and MJC developed the idea and carried out the experiment; CHL collated leaf size data and wrote most of the paper; DCL analysed the global leaf size dataset and wrote corresponding parts of the methods and results; SPH, ICP, MN and BS contributed data and edited the manuscript.

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Supporting Information

Table S1. Sampling regimes in the various source datasets on leaf size in tree and shrub assemblages.

Table S2. Time course of leaf and air temperatures from sunset till sunrise during both nights of the experiment.

Table S3. Summary of model selection criteria for quantile regression models of climatic variables as predictors of the 90th percentile of evergreen leaf size across 165 forest assemblages.

Table S4. Summary of frost damage to seedlings on the night of 7-8 August 2016.

Figure S1. Experimental set-up for leaf temperature measurements at Ruakura.

Table 1. Species used in frost experiment, showing mean width of leaves used for temperature measurements, height at which measurements were made, and temperature characterization of sites naturally occupied by each species (New Zealand Plant Conservation Network, 2016).

Species	Family	Mean leaf width, mm (range)	Height above ground, mm (range)	Average MAT (°C)	Average July min. T (°C)	5% percentile of July min. T (˚C)
Coprosma rhamnoides	Rubiaceae	5.3 (4-7)	506 (410-580)	9.8	0.7	-2.3
Elaeocarpus hookerianus	Elaeocarpaceae	5.8 (4-7)	596 (530-670)	9.7	0.7	-1.9
Myrsine divaricata	Primulaceae	5.8 (5-7)	591 (530-640)	8.5	-0.3	-2.6
Fuscospora cliffortioides	Nothofagaceae	7.5 (7-8)	561 (430-635)	7.1	-2.0	-4.3
Melicytus micranthus	Violaceae	7.9 (7-9)	493 (400-590)	11.3	1.8	-2.6
Lophozonia menziesii	Nothofagaceae	10.8 (8-14)	555 (405-685)	8.5	0.1	-2.8
Myrsine salicina	Primulaceae	15.8 (14-18)	550 (420-690)	10.1	2.0	0.5
Elaeocarpus dentatus	Elaeocarpaceae	19.5 (18-21)	553 (440-620)	12.2	2.6	0.6
Beilschmiedia tawa	Lauraceae	22.5 (20-27)	611 (490-670)	12.2	2.7	0.9
Knightia excelsa	Proteaceae	24.5 (22-26)	580 (510-695)	12.7	4.1	2.1
Melicytus ramiflorus	Violaceae	27.8 (26-32)	575 (485-610)	10.7	2.1	-0.1
Litsea calicaris	Lauraceae	39.5 (34-44)	631 (535-680)	14.2	6.1	3.5
Coprosma grandifolia	Rubiaceae	59.3 (47-65)	630 (505-720)	11.5	2.8	0.2
Corynocarpus laevigatus	Corynocarpaceae	62.0 (56-72)	469 (450-500)	12.7	4.3	1.4
Beilschmiedia tarairi	Lauraceae	63.3 (52-76)	581 (470-680)	14.0	7.0	5.9

Table 2. Summary of model selection criteria for all models as predictors of evergreen leaf size across 165 forest assemblages throughout Asia, Oceania and the Americas. The table shows variation explained by the fixed effects alone (R^2_m), and by fixed and random effects combined (R^2_c). Models are ranked from lowest to highest AIC.

Climatic predictor	**AIC**	Δ_i	R^2_m	R^2_c
MAT	8845.0	0	0.33	0.89
Frost-free period	8862.5	17.5	0.18	0.88
Mean minimum temperature of coldest month	8878.2	33.2	0.27	0.90
Mean maximum temperature of warmest month	8888.6	43.6	0.21	0.89
Isothermality	8904.4	59.4	0.26	0.91
Mean annual precipitation	8939.3	94.3	0.06	0.91
Diurnal temperature range	8941.1	96.1	0.01	0.92
Cramer-Prentice moisture index*	8941.1	96.1	0.02	0.92

* = Actual evapotranspiration / potential evapotranspiration (Cramer & Prentice, 1988)

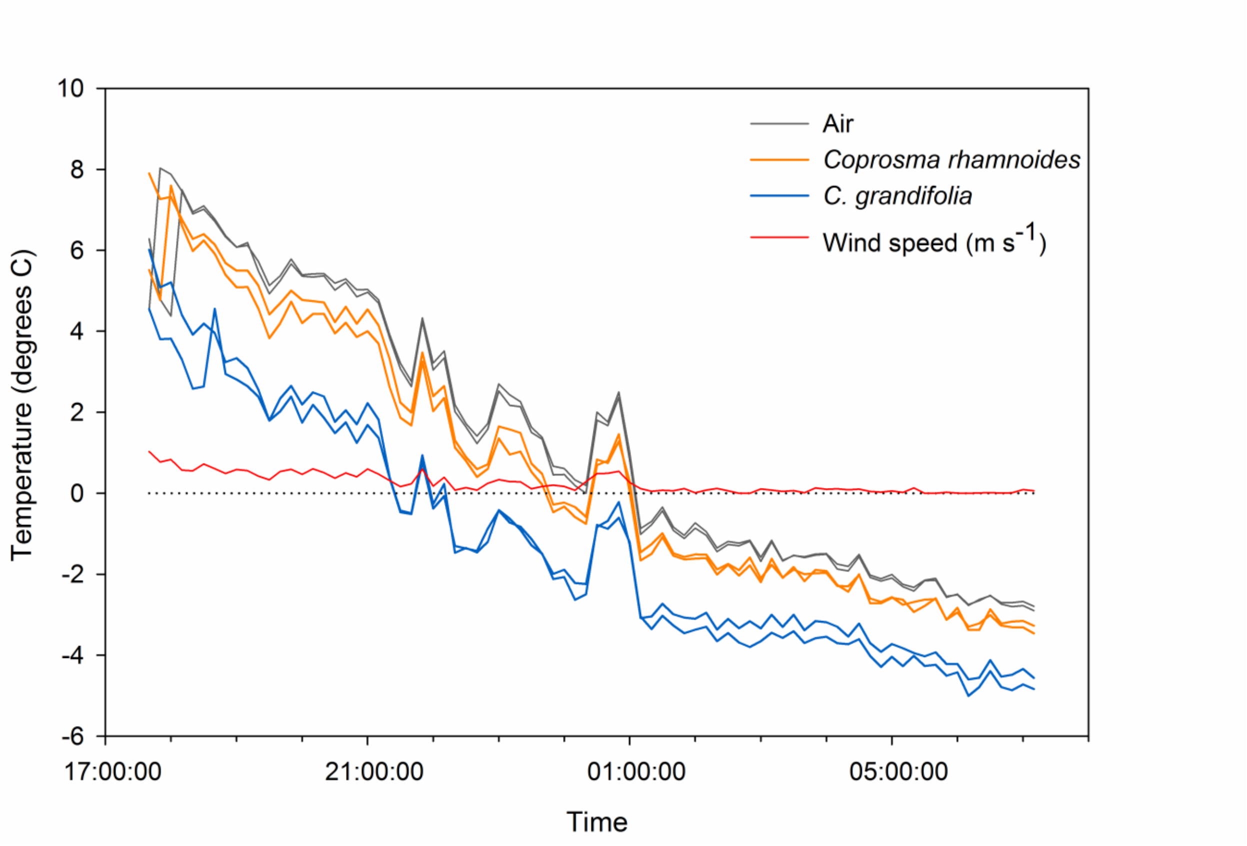
Figure Legends

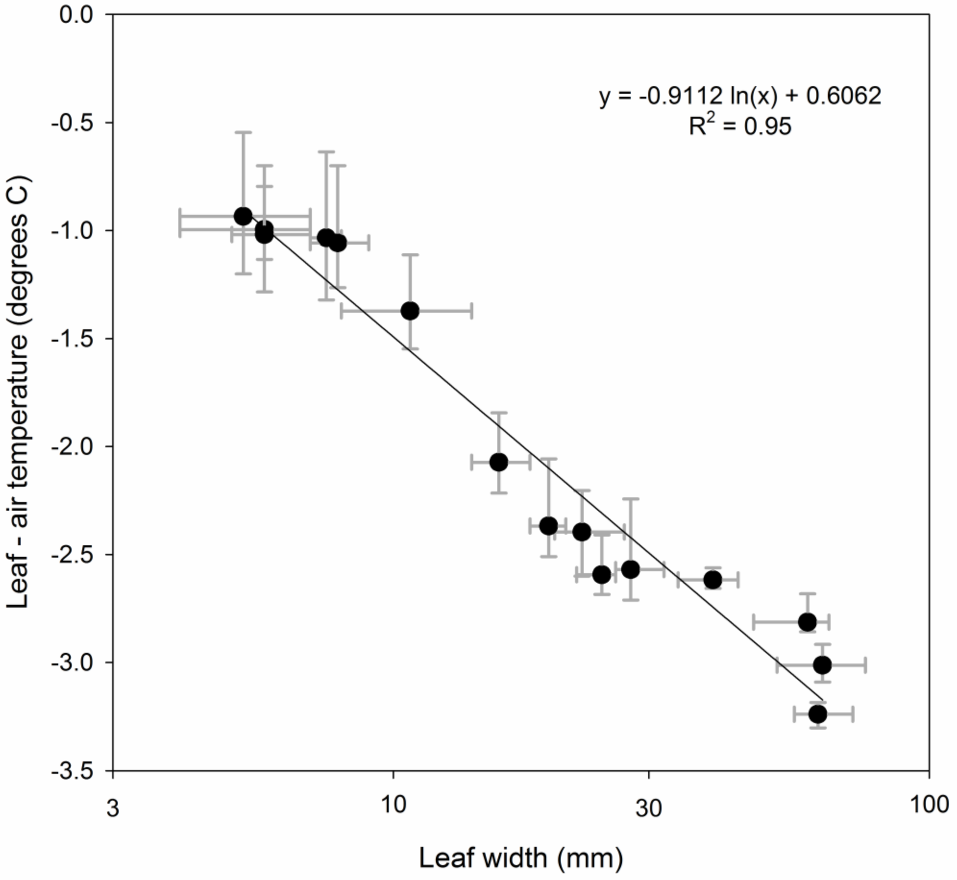
Fig. 1. Temperature traces from thermocouples attached to two *Coprosma rhamnoides* leaves and two *C. grandifolia* leaves, as well as two exposed to free air at the same height above ground. Data shown are from sunset on 7 August till sunrise on 8 August 2016.

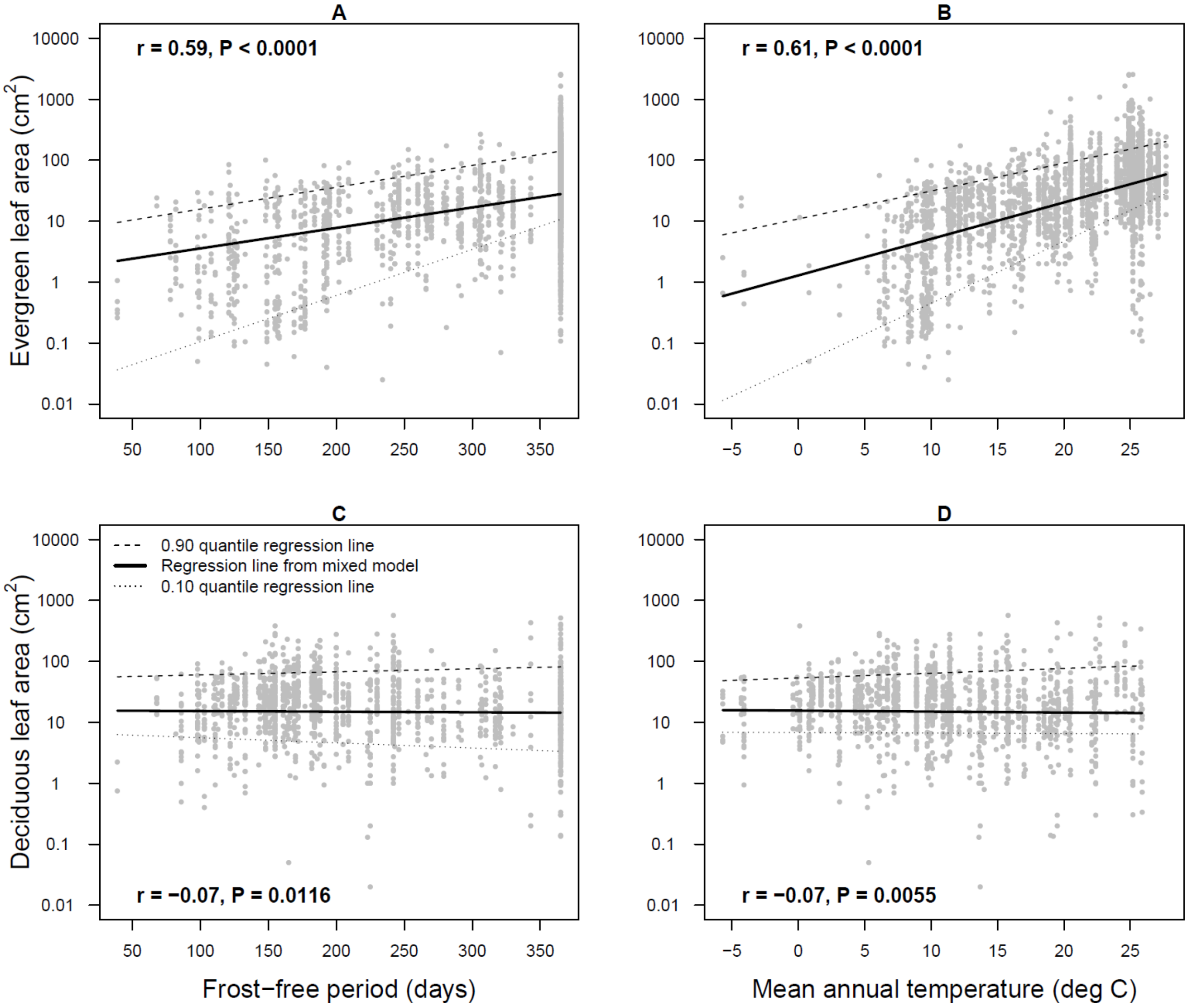
Fig. 2. Relationship of leaf - air temperature differentials with leaf width of 15 New Zealand tree and shrub species exposed to clear night skies. Graph shows mean and range of measurements on one leaf of each of four plants per species. Wind speeds during measurements ranged from 0.00 to 1.03 m s^{-1}.

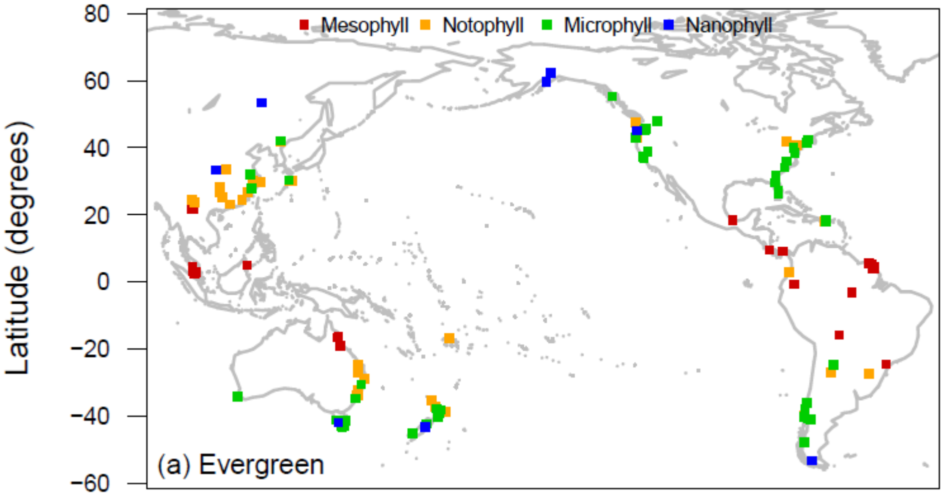
Fig. 3. Relationships of leaf size of tree and shrub species in forest assemblages with frost-free period and mean annual temperature. Panels A-B show evergreen species; C-D show deciduous species. The estimated slopes were drawn from the Generalized Linear Mixed Models that accounted for random region, site, and species effects.

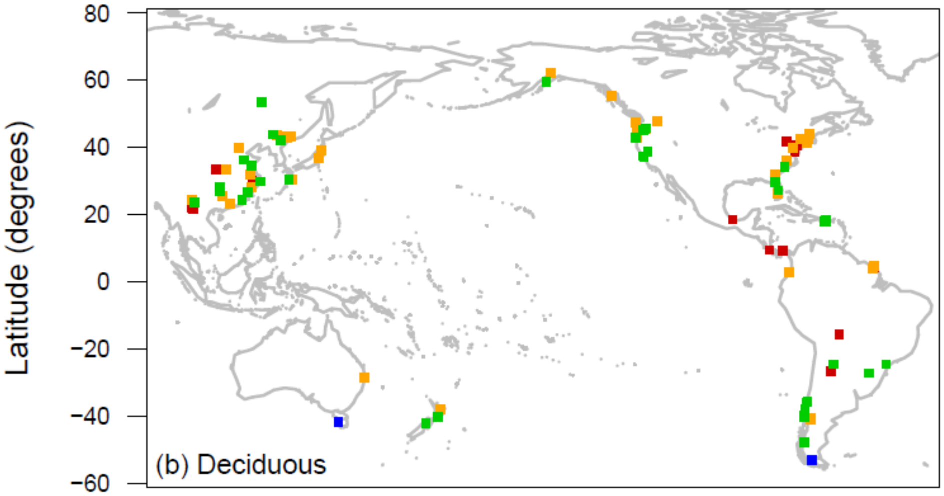
Fig. 4. Distribution of average leaf size in forest assemblages: (a) Evergreens, (b) Deciduous, (c)All. Colours show size classes (sensu Webb, 1968) occupied by mean leaf areas of eudicot and magnoliid tree/ shrub assemblages: blue = nanophyll (<2.25 cm^2), green = microphyll (2.25 - 20.25 cm^2), orange = notophyll (20.25 - 45.0 cm^2) red = mesophyll ($\geq$ 45.0 cm^2).

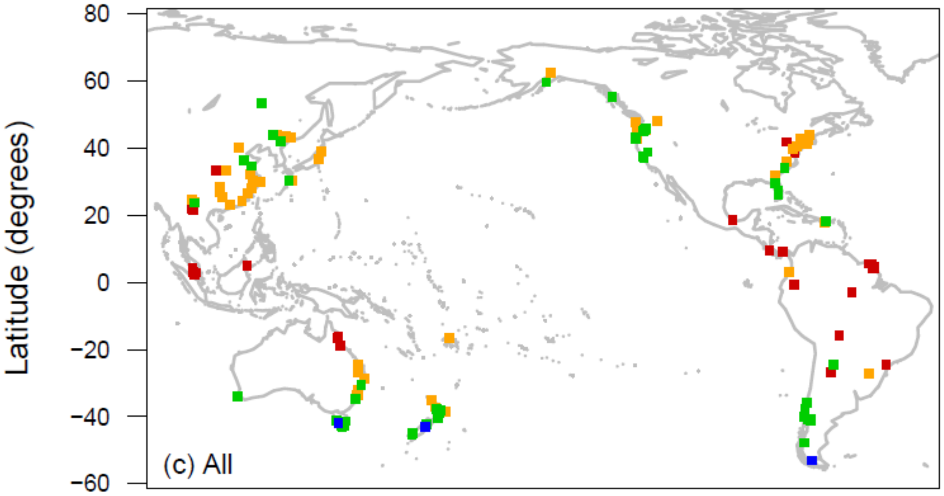

10
8
6
4
2
0
-2
-4
-6
Temperature (degrees C)
17:00:00
21:00:00
01:00:00
05:00:00
Time
Air
Coprosma rhamnoides
C. grandifolia
Wind speed (m s^{-1})


y = -0.9112 ln(x) + 0.6062
R^2 = 0.95
Leaf - air temperature (degrees C)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
3
10
30
100
Leaf width (mm)


A
B
C
D
r = 0.59, P < 0.0001
r = 0.61, P < 0.0001
r = −0.07, P = 0.0116
r = −0.07, P = 0.0055
Evergreen leaf area (cm²)
Deciduous leaf area (cm²)
10000
1000
100
10
1
0.1
0.01
0.90 quantile regression line
Regression line from mixed model
0.10 quantile regression line
50
100
150
200
250
300
350
−5
0
5
10
15
20
25
Frost−free period (days)
Mean annual temperature (deg C)


🟥 Mesophyll
🟧 Notophyll
🟩 Microphyll
🟦 Nanophyll
Latitude (degrees)
80
60
40
20
0
−20
−40
−60
(a) Evergreen


Latitude (degrees)
80
60
40
20
0
−20
−40
−60
(b) Deciduous


Latitude (degrees)
80
60
40
20
0
−20
−40
−60
(c) All