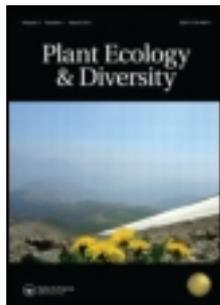


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Expanding the coverage of plant trait databases - A comparison of specific leaf area derived from fresh and dried leaves

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Expanding the coverage of plant trait databases – A comparison of specific leaf area derived from fresh and dried leaves

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Background: Specific leaf area (SLA) is a key plant functional trait, related to leaf life span, nutrient concentrations and photosynthetic rates, among other factors. However, a limiting factor in measuring these traits is that they are taken from fresh leaves. If accurate SLA measurements could be extracted from dried herbarium specimens, values for many more species could be rapidly included in large trait databases.

Aims: To determine whether artificial drying of leaves could influence subsequent calculations of SLA compared to calculations using fresh leaf area.

Methods: Using data from 449 leaves from 123 tropical species, we compared leaf area and SLA of fresh leaves with leaf area and SLA of the same leaves following standard pressing and drying procedures. Drying was carried out in the field using an electric heater.

Results: We found a significant decrease in leaf area following pressing and artificial drying (mean decrease = 8%), but that this effect was less in larger leaves. This decrease in leaf area had no statistically significant effect on calculations of SLA for all species pooled. Comparing plant families, however, we found a significant variation in leaf area decrease (0–30%).

Conclusions: We recommend that researchers continue to follow the established protocol for SLA measurements. However, given the benefits of increasing coverage of trait databases we suggest that herbarium specimens could be included, provided shrinkage is quantified and is less than the wide range of between-species variations already documented.

Keywords: herbarium; leaf lamina; leaves; SLA; tropical rain forest

Introduction

Specific leaf area (SLA), the ratio of leaf area to leaf dry mass, is a key plant functional trait. Data from hundreds of plant species and many ecosystems suggest that SLA is correlated with a number of plant traits (Reich 1993; Turner 1994; Ackerly and Reich 1999; Reich et al. 1999). In general, species with higher SLA have a shorter leaf life span, higher foliar nutrient concentrations and higher photosynthetic rates (Abrams et al. 1994; Garnier et al. 1997; Reich et al. 1997; Poorter and Evans 1998; Cramer et al. 2000; Wright and Westoby 2001; Wright et al. 2002). Furthermore, SLA may be an important component of plant life history strategies, since there is a trade-off between growing a large light-capturing area per mass and building strongly reinforced long-lived leaves (Chapin et al. 1993; Westoby 1998; Grubb 2002). For the same reason SLA plays a key role in the 'leaf economics spectrum' of variation in plant metabolic rates (Wright et al. 2004). Ensuring that SLA is correctly measured is therefore of prime importance (Beerling and Fry 1990).

SLA has been measured for a large number of species. Following a standard protocol is essential to ensure that data are comparable across species and ecosystems (Cornelissen et al. 2003). Key workers in this field have recommended that leaves sampled should be from among the newest

leaves, but fully expanded and without epiphylls or serious herbivore and pathogen damage, and taken from full light positions (Reich et al. 1992; Westoby 1998; Weiher et al. 1999). Studies have also investigated how differences in methodology, such as the time of day when leaves are collected, may influence SLA measurements. For example, spatio-temporal differences in leaf water status and carbohydrate content may affect SLA measurements by up to 20% (Cutler et al. 1977; Picon et al. 1997; Garnier et al. 2001).

The above influences on SLA are becoming increasingly well understood. However, one aspect of SLA measurement that has received little attention is whether fresh or dried leaf area is used in the calculation, primarily because most authors follow standard protocols and use fresh leaf area. Few studies have calculated SLA from dried leaf specimens, often including these observations with those from fresh specimens (e.g. Ackerly et al. 2006). The effect of this process is unquantified, although anecdotal observations suggest that the leaves of some species shrink considerably upon drying, while others do not. Ackerly et al. (2006) compared fresh horticultural and wild specimens with dried herbarium specimens from conspecific plants, but to the best of our knowledge no study has compared the fresh and dried SLA of the same leaves. If SLA

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can be accurately estimated using dried leaves, it becomes very easy to include a vast number of new species and ecosystems in large-scale trait analyses.

Within such large-scale comparative analyses, one often has to take particular care because species that are more closely related are more likely to have trait values that are more similar, because of their shared evolutionary history (Vamosi et al. 2009). Therefore, unless comparing traits within genera or families (e.g. Wright and Westoby 2002), it is important to account for this autocorrelation among species and traits. Furthermore, in an analysis such as ours, because families differed markedly in the construction of their leaves and therefore in the amount of leaf area decrease, it was important to highlight these family-specific effects so that they could be taken into account in future analyses.

Using a diverse tropical flora, we examined the extent to which leaf area decreased when leaves were artificially dried over an electric heater, and tested (i) whether any decrease might bias SLA calculations based on leaf areas taken from dried herbarium specimens, and (ii) whether any decrease was influenced by phylogeny.

Our research questions were:

- (1) Does artificial drying over an electric heater in a plant press cause a decrease in the area of the leaf lamina?
- (2) Do estimates of SLA derived from fresh leaf area differ significantly from those derived from dried leaf area of the same leaf?
- (3) Does phylogeny influence the decrease in leaf area?

Materials and methods

Study site

Yasuni National Park and Biosphere Reserve and the adjacent Huaorani Ethnic Reserve cover 1.6 million ha of forest and form the largest protected area in Amazonian Ecuador. The park is almost level at about 200 m above sea level, but is crossed by numerous ridges rising 25–40 m above the intervening streams. At wider intervals, large rivers flow eastwards to meet the Napo and Amazon. The canopy is 10–25 m high but is punctuated by emergents to 40 and occasionally 50 m tall. Rainfall and temperature are aseasonal at Yasuni (Valencia et al. 2004). Mean annual rainfall is 2800 mm and mean monthly rainfall is hardly ever <100 mm. Mean monthly temperature is 25–27 °C. Data were collected in the environs of the Yasuni Scientific Research Station (ECY, 0° 41' S, 76° 24' W), operated by the Pontificia Universidad Católica del Ecuador.

Leaf data

Branches from herbs, shrubs, small trees, and treelets or juvenile canopy trees were collected from individuals in the shaded understory of undisturbed old-growth terra firme forest along or close to trails around ECY (20–23 September 2010). While SLA protocol stipulates

sun-leaves, our study using shade-leaves provided a conservative test and any difference found is likely to be greater for sun-leaves. Between 10 and 50 samples were collected at any one time, and returned to the laboratory for immediate processing. The most complete fully expanded leaves with minimum herbivory were chosen for analysis. Composite-leafed species were sub-sampled (leaflets) if the whole leaves were too large to be scanned and pressed entire. Three leaves from each individual were taken and scanned in a flatbed scanner with a 4 cm² scale bar (Epson Perfection 2400 Photo, 8-bit greyscale images at 300 ppi) and dried for at least 36 h, or until dry, in a standard plant press over a heat source of two electric rings. Following drying, samples were weighed (Sartorius BL 150 S, Sartorius, Göttingen, Germany), re-measured and re-scanned. Samples that disintegrated during handling were excluded. The identity of each sample was determined. A total of 449 leaves from 123 species were collected. We identified 96% of these to family, 81% to genus and 80% to species, and we ended up with 484 samples from 158 individuals in 123 species.

Data analysis

Fresh and dry leaf areas were estimated using ImageJ software (Abramoff et al. 2004). To determine whether leaves had shrunk during the drying and pressing process, we modelled the leaf area of dried samples as a function of the area of fresh samples using a linear mixed model approach. To determine whether drying had an effect on SLA calculations, we first calculated SLA using (i) the fresh leaf area and (ii) the dried leaf area, using the dry mass of the leaf. We then tested whether the relationship between SLA calculated from dried leaves and SLA calculated from fresh leaves had a significantly different intercept from 0 and slope from 1, using linear regression. If there was no effect on SLA of using dried leaf area, we would expect the relationship between dried-area SLA and fresh-area SLA to have an intercept of 0 and a slope of 1. For these models both the x and y variables were log₁₀ transformed.

Because each leaf sample was not independent (we sampled three leaves per individual), we used a linear mixed modelling approach with leaf sample as a random effect to account for this pseudo-replication at the individual level. The 'lmer' function (lme4 package, Bates et al. 2011) does not return p-values, so these were estimated with the languageR package using the model output from lmer. The tests of regression slope = 1 were carried out using the 'offset' function in base R.

Finally, as a proxy for phylogeny, we tested for a significant difference among plant families in this relationship, modelling the ratio of dry: fresh leaf area as a function of each individual's family with a linear regression.

Results

Specific leaf area

We found that drying and pressing had a significant effect on the area of 449 leaf samples. Dried leaves were on

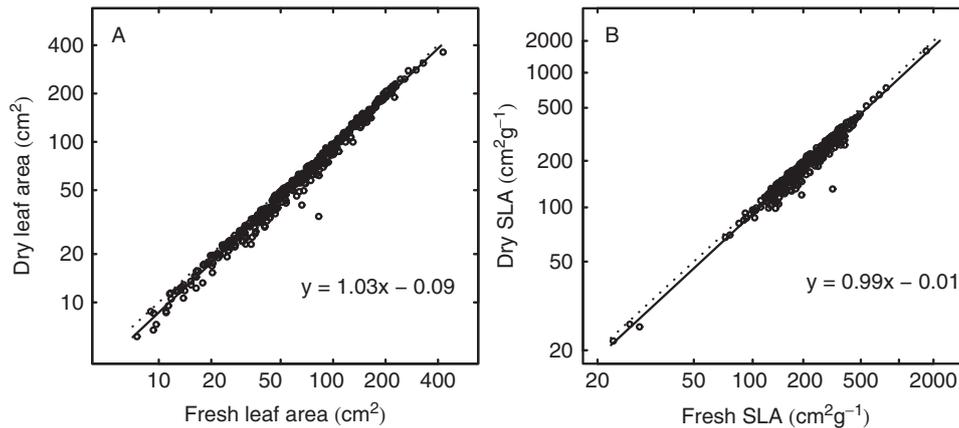


Figure 1. The relationship between dried and fresh leaf area (A), and specific leaf area (B) in 449 leaves: (A) the intercept of the relationship is significantly different from 0 ($P < 0.001$) and the slope (solid line) is significantly different from 1 (dotted line, correlation $r = -0.34$, $P < 0.0001$); (B) the intercept is not significantly different from 0, and the slope is not significantly different from 1 ($r = -0.56$). Note the different scales of the axes (particularly that B is a log scale). Each data point is a single leaf.

average 1.79 cm^2 (8%) smaller in area than fresh leaves, but this discrepancy decreased with leaf size (Figure 1A, model for \log_{10} transformed data: $y = 1.03x - 0.09$, test of slope = 1: $P < 0.001$, difference in slope from 1 = 0.03, CI = 0.02–0.04). The small decrease in leaf area was not enough to significantly modify SLA values calculated from dried versus fresh leaves. SLA values from dried leaf area were slightly, but not significantly, lower than SLA from fresh leaf area, and the slope of this relationship was not significantly different from 1 (Figure 1B, model for \log_{10} transformed data: $y = 0.99x - 0.01$; test of slope = 1: $P = 0.123$, difference in slope from 1 = 0.014, CI = -0.05 to -0.015).

Phylogeny

The area of dried leaves was on average 92% of the area of fresh leaves (range 70–100%). However, there was considerable variation around this value and we found that plant family had a significant effect on the ratio of dry: fresh leaf area (ANOVA, $df = 40$, $SS = 1.36$, $F = 16.312$, $P < 0.0001$). Some families with obviously soft leaves, such as herbaceous Melastomataceae, shrank appreciably, whereas others did not (e.g. Fabaceae and Lecythidaceae, Figure 2).

Within families there was also greater or lesser variation in SLA. For example, the Flacourtiaceae showed much less variation in SLA than the Melastomataceae. This probably reflects in part the variety of life history strategies present in each family.

Discussion

We found that a typical artificial drying and pressing procedure had a significant effect on the area of leaves from 123 species from a diverse tropical, predominantly woody, flora. The area of dried leaves compared to fresh leaves varied from 70 to 100%, with a mean of 92% (1.79 cm^2 area lost). Whether a similar relationship will be found in other

ecosystems remains to be seen. However, this decrease in leaf area did not appear to effect calculations of SLA, and we found a small but statistically non-significant difference between SLA calculated from fresh leaf area rather than from dried leaf area. At first glance it seemed that measurements of SLA from herbarium specimens might be equivalent to those from live plants. However, as noted above, we found considerable variation in the proportion of leaf shrinkage on drying. Two factors were found to influence this process. First, leaf size: small leaves decreased in area more than large leaves. Second, plant family: there were significant differences in the decrease in area between leaves from different families.

Effect of leaf size

Why would small leaves shrink more than larger leaves when dried in a plant press? If small leaves were less tough, they might shrink more. We found a significant negative relationship between SLA and leaf size in our study (linear regression of SLA from fresh leaves against area of fresh leaves: intercept: 252.5, slope: -0.38 , $P < 0.001$). As such, there seems to be an overall decrease of SLA with leaf size (i.e. a trend to invest more dry mass in every unit of leaf area with increasing leaf area), which would indicate that small leaves may indeed be less tough (Milla and Reich 2007; Ackerly et al. 2002). In fact, although there was much variation between species, this average negative relationship between SLA and leaf area occurred both within and across species, and suggested an upper limit to leaf size, given the greater cost of constructing and maintaining larger leaves (Milla and Reich 2007).

Effect of phylogeny

Phylogeny is well known to have a large influence on a number of plant traits (e.g. Moles et al. 2005), including leaf size, shape and structure (Ackerly and Donoghue

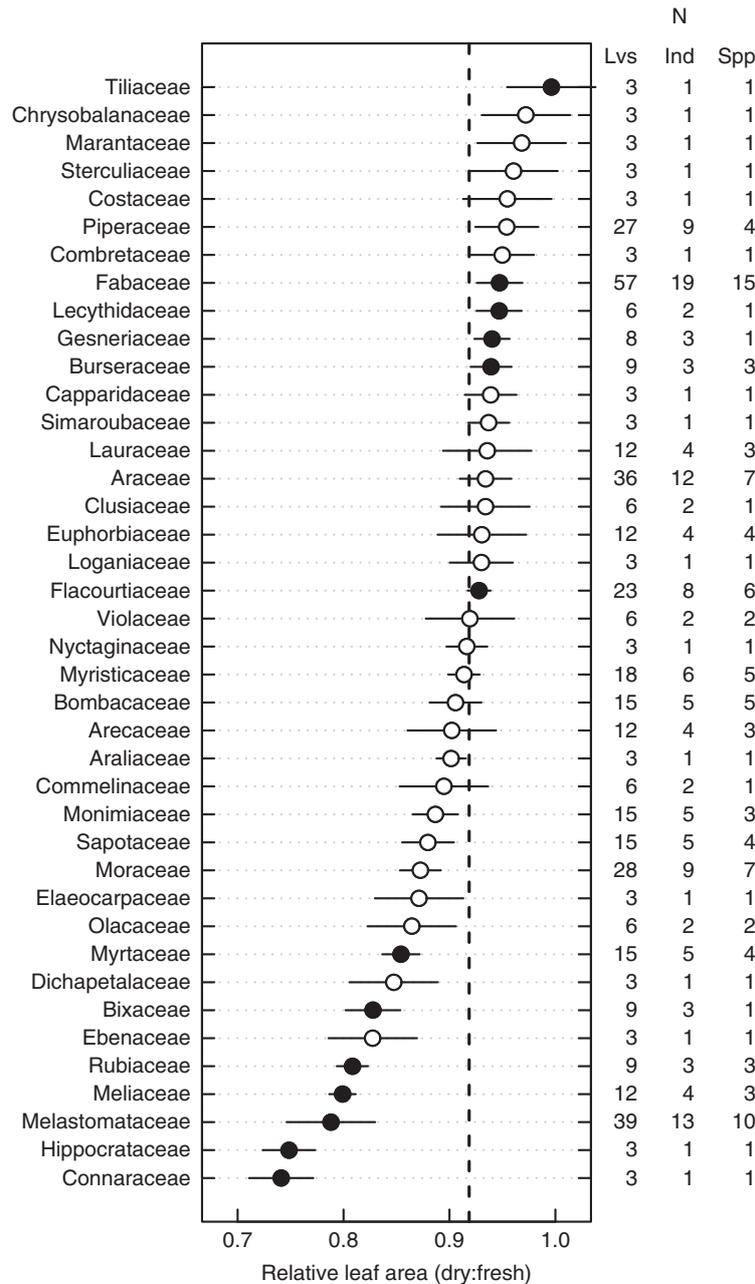


Figure 2. The differences between families in terms of leaf shrinkage following artificial drying and pressing. For each family we present the model coefficient (\pm SE) for a linear regression model of percent shrinkage (ratio of dry:fresh leaf area) as a function of family. Significant differences from the grand mean of 0.92 (dashed line) are shown as filled points. Sample sizes (N) are shown for each family for leaves (Lvs), individuals (Ind) and species (Spp).

1998; Ackerly and Reich 1999). Knowing which taxa are likely to undergo greater shrinkage upon artificial drying is important information. In our study, species with obviously soft or fleshy leaves, such as herbaceous Melastomataceae, were among those that shrank considerably. The leaves of herbaceous species are more likely to shrink upon artificial drying because they tend to be softer and less rigidly constructed. Therefore, taxa with high SLA, such as herbaceous or deciduous woody species, may be less suitable for taking SLA measurements from herbaria.

Phylogeny not only has an effect on leaf construction and SLA but also on leaf size, and both are significantly

correlated in phylogenetic independent contrasts, although there is less covariation between leaf size and traits, such as leaf life span (Ackerly and Reich 1999). For example, differences between gymnosperms and angiosperms drives leaf trait variation at broad phylogenetic scales, and correlated evolutionary change tends to occur within plant lineages (Ackerly and Reich 1999).

Sun- versus shade-leaves

Sun-leaves differ from shade-leaves in numerous ways as a response to the high and low light environments in which

they exist. For example, sun-leaves tend to be thicker, with a large number of small stomata and a thick layer of palisade cells (Givnish 1988). Specific leaf area measurements should be taken from fully sunlit leaves, or at least as near to full sunlight as the species grows in (Cornelissen et al. 2003). In the present study we were not able to access such leaves for a number of species and so chose to document SLA in shade leaves for a greater range of species. While this will certainly influence the exact SLA values we obtained for each species, since we were interested in the effects of drying on individual leaves, the SLA values we obtained relative to drying should nonetheless be informative.

Recommendations for SLA measurements

Given our results, we recommend that all researchers should follow the standard protocol for taking SLA measurements (Cornelissen et al. 2003). However, in cases where species values cannot be obtained in the field, herbarium specimens may be a viable alternative. Given the considerable range of SLA documented between species, the effect of using dried leaves or herbarium specimens is likely to be comparatively negligible, and the use of herbarium specimens for plant trait databases could therefore be considered.

If leaf trait values are to be taken from herbarium specimens there are a number of important points to bear in mind. First, whilst measuring leaf area from herbarium specimens can be harmless, taking samples for leaf mass are likely to be destructive. In this case small areas of specimens could be sampled rather than the whole leaf; or even better, trait samples could be collected as standard with every herbarium specimen, thus avoiding the need to remove part of the specimen destined for archiving (see also Pendry et al. 2007). Second, while our samples were not exclusively full-sun leaves (and we could only obtain such a large sample size by including shade-leaves), SLA and other traits should strictly come from full-sun leaves. Ensuring that herbarium specimens used for trait work are sun-leaves is essential, but determining whether this is the case could be problematic. Information from the herbarium label will be critical to establish the light environment of each specimen. Third, Cornelissen et al. (2003) prescribed a minimum number of replicates per species (one leaf from each of 10 individuals), and ensuring that this is adhered to is important. Finally, as we have shown, it is important to account for leaf size and family, and to make some estimate of leaf shrinkage from species as closely related to the study species as possible.

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Notes on contributors

Simon Queenborough is a botanical ecologist with interests in the evolution and ecology of breeding systems, species diversity and comparative ecology, and works mainly in tropical forest and arable ecosystems.

Cristina Porras is currently studying biology with a view to undertaking graduate research in tropical ecology.

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