3.1 Specific leaf area

Specific leaf area (SLA) is the one-sided area of a fresh leaf, divided by its oven-dry mass. (Note that leaf mass per area (LMA), specific leaf mass (SLM) and specific leaf weight (SLW), are simply 1/SLA). SLA is frequently used in growth analysis because it is often positively related to potential RGR across species. SLA tends to scale positively with mass-based light-saturated photosynthetic rate and with leaf nitrogen (N) concentration, and negatively with leaf longevity and C investment in quantitatively important secondary compounds such as tannins or lignin. In general, species in permanently or temporarily (e.g. deserts during the rainy season) resource-rich environments tend, on average, to have a higher SLA than do those in resource-poor environments, although there can also be considerable variation in SLA among co-occurring species.

Specific leaf area is a function of leaf dry-matter content (see Section 3.3), and L_M (see Section 3.4). Both components can contribute to SLA to different degrees, depending on the habitat and the plant group in question. In cool-temperate herbaceous datasets, low SLA of slow-growing species tends to be related to high leaf dry-matter content, more than to high leaf thickness. When woody perennials are dominant, L_M can be equally influential. Some species that normally grow in deeply shaded, and thus presumably resource-limited, micro-habitats (e.g. *Oxalis*) have a high SLA and low L_M. In areas with severe soil-nutrient limitations, slow-growing plants with sclerophyllous leaves (with thick epidermal walls and cuticle, abundant sclerification, high ratio of crude fibre to protein) are common. In these, low SLA is associated with high leaf dry-matter content more than with high L_M. In contrast, in the succulent plants that are common in some seasonally dry subtropical to tropical areas, low SLA is associated with low leaf dry-matter content and high L_M. As a consequence of these variations, SLA and its components are often, but not always, related to each other and to productivity gradients in a simple way. We then recommend additional measurement of the two component variables, namely, leaf dry-matter content and L_M, as well as SLA.

What and how to collect?

Select the relatively young (presumably more photosynthetically active) but fully expanded and hardened leaves from adult plants (unless the research is focussed on e.g. seedlings, or expanding or senescent leaves). Wherever possible, avoid leaves with obvious symptoms of pathogen or herbivore attack, or with a substantial cover of epiphylls. SLA is strongly affected by light intensity. Therefore, for many research questions it is best (giving the fairest comparison across individuals or species) to sample outer canopy leaves (also called 'sun leaves') from plants growing under relatively optimal conditions. For species that typically grow in the overstory, take leaves from plant parts most exposed to direct sunlight. For true shade species (those that never grow in full sunlight), collect leaves from the least shaded parts found (but not from those that look light-stressed or bleached). Any rachis (stalk-like midrib of a compound leaf) and all veins are considered part of the leaf for a standardised SLA measurement (but see *Special cases or extras* below in the present Section, for a discussion on this and on whether petioles should be included in the measurement). We recommend collecting whole twig sections with the leaves still attached and not removing the leaves until just before processing.

Storing and processing

Plants in the field may be dehydrated to an unknown extent, which may lead to shrinkage of the leaves and therefore somewhat unreliable measurements of LA (see *Special cases or extras* below in the present Section). This is more of a problem for soft-textured high-SLA leaves than for low-SLA, sclerophyllous leaves. Ideally, the samples (twigs with leaves attached) should be cut and immediately placed into test tubes or flasks, with the cut end submerged in deionised water. If this is not feasible, wrap the samples in moist paper and put them in sealed plastic bags. In that case, breathe into the bag before closing it to enhance CO_2 concentration and air humidity, which will minimise transpirational water loss, and store the bags in the dark. Tissues of some xerophytic species (e.g. bromeliads, cacti, some species with very small, highly resinous leaves) rot very quickly when moist and warm; therefore, they are better stored dry in paper bags. If in doubt (e.g. in mildly succulent species), and if recollecting would be difficult, try both moist and dry storage simultaneously and use the dry-stored leaves in the case of rotting of the moist-stored ones. Store the collected samples in a cool box or fridge (never in a freezer) in the dark, until further processing in the laboratory. If no cool box is available and temperatures are high, it is better to store the samples in plastic bags without any additional moisture. Measure as soon as possible after collecting and rehydrating, preferably within 24 h. If storage is to last for more than 24 h, low temperatures (2–6°C) are essential to avoid rotting.

Rehydration is preferable for most plants and essential for leaves subjected to dry storage or for 'soft' leaves, such as those with SLA values higher than 10–15 $m^2\ kg^{-1}$. In situations where the rehydration procedure described above cannot be applied, storage in sealed, moist plastic bags (with or without addition of damp paper) for 12 h is an acceptable option, although generally yields approximately ~5% lower values than does the complete rehydration method. Xerophytic and especially succulent leaves should not be rehydrated for more than 6 h, whatever the storage or rehydration method used might be. If this process fails, we recommend collecting leaves of these species in the morning after a rain event, or a few hours after generous watering.

Measuring

Each leaf (including or excluding petiole, see *Special cases and extras* below in the present Section) is cut from the stem and gently patted dry before measurement. Projected area (as in a photograph) can be measured with specialised leaf-area meters such as Delta-T (Delta-T Devices Ltd. Cambridge, UK) or LI-COR (LiCor, Inc., Lincoln, Nebraska, USA). Always calibrate the area meter by using pieces of known area before measuring leaves and always check (e.g. on the monitor) that the whole leaf is positioned flat and completely within the scanning area. If you are to use a portable LA meter, make sure that the estimation error is not too high for your purposes, by running a preliminary check against LAs scanned in the laboratory, using a range of different leaf sizes. Images of leaves can also be electronically captured in the field or in the laboratory, and stored for later processing, e.g. with a digital camera. Leaves are pressed gently under a glass plate, including a ruler or an object of known size in the image allows for size calibration. A camera mounted on a tripod, with two lamps shining from different sides and no flash gives the best results. A third option is to determine LA with a flatbed scanner (with the advantage that many scanners can draw their power via USB, e.g. from a laptop). Scan in colour mode to obtain maximal information for the threshold level between what is to be considered leaf and background Coloured scans will also allow for *post hoc* measurement of other features of interest. In all cases, make sure the leaves are not curled-up or overlapping. Try to position the leaves flat as possible in the position that gives the largest area, but without squashing them to the extent that the tissue might get damaged. Cutting leaves into smaller pieces may facilitate flattening. In both cases, LA can be measured with image analysis software. Freely downloadable programs are e.g. Leafarea (A. P. Askew, University of Sheffield, UK, downloadable from the Nucleo DiverSus toolbox, see Box 1) or, for more complex analyses including other plant organs, ImageJ (from the US National Institutes of Health; http://www.nih.gov/) and GIMP (from the GNU Project; http://www.gnu.org/). Transform images to the HSV colorspace for a better separation of leaf and background. Additional details on image processing can be found at Prometheus Wiki (see Box 1).

After the area measurement, put each leaf sample in the oven (petioles and laminae either separately or in the same envelope, according to the objective of the study, see *Special cases or extras* below in the present Section), ideally at 70°C for at least 72 h, or at 80°C for 48 h (avoid higher temperatures); then determine the dry mass. Be aware that, once taken from the oven, the samples will take up some moisture from the air. Put them therefore in a desiccator with silica gel until weighing, or else back in the oven to re-dry. Weighing several tiny leaves as if they were one and then dividing the weight by the number of leaves will generally improve the accuracy of the weighing. When converting SLA into LMA values or *vice versa*, always do so for each individual replicate, rather than for the average of several replicates.

Special cases or extras

(1) *Petioles*. An important issue is whether or not petioles should be included in SLA measurements. The appropriate decision depends on the research question at hand. Some authors consider that the petiole is an integral part of the leaf because it is shed at abscission together with the leaf, and because it provides support and a vascular system without which the leaf cannot be displayed. Therefore, they include petioles in SLA measurements. Other authors consider that the petiole should not be included in the SLA because the main function of the petiole is the spatial positioning and hydraulic support of the leaf, thus resembling the function of the stem, whereas the main function of the leaf blade is light interception and C fixation. The fraction of leaf dry mass represented by the petiole varies from ~zero to almost 50%; therefore, inclusion of the petiole may reduce the calculated SLA drastically. Although inclusion or not of the petiole may sometimes not be crucial within a single study, it can be a source of considerable and systematic error when comparing different studies, or even in certain same-site comparisons of species with very different leaf structures. Therefore, the best (albeit more time-consuming) option is to measure leaf blade and petioles separately, so that SLA can be calculated in both ways, thereby facilitating comparisons with other studies. When using digital images, we suggest to scan or photograph petioles and the rest of the leaves of each replicate in the same image, but in clearly different sectors, so that they can be measured together or separately according to the objectives of the study; then oven-dry and weigh petioles belonging to a replicate separately from the rest of the leaves from the same replicate. In general, make the decision that best suits your study objectives; however, specify in your publication whether petioles are included or not.

(2) *Compound leaves*. Sometimes species have a lower SLA because of their thick rachis. The decision of whether to include the rachis or not, and the practical steps in each case, are similar to those on petioles (see Point 1 above in the present Section). As a default option, we recommend considering the rachis as part of the leaf for the SLA calculation, and including this clearly when reporting the study. Be aware that in some plants, the rachis can be more than 10 times heavier that the sum of the leaflets. Another decision to make in the case of compound leaves is whether to measure the SLA of a typical individual leaflet or that of all leaflets taken together. As in the case of rachis and petiole, the decision largely depends on the objectives of the study. We recommend taking both measurements, and above all, we recommend to clearly specify in the publication whether the area reported is that of an individual leaflet or the whole leaf.

(3) *LA shrinkage*. Leaves decrease in size when they desiccate. Shrinkage is defined as a percentage, calculated as 100 × (1 − dry area / saturated area). Shrinkage averages ~20% and can reach 80% for some species. The maximum area shrinkage reflects differences in leaf structure and is correlated with other leaf traits including cuticular conductance, pressure–volume parameters such as modulus of elasticity and turgor loss point, leaf dry-matter content (LDMC), and L_M, as well as plant growth form and deciduousness. LA shrinkage is both a potential problem (causing biases in leaf-area measurements for herbarium material and fossils) and an easily measured trait that reflects multiple structural and hydraulic properties of a leaf. To measure this trait, collect and measure leaves following the exact same protocol as for LA (Section 3.2) and LDMC (Section 3.3). Measure fresh projected LA. Cut leaf into pieces in the cases when the leaf is not flat. This step is crucial because some leaves may experience very little shrinkage in area (5%), so the accuracy of the subsequent measurement of the saturated area is crucial. Then oven-dry the leaf, pressed flat in envelopes or in a plant press. Measure projected LA on the dried leaf, taking care that the same surface is measured. This may be difficult or impossible for some needle-like leaves, and especially thick leaves may need to be broken into several smaller flat pieces. Calculate shrinkage using the formula given above.

(4) *Projected v. total LA*. In 'standard' leaves, LA is measured as a one-sided projected LA However, in non-flat leaves, the projected LA is smaller than the total one-sided LA. Projected LA is partly related to light interception, whereas total LA is related to the total amount of photosynthetically active tissue. There are, however, cases, such as leaf-rolling in some grass species, where both light interception and gas exchange are reduced. Clearly, the choice of measurement depends on the research question, although knowledge of both would provide the best insight.

(5) *Needle-like leaves*. Needle-like leaves are a specific case where projected and total LAs are different. Projected LA could be measured following the standard routines; however, because the leaves are generally narrow, make sure that your equipment is sensitive enough to adequately measure such leaves. For a rough measurement, you can measure leaf length with a ruler and leaf width with a calliper and subsequently multiply 2 × length × width.

(6) *Tiny leaves*. Four issues from some species (e.g. *Callitris* sp.) have very tiny scales closely appressed to fine soft twigs. In such cases, you might treat the terminal twiglets as a leaf analogue, because they are shed as a single unit.

(7) *Leaves of grasses and grass-like plants*. Usually, only the lamina is considered, excluding the leaf sheath. However, as in the case of petioles (see Point 1 above in the present Section), the decision on which measurement to take depends on the research objectives. Several species have leaves that tend to curl, or even roll up. They are generally much easier managed by cutting leaves into shorter pieces of 5–10 cm.

(8) *Succulent and leafless plants*. For plants whose main photosynthetic organs are not true leaves, take the plant part that is the functional analogue of a leaf and treat as above. For some species with photosynthetic spines or non-succulent stems (e.g. *Ulex*, *Senna aphylla*), this could mean taking the top 2 cm of a young twig. For cacti and other succulents, we recommend taking a whole leaf or equivalent (e.g. a cladode in *Opuntia*) where possible. This sometimes poses practical difficulties, e.g. a whole *Agave* leaf or a rib of a columnar cactus are often too big to process or even to collect. In such cases, we recommend taking several 'pastry cutter' portions (of known area) of young but fully hardened leaves (e.g. in *Agave*) or 'ribs' (in cacti), including epidermis and mesophyll on both sides, plus the internal succulent parenchyma. Although this internal parenchyma does not always contain chlorophyll and therefore some authors recommend not considering it in SLA measurements, it has an essential role in the CAM metabolism of succulent plants (see also Section 3.12). The younger stems of some rushes and sedges (*Hesperochiris*, *Juncus*) and the 'branches' of horsetails (*Equisetum*) or similar green leafless shoots can be treated as leaves too. Because many of these species occur in a range of environments, it is important to specify the exact method used in each case.

(9) *Ferns*. For ferns, only collect fronds (fern 'leaves') without the spore-producing sori, often seen as green or brown structures of various shapes at the lower side or margin of the frond.

(10) *Leaves of tall trees*. Upper-canopy leaves of sun-exposed trees should be preferred. If these cannot be easily reached, some workers rely on professional climbers, slingshots or guns. In not extremely tall trees, an alternative could be to consider exposed leaves halfway the crown length, at the outer half of the crown (inner leaves are sometimes older), which could be accessed with a pruner on an extension pole.

(11) *Very large leaves*. Once they have been placed in plastic bags, large leaves may be put in a hard-cover folder to avoid wrinkling and folding. If leaves are larger than the window of the area meter, cut the leaf up into smaller parts and measure the cumulative area of all parts. Leaves with very thick veins or rachis can cast a lateral shade on the LA meter, thus overestimating the LA. In the case of a thick central vein, remove with a scissor the protruding upper or lower part of the vein and scan the leaves without that removed part, but include it in the dry-mass measurement. In the case of a thick rachis, remove the rachis and measure its diameter and length halfway, and calculate the rachis area as the product of the two. Then scan the leaves without rachis but include the rachis in the dry mass. If you want to rely on subsamples of leaves, make sure that there is not too large of a variation in SLA over the leaf.

(12) *Heterophyllous plants*. In the case of species with two or more types of leaves with contrasting shape and anatomy, such as e.g. plants with both rosette and stem leaves, collect leaves of both types in proportion to their estimated contribution to total LA of the plant, so as to obtain a representative SLA value of the individual.

(13) *Low-tech options for the measurement of SLA and LA in the field*. There are situations in which taking fresh leaves to the laboratory for scanning is not feasible, or portable scanning devices cannot be transported to or powered in the field site. One solution in these cases is to use a digital camera (see *Measuring* above in the present Section). Another practical and inexpensive alternative is obtaining leaf fragments of known area (e.g. with a punch-borer), avoiding thick veins, and placing the fragments in an envelope for drying (take several patches per replicate, because they tend to weigh very little). This method is a quick and accurate way to compare leaf laminae. However, it overestimates SLA as compared with measurements of whole leaves, especially in the case of large leaves with thick veins, ribs and petioles. Therefore, SLA measurements obtained in this way should not be compared with or combined with those taken on whole leaves, at least not without a specific calibration. Another alternative is to obtain plastic or paper prints or cut-outs of the leaves in the field, and measure their area later. This method works well for medium- to large-sized leaves, entire leaves, and leaves that are not too narrow (e.g. xerophytic grasses).

References on theory, significance and large datasets: Reich *et al.* (1992, 1999); Garnier and Laurent (1994); Poorter and Garnier (1999); Wilson *et al.* (1999); Castro-Díez *et al.* (2000); Niinemets (2001); Westoby *et al.* (2002); Díaz *et al.* (2004); Paula and Pausas (2006); Wright *et al.* (2007); Poorter *et al.* (2009); Hodgson *et al.* (2011); Blonder *et al.* (2012); Juneau and Tarasoff (2012).

More on methods: Chen and Black (1992); Garnier *et al.* (2001*a*, 2001*b*); Vendramini *et al.* (2002); Vile *et al.* (2005); Niinemets *et al.* (2007).