- Method:
    1. Obtained by measurements
    2. Observations
    3. From published data
- Unit: categories
- Plant lifespan categories:
    1. Annuals
    2. Summer annuals
    3. Winter annuals
    4. Strict monocarpic biennials and poly-annuals
    5. Perennials
    6. Poly-annuals < 5 years (short-lived perennials)
    7. Poly-annuals 5-50 years (medium-lived perennials)
    8. Poly-annuals >50 years (long-lived perennials)
- Age of first flowering categories:
    1. < 1 year
    2. 1 and 5 years
    3. > 5 years

Optional:
- Original value plant lifespan: years
- Value: minimum, maximum (in years)
- Comment field: Any information of importance to the trait

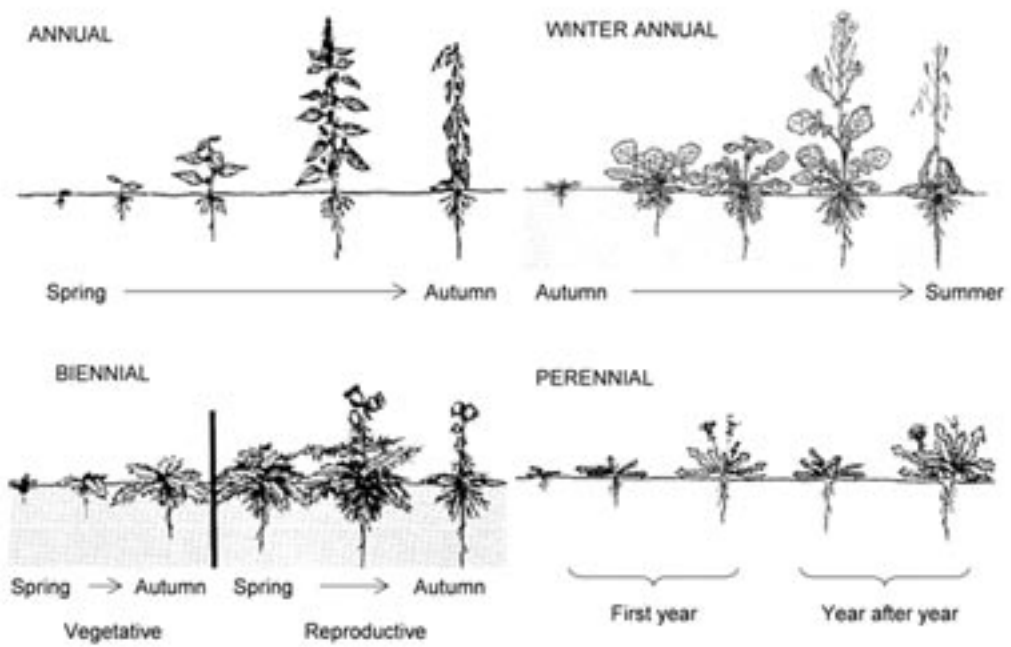


***Figure 3.4.*** *An example of the life cycle of an annual, winter annual, biennial and perennial plant species (after Fermania 2000).*

# 2. LEAF TRAITS

*D. Kunzmann*

**Introduction**

Interspecific variation in leaf size has been connected with climatic variation, geology, altitude or latitude, where heat stress, cold stress, drought stress and high-radiation stress all tend to select for relatively small leaves. Hence, leaf size has important consequences for the leaf energy and water balance. Leaf size variation can also be linked to allometric factors (plant size, twig size, anatomy and architecture) and ecological strategy with respect to environmental nutrient stress and disturbances within climatic zone, while phylogenetic factors can also play an important role (Cornelissen *et al*. 2003).

In many cases the specific leaf area (SLA) of a species is positively correlated with its potential relative growth rate and mass-based maximum photosynthetic rate (Cornelissen *et al*. 2003). Lower values of SLA tend to correspond with a long leaf lifespan and species with a relatively high investment in leaf 'defences' (particularly structural ones). Some shade-tolerant woodland understorey species are known to have remarkably high SLA, as well as species in resource-rich environments compared to those in environments with resource stress (Cornelissen *et al*. 2003).

SLA is the one-sided area of a fresh leaf divided by its oven-dry mass, hence leaf mass is one component of the SLA measurements, expressed as leaf dry mass (see Wright *et al*. 2002). Note that this expression does not mean the same as leaf mass per area or specific leaf mass (SLM; Pynakow *et al*. 1999).

As a measure for the tissues density the trait leaf dry matter content (LDMC) will be measured. Tissue density plays a central role in the nutrient utilisation of a species by determining the rate of biomass turnover (i.e. low tissue density is associated with high growth rate). Although variation in tissue density is often correlated with differences in life history traits among species, for the bulk of the organ tissue density is relatively constant for each species (Niklas 1994, Enquist *et al*. 1999). In general the definition for 'density' is the mass of an object divided by its volume, the density of a plant organ is therefore the mass of the plant organ divided by its volume. Hence, the density of the dry matter of an organ is its dry mass divided by its volume and the dry matter concentration of an organ is the mass of its dry matter divided by volume of the organ itself. An indirect measure of dry matter concentration is the dry matter content. The dry matter content is defined as the ratio of organ dry mass to fresh mass (Shipley & Vu 2002), which can also be referred to as tissue density, defined as the dry weight per unit volume (see Ryser 1996, Westoby 1998, Wilson *et al*. 1999).

Leaves with a high LDMC tend to be relatively tough, and are as such assumed to be more resistant to physical hazards (e.g. herbivory, wind, hail) compared to leaves with a low LDMC. Species with a low LDMC tend to be associated with productive often high-disturbance environments (Cornelissen *et al*. 2003). The LDMC is the ratio dry leaf mass to fresh leaf mass after the definition of Ryser (1996) with the assumption that leaf tissue density ≈ leaf dry matter content. Thus a tight relationship between volume and fresh mass of the leaf is assumed (see Garnier & Laurent 1994). In general the LDMC is negatively correlated to potential relative growth rate and positively with leaf lifespan, however these correlation are weaker than compared to for instance the correlation between leaf

lifespan and SLA (Cornelissen *et al*. 2003).
The LDMC can be used in cases where the leaf area is difficult to measure (Cornelissen *et al*. 2003), even though LDMC and SLA are not the same, the average density of the leaf tissues is related to the LDMC and tends to scale with 1/SLA.

*Trait definition*

Leaf size: Leaf size is the one-sided projected surface area of an individual leaf or lamina expressed in $mm^2$.

SLA: Specific leaf area (SLA) is the ratio of fresh leaf area to leaf dry mass: SLA = leaf area / leaf dry mass, expressed in $mm^2\ mg^{-1}$

Leaf mass: Is the dry weight of a leaf, expressed in mg.

LDMC: Leaf dry matter content (LDMC), a measure of tissue density, is the ratio dry leaf mass to fresh leaf mass and is expressed in mg/g.

## 2.1. LEAF SIZE, SLA, LEAF MASS & LDMC

**What and how to collect**

For the collection of leaves, the individuals of herbaceous and small woody species should be randomly selected and should have their foliage exposed to the light (i.e. sunny spot). Whole leaves (including the petiole) should be collected and for tall woody species the leaves most exposed to direct sunlight ('outer canopy' leaves) should be sampled (Cornelissen *et al*. 2003).
As most leaf traits are rather variable within plants, it is recommended that for each species 2 randomly selected leaves exposed to the light should be collected from each of 10 different individuals for each sample site. If it is impossible to collect leaves from 10 different individuals, i.e. due to small populations or rarity of the species, more than 2 leaves could be collected from the minimum of 3 individuals per species per sample site. For small species it is recommended to collect complete plants or branches. As LDMC can vary during the day, it is recommended to sample the leaves before (or close to) sunset or after sunrise (Cornelissen *et al*. 2003, see also Garnier *et al*. 2001).
Note that to economise on collecting time, the same leaves could be used to determine leaf size, SLA, leaf mass and LDMC.

**Storing and processing**

When collected, the leaf samples should be wrapped in moist (filter)paper, sealed in plastic bags, and transported to the laboratory in cooler boxes to prevent weight (or turgor) loss. In the laboratory the leaves should be stored in the plastic bags in the fridge at 5°C until further measurements. The samples that are kept in the fridge should be stored as flat as possible when SLA needs to be obtained from the leaves. If no cool box is available and temperatures are high, it is better to store the samples in plastic bags without any additional moisture. If storage during rehydration is to last for more than 24h, low temperatures (2-6°C) are essential to avoid rotting (Cornelissen *et al*. 2003). The leaves of some xerophytic species (e.g. bromelias, cacti) decompose very quickly when stored too wet and should therefore be stored dry in paper bags. A 1-3 hour rehydration period is

suggested for these leaves before measurements.
When uncertain about the best storage method, store plant material under both dry and wet circumstances. For 'soft' leaves, such as those of many herbaceous and deciduous woody species, the leaves should be rehydrated with de-ionised water when kept under dry conditions prior to measurements in order not to underestimate the measurements. Note that the measurements should preferably be carried out as soon as possible (within 24 hours) after collecting (Cornelissen *et al*. 2003). If this is not possible, the leaves should be stored between moist filter paper in sealed plastic bags in the freezer (-18 to -35°C) until further measurements. When ready to measure the leaves, the frozen leaves should be defrosted in water and remain in the water until the fresh weight and area measurement are finalised. Note that this method corresponds to the 'fresh leaf method' (with a few modifications). Note also that not all leaves are suitable for freezing, for instance it is not advisable to freeze certain water plants, succulents and big thin leaves of hygrophytes (e.g. *Cicerbita, Adenostyles, Aconitum*).

**How to measure**
For new measurements of any of the leaf traits, LEDA prefers the measurements carried out with the whole leaf, i.e. with petiole and rachis.

Leaf size: Individual leaf laminas (or leaflets in compound leaves) should be measured with the petiole and rachis (i.e. is the whole leaf), but data of measurements withhout the petiole and rachis will be accepted (see also Special cases). The average leaf size of the leaves collected from one individual will represent one statistical observation (Cornelissen *et al*. 2003).

SLA: Each leaf (including petiole) is gently rubbed dry before measurement. The projected area (as in a photo) can be measured with specialised leaf area meters (e.g. Li-Cor), or, if a leaf area meter is not available, an alternative is to scan leaves with a flatbed scanner (Cornelissen *et al*. 2003). From the leaf a computer image is generated and the area can be measured using appropriate analysis software (e.g. Lafore Fig. 3.5; Lehsten 2002). Documentation of sampled leaves by reference pictures of scanned leaves, scanned at 300 DPI, is preferred and the readings of the area meter should be checked by using coins or pieces of paper of known area before measuring leaves. The latter also applies to leaf areas measured using a flatbed scanner. LEDA prefers to use a flatbed scanner, because in practice the measurements with this scanner are more exact, and can be used in the field with electricity from a laptop. Where none of these facilities are available, the area can be estimated by weighing paper or plastic cut-outs of similar shape and size and then multiplying by the known area/weight ratio of the paper, as long as the paper or plastic is of a constant quality.
When measuring the leaves, the leaves should be positioned as flat as possible (e.g. by using a glass cover), in the position that gives the largest area, but without squashing and damaging the leaves.
The use of the methods mentioned above may give a large error for small or narrow leaves or needles, partly due to the pixel size of the projected images (Cornelissen *et al*. 2003). For such leaves it is recommended to calibrate the

image analysis equipment with objects of similar shape, size and colour (e.g. green paper cuttings of the desired dimensions) and treat several leaves as if they were one (Cornelissen *et al*. 2003). For very small leaves and needles the projected area can best be obtained by placing the leaves on millimetre grid paper and estimated the area by using a binocular microscope (10x magnification), after which large drawings of both the leaves and millimetre squares could be compared using the leaf area meter (Cornelissen *et al*. 2003).
On the other hand, very large leaves might not fit in the area meter or on the flatbed scanner. In this case the leaf needs to be cut up in smaller leaf parts and the total area is determined by taking the cumulative area of all parts (Cornelissen *et al*. 2003).

Leaf mass: After the leaf area is measured, each leaf sample is dried in the oven at 70°C for 48-72 hours and subsequently the dried leaves weighed to determine their dry mass (=leaf mass). If the leaf samples cannot be weighed immediately after cooling down, put them in the desiccator until weighing, or else back in the oven to dry off again. As is the case for leaf area, the weighing of several tiny leaves as if they were one will improve the accuracy, depending on the type of balance used (Cornelissen *et al*. 2003).

LDMC: For measurements of LDMC a combination of the standardised protocol of the 'fresh leaf method' from Wilson *et al*. (1999) and Cornelissen *et al*. (2003) will be used. The rehydration (or saturated) method for LDMC of Garnier *et al*. (2001) is not used in the LEDA Traitbase, but is one of the methods that can be chosen for data obtained from published sources. When measuring the LDMC, the leaves with and without the petiole should be measured to be able to compare with other published data sets as in general both leaf 'states' are measured.
After collection the leaves are weighed (fresh weight) after which the sample was dried in a paper bag or envelope at 70°C for 48-72 hours and subsequently re-weighed to obtain the oven-dry weight of the leaf (dry weight). Note that before weighing the leaves, the leaf lamina should be blotted dry with tissue paper to remove any surface water (Wilson *et al*. 1999). LDMC (expressed in mg $g^{-1}$) is the oven-dry mass (mg) of a leaf divided by its fresh mass (g).

*Special cases*

- For leafless plants the functional analogue of a leaf is sampled and treated as a leaf. For instance for spiny species such as *Ulex*, the top 2 cm of a young twig should be sampled, whereas for cacti and other succulents it is recommend to cut off a slice ('the scalp') of the epidermis plus some parenchyma of a relatively young part. Also the younger stems of some rushes and sedges (*Juncus, Eleocharis*) and green leafless shoots and/or the 'branches' of horsetails (*Equisetum*) can be treated as leaves (Cornelissen *et al*. 2003). Data collectors have to decide what they consider to be the leaf analogue, but note that it is important to record the exact method used when this is the case.
- For heterophyllous species which have, for instance, both rosette and stem leaves, both leaf types should be collected in proportion to the total leaf number in

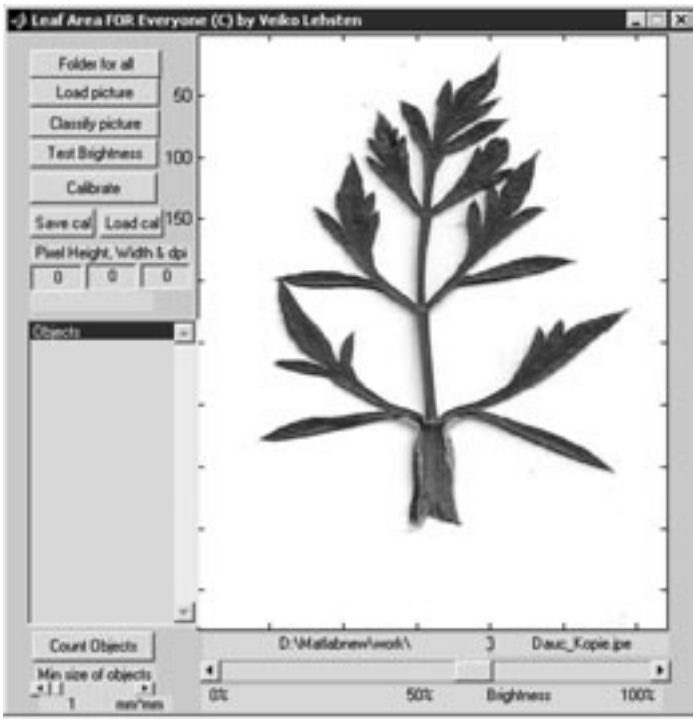


***Figure 3.5.*** *Lafore scan software for image classification for plant leaf investigations, with an example of Daucus carota (Lehsten 2002).*

order to obtain a representative SLA and leaf size (Cornelissen *et al*. 2003).

- It might be relevant to determine SLA on the basis of actual (rather than projected) one-sided leaf area, as an additional measurement. In needles (e.g. *Pinus*) or rolled-up grass leaves (e.g. some *Festuca*) this makes a large difference. By taking the ratio of the upper half of the circumference and leaf width of a leaf cross section, using a microscope, a true one-sided leaf area may be estimated.
- It should be noted that interspecific rankings of SLA are rather robust to methodological factors (e.g. with or without petioles). For comparisons on a coarse scale SLA data from several sources may be combined, only as long as (at least) possible methodological artefacts are acknowledged (Cornelissen *et al*. 2003).
- Whole-leaf sizes may be added as they can be relevant for some allometric analyses. For whole leaf measurements of compound leaves, all leaflets should be included as well as any petiole and rachis. Note that these whole-leaf measurements are part of SLA measurements (Cornelissen *et al*. 2003).
- Record leaf size for leafless plant species as zero and not as a missing value as it is an important functional trait. Note that from certain data analyses these zero values may need to be excluded (Cornelissen *et al*. 2003).

- For leaves with massive midrib support structures (e.g. *Petasites hybridus*; Fig. 3.6) excise a lamina sample from the leaves (Wilson *et al*. 1999).
- For resinous and succulent xerophyte species, rehydration in the laboratory may prove difficult. For these species an alternative method could be to collect the leaves the morning after a rainfall event (Cornelissen *et al*. 2003).
- For new measurements of aquatic plants LEDA prefers the collecting of land forms instead of aquatic forms (e.g. for *Callitriche, Apium, Ranunculus aquatilis*, *Hippuris, Potamogeton*). In case of water plants with emerged or floating leaves it is preferred that only these leaf types should be measured (e.g. *Nuphar, Nymphaea, Potamogeton, Stratiotes*). In case of submerged water plants LEDA prefers the leaf samples from closely to the water surface (e.g. *Najas, Ruppia, Potamogeton, Ceratophyllum*). Note that the SLA of these submerged leaves can vary depending on the water depth, measurements of submerged water plants could have a lower data quality.

***Figure 3.6.*** *Special case example Petasites hybridus with leaves with massive midrib support structures (Photo: see Source list).*

**Minimal requirements**

Measurement obtained from literature (or other published sources) data of Leaf size, SLA, LDMC and Leaf mass can not be accepted by the LEDA Traitbase when the mean or median is given without the number of replicates (N) and the standard deviation or standard error. For information obtained from literature sources, details of the method used (i.e. leaf area meter or scanner) and the part of the leaf that is measured (leaf state: whole leaf or without petiole and/or rachis) are obligatory.

When data obtained by measurements are entered in the Traitbase, the mean or the median with the standard deviation or standard error with a minimum number of 3 replicates of individuals is obligatory. A minimum of 2 leaves should be collected within each individual, with the exception of species that only produce one leaf. Leaf trait data obtained from greenhouse or garden experiments are only accepted when all obligate fields can be completed.

The leaf traits Leaf size, SLA and Leaf mass will be expressed in mm2 $mg^{-1}$, $mm^2$, mg, respectively. Data collected from literature or other sources expressed in other units will need to be converted to the above mentioned units before entering the data to the Traitbase. Leaf size data obtained from greenhouse or garden experiments are only accepted when all obligate fields can be completed, including additional information on the use of e.g. fertilisers during the experiments. The lack of information on one of the obligate points mentioned above will result in rejection of the data.

**Data structure**

To collect: 2 leaves of 10 different individuals = 20 leaves in total per species (per site)

Obligate:

- Type of variable: Numerical
- Number of individuals per sample (n): 10
- Number of replicates per individual (N): 2
- Unit:
  Leaf mass = mg
  SLA = $mm^2$ $mg^{-1}$
  Leaf size = $mm^2$
  LDMC = mg $g^{-1}$
- Values: N, mean, median, minimum, maximum, standard deviation, standard error
  *Note*: The average leaf trait data for each individual plant (which is in general 2 leaves) is taken as one statistical observation when calculating mean, standard deviation or standard error (Cornelissen *et al*. 2003).
- Validity range:
  Leaf mass = 0-1.000.000
  SLA = 0-100
  Leaf size = 0-3.000.000
  LDMC = 0-1000
- Leaf state (what measured):
  1. Whole leaf (with petiole and rachis)
  2. Leaf without petiole and rachis
  3. Unknown

- Leaf specific method (rehydration): *
    1. Leaf rehydration
    2. No leaf rehydration
    3. Unknown
- Plant stage: *
    1. Adult
    2. Juvenile
    3. Seedling
    4. Unknown

Optional:
- o Balance error: mg
- o Comment field: Any information of importance to the trait

* This information is not obligate for leaf size and leaf mass.

# 3. STEM TRAITS

*D. Kunzmann*

Stem traits included in the LEDA Traitbase are Woodiness (or stem specific density), shoot growth form (including branching), leaf distribution along the stem.

## 3.1. WOODINESS & STEM SPECIFIC DENSITY

**Introduction**

Tissue density plays a central role in the nutrient utilisation of a species by determining the rate of biomass turnover (i.e. low tissue density is associated with high growth rate). Although variation in tissue density is often correlated with differences in life history traits among species, for the most of the organ tissue density is relatively constant for each species (Niklas 1994, Enquist *et al*. 1999).

In general the definition for 'density' is the mass of an object divided by its volume, the density of a plant organ is therefore the mass of the plant organ divided by its volume. Hence, the density of the dry matter of an organ is its dry mass divided by its volume. The dry matter concentration of an organ is the mass of its dry matter divided by volume of the organ itself. An indirect measure of dry matter concentration is the dry matter content (or mass fraction of dry matter in the international system of units), with the dry matter content defined as the ratio of organ dry mass to fresh mass (Shipley & Vu 2002). The dry matter content of an organ is referred to as tissue density (see Ryser 1996, Westoby 1998), which is defined as the dry weight per unit volume (Wilson *et al*. 1999).

A stem provides the structural strength that a plant needs to stand upright and the durability it needs to live sufficiently long. Stem density appears to be central in a trade-off between plant (relative) growth rate (high rate at low stem densities) and stem defences against pathogens, herbivores or physical damage by abiotic factors (high defence at high stem densities). In combination with plant size related traits, stem density also plays an important role in the aboveground storage of carbon (Cornelissen *et al*. 2003, see also Niklas 1993, 1995). The persistence, the stiffness and longevity of stems depends on