- 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

their tissue density (Wilson 1995). Therefore, stem tissue density plays an indirect role to the placement of flowers and fruits in space and time (Niklas 1994, Waller & Steingraeber 1995), e.g. for wind-pollinators and wind-dispersers.
Woodiness is an easy trait, to determine tissue density of each species in three very coarse categories; woody, semi-woody and non-woody or herbaceous. Note that the term woodiness means also the occurrence and distribution of wood along the stem (e.g. semi-woody ≈ woody at base; see Woodiness categories. The stem density (or wood density) is determined by dividing the dry mass of a stem segment by its fresh volume (Weiher *et al*. 1999). This value is referred to as stem specific density (SSD), which quantifies woodiness and stem water content (Castro- Díez *et al*. 1998, Hacke *et al*. 2001, Cornelissen *et al*. 2003 (see also Twig Dry Matter Content (TDMC) and twig drying time in Cornelissen *et al*. (2003)). In the LEDA Traitbase woodiness is an obligate trait obtained by observation, while the SSD measurements are an optional choice.

Trait definition
Woodiness: The occurrence and distribution of wood along the stem.
SSD: Stem specific density quantifies woodiness and stem-water content and is determined by dividing the oven dry mass of a stem segment by its fresh volume, expressed in g cm^{-3}.

What and how to collect for Woodiness
The trait woodiness will be obtained from published sources. In many published sources (e.g. floras) woodiness is a nominal trait with three main categories (for definitions of hard wood and soft wood see Diaz *et al*. (2004)):

1. Woody = including (1.1) hard wood (defined as ≥ 0.60 g cm^{-3} wood density e.g. Quercus species) and (1.2) soft wood (defined as < 0.60 g cm^{-3} wood density e.g. conifers and *Salix* species). Note: In forestry literature wood density is often referred to as an estimate of Air-dry weight (ADW) per volume vs. Oven-dry weight (ODW) per volume as a measure of SSD.
2. Semi-woody = including species that are often 'woody at base' such as *Solanum dulcamara* or *Rubus* species (Fig. 3.7); the wood density of semi-woody species is not clear due to too few available measurements. It will be expected, that you can find species with hard woody shoots and with soft woody shoots at their base.
3. Herbaceous = non-woody, including all other species (most herbaceous and graminoid species).

In the absence of quantitative data sets the above mentioned nominal categories (hard wood, soft wood, semi wood, herbaceous) should be used to make a rough estimate.

Figure 3.7. *Semi-woody examples Rubus spec. (a) and Solanum dulcamara (b) (Photo: see Source list).*

What and how to collect for stem specific density (SSD)

A minimum of 5 replicates (i.e. individuals) of representative healthy adults should be sampled, as described for canopy height (foliage exposed to sunlight). For herbaceous and woody species with a main stem diameter of ≤ 6 cm, a branchless section of at least 10 cm long section is cut out (knife) at approximately one third of the total stem height measured from the base of the stem (Note: Remove branches when necessary). For shorter stems the whole stem is used with the apical part and loose bark removed. Any firmly attached bark or equivalent phloem tissue is considered to be an integral part of the functioning stem and therefore it needs to be included in stem density measurement. Note that if this method causes unacceptable damage to shrubs or small trees, the 'slice method' may therefore be a compromise alternative (Cornelissen *et al*. 2003).

For woody (or thick succulent) plants with stem diameters of > 6 cm, a pie-shaped slice from the trunk is removed at approximately at one third of trunk height when measured from the base, or at approximately 1.3 m for trees over 4 m in height. The pie-shaped slice (2 to 10 cm in height) needs to represent a cross-section area of approximately $1/8^{th}$ of the total cross-section. Hard-wooded samples should be stored cool in a sealed plastic bag, whereas the herbaceous samples (more sensitive to dehydration) are stored cool between moist filter paper in plastic bags until future use.

How to measure stem specific density (SSD)

Depending on the species, the volume can be determined in several ways:

1. Volume replacement method (preferred by LEDA): Stem specific density (SSD) is determined by the oven-dry mass of a section of a plant's main stem divided by the volume of the same section when still fresh, expressed in mg mm^{-3} (corresponding with kg dm^{-3}) with values 1 (only tropical hardwood have values of >1). Large spaces in relation to the stem diameter are considered air or water spaces, and as such do not belong to the stem tissue, whereas smaller spaces do.

Accordingly, the central hollow of a hollow stem is not included in the volume, but smaller xylem vessels will be included. The volume can be determined by the volume replacement method. With this method the volume of a fresh stem sample (rubbed dry) is measured by immersing the stem section in a volumetric flask filled with water and the increase of volume is measured. During the 5 second interval, the larger but not yet the smaller spaces should fill with water. After volume measurement the sample is dried in the oven at 70°C for 48 to 72 hours and the oven-dry weight determined (Cornelissen *et al*. 2003). To determine wood density of lower shrubs (often with multiple stems), herbs, grasses or seedlings the use of 5-10 short stem segments (0.3 - 2.5 cm long) per individual will be accepted (e.g. grasses (Ryser 1996), shrubs (Hacke *et al*. 2000), seedlings (Castro- Díez *et al*. 1998)).

2. Actual measurement. For very small samples, or species with unusual tissue, this volume replacement method may not work. For those species the mean diameter (D) and the length (L) of the cylindrical sample is measured with a calliper or ruler. If the stem is very thin, the stem diameter should be determined using a cross-section under the microscope (e.g. small annuals). The cylinder volume (V) is subsequently calculated using: $V = (0.5\ D)^2 * \pi * L$. In the case of hollow stems, estimate the diameter of the hollow and subtract the cross-sectional area of the hollow from the stem cross-section before calculating the volume.
3. Tree cores. An additional forestry method to determine stem density is the use of tree cores. Although this method does not always use a representative part of the stem volume, similar data from tree cores are acceptable for use in broader comparisons where small deviations are not critical. The mass component of wood density is often measured at 12% moisture content, and density reported as 'air dry weight' (ADW) or 'air-dried timber'. Stem specific density as described in this protocol is called 'oven dry weight' (ODW) in technical timber journals. Data obtained by ODW, directly measurement or derivation from ADW, can be used as stem specific density for trees. After Reyes *et al*. (1992), ADW can be transformed to ODW or SSD as follows: $SSD = 0.800\ ADW + 0.0134$ ($R^2 = 0.99$). For further details see Cornelissen *et al*. (2003).

The relationships between woodiness, and stem specific density of herbaceous or woody tissue is as follows:

Woodiness categories (**obligate**)		Stem specific density categories (optional)		
1. Woody	1.1. Hard wood	Hard wood		≥ 0.60 g/cm^3
	1.2. Soft wood	Soft wood		< 0.60
2. Semi-woody		Semi-woody	hard woody at base	≥ 0.60
			soft woody at base	< 0.60 [1]
3. Herbaceous (non-woody)		Herbaceous		< 0.60 [2]

[1] Also records known of SSD of ≥ 0.26 (e.g. Castro-Díez *et al*. (1998)).
[2] Also records known of SSD of ≤ 0.26 (e.g. Shipley & Vu (2002)).

Special cases

- In some plant species without a well-defined stem (i.e. rosette plants, grasses, sedges), the central aboveground area from which the leaves grow is isolated and treated as the stem. The stem density is reported as zero if the plant species has no recognisable aboveground support structure (Cornelissen *et al*. 2003).
- When plants are branching at ground level, the apparent main branch or a random branch should be selected to measure (Hacke *et al*. 2000, Cornelissen *et al*. 2003).
- To compare SSD with other traits such as relative growth rate, it is interesting to measure adult plants as well as seedlings (see Castro- Díez *et al*. 1998).

Minimal requirements

In the case of SSD the mean or the median with the standard deviation or the standard error has to be given with a minimum of 3 replicates (i.e. 3 different individuals per species). A criterion for data rejection is the absence of the number of replicates and/or the standard deviation or standard error.

For SSD information on the stage measured (seedling or adult) is obligatory. Woodiness or SSD data obtained from greenhouse or garden experiments are only accepted when all obligate fields can be completed. In the case the data are obtained from literature, the LEDA Traitbase accepts data in other unit's but these have to be converted to mg/mm^3 before entry into the database.

Data structure

Woodiness

To collect: 1 observation per species for Woodiness

Obligate:
- Type of variable: nominal and ordinal
- Unit: categories
- Woodiness categories:
  1. Woody
     1.1. Hard wood
     1.2. Soft wood
  2. Semi-woody
  3. Herbaceous (non-woody)

Optional:
- o Original woodiness value: $g\ cm^{-3}$

SSD

To collect: 1 stem piece from 5 different individuals per species = 5 stem pieces in total per species

Optional:
- o Type of variable: numerical
- o Number of individuals per sample size (n): 5
- o Number of replicates (N): 1

  Note: For multiple stem plants or branched herbs it is preferred to collect more replicates per individual

- o Unit: g cm^{-3}
- o Values: N, mean, median, minimum, maximum, standard deviation, standard error
- o Validity range: from 0-1.5
- o Trait specific methods:
    1. Volumetric flask method
    2. Volume calculation method
    3. Unknown
- o Plant stage:
    1. Adult
    2. Juvenile
    3. Seedling
    4. Unknown
- o Comment field: Any information of importance to the trait

3.2. SHOOT GROWTH FORM (including branching)

Introduction
Shoot growth form is related to the LEDA traits plant growth form and leaf distribution along the stem (Section 3, Chapter 1.1 and 3.3, respectively). Barkman (1988) distinguished between the concepts 'growth form' and 'life form'. Growth form describes plant types with the same growth morphology or architecture, whereas life form describes the morphological and/or physiological adaptation to a certain ecological factor. To determine shoot growth form the LEDA Traitbase will follow a modified version of shoot classification systems of Kleyer (1995). The categories of shoot form are supplemented by a binary classification of branching (i.e. yes or no). For more details of branching classification systems see Bell (1991).

Trait definition
Shoot growth form: Shoot form describes canopy structure of shoots, including branching.

How and what to measure
To estimate the shoot form of each species, a nominal classification system will be used (defined by Kleyer 1995, revised and refined for aquatic plants using Sculthorpe 1967). Note that the shoot of many rosette plants is very short (e.g. *Bellis perennis* up to 1 cm; Kutschera & Lichtenberger 1992), but the inflorescence is erect; therefore it would fit in category 2, stem erect.

In this classification the categories 1-4 are for terrestrial plants, 5-8 are for aquatic plants:

1. Lianas, climbers and scramblers (e.g. *Hedera helix*)
2. Stem erect (e.g. *Fagus sylvatica*)
3. Stem ascending to prostrate (e.g. *Veronica prostata, Calluna vulgaris*)
4. Stem prostrate (e.g. *Lysimachia nummularia*)
5. Free-floating plants (e.g. *Lemna, Salvinia, Stratiotes*)

6. Emergent, attached to the substrate (e.g. *Butomus, Typha, Glyceria*)
7. Floating leaves, attached to the substrate (e.g. *Nuphar, Nymphoides, Luronium*)
8. Submerged, attached to the substrate (e.g. *Elodea, Najas, Isoetes, Vallisneria*)

Special cases

- Facultative epiphytes (e.g. *Phyllitis scolopendrium*) or facultative nutrition's such as holo- and hemi-parasites (e.g. *Orobanche* spec.) will be categorised in the same way as well as all other species (see Fig. 3.8).

Figure 3.8. *Examples of the special cases for shoot growth form Phyllitis scolopendrium (A) and Orobanche hederae (B) (Photo: see Source list).*

Minimal requirements

Shoot form data obtained from greenhouse or garden experiments are only accepted when all obligate fields can be completed. In the LEDA Traitbase information about the sub-trait branching is obligatory.

Branching

Branching is a simple binominal trait giving information about the capacity of a plant species to fill lateral space above ground, thus it is an indicator of competitive capacity.
Most of the data on branching in the BIOPOP database incorporated in LEDA were derived from the scientific drawings in Jäger & Werner (2000). A species was classified as a branching species when lateral shoots were produced above the epicotyl and below the inflorescence. When branching was unclear it was primarily decided according to the capacity of the species to fill lateral space, i.e. if a species is only branching above the most basal flower but the inflorescence contains photosynthetic active leaves, it is classified as branching (e.g. *Verbascum lychnitis*). Tussock grasses (Hegi 1998), species able to produce vegetative parts (i.e. daughter rosettes) that grow very near to the main ramet, were regarded as branching species (e.g. *Saxifraga paniculata*). Note that the presence or absence (yes/no) of branching is possible with all shoot growth form categories.

Data structure

To collect: 1 observation per species

Obligate:
- Type of variable: nominal in classes
- Number of samples (n): 1 observation per species
- Number of replicates (N): -
- Unit: category
- Shoot growth form categories:
  1. Lianas, climbers and scramblers
  2. Stem erect
  3. Stem ascending to prostrate
  4. Stem prostrate
  5. Free-floating plants
  6. Emergent, attached to the substrate
  7. Floating leaves, attached to the substrate
  8. Submerged, attached to the substrate
- Branching:
  1. Yes
  2. No
  3. Unknown

Optional:
- o Original shoot growth form
- o Comment field: Any information of importance to the trait

3.3. LEAF DISTRIBUTION ALONG THE STEM

Introduction

This trait is related with the traits plant - and shoot growth form (Section 3, Chapter 1.1 and 3.2) as these are also associated with plant strategy, climatic factors and land use (Cornelissen *et al*. 2003). For instance, a certain height and or the position of leaves may be an adaptation or a respons to grazing by different herbivores, for instance rosettes growth forms are associated with high grazing pressure by mammalian herbivores (Conelissen *et al*. 2003).

This simple trait describes the distribution of leaves along the stem of an adult plant (see for more details Troll 1935). This trait gives information about the canopy structure of a plant, or more precisely the distribution of photosynthetically active organs. Leaf distribution along the shoot also gives information on the partitioning of allocated biomass between leaves and stem (see also Niklas & Enquist 2002).

Trait definition

Leaf distribution along the stem: Is the distribution of leaves along the stem of an adult plant.

How and what to measure

To estimate the distribution of leaves the following six nominal categories will be used, but note that it is possible to use different categories for the same species:

1.	Rosette / tufted plant (leaves concentrated near soil or water surface)	(e.g. *Menyanthes, Primula vulgaris*, *Festuca ovina, Trapa natans,Stratiotes*)
2.	Semi-rosette	(e.g. *Crepis* spec*., Ajuga, Antennaria, Aegopodium, Pedicularis palustris*)
3.	Leaves distributed regularly along the stem	(e.g. *Helianthus tuberosus* (Fig. 3.9a), *Origanum vulgare, Myriophyllum* and *Elodea*) Note that this also includes multiple-stemmed shrubs or trees, winding herbs e.g. *Convolvulus*).
4.	Shoot scarcely foliated	(e.g. *Orobanche spec., Chondrilla juncea* (Fig. 3.9b))
5.	Tufts and crowns, leaves concentrated as a rosette at the top of taller shoot or vegetative stem	(e.g. *Daphne mezereum* (Fig. 3.9c), but also *Trientalis* and trees with concentrated crowns)
6.	Other	(e.g. plant without obvious stems such as *Lemna minor* (Fig. 3.9d), *Wolffia*)

Special cases

- Facultative epiphytes (e.g. *Phyllitis scolopendrium*), plants needing other support plants (e.g. *Hedera*) or nutrition's as Holo- and Hemi parasites (e.g. *Orobanche* spec.) will be categorised in the same way as all other species.

Minimal requirements

Leaf distribution data obtained from garden experiments are only accepted when all obligate fields can be completed.

Data structure

To collect: 1 observation per species

Obligate:
- Type of variable: nominal classes
- Number of samples: 1 observation
- Number of replicates: -
- Unit: categories
- Leaf distribution categories:
    1. Rosette / tufted plant
    2. Semi-rosette
    3. Leaves distributed regularly along the stem
    4. Shoot scarcely foliated
    5. Tufts and crowns, leaves concentrated as a rosette at the top of taller shoot or vegetative stem
    6. Other

Optional:
- o Original shoot growth form
- o Comment field: Any information of importance to the trait

Figure 3.9. *Examples of some of the leaf stem distribution categories; (A) Leaves distributed regularly along the stem - Helianthus tuberosus, (B) Shoot scarcely foliated - Chondrilla juncea, (C) Tufts and crowns, leaves concentrated as a rosette at the top of taller shoot or vegetative stem - Daphne mezereum, (D) No obvious stem structure - Lemna minor (Photo: see Source list).*