

## A handbook of protocols for standardised and easy measurement of plant functional traits worldwide

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**Abstract.** There is growing recognition that classifying terrestrial plant species on the basis of their function (into 'functional types') rather than their higher taxonomic identity, is a promising way forward for tackling important ecological questions at the scale of ecosystems, landscapes or biomes. These questions include those on vegetation responses to and vegetation effects on, environmental changes (e.g. changes in climate, atmospheric chemistry, land use or other disturbances). There is also growing consensus about a shortlist of plant traits that should underlie such functional plant classifications, because they have strong predictive power of important ecosystem responses to environmental change and/or they themselves have strong impacts on ecosystem processes. The most favoured traits are those that are also relatively easy and inexpensive to measure for large numbers of plant species. Large international research efforts, promoted by the IGBP–GCTE Programme, are underway to screen predominant plant species in various ecosystems and biomes worldwide for such traits. This paper provides an international methodological protocol aimed at standardising this research effort, based on consensus among a broad group of scientists in this field. It features a practical handbook with step-by-step recipes, with relatively brief information about the ecological context, for 28 functional traits recognised as critical for tackling large-scale ecological questions.

### Introduction and discussion

This paper is not just another handbook on ecological methodology, but serves a particular and urgent demand as well as a global ambition. Classifying plant species according to their higher taxonomy has strong limitations when it comes to answering important ecological questions at the scale of ecosystems, landscapes or biomes (Woodward and Diament 1991; Keddy 1992; Körner 1993). These questions include those on responses of vegetation to environmental variation or changes, notably in climate, atmospheric chemistry, land use and natural disturbance regimes. Reciprocal questions are concerned with the impacts of vegetation on these large-scale environmental parameters (see Lavorel and Garnier 2002 for a review on response and effect issues). A fast-growing scientific community has come to the realisation that a promising way forward for answering such questions, as well as various other ecological questions, is by classifying plant species on functional grounds (e.g. Diaz *et al.* 2002). Plant functional types and plant strategies, the units within functional classification schemes, can be defined as groups of plant species sharing similar functioning at the organismic level, similar responses to environmental factors and/or similar roles in (or effects on) ecosystems or biomes (see reviews by Box 1981; Chapin *et al.* 1996; Lavorel *et al.* 1997; Smith *et al.* 1997; Westoby 1998; McIntyre *et al.* 1999a; McIntyre *et al.* 1999b; Semenov and van der Maarel 2000; Grime 2001; Lavorel and Garnier 2002). These similarities are based on the fact that they tend to share a set of key functional traits (e.g. Grime and Hunt 1975; Thompson *et al.* 1993; Brzeziecki and Kienast 1994; Chapin *et al.* 1996; Noble and Gitay 1996; Thompson *et al.* 1996; Diaz and Cabido 1997; Grime *et al.* 1997; Westoby 1998; Weiher *et al.* 1999; Cornelissen *et al.* 2001; McIntyre and Lavorel 2001; Lavorel and Garnier 2002; Pausas and Lavorel 2003).

Empirical studies on plant functional types and traits have flourished recently and are rapidly progressing towards an understanding of plant traits relevant to local vegetation and

ecosystem dynamics. However, functional classifications are not fully resolved with regard to application in regional to global scale modelling, or to the interpretation of vegetation–environment relationships in the paleo-record. Recent empirical work has tended to adopt a 'bottom-up' approach where detailed analyses relate (responses of) plant traits to specific environmental factors. Some of the difficulties associated with this approach regard the identification of actual plant functional groups from the knowledge of relevant traits and the scaling from individual plant traits to ecosystem functioning. On the other hand, geo-biosphere modellers as well as paleo-ecologists have tended to focus on 'top-down' classifications where functional types or life forms are defined *a priori* from a small set of postulated characteristics. These are often the characteristics that can be observed without empirical measurement and only have limited functional explanatory power. The modellers and paleo-ecologists are aware that their functional type classifications do not suffice to tackle some of the pressing large-scale ecological issues (Steffen and Cramer 1997).

In an attempt to bridge the gap between the 'bottom-up' and 'top-down' approaches (see Canadell *et al.* 2000), scientists from both sides joined a workshop (at Isle sur la Sorgue, France, in October 2000) organised by the International Geosphere–Biosphere Programme (IGBP, project Global Change and Terrestrial Ecosystems). One of the main objectives of the workshop was to assemble a minimal list of functional traits of terrestrial vascular plants that (1) can together represent the key responses and effects of vegetation at various scales from ecosystems to landscapes to biomes to continents, (2) can be used to devise a satisfactory functional classification as a tool in regional and global-scale modelling and paleo-ecology of the geo-biosphere, (3) can help answer some further questions of ecological theory, nature conservation and land management (see Table 1 and Weiher *et al.* 1999) and (4) are candidates for relatively easy, inexpensive and standardised

measurement in many biomes and regions on Earth. Another main objective of the workshop was to initiate the production of a series of trait-measuring protocols for worldwide use, in the form of an easy-to-use recipe book. Some previous publications (e.g. Hendry and Grime 1993; Westoby 1998; Weiher *et al.* 1999; Lavorel and Garnier 2002) and unpublished reports (by J. G. Hodgson, S. Díaz, G. Montserrat-Martí, K. Thompson and J. P. Sutra) have made important contributions towards these four objectives and provided important information for the current handbook. Our new protocol handbook has the advantages of (1) being based on consensus among a broad scientific community about which traits are critical for the ecological challenges ahead as well as practically feasible (see Table 2) and (2) giving comprehensive and detailed step-by-step recipes for direct and, to the extent possible, unambiguous use in any terrestrial biome.

Most of the functional traits in this handbook are 'soft traits', i.e. traits that are relatively easy and quick to quantify (Hodgson *et al.* 1999). They are often good correlates of other 'hard traits', which may be more accurate indicators of plant functions responsible for responses or effects at the ecosystem or biome scale, but which cannot be quantified for large numbers of species in many regions of the world (Hodgson *et al.* 1999; Weiher *et al.* 1999; Lavorel and Garnier 2002). For instance, the combination of seed mass and seed shape (both 'soft traits') was found to be a good predictor of seed persistence ('hard trait') in temperate-zone seedbanks, small and relatively round seeds surviving the longest periods of burial in the soil (Thompson *et al.* 1993; Funes *et al.* 1999). It is beyond the scope of this handbook to discuss in detail why each particular trait was selected and how it relates to the various hard traits and ecosystem properties. Some of this information can be found in a recent paper based partly on findings from the same IGBP workshop (Lavorel and Garnier 2002). Table 2 summarises the known or assumed links of the traits selected with important environmental change parameters and responses, plant fitness parameters and effects on ecosystems.

While we call the trait list chosen the 'minimal list' and strongly encourage researchers to go out and measure as many of these as possible for their particular species set, this trait list is not a minimum for individual sites and research projects. We emphasise that any of the traits measured in the standardised way on a range of species will be of great value for tackling some of the ecological questions mentioned. Also, it is logical that different traits will be favoured by different researchers, partly because of familiarity and research facilities and some (e.g. fire-related) traits will have particular appeal in certain regions. At the same time, the more traits covered, the greater will be the hypothesis-testing power of any particular database, both within itself and as a contributor to ecological questions at the scale of biomes or our planet. We also need to emphasise that the trait list

covered here is not complete and is based on consensus and compromise. We strongly encourage researchers to combine soft-trait measurements according to our 'minimal list' with measurement of further (often 'harder') traits with proven large-scale ecological significance not covered here. These include, for instance, plant or leaf tolerance of drought, anoxia and high salt concentrations; presence/absence of stem and root aerenchyma; wood anatomy (e.g. true vessels versus tracheids); ramet (plant) longevity; age until sexual maturation; plant biomass; ramet (plant) architecture; stomatal sizes, densities or indices; concentrations of foliar (or root, shoot) lignin, cellulose, phenols, volatile organic compounds, ash and other chemistry; foliar chlorophyll content; photosynthetic capacity, leaf pubescence and hair types; leaf thickness; seed germination requirements including serotiny; pollination mode; potential relative growth rate; reproductive output and phenology; and litter quality. Combination of some of these traits with traits from our proposed list and with biogeographical data may help to test the wider significance and validity of currently known patterns and trade-offs and to identify and test new ones. Many of the above list are 'hard traits' still in need of 'soft' surrogate traits.

For this handbook, we have chosen to give only the very minimum of ecological introduction to each trait, with an accompanying separate list of references that contain further details on its ecological theory and significance. The recipes themselves aim to provide one brief, standardised, 'minimum' methodology, while under the heading 'Special cases or extras' pointers are given to interesting additional methods and parameters. We expect that the strong focus on the practicalities and standardisation of the trait recipes will help this handbook to become a standard companion in laboratories, on field trips and bed-side tables all over the world.

## The protocol handbook

### 1. Selection of plants and statistical considerations

#### 1.1. Selection of species in a community or ecosystem

The following instruction is a facultative guideline; see the Note below for alternatives.

The most abundant species of a given ecosystem are selected, with the following two underlying objectives:

(i) to obtain a good representation of the ecosystem or plant community under study; and

(ii) to provide enough information to scale-up the values of traits from the plant to the community level. This requires knowledge of the relative proportions of species.

The most abundant species are arbitrarily defined as those species that, together, make up about 70–80% of the standing biomass of the community. This can be estimated by people familiar with the ecosystem, if no biomass or abundance data



are available. In forest and other predominantly woody vegetation the most abundant species of the lower (shrub and/or herbaceous) vegetation strata may also be included, even if their biomass is much lower than that of the woody species. In predominantly herbaceous vegetations, the species contribution to a particular community varies with time during a growing season. As a first step, we suggest that the floristic composition be determined at the time of peak standing biomass of the community. Be aware of species with a short life cycle outside peak biomass time. Summer or winter annuals, which have very short life cycles, will need to be sampled at the time they are available, which may not coincide with that of the bulk of the species. In very diverse vegetation types without a clear dominance hierarchy of biomass, such as the South African fynbos, as many species as possible should be selected, depending on logistic feasibility.

[Note: It is important to note that a species set that is not representative for the particular ecosystem under study can still provide useful data for analyses both at the local, regional and global scale. Important examples are subsets consisting of woody species or herbaceous species only. Also, rarer species often do not produce much biomass, but may be useful for certain analyses, for instance those addressing questions relating to diversity or species richness. For questions about evolution, the choice of species may be based on a good representation of different phylogenetic groups rather than predominance in ecosystems. The preferred choice of species may not overlap entirely for both purposes (Díaz and Cabido 1997; Díaz *et al.* 1998). The important message is that most species by trait datasets will be valuable!]

### 1.2. Selection of individuals within a species

Traits should be measured on robust, well grown plants, located in well-lit environments, preferable totally unshaded. This is particularly important for some leaf traits that are known to be very plastic in response to light. This will obviously create sampling problems for species found, for instance, in the understorey of forests, or those in the bryophyte layer of grasslands. In such cases, plants are chosen in the least-shaded sites for that species. Plants strongly affected by herbivores or pathogens are excluded.

The selection of individuals can be done by the transect method: every  $x$  metres ( $x$  depending on the spatial scale of the particular vegetation under study), select an individual that falls on a line (i.e. by using a string or tape measure). If no individual falls on the line at the predetermined point, then find the individual that is closest to that point. If different plant types occur at different spatial scales within the ecosystem (as may be the case for trees versus herbaceous plants), the distance between points along the line may vary accordingly.

[Note: This is a systematic (rather than random) approach. It has the drawback of being biased if the distance chosen between points is related to a distance at which there is an intrinsic change in the vegetation pattern. Such potential problems can be checked by careful observations of the vegetation pattern (e.g. plant species composition, vertical structure and height) along the line. If one detects or suspects problems with fixed distance between points, an alternative is to use the transect method with random intervals between measurements. This makes mapping and spatial analysis difficult, but does avoid most kinds of sampling bias.]

Deciding where to lay out the different transects (selecting at random or following a system) is left to the judgement and experience of the collectors in the field, as long as they aim to capture the most representative species in terms of abundance or biomass.

What an individual is may be difficult to define in many species, so the fundamental unit on which measurements are taken is the ramet, i.e. the recognisable separate aboveground unit. This choice is both pragmatic, as genets would be difficult to identify in the field, and ecologically sound, as the ramet is likely to be the unit of most interest for most functional-trait-related questions addressed worldwide.

### 1.3. Statistical considerations

Most of the information obtained for the traits described in this handbook will be used in a comparative way, classifying species in different functional groups, or analysing relationships between variables across species within or even among biomes. This almost inevitably implies that this type of research is prone to the classical conflict between scale and precision: the more species within an ecosystem are covered, the better, but given constraints of time and labour, this will come at the cost of less replicates for each individual species.

Reasoning along the lines that there is more variation across species than within, the extreme solution would be to sample as many species as feasible, with only one replicate per species. However, in general a more conservative approach is used, in which each species is represented by a given number of replicates. The number of individuals selected (with the required characteristics described in 1.2) will depend on the natural variability in the trait of interest. To obtain an impression of the variability for a number of quantitative traits described in this handbook, we analysed field data collected for a range of species. From all the replicates measured per species we obtained an estimate of the standard deviation and the mean in the sampled population and divided the first by the latter to arrive at an estimate of the coefficient of variation (CV). Because of the low number of replicates generally used, each of the individual estimates bears an uncertainty, but by looking at the range of CVs obtained across a wide range of species, we

**Table 3. List of traits discussed in this protocol**

The preferred units are given (except for traits that are categorical, which are marked 'cat.'). and the range of values that can normally be encountered in field-grown plants. Recommended sample size indicates the minimum and preferred number of individuals to be sampled, so as to obtain an appropriate indication about the values for the trait of interest. When two numbers are given, the first indicates the number of individuals and the second the number of leaves or root pieces collected per individual. The expected range in CV% gives the 20th and 80th percentile of the distribution of the coefficient of variation (standard deviation scaled to the mean) as observed in a number of datasets obtained for a range of field plants from different biomes (Poorter and De Jong 1999; Pérez Harguindeguy *et al.* 2000; Garnier *et al.* 2001a; Gurvitch *et al.* 2002; Craine and Lee 2003; Craine *et al.* 2003; Garnier *et al.*, unpubl. data; authors' own unpublished datasets). Under Logical combinations, traits that should be logically measured on the same individual are indicated by the same letter, and those parameters for which a range of useful data have been published in the available literature are indicated by a '+'

Variable	Preferred unit	Range of values	Recommended sample size ( <i>N</i> ) minimum preferred	Range in CV (%)	Logical combinations	Available literature
<i>Traits that can be measured on any plants in the population that meet the trait criteria</i>						
<b>Vegetative traits</b>						
Growth form	cat.		3	5		+
Life form	cat.		3	5		+
Plant height	m	0.01–100	10	25	17–36	+
Clonality	cat.		5	10		+
Spinescence	cat.		3	5		+
Flammability	cat.		5	10		
Leaf life-span	month	0.5–200	3, 12	10, 12	?	a
Leaf phenology	month	0.5–12	5	10	?	a
<b>Regenerative traits</b>						
Dispersal mode	cat.		3	3		b
Dispersule shape	unitless	0–1	3, 5	10, 5	?	b
Dispersule size (mass)	mg	10 <sup>-3</sup> –10 <sup>7</sup>	3, 5	10, 5	?	b
Seed mass	mg	10 <sup>-3</sup> –10 <sup>7</sup>	3, 5	10, 5	?	b
Resprouting capacity	unitless	0–100	5	25	?	+
<i>Traits that may all be measured on the same plant individuals (note that belowground traits of small species are best sampled by whole-plant excavation)</i>						
<b>Leaf traits</b>						
Specific leaf area (SLA)	m <sup>2</sup> kg <sup>-1</sup> (mm <sup>2</sup> mg <sup>-1</sup> )	2–80	5, 2	10, 2	8–16	c
Leaf size (individual leaf area)	mm <sup>2</sup>	1–10 <sup>6</sup>	5, 2	10, 2	17–36	d
Leaf dry matter content (LDMC)	mg g <sup>-1</sup>	50–700	5, 2	10, 2	4–10	c
Leaf nitrogen concentration (LNC)	mg g <sup>-1</sup>	10–50	5, 2	10, 2	8–19	d
Leaf phosphorus concentration (LPC)	mg g <sup>-1</sup>	0.5–5	5, 2	10, 2	10–28	d
Physical strength of leaves	N (or N mm <sup>-1</sup> )	0.02–4 (or 0.2–40)	5	10	14–29	
Photosynthetic pathway	cat.		3	3		+
Leaf frost sensitivity	%	2–100	5	10	9–26	
<b>Stem traits</b>						
Stem specific density (SSD)	mg mm <sup>-3</sup> (kg dm <sup>-3</sup> )	0.4–1.2	5	10	5–9	e
Twig dry matter content (TDMC)	mg g <sup>-1</sup>	150–850 ?	5	10	?	
Twig drying time	day	?	5	10	?	
Bark thickness	mm	?	5	10	?	e
<b>Below-ground traits</b>						
Specific root length (SRL)	m g <sup>-1</sup>	10–500	5, 10	10, 10	15–54	f
Fine root diameter	mm	?	5, 10	10, 10	5–16	f
Root depth distribution	g m <sup>-3</sup>	?	5	10	?	f/g
95% rooting depth	m	0–5 (10)	5	10	?	f/g
Nutrient uptake strategy	cat.		5	10		+

can get a fairly good estimate of the overall variability. Interestingly, these distributions are fairly constant for a given parameter across habitats, but the observed range in variability differs strongly for different parameters.

Table 3 shows the various traits that will be discussed in this handbook, along with the preferred units and the range of values that can be expected. The CV values normally found are based on the 20th and 80th percentile of the distribution as obtained above. Furthermore, Table 3 shows the minimum and preferred number of replicates, based on

common practice. However, a statistical power analysis based on the assumed difference in values between plants and the variability as given by the CV is required to calculate a more precise number, depending on the interest of the researcher. Remember that in most correlation analyses, data are compared across species averages and variability within a species is ignored. To obtain an impression of the relative contribution of variability across and within species, an ANOVA can be used, with species and replicates within species as random factors.

## 2. Vegetative traits

### 2.1. Whole-plant traits

#### Growth form

##### *Brief trait introduction*

Growth form, mainly determined by canopy structure and canopy height, may be associated with plant strategy, climatic factors and land use. For instance, the height and positioning of the foliage may be both adaptations and responses to grazing by different herbivores, rosettes and prostrate growth forms being associated with high grazing pressure by mammalian herbivores.

##### *How to record?*

This is a categorical trait assessed through straightforward field observation or descriptions or photos in the literature. Growth forms 1–6, 18 and 19 are always herbaceous. Assign a species according to one of the following growth form categories:

- (1) *short basal*: leaves <0.5 m long concentrated very close to the soil surface, e.g. rosette plants or prostrate growth forms (compare with 5, 6 and 11);
- (2) *long basal*: large leaves (petioles) >0.5 m long emerging from the soil surface (e.g. bracken *Pteridium aquilinum* or certain agaves), but not forming tussocks (cf. 6);
- (3) *semi-basal*: significant leaf area deployed both close to the soil surface and higher up the plant;
- (4) *erect leafy*: plant essentially erect, leaves concentrated in middle and/or top parts;
- (5) *cushions* (=pulvinate): tightly packed foliage held close to soil surface, with relatively even and rounded canopy boundary;
- (6) *tussocks*: many leaves from basal meristem forming prominent tufts;
- (7) *dwarf shrubs*: woody plants up to 0.8 m tall;
- (8) *shrubs*: woody plants taller than 0.8 m with main canopy deployed relatively close to the soil surface on one or more relatively short trunks;
- (9) *trees*: woody plants with main canopy elevated on a substantial trunk;
- (10) *leafless shrubs or trees*: with green, non-succulent stems as the main photosynthetic structures;
- (11) *short succulents* (plant height <0.5 m): green globular or prostrate ‘stems’ with minor or no leaves;
- (12) *tall succulents* (>0.5 m): green columnar ‘stems’ with minor or no leaves;
- (13) *palmoids*: plants with a rosette of leaves at the top of a stem (e.g. palm trees and other monocotyledons, certain alpine Asteraceae such as *Espeletia*);
- (14) *epiphytes*: plants growing on the trunk or in the canopy of shrubs or trees (or telegraph wires);

(15) *climbers and scramblers*: plants that root in the soil and use external support for growth and leaf positioning; this group includes lianas;

(16) *hemi-epiphytes*: plants that germinate on other plants and then establish their roots in the ground, or plants that germinate on the ground, grow up the tree and disconnect their soil contact. This group also includes tropical ‘stranglers’ (e.g. some figs);

(17) *hemiparasites or holoparasites* (see under *Nutrient uptake strategy*) with haustoria tapping into branches of shrubs or trees, to support green foliage (mistletoes, e.g. Loranthaceae, Viscaceae; also Cuscutaceae);

(18) *aquatic submerged*: all leaves submerged in water;

(19) *aquatic floating*: most of the leaves floating on water; and

(20) *other growth forms*: give a brief description.

*References on theory and significance*: Cain (1950); Ellenberg and Müller-Dombois (1967); Whittaker (1975); Barkman (1988) and references therein; Rundel (1991); Richter (1992); Box (1996); Ewel and Bigelow (1996); Cramer (1997); Díaz and Cabido (1997); Lüttge (1997); Medina (1999); McIntyre and Lavorel (2001).

*More on methods*: Barkman (1988), and references therein.

#### Life form

##### *Brief trait introduction*

Life form is another classification system of plant form designed by Raunkiaer (1934) and adequately described by Whittaker (1975): ‘instead of the mixture of characteristics by which growth forms are defined (...), a single principal characteristic is used: the relation of the perennating tissue to the ground surface. Perennating tissue refers to the embryonic (meristematic) tissue that remains inactive during a winter or dry season and then resumes growth with return of a favourable season. Perennating tissues thus include buds, which may contain twigs with leaves that expand in the spring or rainy season. Since perennating tissue makes possible the plant’s survival during an unfavourable season, the location of this tissue is an essential feature of the plant’s adaptation to climate. The harsher the climate, the fewer plant species are likely to have buds far above the ground surface, fully exposed to the cold or the drying power of the atmosphere’. Furthermore, for species that may be subject to unpredictable disturbances, such as periodic grazing and fire, the position of buds or bud-forming tissues allows us to understand the likelihood of their surviving such disturbances. It is important to note that the categories below refer to the highest perennating buds for each plant.

##### *How to record?*

Life form is a categorical trait assessed from field observation, descriptions or photos in the literature. Many

floras give life forms as standard information on plant species. Five major life forms were initially recognised by Raunkiaer, but his scheme was further expanded by various authors (e.g. Ellenberg and Müller-Dombois 1967). Here we present one of the simplest, most widely used schemes:

(1) *phanerophytes*: plants that grow taller than 0.50 m and whose shoots do not die back periodically to that height limit (e.g. many shrubs, trees and lianas);

(2) *chamaephytes*: plants whose mature branch or shoot system remains below 0.50 m, or plants that grow taller than 0.50 m, but whose shoots die back periodically to that height limit (e.g. dwarf shrubs);

(3) *hemicryptophytes*: periodic shoot reduction to a remnant shoot system, so that buds in the 'harsh season' are close to the ground surface (e.g. many grasses and rosette forbs);

(4) *geophytes*: annual reduction of the complete shoot system to storage organs below the soil surface [e.g. many bulb flowers and *Pteridium* (bracken)];

(5) *therophytes*: plants whose shoot and root system dies after seed production and which complete their whole life cycle within 1 year (e.g. many annuals in arable fields);

(6) *helophytes*: vegetative buds for surviving the harsh season are below the water surface, but the shoot system is mostly above the water surface (e.g. many bright-flowered monocotyledons such as *Iris pseudacorus*); and

(7) *hydrophytes*: the plant shoot remains either entirely under water [e.g. *Elodea* (waterweed)] or partly below and partly floating on the water surface [e.g. *Nymphaea* (waterlily)].

#### *Special cases or extras*

Climbers, hemi-epiphytes and epiphytes may be classified here as phanerophytes or chamaephytes, since their distinct growth forms are classified explicitly above under *Growth form*.

*References on theory and significance*: Raunkiaer (1934); Cain (1950); Ellenberg and Müller-Dombois (1967); Whittaker (1975); Box (1981); Ellenberg (1988).

### Plant height

#### *Brief trait introduction*

Plant height is the shortest distance between the upper boundary of the main photosynthetic tissues on a plant and the ground level, expressed in metres. Plant height is associated with competitive vigour, whole plant fecundity and with the time intervals plant species are generally given to grow between disturbances (fire, storm, ploughing, grazing). There are also important trade-offs between plant height and tolerance or avoidance of environmental (climatic, nutrient) stress. On the other hand, some tall plants may successfully avoid fire reaching the green parts and meristems in the canopy. Height tends to correlate

allometrically with other size traits in broad interspecific comparisons, for instance aboveground biomass, rooting depth, lateral spread and leaf size.

#### *What and how to measure?*

The same type of individuals as for leaf traits (see below) should be sampled, i.e. healthy adult plants that have their foliage exposed to full sunlight (or otherwise plants with the strongest light exposure for that species). However, because plant height is much more variable than some of the leaf traits, measurements are taken preferably on at least 25 individuals per species.

The height to be measured is the height of the foliage of the species, not the height of the inflorescence (or seeds, fruits) or main stem if this projects above the foliage. Measure plant height preferably towards the end of the growing season (but during any period in the non-seasonal Tropics), as the shortest distance between the highest photosynthetic tissue in the canopy and ground level. The height recorded should correspond to the top of the general canopy of the plant, discounting any exceptional branches. In the case of epiphytes or certain hemi-parasites (which penetrate tree or shrub branches with their haustoria), height is defined as the shortest distance between the upper foliage boundary and centre of their basal point of attachment. These and other species that use external support, for instance twiners, vines, lianas and hemi-epiphytes, are measured, but may have to be excluded from certain analyses, for instance those relating to carbon allocation towards mechanical support.

For estimating the height of tall trees the following options are available:

(1) a telescopic stick with metre marks;

(2) measuring the horizontal distance from the tree to the observation point ( $d$ ) and the angles between the horizontal plane and the tree top ( $\alpha$ ) and between the horizontal plane and the tree base ( $\beta$ ). The tree height ( $H$ ) is then calculated as:  $H = d \times [\tan(\alpha) + \tan(\beta)]$ . This method is appropriate in flat areas; and

(3) measuring the following three angles: (i) between the horizontal plane and the tree top ( $\alpha$ ); (ii) between the horizontal plane and the top of an object of known height ( $h$ ; e.g. a pole or person) that is positioned vertically next to the trunk of the tree ( $\beta$ ); and (iii) between the horizontal plane and the tree base (which is the same as the base of the object or person) ( $\gamma$ ). The tree height ( $H$ ) is then calculated as:  $H = h \times [\tan(\alpha) - \tan(\gamma)] / [\tan(\beta) - \tan(\gamma)]$ . This method is appropriate on slopes.

#### *Special cases or extras*

(i) For plants with major leaf rosettes and proportionally very little photosynthetic area higher up (e.g. *Capsella bursa-pastoris*, *Onopordon acanthium*), plant height is based on the rosette leaves.

(ii) In herbaceous species, the potential space occupied can be assessed by using an additional measure called ‘stretched length’. Select a stem (or a tiller in the case of graminoids) whose youngest expanded leaf is fully active (i.e. still green, not eaten and not attacked by any pathogen) and stretch this axis to its maximum height. The distance between the base of the plant and the top of the youngest fully expanded leaf is taken as the ‘stretched length’.

*References on theory and significance:* Beard (1955); Jarvis (1975); Gaudet and Keddy (1988); Niinemets and Kull (1994); Niklas (1994); Gartner (1995); Givnish (1995); Westoby (1998); Gitay *et al.* (1999); Thomas and Bazzaz (1999); Reich (2000); Grime (2001).

*More on methods:* Westoby (1998); McIntyre *et al.* (1999b); Weiher *et al.* (1999).

### Clonality (and belowground storage organs)

#### *Brief trait description*

Clonality is the ability of a plant species to reproduce itself vegetatively, thereby producing new ‘ramets’ (aboveground units) and expanding horizontally. Clonality can give plants competitive vigour and the ability to exploit patches rich in key resources (e.g. nutrients, water, light), while it may promote persistence after environmental disturbances. Clonal behaviour may also be an effective means of short-distance migration under circumstances of poor seed dispersal or seedling recruitment. Clonal organs, especially belowground ones, may also serve as storage organs and the distinction between both functions is often unclear. The tubers and bulbs of geophytes (see 3b, 3c in list below) probably function predominantly for storage and are relatively inefficient as clonal organs.

#### *How to collect and classify?*

For aboveground clonal structures, observe a minimum of five (preferably at least 10) plants that are far enough apart to be unlikely to be connected. For belowground structures, dig up a minimum of five (preferably 10) healthy looking plants during the growing season, from typical sites for each of the predominant ecosystems studied. In some cases (large and heavy root systems), only partial excavation may give sufficient evidence for classification. If possible, use the same plants used to determine 95% rooting depth and Nutrient uptake strategy (see below). The species is considered clonal if at least one plant clearly has one of the clonal organs listed below.

Assign a species according to one of the following three categories here, with subcategories (based mostly on Klimeš and Klimešová 2000):

(1) *non-clonal*;

(2) *clonal aboveground*:

(a) *stolons*: horizontal stems [e.g. *Fragaria vesca* (strawberry), *Lycopodium annotinum* (clubmos)];

(b) *gemmiparous*: adventitious buds on leaves (e.g. *Cardamine pratensis*); and

(c) *other vegetative buds or plant fragments* that can disperse and produce new plants (including axillary buds, bulbils and turions). This category also includes pseudovivipary (vegetative propagules in the inflorescence as in *Polygonum viviparum*), gemmipary (adventitious buds on leaves as in *Cardamine pratensis*) and larger plant fragments that break off and develop (as in *Elodea canadensis*);

(3) *clonal belowground*:

(a) *rhizomes*: more or less horizontal belowground stems [e.g. *Pteridium aquilinum* (bracken)];

(b) *tubers*: modified belowground stems or rhizomes often functioning as storage organs. Tubers are shaped short, thick and (irregularly) rounded, often covered with modified buds but not by leaves or scales [e.g. *Solanum tuberosum* (potato), *Dahlia*];

(c) *bulbs*: relatively short, more or less globose belowground stems covered by fleshy overlapping leaves or scales, often serving as storage organs. There are many representatives among the monocotyledons [e.g. *Tulipa* (tulip); *Allium* (onion); some sedges, Cyperaceae]. Daughter bulbs represent (modest) clonal growth; and

(d) *adventitious root buds* on main root (e.g. *Alliaria petiolata*) or lateral roots (e.g. *Rumex acetosella*).

*References on theory and significance:* De Kroon and van Groenendael (1997); Klimeš *et al.* (1997); Van Groenendael *et al.* (1997); Klimeš and Klimešová (2000).

*More on methods:* Böhm (1979); Klimeš *et al.* (1997); Van Groenendael *et al.* (1997); Weiher *et al.* (1998); Klimeš and Klimešová (2000).

### Spinescence

#### *Brief trait description*

A spine is usually a pointed modified leaf, leaf part or stipule, while a thorn is a hard, pointy modified twig or branch. A prickle is a modified epidermis. The type, size and density of spines, thorns and/or prickles play an obvious role in anti-herbivore defence. Different types, sizes and densities of spines, thorns and prickles may act against different potential herbivores, mostly vertebrate ones. They can play additional roles in reducing heat or drought stress. Spiny plants may also provide other plant species with refuges from herbivores.

#### *How to measure?*

This is a categorical trait assessed through straightforward field or herbarium observation or descriptions in the literature. Spines, thorns and prickles are summarised here as ‘spine equivalents’. Only those on vegetative plant parts (stems, branches, twigs, leaves) are

**Table 4. Classes for components of overall flammability of plant species**

Flammability itself is calculated as the average class value (rounded to 1 decimal) over all component traits. Flammability increases from 1 to 5

	Flammability class				
	1	2	3	4	5
Twig dry matter content (mg g <sup>-1</sup> )	<200	200–400	400–600	600–800	>800
Twig drying time (day)	≤5	4	3	2	≤1
Leaf dry matter content (mg g <sup>-1</sup> )	<150 mg	150–300	300–500	500–700	>700
Degree of ramification (branching) (number of ramification orders)	No branches	Only 1st order ramification	2 orders of ramification	3 orders of ramification	≤4 orders of ramification
Leaf size (lamina area) (mm <sup>2</sup> )	>25000	2500–25000	250–2500	25–250	<25
Standing fine litter in driest season	None	Some	Substantial (with dead leaves/twigs or flaking bark)	More dead than live fine mass aboveground	Shoot dies back entirely, standing as one litter unit
Volatile oils, waxes and/or resins	None	Some	Substantial	Abundant	Very abundant

considered. Spine equivalents are defined as ‘soft’ if, when mature, they can be bent easily by pressing sideways with a finger. Low density is defined as <100 spine equivalents m<sup>-2</sup> twig or leaf (roughly <1 per palm of a big hand) and high density as >1000 m<sup>-2</sup> (>10 per palm). Assign a species according to one of the following categories:

(0) no spines, thorns or prickles;

(1) low or very local density of soft spine equivalents <5 mm; plant may sting or prickle a little when hit carelessly;

(2) high density of soft spine equivalents or intermediate density of spine equivalents of intermediate hardness; or else low density of hard, sharp spine equivalents >5 mm; plant hurts when hit carelessly;

(3) intermediate or high density of hard, sharp spine equivalents >5mm; plant hurts a lot when hit carelessly;

(4) intermediate or high density of hard, sharp spine equivalents >20 mm; plant may cause significant wounds when hit carelessly; and

(5) intermediate or high density of hard, sharp spine equivalents >100 mm; plant is dangerous to careless large mammals including humans!

*References on theory and significance:* Milton (1991); Grubb (1992); Cooper and Ginnett (1998); Pisani and Distel (1998); Olff *et al.* (1999); Hanley and Lamont (2002); Rebollo *et al.* (2002).

### Flammability

#### Brief trait description

In the strict sense, flammability (or ignitability) indicates how easily a plant ignites (i.e. starts to produce a flame), while heat conductivity (combustibility) determines how quickly the flames can spread within the plant. For simplicity and because of the generally positive links between these two parameters at the species level, we consider (overall) flammability to represent both parameters here. Flammability is an important contributor to fire regimes in (periodically) dry regions and therefore it has important ecological impacts (promoting ecosystem dynamics) as well

as economic consequences. The flammability of a plant depends on (1) the type or quality of the tissue and (2) the architecture and structure of the plant and its organs (which is mainly related to heat conductivity).

[Note: The flammability of a given species can be overridden by the combustibility of the entire plant community (e.g. amount of litter, community structure and continuity, organic matter content of the soil) and climatic conditions (e.g. after a long, very dry period many plants would burn independently of their flammability).]

#### How to define and assess?

Flammability is a compound, unitless trait. We first give brief protocols or definitions for the individual components of flammability (see Bond and Van Wilgen 1996 for an overview). Five classes are defined for each component trait. Overall flammability is subsequently calculated as the average (rounded to one decimal) of the class scores for each individual component (see Table 4). For this calculation, twig drying rate (which is probably closely negatively linked with *twig dry matter content*, TDMC; see below) is optional. Do enter values or classes for each component trait into the database as well, since they may themselves be of additional interest for contexts other than flammability. The following component traits are measured:

(1) *Water content of branches, twigs and leaves.* Flammability is expected to be greater in species with higher twig dry matter content (TMDC) and high leaf dry matter content (LDMC) and is probably also a function of the drying rate (here represented inversely by drying time from saturation to dry equilibrium). Detailed protocols for *TDMC*, *twig drying time* and *LDMC* are elsewhere in this handbook.

(2) *Canopy architecture.* Plants with complex architecture, i.e. extensive branching, tend to be more heat conductive. The degree (number of orders) of ramification (branching) is used here as a close predictor of canopy architectural complexity (see Fisher 1986) and ranges from zero (no branches) to 5 (four or more orders of ramification).

(3) *Surface:volume ratios*. Smaller twigs (i.e. twigs of smaller cross-sectional area) and smaller leaves should have a higher surface:volume ratio (and thus, faster drying rate) and therefore be more flammable. Since twig and leaf size tend to be correlated in interspecific comparisons, according to allometric rules (Bond and Midgley 1988; Cornelissen 1999), we use leaf size here to represent both traits. A complication is that some species are leafless during the dry season, but on the other hand the leaf litter is likely to still be around in the community and affect flammability during the dry season. See under *Leaf size* for the detailed protocol.

(4) *Standing litter*. The relative amount of fine dead plant material (branches, leaves, inflorescences, bark) still attached to the plant during the dry season is critical, since litter tends to have very low water content and thus enhance plant flammability. 'Fine' litter means litter with diameter or thickness less than 6 mm. We consider decorticating (flaking) bark to be an important component of standing litter, since it increases the probability of ground fires carrying up into the canopies and developing crown-fire [e.g. in *Eucalyptus* (gum trees)]. We define five subjective classes from no fine standing litter, via 'substantial' fine standing litter to 'the entire aboveground shoot died back as one standing litter unit'.

(5) *Volatile oils, waxes and resins* in various plant parts contribute to flammability. This is a subjective, categorical trait ranging from none to 'very high concentrations'. Check for aromatic (or strong, unpleasant) smells as well as sticky substances that are released on rubbing, breaking or cutting various plant parts. Scenting flowers are not diagnostic for this trait.

This protocol is a new design, therefore we strongly recommend testing and calibrating it against 'hard' measurements of ignitability, fire spread and combustibility described below under *Special cases or extras!*

#### *Special cases or extras*

(i) Ignitability can be measured directly by measuring the time required for a plant part to produce a flame when exposed to heat from a given heat source located at a given distance. Ignitability experiments are usually performed several times (e.g. 50) and the different fuels are ranked by taking into account both the proportion of successful ignitions (inflammation frequency) and the time required to produce flames (inflammation delay). Tissues producing flames quickly in most of the trials are ranked as extremely ignitable, while tissues that rarely produce flames and/or take a long time to produce them are considered of very low ignitability. These experiments are run in the laboratory under controlled conditions (moisture and temperature) by locating a heat source (e.g. electric radiator, epiradiator, open flame) at a given distance (few centimetres) from the sample. If the heat source has no flame (electric radiator or epiradiator), a pilot flame is also needed to initialise the flames from the gas

originated from the heated sample. The values used to rank species according to ignitability depend on the type and power of the heat source, on the distance of the heat source to the sample, on the shape and size of the samples and on the relative humidity of the environment in the days prior to the test; these experimental conditions should be kept constant for all trials and samples. We propose as a standard the method of Valette (1997), who used an open flame at 420°C, placing the plant material at 4 cm from the flame. A standard quantity of 1 g of fresh material is used.

(ii) Plant tissue combustibility can be assessed by the heat content (calorific value, kJ g<sup>-1</sup>), which is a comprehensive measure of the potential thermal energy that can be released during the burning of the fuel. It is measured with an adiabatic bomb calorimeter by using fuel pellets of approximately 1 g, while the relative humidity of the environment in the days prior to the test should be standardised as well. According to Bond and van Wilgen (1996), heat content varies relatively little among species and is only a modest contributor to interspecific variation in flammability.

(iii) In relation to the surface area:volume ratio, other structural variables have been used to characterise the flammability and combustibility, especially the proportion of biomass of different fuel classes (size distribution). Typically, the fuel classes used are the biomass fractions of (a) foliage, (b) live fine woody fuel (<6 mm of diameter; sometimes subdivided in <2.5 and 2.5–6 mm), (c) dead fine woody fuel (<6 mm) and (d) coarse woody fuel (6–25, 26–75, >75 mm). The summed proportion of live and dead fine fuels (foliage and woody of <6 mm) may be the best correlate of overall surface area:volume ratio.

(iv) Fuel bulk density (fuel weight: fuel volume) and fuel porosity (ratio of canopy volume to fuel volume) have also been used to characterise heat conductivity, mainly at the population and community levels. Furthermore, high litter fall and low decomposition rate will increase the combustibility of the community. These two factors are related to the species but also they are strongly related to site and climatic conditions.

*References on theory and significance:* Mutch (1970); Bond and Midgley (1995); Bond and Van Wilgen (1996); Schwilk and Ackerly (2001); Lavorel and Garnier (2002).

*More on methods:* Brown (1970); Papió and Trabaud (1990); Hogenbirk and Sarrazin-Delay (1995); Valette (1997); Dimitrakopoulos and Panov (2001).

## 2.2. Leaf traits

### Specific leaf area (SLA)

#### *Brief trait introduction*

Specific leaf area is the one-sided area of a fresh leaf divided by its oven-dry mass, expressed in m<sup>2</sup> kg<sup>-1</sup> or (correspondingly) in mm<sup>2</sup> mg<sup>-1</sup>. [Note: leaf mass per area

(LMA), specific leaf mass (SLM) or specific leaf weight (SLW), often used in the literature, is simply  $1/\text{SLA}$ .] SLA of a species is in many cases a good positive correlate of its potential relative growth rate or mass-based maximum photosynthetic rate. Lower values tend to correspond with relatively high investments in leaf 'defences' (particularly structural ones) and long leaf lifespan. Species in resource-rich environments tend to have larger SLA than those in environments with resource stress, although some shade-tolerant woodland understorey species are known to have remarkably large SLA as well.

#### *What and how to collect?*

Go for the relatively young (presumably photosynthetically more productive) but fully expanded and hardened leaves from adult plants without obvious symptoms of pathogen or herbivore attack and without substantial cover of epiphylls. Any petiole or rachis (stalk-like midrib of a compound leaf) and all veins are considered part of the leaf for standardised SLA measurement (but see under *Special cases or extras*). We recommend collecting whole twig sections with the leaves still attached and not removing the leaves until just before measurement (see below). For herbaceous and small woody species, take whole leaves from plants in full-light situations (not under tree cover, for instance). For tall woody species, take leaves from plant parts most exposed to direct sunlight during the sampling period ('outer canopy' leaves). Leaves of true shade species, never found in full sunlight, are collected from the least shady places found. Take at least 10 leaves per species (20 leaves from 10 individuals would be preferable, particularly if variability seems high or if a high precision is critical for a particular study, or if leaf size is measured on the same leaves; see under 1.3). For most species, this corresponds to 10 different individual plants; however, if this is impossible some leaves can be taken from the same individual. Since SLA may vary during the day, we recommend to sample leaves at least 2–3 h after sunrise and 3–4 h before sunset.

#### *Storing and processing*

Wrap the samples (twigs with leaves attached) in moist paper and put them in sealed plastic bags, so that they remain water-saturated. Store these in a cool box or fridge (never in a freezer!) 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. If storage is to last for more than 24 h, low temperatures (2–6°C) are essential to avoid rotting. Tissues of some xerophytic species (e.g. bromeliads, cacti) rot very quickly when moist and warm and 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 case

of rotting of the moist-stored ones. For 'soft' leaves, such as those of many herbaceous and deciduous woody species (SLA values higher than 10–15 mm<sup>2</sup> mg<sup>-1</sup>), rehydration for at least 6 h before measurement is essential in order not to underestimate SLA. For rehydration, place the cut end of the stem in deionised water (e.g. in test tubes) in the dark. If storage was dry until measurement, such rehydration is especially important for any species (however, in the case of species sensitive to rotting rehydration should be for maximum 12 h). See Garnier *et al.* (2001b) for good alternative rehydration methods. Measure as soon as possible after collecting (preferably within 48 h).

#### *Measuring*

Each leaf (including petiole) is cut from the stem and gently rubbed dry before measurement. Projected area (as in a photo) can be measured with specialised leaf area meters such as Delta-T (Cambridge, UK) or LiCor (Lincoln, Nebraska, USA). Always check the readings of 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 within the scanning area. If a leaf area meter is not available, an alternative would be to scan leaves as a computer image and measure the area by using image analysis software. Estimating area by weighing paper or plastic cut-outs of similar shape and size and then multiplying by the known area: weight ratio of the paper, may be useful where none of these facilities are available, as long as the paper or plastic is of a constant quality. Try to position the leaves as flat as possible (e.g. by using a glass cover), in the position that gives the largest area, but without squashing them to the extent that the tissue might get damaged. Curled-up leaves may be cut into smaller pieces to facilitate flattening them.

For very small or very narrow leaves or needles, the measuring error by any of these methods may be great, partly because of the pixel size of the projected images. In such cases, we recommend a combination of calibrating the image analysis equipment with objects of similar shape, size and colour [e.g. by cutting up a piece of green paper of known (total) area into several pieces of the desired dimensions] and treating a number of leaves as if they were one. For tiny leaves or needles (a few mm<sup>2</sup> or less), projected areas may need to be estimated by putting them on paper with a millimetre grid, and then using a magnifying glass or binocular microscope ( $\times 10$  magnification). Large drawings of both the leaves and millimetre squares could be compared with the leaf area meter.

For very large leaves that exceed the window of the area meter, do not take one leaf section only. Instead, cut the leaf up into smaller parts and measure the cumulative area of all parts.

Since the projected area does not correspond with half of the true area in significantly non-flat leaves, we strongly

recommend additional measurement of the ratio between the two for such species, so that datasets for both types of areas can be derived. See below under *Special cases or extras*.

After area measurement, place each leaf sample in the oven at 60°C for at least 72 h (or else at 80°C for 48 h), then weigh the dry mass. Be aware that, once taken from the oven, the samples will take up moisture from the air. If they cannot be weighed immediately after cooling down, put them in a desiccator with silica gel until weighing, or else back in the oven to dry off again. As for area, weighing several tiny leaves as if they were one will improve the accuracy, depending on the type of balance used.

For calculating mean, standard deviation or standard error, the average SLA for each individual plant (which is not always each leaf) is one statistical observation.

#### *Special cases or extras*

(i) While we recommend measuring SLA *at least* the above way in order to achieve standardisation (and for reasons given by Westoby 1998), for particular purposes a second series of measurements may be *added*. For instance, SLA of the lamina-only (with or without major veins; leaf discs) may be of interest (quality of the productive leaf tissues), or in evergreen leaves the average SLA of leaf cohorts formed in different years may be used (whole-plant leaf quality). For particular species, SLA based on the total photosynthetic area, which is a function of both projected area and leaf shape, may be of additional interest.

(ii) For leafless plants, take the plant part that is the functional analogue of a leaf and treat as above. For some spiny species (e.g. *Ulex*) this could mean taking the top 2 cm of a young twig, while for cacti and other succulents we recommend cutting off a slice ('the scalp') of the epidermis plus some parenchyma of a relatively young part. The younger stems of some rushes and sedges (*Juncus*, *Eleocharis*) and the 'branches' of horsetails (*Equisetum*) or similar green leafless shoots can be treated as leaves too. Many other examples exist where the data collectors have to decide what they consider to be the leaf analogue. It is important to record the exact method used in such cases.

(iii) For heterophyllous species, for instance plants with both rosette and stem leaves, collect leaves of both types in proportion to their estimated contribution to total leaf area of the plant, in order to obtain a representative species SLA value.

(iv) For certain purposes it is relevant to additionally determine SLA on the basis of actual (rather than projected) one-sided leaf area. This makes a big difference for needles (e.g. *Pinus*) or rolled-up grass leaves (e.g. some *Festuca*). True one-sided leaf area may be approximated in leaf cross-sections (with a microscope) by taking the circumference divided by two and subsequently divide this value by the leaf width.

(v) Note that interspecific rankings of SLA are rather robust to methodological factors (e.g. with or without petioles) and, for coarse-scale comparisons, SLA data from several sources may be combined as long as possible methodological artefacts are at least acknowledged.

*References on theory and significance:* Dijkstra (1989); Bongers and Popma (1990); Witkowski and Lamont (1991); Lambers and Poorter (1992); Poorter and Bergkotte (1992); Popma *et al.* (1992); Reich *et al.* (1992, 1997, 1998, 1999); Garnier and Laurent (1994); Niinemets and Kull (1994); Shipley (1995); Cornelissen *et al.* (1996); Hunt and Cornelissen (1997); Poorter and Van der Werf (1998); Westoby (1998); Cornelissen *et al.* (1999); Poorter and Garnier (1999); Poorter and de Jong (1999); Weiher *et al.* (1999); Wilson *et al.* (1999); Castro-Díez *et al.* (2000); Wright *et al.* (2001); Garnier *et al.* (2001a); Lamont *et al.* (2002); Westoby *et al.* (2002).

*More on methods:* Chen and Black (1992); Westoby (1998); Weiher *et al.* (1999); Garnier *et al.* (2001b).

#### Leaf size (individual leaf or lamina area)

##### *Brief trait introduction*

Leaf size is the one-sided projected surface area (see under *Specific leaf area*) of a single or an average leaf or leaf lamina, expressed in mm<sup>2</sup>. Leaf size has important consequences for the leaf energy and water balance. 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. Within climatic zones, 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, while phylogenetic factors can also play an important role.

##### *What and how to collect?*

For the leaf collecting protocol see under *Specific leaf area*. Leaf size is rather variable within plants and we recommend collecting 20 leaves, ideally being two random but well-lit leaves from each of 10 individual plants. Two leaves from each of five individuals or even five leaves from each of four individuals are alternative options, but only if the species is scarce.

##### *Storing and processing*

For storing leaves, see under *Specific leaf area*.

##### *Measuring*

Measure individual leaf laminae (or leaflets in compound leaves) without petiole or rachis (but see under *Special cases and extras*). Note that this area may be different from the area used to determine SLA.

For calculating mean, standard deviation or standard error, the average leaf size for each individual plant is one statistical observation.

#### *Special cases or extras*

(i) While we recommend measuring leaf size *at least* the above way in order to achieve standardisation, a second series of whole-leaf sizes may be added. The sizes of whole leaves are relevant for certain allometric analyses, for instance. For each measurement, include all leaflets in the case of a compound leaf as well as any petiole or rachis. Note that whole-leaf size is one of the measurements taken for SLA.

(ii) Since leaflessness is an important functional trait, record leaf size as zero for leafless species (not as a missing value). However, be aware that these zeros may need to be excluded from certain data analyses.

(iii) For heterophyllous plants, for instance plants with both rosette and stem leaves, collect leaves of both types in proportion to their estimated contribution to total leaf number of the plant, in order to obtain a representative species leaf size.

(iv) 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.

(v) Be aware that there is a lot of leaf size data in the, often older, literature. Whether this can be used without clear data about the methodology, will depend on the level of precision needed for the particular analysis. Certain coarse-scale (global) analyses may be robust to relatively small methodological deviations.

(vi) An additional related trait of ecological interest is leaf width (Parkhurst and Loucks 1972; Givnish 1987; Fonseca *et al.* 2000). Narrow leaves, or divided leaves with narrow lobes, tend to have more effective heat loss than broad leaves, which is adaptive in warm, sun-exposed environments. Leaf width is measured as the maximum diameter of an imaginary circle that can be fitted anywhere within a leaf (Westoby 1998).

*References on theory and significance:* Parkhurst and Loucks (1972); Orians and Solbrig (1977); Givnish (1987); Bond and Midgley (1988); Körner *et al.* (1989); Popma *et al.* (1992); Richter (1992); Niinemets and Kull (1994); Niklas (1994); Box (1996); Ackerly and Reich (1999); Cornelissen (1999); Moles and Westoby (2000); Westoby *et al.* (2002).

*More on methods:* Cornelissen (1992); Niinemets and Kull (1994); Cornelissen (1999).

#### Leaf dry matter content (LDMC)

##### *Brief trait introduction*

Leaf dry matter content is the oven-dry mass (mg) of a leaf divided by its water-saturated fresh mass (g), expressed

in  $\text{mg g}^{-1}$ . (It is  $1 - \text{leaf water content}$  expressed on a fresh mass basis).

Leaf dry matter content is related to the average density of the leaf tissues and tends to scale with  $1/\text{SLA}$ . It has been shown to correlate negatively with potential relative growth rate and positively with leaf life-span, but the strengths of these relationships are usually weaker than those involving SLA. Leaves with high LDMC tend to be relatively tough (see *Physical strength of leaves below*) and are thus assumed to be more resistant to physical hazards (e.g. herbivory, wind, hail) than leaves with low LDMC. Some aspects of leaf water relations and flammability (see under *Flammability*) also depend on LDMC. Species with low LDMC tend to be associated with productive, often highly disturbed environments. In cases where leaf area is difficult to measure (see above), LDMC may give more meaningful results than SLA, although the two traits may not capture exactly the same functions.

##### *What and how to collect?*

Follow exactly the same procedure as for *Specific leaf area* (see above). In most cases, the same leaves will be used for the determination of both SLA and LDMC. As for SLA, since LDMC may vary substantially during the day, it is recommended to sample leaves in the field at least 2–3 h after sunrise and 3–4 h before sunset.

##### *Storing and processing*

Similar as for SLA, except that rehydration prior to measurement is compulsory. For xerophytic species particularly sensitive to rotting (see under *Specific leaf area*), we recommend dry storage and between 6 and 12 h of rehydration before measurement.

##### *Measuring*

Following the rehydration procedure, the leaves are cut from the stem and gently blotted dry with tissue paper to remove any surface water before measuring water-saturated fresh mass. Each leaf sample is then dried in an oven (see under *Specific leaf area*) and its dry mass subsequently determined.

For calculating mean, standard deviation or standard error, the average LDMC for all the measured leaves of one individual plant (which is not always a single leaf) is one statistical observation.

##### *Special cases or extras*

(i) Most comments for SLA apply also to LDMC.

(ii) In some species such as resinous and succulent xerophytes, rehydration in the laboratory may prove difficult. An alternative method is to collect leaf samples in the field in the morning following a rainfall event.

*References on theory and significance:* Eliáš (1985); Garnier (1992); Garnier and Laurent (1994); Cornelissen *et al.* (1996, 1997); Ryser (1996); Grime *et al.* (1997); Cunningham *et al.* (1999); Hodgson *et al.* (1999); Niinemets (1999, 2001); Poorter and Garnier (1999); Roderick *et al.* (1999); Ryser and Aeschlimann (1999); Wilson *et al.* (1999); Ryser and Urbas (2000); Garnier *et al.* (2001a); Shipley and Vu (2002); Vendramini *et al.* (2002); Wright and Westoby (2002).

*More on methods:* Weiher *et al.* (1999); Wilson *et al.* (1999); Garnier *et al.* (2001b); Vendramini *et al.* (2002).

### Leaf nitrogen concentration (LNC) and leaf phosphorus concentration (LPC)

#### *Brief trait introduction*

Leaf nitrogen concentration (LNC) and LPC are the total amounts of N and P, respectively, per unit of dry leaf mass, expressed in mg g<sup>-1</sup>. Interspecific rankings of LNC and LPC are often correlated. Across species, LNC tends to be closely correlated with mass-based maximum photosynthetic rate. High LNC or LPC is generally associated with high nutritional quality to the consumers in food webs. However, LNC and LPC of a given species tend to vary significantly with the N and P availability in their environments. The LNC:LPC (N:P) ratio is used as a tool to assess whether the availability of N or P is more limiting for carbon cycling processes in ecosystems.

#### *What and how to collect?*

See under *Specific leaf area* for the leaf collecting procedure. Initial leaf saturation is not necessary. However, any petiole or rachis is cut off before LNC and LPC analysis. Therefore, leaves used for leaf-size analysis can be taken. In that case, oven dry these (72 h at 60°C or 48 h at 80°C). Oven-dried leaves used for SLA analyses may be used too, after removing any petiole or rachis. For replication see under *Leaf size*. Note that replication is at the individual plant level, so one replicate sample should be one or more (pooled) leaves from one plant.

#### *Storing and processing*

After oven-drying the leaves without petiole or rachis (see above), store the material air-dry and dark until use, up to a maximum of 1 year. Grind each replicate leaf or replicate group of leaves separately. Manual grinding with mortar and pestle is okay for smaller numbers of samples, but poses a serious health risk for larger quantities (repetitive strain injury). Effective, inexpensive mechanic grinders are available. Make sure to avoid inter-sample contamination by cleaning the grinder carefully between samples. Use a ball mill for small samples. Dry the ground samples again in the oven at 60 or 80°C for at least 12 h prior to analysis.

#### *Measuring*

A number of techniques are available to measure N and P concentrations in ground plant material. Kjeldahl analysis, including acid digestion followed by colorimetric (flow-injection) analysis, is widely used (e.g. Allen 1989). Other methods employ a combination of combustion element analysis, converting organic matter into N and CO<sub>2</sub> and mass spectrometry or gas chromatography. We take the view that most laboratories use one of such standard methods, which should give reasonably accurate LNC and LPC. We recommend running a standard reference material with known LNC and LPC along with the samples, for instance standard hay powder, CRM 129 from the Laboratory of the Government's Chemist, The Office for Reference Materials, Teddington, United Kingdom. Be aware that LNC and LPC have been recorded in numerous ecological, agricultural and forestry studies in many parts of the world and a literature search for existing data may save a lot of effort and money. However, the methodology used needs to be judged critically in such cases.

#### *Special cases or extras*

(i) While we recommend measuring LNC and LPC *at least* on leaf samples as described here (the lamina or leaflet being the unit of interest in relation to photosynthetic capacity), for particular purposes a second series of measurements may be added. For instance, LNC or LPC of the whole leaf (including petiole or rachis) may be of interest (link with SLA; allometric relationships), or in evergreen leaves the average LNC or LPC of leaf cohorts formed in different years may be used (whole-plant nutritional leaf quality).

(ii) For leafless or heterophyllous plants, use similar material as recommended for SLA.

(iii) Be aware that LNC and LPC can be influenced strongly by the availability of N and P in the soil. For an overall species value, we recommend sampling in the predominant ecosystems in a particular area and taking the average of all ecosystem mean values.

(iv) In woody species, most of the N tends to be organically bound. In herbaceous species in nutrient-rich soils, part of the N can be present in the form of nitrate. However, most of this would be in the petiole, which is not included in LNC measurement.

*References on theory and significance:* Garten (1976); Chapin (1980); Field and Mooney (1986); Grimshaw and Allen (1987); Hirose and Werger (1987); Bongers and Popma (1990); Grime (1991); Lambers and Poorter (1992); Poorter and Bergkotte (1992); Reich *et al.* (1992, 1997); Schulze *et al.* (1994); Huante *et al.* (1995); Marschner (1995); Aerts (1996); Koerselman and Meuleman (1996); Nielsen *et al.* (1996); Cornelissen and Thompson (1997); Cornelissen *et al.* (1997); Grime *et al.* (1997); Thompson

*et al.* (1997a); Aerts and Chapin (2000); Garnier *et al.* (2001a); Wright *et al.* (2001).

*More on methods:* Allen (1989); Anderson and Ingram (1993); Hendry and Grime (1993).

### Physical strength of leaves

#### *Brief trait description*

The physical strength of leaves can be defined and measured in different ways. Here we define *leaf resistance to fracture* (also called ‘force of fracture’ or ‘work to shear’) as the mean force needed to cut a leaf or leaf fragment at a constant angle (20°) and speed (e.g. Wright and Cannon 2001), expressed in Newtons (N) or its analogue,  $\text{J m}^{-1}$ . *Leaf tensile strength* is the force needed to tear a leaf (fragment) divided by its width (e.g. Cornelissen and Thompson 1997), expressed in  $\text{N mm}^{-1}$ . These related traits are good indicators of the relative carbon investment in structural protection of the photosynthetic tissues. Physically stronger leaves are better protected against abiotic (e.g. wind, hail) and biotic mechanical damage (e.g. herbivory), contributing to longer leaf lifespans. However, other defences against herbivores are important too (e.g. spines, secondary metabolites for chemical defence). Physical investments in leaf strength tend to have afterlife effects in the form of poor litter quality for decomposition.

#### *What and how to collect?*

For the selection and collecting procedure see under *Specific leaf area*. If possible, collect two young but fully expanded and hardened leaves from each of 10 plant individuals.

#### *Storing and processing*

Follow the procedure described for SLA and store leaves in a cool box or fridge. Measure as soon as possible after collecting, certainly within a few days for species with ‘delicate’ leaves. (Tougher leaves tend to keep their strength for a few weeks; I. J. Wright, pers. comm.) If this is not possible (for instance if samples have to be sent away), an alternative is to air-dry the samples immediately after collecting. But in such cases make sure the leaves do not break at any time.

#### *Measuring*

For fresh samples, proceed to measuring straight away. For air-dried samples, first rehydrate by wrapping in moist paper and put in a sealed plastic bag in the fridge for 24 h. (Gentle spraying may be better for some xerophytic, rotting-sensitive species; see under *Specific leaf area*.) Here we describe two methods that have produced good results and for which purpose-built equipment is available for use. In order to promote standardisation of large (regional or global) datasets, we strongly recommend cross-calibration

between both methods by (1) measuring certain leaf populations both ways and (2) including measurements with international-standard-cotton strips (‘Soil burial cloth’, supplier Shirley Dyeing and Finishing Ltd, Unit B6, Newton Business Park, Talbot Road Hyde, Cheshire SK14 4UQ, UK).

(1) *Leaf resistance to fracture.* For measuring the average force needed to fracture a leaf at a constant shearing angle of 20° and speed, Wright and Cannon (2001) described and illustrated an apparatus, a calibrated copy of which is available for use at CNRS in Montpellier, France (contact Eric Garnier, email [garnier@cefe.cnrs-mop.fr](mailto:garnier@cefe.cnrs-mop.fr)). Leaves are cut at right angles to the midrib, at the widest point along the lamina (or halfway between base and tip if this is difficult to determine).

(2) *Leaf tensile strength.* Cut a leaf fragment from the central section of the leaf, but away from the midrib (central vein) unless the latter is not obvious (e.g. some grasses Poaceae, some Liliaceae). For tiny leaves, the whole leaf may need to be measured. The length of the fragment follows the longitudinal axis (direction of main veins). The width of the leaf or leaf fragment depends on the tensile strength and tends to vary between 1 mm (extremely tough species) and 10 mm (delicate species). Measure the exact width of the leaf sample. Then fix both ends of the sample in the clamps of the ‘tearing apparatus’ described by Hendry and Grime (1993). Try to do this gently, without damaging the tissues, if at all possible. (Slightly succulent leaves may be clamped tightly without much tissue damage using strong double-sided tape.) Then pull slowly, with increasing force, until it tears. The spring balance holds the reading for the force at the moment of tearing. A very similar calibrated copy of the apparatus described and illustrated in Hendry and Grime (1993) is available for use in Argentina (contact Sandra Díaz; address above, email [sdiaz@gtwing.efn.uncor.edu](mailto:sdiaz@gtwing.efn.uncor.edu)). For conversion, remember that 1 kg = 10 N. Divide the total force by the width of the leaf fragment to obtain leaf tensile strength.

Leaves too tender to provide an actual measurement with the apparatus have an arbitrary tensile strength of zero. For leaves too tough to be torn, first try a narrower sample (down to 1 mm if necessary and possible). If still too tough, then tensile strength equals the maximum possible value in apparatus (assuming sample width of 1 mm). Some leaves are so tough that they defy being cut by the apparatus at all. In the case of highly succulent leaves (or modified stems), which would be squashed if clamped into the apparatus, carry out the measurements on epidermis fragments.

(3) *Other methods.* With some slight creative adjustments, specialised equipment to tear cotton strips used in soil decomposition assays (e.g. Mecmesin Ultra Test Tensiometer, Mecmesin, UK) can also be applied to directly measure leaf tensile strength (J. H. C. Cornelissen, unpubl. data). Good, alternative leaf-shearing methods are also

available (e.g. Wright and Illius 1995). In all such cases, interspecific comparisons are possible, but for broad comparisons combining different methods, we strongly recommend calibration against one of the above devices as well as including cotton strips (see above).

For calculating mean, standard deviation or standard error, the average leaf strength value (by any method) for each individual plant (which is not always each leaf) is one statistical observation.

#### *Special cases or extras*

(i) Some plants have organs other than leaves as the major photosynthetic organs (e.g. Cactaceae). In those cases, we consider the photosynthetic organ as a leaf, and treat it accordingly. For leafless plants with non-succulent photosynthetic stems, we consider the terminal, greenest, most tender stems as leaves (see under *Specific leaf area*).

(ii) An additional test of leaf strength is leaf puncturability (Aranwela *et al.* 1999), which provides data for the resistance of the actual leaf tissues (particularly the epidermis) to rupture, excluding toughness provided by midribs and main veins. Different point penetrometers have been used (there is no standard design), all of which have some kind of fine needle (diameter *c.* 1–1.5 mm) attached to a spring-loaded balance or a counterweight (being a container gradually filled with water and weighed after penetration). Express the data in  $\text{N mm}^{-2}$ . Consistency across the leaf tends to be reasonable as long as big veins are avoided. Three measurements per leaf are probably sufficient. This test does not work well for many grasses and other monocots.

(iii) Another interesting additional parameter of leaf strength is leaf tissue toughness, derived by dividing leaf resistance to fracture or leaf tensile strength by the (average) thickness of the leaf sample (Hendry and Grime 1993; Wright and Cannon 2001).

*References on theory and significance:* Grubb (1986); Coley (1988); Vincent (1990); Choong *et al.* (1992); Turner (1994); Wright and Illius (1995); Choong (1996); Wright and Vincent (1996); Cornelissen and Thompson (1997); Cornelissen *et al.* (1999); Lucas *et al.* (2000); Pérez-Harguindeguy *et al.* (2000); Wright and Cannon (2001); Wright and Westoby (2002).

*More on methods:* Hendry and Grime (1993); Wright and Illius (1995); Aranwela *et al.* (1999); Wright and Cannon (2001).

### Leaf lifespan

#### *Brief trait description*

Leaf lifespan (longevity) is defined as the time period during which an individual leaf (or leaf analogue) or part of a leaf (see *Monocotyledons*, below) is alive and physiologically active. It is expressed in months. Long leaf

lifespan is often considered a strategy to conserve nutrients in habitats with environmental stress. It is also central in the important trade-off between plant growth rate and plant protection ('defences') or nutrient conservation. Species with longer-lived leaves tend to invest significant resources in leaf protection and (partly as a consequence) grow more slowly than species with short-lived leaves; they also conserve internal nutrients longer. The litter of (previously) long-lived leaves tends to be relatively resistant to decomposition.

#### *Measuring*

Different methods are required for different kinds of phenological patterns and leaf demographic patterns. In all cases, select (parts of) healthy, adult plants exposed to full sunlight or as close as possible to full sunlight for the particular species.

#### (a) *Dicotyledons*

Method 1 (see below) is best but is most labour-intensive and takes a longer time period. Methods 2–4 can replace Method 1 if the criteria are met. If they are not, Method 1 is the only viable option.

(1) *Periodic census of tagged leaves.* This is the best but most labour-intensive method. Tag individual leaves (not leafy cotyledons!) as they unfold for the first time at a census interval and record periodically (at intervals roughly 1/10 of 'guesstimated' lifespan) whether they are alive or dead. Sample all leaves from at least two shoots or branches from at least three individuals, preferably more. Census a minimum of 36 leaves per species, preferably at least 120. Calculate the lifespan for each individual leaf and take the average.

(2) *Count leaves produced and died over a time interval.* This is a good method under some conditions. Count (for each shoot or branch) the total number of leaves produced and died over a time interval that represents a period of apparent equilibrium for leaf production and mortality (see below). We recommend about eight counts over this time interval, but a higher frequency may be better in some cases. Then estimate mean leaf lifespan as the mean distance in time between the accumulated leaf production number and the accumulated leaf mortality number (facilitated by plotting leaf production and leaf death against time). This is a good method if the census is long enough to cover any kinds of seasonal periodicity (so typically it needs to be several months up to a year if seasonal periodicity occurs) and the branch or shoot is in quasi-equilibrium in terms of leaf production and mortality. This period can be much shorter for fast-growing plants such as tropical rainforest pioneers, woody pioneers in temperate zone or many herbs.

This technique is useful for plants in their exponential growth phase and for plants with very long leaf lifespan (because one gets data much more quickly).

(3) *Counting 'cohorts'* for many conifers and only some woody angiosperms. For woody angiosperms it is important to be very familiar with the species. This method is very easy and quick, but can only be used under strict conditions. These conditions are that a species is known to produce foliage at regular, known intervals (such as once per year) and that each successive cohort can be identified either by differences in foliage properties or by scars or other marks on the shoot or branch. In that case, it is simple to count, branch by branch, the number of cohorts with more than 50% of original foliage until one gets to the cohort with less than 50% of original foliage and use that as the estimate of mean lifespan. This works *if* there is little leaf mortality for younger cohorts and most mortality occurs in the year of peak 'turnover'. Many conifers, especially *Pinus* and *Picea*, show this pattern. This method gives a slight over-estimate, since there is some mortality in younger cohorts and usually no or very few survivors in the cohorts older than this 'peak turnover' one. This method can also work (a) if there is some mortality in younger cohorts and a roughly equal proportion of survivors in cohorts older than the first cohort with >50% mortality, or (b) if one estimates percentage mortality cohort by cohort. This can be tricky. For instance, some conifers may appear to be missing needles (judging from scars) that were never there in the first place because of reproductive structures. Be aware that in Mediterranean-type climates some species experience two growing seasons.

(4) *Phenology* for species that produce most of leaves in a single 'cohort' within a small time period and 'drop' them all within a small time period. See also below under *Leaf phenology*. The main examples are deciduous trees in the cold-temperate biome and some rain-green plants in (semi-)arid regions, such as ocotillo, *Fouquieria splendens*. Track the phenology twice a month. Binoculars can be useful here. At each visit, estimate (very crudely) the percentage of the potential maximum canopy foliage that is occupied by each of the following: (a) new expanding leaves; (b) young, fully expanded leaves; (c) mature leaves; (d) mix of green and senescing leaves; (e) mostly senescing or senescent leaves. From these data, derive the following two time intervals: (1) from the first time that 20% of potential canopy foliage has unfolded until the first time that 20% of the leaves have senesced; (2) the last time that 20% of potential canopy foliage has unfolded until the time that the last 20% of the leaves have senesced. Mean leaf lifespan is the average of these two intervals.

#### (b) *Monocotyledons*

For some monocot species, the longevity of entire blades can be measured as described above. However, in some grasses and related taxa, the blade continues to grow new tissue while senescing old tissue over time, making the mean lifetime much less meaningful than for a leaf blade that is 'even-aged' throughout its entire area. In this case, one can

assess the production and mortality of specific zones of the blade (akin to Method 2 above), to estimate the tissue longevity.

#### *Special cases or extras*

(i) For very long-lived leaves in seasonal biomes, try to recognise individual years as stem or branch growth segments—look for dense structures or lines across branches, indicating slow growth in winter or dry periods. The first annual segment that has significant numbers of dead leaves or leaf scars could be interpreted as the leaf lifespan. Alternatively, give the minimum leaf lifespan within the census period (e.g. >24 months).

(ii) For leafless plants on which photosynthetic tissues do not die and fall off as separate units, follow Method 2 for specific zones of the photosynthetic tissues, as specified for grasses.

*References on theory and significance:* Chabot and Hicks (1982); Southwood *et al.* (1986); Coley (1988); Harper (1989); Williams *et al.* (1989); Kikuzawa (1991); Reich *et al.* (1992); Aerts (1995); Cornelissen (1996a); Ryser (1996); Cornelissen and Thompson (1997); Diemer (1998); Garnier and Aronson (1998); Ackerly (1999); Kikuzawa and Ackerly (1999); Westoby *et al.* (2000); Craine and Reich (2001); Villar and Merino (2001); Wright and Cannon (2001); Wright *et al.* (2002); Navas *et al.* (2003).

*More on methods:* Jow *et al.* (1980); Southwood *et al.* (1986); Williams *et al.* (1989); Reich *et al.* (1991); Diemer (1998); Craine *et al.* (1999); Dungan *et al.* (2003); Navas *et al.* (2003).

#### *Leaf phenology (seasonal timing of foliage)*

##### *Brief trait description*

We define leaf phenology as the number of months per year that the leaf canopy (or analogous main photosynthetic unit) is green. Certain groups of competition avoiders may have very short periods of foliar display (and short life cycles in some annuals) outside the main foliage peak of the more competitive species. Species that colonise gaps after major disturbance events may belong to this group too. Deciduous species avoid losing precious foliar resources by resorbing them and then dropping the leaves before the onset of a drought season or winter. Evergreen species have the advantage of a year-round ability to photosynthesise and they manage important growth at the beginning of the favourable season, before the seasonally green species start competing for light. Many spring geophytes below deciduous tree canopies display a similar strategy.

##### *Measuring*

Track five plant individuals for phenological status several times throughout the year. We recommend a census for all species in the survey at least once a month during the

favourable season (preferably including a census shortly before and shortly after the favourable season) and, if possible, one during the middle of the unfavourable season. During periods of major change, two visits a month are better still. The months in which the plants are estimated to have at least 20% of their potential peak-season foliage area, are interpreted as ‘green’ months.

This census can be combined with assessment of *Leaf lifespan* (see above). Most species with individual leaf lifespans >1 year will be green throughout the year. Note that in some evergreen species from the aseasonal tropics, individual leaf lifespans can be as short as a few months only.

*References on theory and significance:* Lechowicz (1984); Kikuzawa (1989); Aerts (1995); Reich (1995); Cornelissen (1996b); Diemer (1998); Jackson *et al.* (2001); Castro-Díez *et al.* (2003); Lechowicz (2002).

*More on methods:* Diemer (1998); Castro-Díez *et al.* (2003).

### Photosynthetic pathway

#### *Brief trait description*

Three main photosynthetic pathways operate in terrestrial plants, each with their particular biochemistry: C<sub>3</sub>, C<sub>4</sub> and CAM (crassulacean acid metabolism). These pathways have important consequences for optimum temperatures for photosynthesis and growth (higher in C<sub>4</sub> than in C<sub>3</sub> plants), water and nutrient use efficiencies and responsiveness to elevated CO<sub>2</sub>. Compared with C<sub>3</sub> plants, C<sub>4</sub> plants tend to perform well in warm, sunny and relatively dry and/or salty environments (e.g. in tropical savanna-like ecosystems), while CAM plants are generally very conservative with water and occur predominantly in dry ecosystems. Some submerged aquatic plants have CAM too. There are obligate CAM species and facultative ones, which may switch between C<sub>3</sub> and CAM, depending on environmental factors (e.g. epiphytic orchids in high-elevation Australian rainforest, see Wallace 1981). Two main identification methods are available, carbon isotope composition and anatomical observations. Which to choose (a combination would be the most reliable) depends on facilities or funding or on which expertise is locally available. Carbon isotope composition, which can be used to detect differences in biochemical composition, can also be affected by environmental factors, intraspecific genetic differences and/or phenological conditions, but such intraspecific variability is generally small enough not to interfere with the distinction between photosynthetic pathways. In many plant families only C<sub>3</sub> metabolism has been found. It is useful to know in which families C<sub>4</sub> and CAM have been found, so that species from those families can be screened systematically as potential candidates for these pathways; see Tables 5 and 6. We describe both a ‘hard’ and a ‘soft’ method, since many labs will have facilities and expertise for

only one of these methods, while both will give reliable results at least for the C<sub>4</sub> v. C<sub>3</sub>/CAM distinction.

#### *What and how to collect?*

Collect the fully expanded leaves or analogous photosynthetic structures of adult, healthy plants growing in full sunlight or as close to full sunlight as possible. We recommend sampling at least three leaves from each of three individual plants. If conducting anatomical analysis (see under *Anatomical analysis*), store at least part of the samples fresh (see under *Specific leaf area*).

#### *(a) Carbon isotope analysis*

##### *Storing and processing*

Dry the samples immediately after collecting. Once dry, the sample can be stored for long periods of time without affecting its isotope composition. If this is not possible, the sample should first be stored moist and cool (see under *Specific leaf area*) and then be dried as quickly as possible at 70–80°C to avoid loss of organic matter (through leaf respiration or microbial decomposition). Although not the preferred procedure, samples can also be collected from a portion of a herbarium specimen. Be aware that insecticides or other sprays to preserve the voucher can affect the isotope composition.

Bulk the replicate leaves or tissues for each plant, then grind the dried tissues thoroughly to pass through a 40-µm or finer mesh screen. It is often easier with small samples to grind all of the material with mortar and pestle. Only small amounts of tissue are required for a carbon isotope ratio analysis. In most cases, less than 3 mg of dried organic material is used.

##### *Measuring*

Carbon isotope ratios of organic material ( $\delta^{13}\text{C}_{\text{leaf}}$ ) are measured with an isotope ratio mass spectrometer (IRMS, precision between 0.03 and 0.3‰, dependent on the IRMS used). Carbon isotope ratios ( $\delta^{13}\text{C}$ ) are calculated as

$$\delta^{13}\text{C} = 1000 \times [(R_{\text{sample}}/R_{\text{standard}}) - 1],$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the <sup>13</sup>C:<sup>12</sup>C ratios of the sample and the standard (PeeDee Belemnite), respectively (Farquhar *et al.* 1989).

After isotopic analysis, the photosynthetic pathway of the species can be determined on the basis of the following (see Fig. 1):

- C<sub>3</sub> photosynthesis, when  $\delta^{13}\text{C} = -21$  to  $-35\text{‰}$ ,
- C<sub>4</sub> photosynthesis, when  $\delta^{13}\text{C} = -10$  to  $-14\text{‰}$ ,
- facultative CAM, when  $\delta^{13}\text{C} = -15$  to  $-20\text{‰}$ , and
- obligate CAM, when  $\delta^{13}\text{C} = -10$  to  $-14\text{‰}$ .

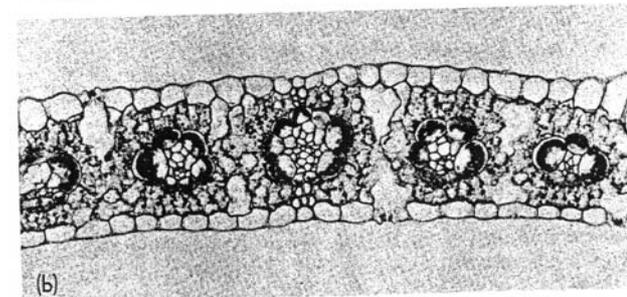
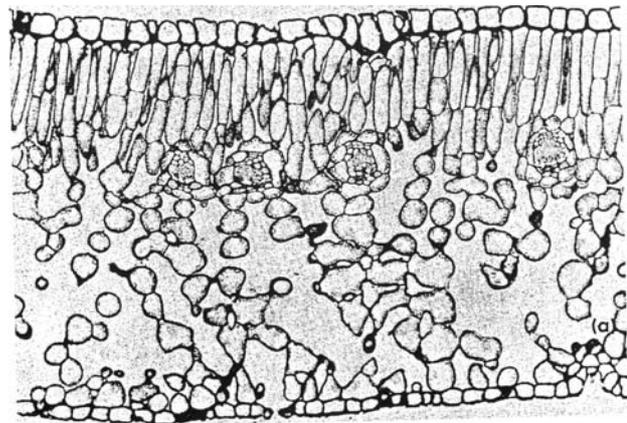
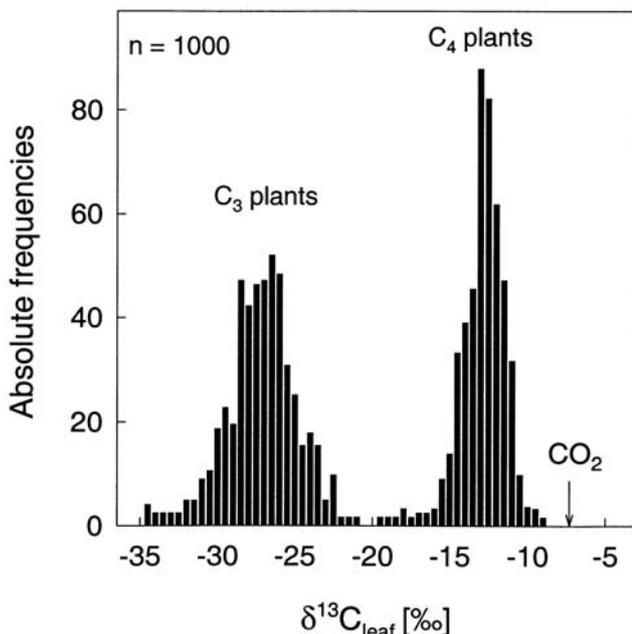
Separating C<sub>4</sub> and CAM plants can be difficult based on  $\delta^{13}\text{C}$  alone. However, as a rule of thumb, if  $\delta^{13}\text{C}$  is between  $-10$  and  $-15\text{‰}$  and the photosynthetic tissue is succulent,

**Table 5. List of families in which C<sub>4</sub> photosynthesis has been reported**Genera in which both C<sub>3</sub> and C<sub>4</sub> metabolism occur are given in parentheses (Osmond *et al.* 1980; Sage 2001)

Acanthaceae	Euphorbiaceae ( <i>Euphorbia</i> )
Aizoaceae ( <i>Mollugo</i> )	Hydrocharitaceae
Amaranthaceae ( <i>Alternanthera</i> )	Molluginaceae
Asteraceae (=Compositae) ( <i>Flaveria</i> )	Nyctaginaceae ( <i>Boerhaavia</i> )
Boraginaceae ( <i>Heliotropium</i> )	Poaceae (=Gramineae) ( <i>Alloteropsis</i> , <i>Panicum</i> )
Capparidaceae	Polygonaceae
Caryophyllaceae	Portulacaceae
Chenopodiaceae ( <i>Atriplex</i> , <i>Bassia</i> , <i>Kochia</i> , <i>Suaeda</i> )	Scrophulariaceae
Cyperaceae ( <i>Cyperus</i> , <i>Scirpus</i> )	Zygophyllaceae ( <i>Kalistromia</i> , <i>Zygophyllum</i> )

**Table 6. List of families in which CAM has been reported (Kluge and Ting 1978; Zotz *et al.* 1997; Crayne *et al.* 2001)**

Agavaceae	Geraniaceae
Aizoaceae	Lamiaceae (=Labiatae)
Asclepiadiaceae	Liliaceae
Asteraceae (=Compositae)	Oxalidaceae
Bromeliaceae	Orchidaceae (photosynthetic roots)
Cactaceae	Piperaceae
Clusiaceae	Portulacaceae
Crassulaceae	Rapateaceae?
Cucurbitaceae	Vitaceae
Didieraceae	Also some ferns have CAM
Euphorbiaceae	

**Fig. 2.** Comparison of leaf anatomy of (a) a typical C<sub>3</sub> plant (top) and (b) a typical C<sub>4</sub> plant (bottom).**Fig. 1.**  $\delta^{13}\text{C}$  of C<sub>3</sub> and C<sub>4</sub> plants (redrawn from O'Leary 1981).

then the plant is CAM. In such cases, anatomical observations would be decisive (see below).

#### (b) Anatomical analysis

C<sub>3</sub> and C<sub>4</sub> plants show consistent differences in leaf anatomy, best seen in a cross-section. With a razor blade or microtome, make cross-sections of leaf blades of at least

three plants per species, making sure to include some regular veins (particularly thick and protruding veins are not examined). Basically, C<sub>3</sub> plants have leaves in which all chloroplasts are essentially similar in appearance and spread over the entire mesophyll (photosynthetic tissues). The mesophyll cells are not concentrated around the veins and are usually organised in layers going from upper to lower epidermis (see Fig. 2a). The cells directly surrounding the veins (transport structures with generally thicker-walled phloem and xylem cells), called bundle-sheath cells, contain no chloroplasts. C<sub>4</sub> plants exhibit 'Kranz anatomy', viz. the veins are surrounded by a distinct layer of bundle-sheath cells (see Fig. 2b). These cells are often thick-walled and show large concentrations of chloroplasts, which contain large (visible) concentrations of starch. The mesophyll cells are usually concentrated around the bundle-sheath cells and

contain less conspicuous chloroplasts with grana (stacks of membranes containing chlorophyll) but no obvious starch concentration. These differences can usually be identified easily under a regular light microscope. There are many plant physiology and anatomy textbooks with further pictures, for instance Bidwell (1979, p. 359), Fahh (1990, pp. 224–245), Taiz and Zeiger (1991, p. 235), Mohr and Schopfer (1995, p. 248) and Lambers *et al.* (1998, p. 65:  $C_4$  anatomy).

If Kranz anatomy is observed, the species is  $C_4$ . If not, it is  $C_3$  unless the plant is particularly succulent and belongs to one of the families with CAM occurrence. In the latter case, it could be classified as (possible) CAM. If living plants are within easy reach, an additional check might be to determine the pH of the cytoplasm fluid (after crushing) from fresh leaf samples in the afternoon and repeat this procedure (with new fresh samples from the same leaf population) in the very early morning. Since Crassulacean (mostly malic) acid builds up during the night, CAM species show a distinctly lower pH after the night than in the afternoon. However, carbon isotope discrimination would be needed to verify CAM metabolism unambiguously [see under *Carbon isotope analysis* above].

#### *Special cases or extras*

(i) A range of methods is available for making the microscope slides permanent, but beware that some may result in poorer visibility of the chloroplasts. One method to retain the green colour of the chloroplasts is to soak the plant or leaves in a mixture of 100 g  $\text{CuSO}_4$ , 25 mL 40% formal alcohol, 1000 mL distilled water and 0.3 mL 10%  $\text{H}_2\text{SO}_4$  for 2 weeks, then in 4% formal alcohol for 1 week, subsequently rinse with tap water for 1–2 h and store in 4% formal alcohol until use.

(ii) Photographs of the slides are an alternative way to keep them for later assessment.

*References on theory and significance:* Kluge and Ting (1978); Osmond *et al.* (1980); O'Leary (1981); Wallace (1981); Farquhar *et al.* (1989); Poorter (1989); Earnshaw *et al.* (1990); Ehleringer (1991); Ehleringer *et al.* (1997); Lüttge (1997); Zotz and Ziegler (1997); Lambers *et al.* (1998); Wand *et al.* (1999); Pyankov *et al.* (2000); Sage (2001); Hibberd and Quick (2002).

*More on methods:* Farquhar *et al.* (1989); Ehleringer (1991); Belea *et al.* (1998); Pierce *et al.* (2002).

### Leaf frost sensitivity

#### *Brief trait introduction*

Leaf frost sensitivity is related to climate and plant geographical distribution. Leaves of species from warmer regions and/or growing at warmer sites along a steep regional climatic gradient have shown greater frost sensitivity than those of species from colder regions and/or growing at colder sites within a regional gradient (Gurvich

*et al.* 2002). Leaf sensitivity to freezing can be assessed by the electrolyte leakage technique, expressed here as percentage of electrolyte leakage (PEL). When a cell or tissue experiences an acute stress, one of the first responses is a change in the physical properties of membranes. This alters the cell's ability to control electrolyte loss. Electrolyte leakage from a tissue, an indicator of membrane permeability, can be easily assessed by measuring changes in electrolyte concentration (conductivity) of the solution in which the tissue is submerged. The technique has been shown to be suitable for a wide range of leaf types (tender and sclerophyllous) and taxa (monocotyledons and dicotyledons) and not to be affected by cuticle thickness.

#### *What and how to collect?*

Collect young, fully expanded sun leaves with no sign of herbivory or pathogen damage, during the peak growing season (see under *Specific leaf area*). If a species grows along a wide environmental gradient and the objective is an interspecific comparison, collect the leaves from the point of the gradient where the species is most abundant. If many species are considered, try to collect them within the shortest possible time interval, to minimise differences due to acclimation to different temperatures in the field. Collect leaves from at least five randomly chosen adult individuals of each species.

#### *Storing and processing*

Store the leaf material in a cool container until processed in the lab (see under *Specific leaf area*). Process the leaves on the same day of harvesting in order to minimise natural senescence processes. For each plant, cut four 5-mm-diameter round leaf fragments (i.e. two treatments with two fragments per Eppendorf each, see below), avoiding the main veins. In some cases, for example species with needle-like leaves, it is impossible to cut 5-mm-diameter fragments. In those cases, cut fragments of the photosynthetically active tissue adding up to a similar area. Rinse the leaf fragments for 2 h in deionised water on a shaker and then blot them dry and submerge them in 1 mL of deionised water in Eppendorf tubes. Making sure that leaves are fully submerged is an important aspect of the technique. Place two 5-mm leaf fragments per tube. Prepare six replicates (one replicate = one tube containing two leaf fragments) per treatment per species, corresponding with the number of plants sampled.

#### *Measuring*

Apply two treatments to the leaf fragments contained in the tubes: (1) incubation at 20°C (or at ambient temperature, as stable as possible) for the control treatment and (2) incubation at –8°C in a calibrated freezer, for the freezing treatment. Apply treatments without any acclimation.

Incubations have to be carried out for 14 h in complete darkness, to avoid light-induced reactions.

After applying the treatment, let the samples reach ambient temperature and then measure the conductivity of the solution. Measure conductivity by taking a sample of the solution in each Eppendorf tube and placing it into a standard previously calibrated conductivity meter (such as the Horiba C-172). After submitting the tubes to a boiling bath for 15 min, which causes the total disruption of the cell membranes, measure conductivity again. Small perforations have to be made in the caps of the Eppendorf tubes to prevent them from bursting open at boiling temperatures.

First, calculate PEL (=percentage of electrolyte leakage) separately for the frost treatment and the control of each individual replicate plant as follows:

$$\text{PEL} = (e_s/e_t) \times 100,$$

where  $e_s$  is the conductivity value of the sample immediately after the treatment and  $e_t$  is the conductivity value of the same sample after placing it in the boiling bath. High values of PEL indicate an important disruption of membrane properties and thus cell injury. Therefore, the higher the PEL value, the higher the frost sensitivity.

Second, PEL under the control treatment can vary substantially among species, due to intrinsic differences among species, experimental manipulations and probably the way in which leaf fragments were cut. To control for these and other sources of error, calculate a Corrected PEL value, by simply subtracting the PEL value under the control treatment from that under the freezing treatment. Corrected PEL can therefore be calculated as:

PEL in the freezing treatment – PEL in the control treatment.

For calculating mean, standard deviation or standard error for a species, one average corrected PEL for each individual plant counts as one statistical observation.

#### *Special cases or extras*

(i) The technique is not suitable for halophytes and succulents.

(ii) We strongly recommend including an *additional* treatment, viz. incubation at  $-40^\circ\text{C}$ , with all other methodology as described above. This extreme low temperature will be particularly relevant for plants from very high latitudes and altitudes. However, we are aware that this method will not allow for acclimation and recommend repeating the protocol with leaves collected at the very end of the growing season.

(iii) The same basic technique, with a modification in the treatment temperature, has been successfully applied to leaf sensitivity to unusually high temperatures (*c.*  $40^\circ\text{C}$ , details in Gurvich *et al.* 2002).

*References on theory and significance:* Levitt (1980); Blum (1988); Earnshaw *et al.* (1990); Gurvich *et al.* (2002).

*More on methods:* Earnshaw *et al.* (1990); Gurvich *et al.* (2002).

### 2.3 Stem traits

#### Stem specific density (SSD)

##### *Brief trait introduction*

Stem specific density is the oven-dry mass of a section of a plant's main stem divided by the volume of the same section when still fresh. It is expressed in  $\text{mg mm}^{-3}$ , which corresponds with  $\text{kg dm}^{-3}$ . A dense stem provides the structural strength that a plant needs to stand upright and the durability it needs to live sufficiently long. The rules of allometry generally dictate greater stem densities for taller plants, but only in very broad terms. Stem density appears to be central in a trade-off between plant (relative) growth rate (high rate at low SSD) and stem defences against pathogens, herbivores or physical damage by abiotic factors (high defence at high SSD). In combination with plant size-related traits, it also plays an important global role in the aboveground storage of carbon.

##### *What and how to collect?*

The same type of individuals as for leaf traits and plant height should be sampled, i.e. healthy adult plants that have their foliage exposed to full sunlight (or otherwise plants with the strongest light exposure for that species). Collect material from a minimum of five individual plants. For herbaceous species or woody species with thin main stems (diameter  $<6$  cm), cut out (knife, saw) at least a 10-cm-long section at about one-third of the stem height or length. (If this causes unacceptable damage to shrubs or small trees, the 'slice method' may be a compromise alternative; see below.) If possible, select a relatively regular, branchless section, or else cut off the branches. For shorter stems, take the whole stem but cut off the youngest apical part. Remove any loose bark pieces that appear functionally detached from the stem. We consider any firmly attached bark or equivalent phloem tissue to be an integral part of the functioning stem and therefore it needs to be included in stem density measurement. For woody (or thick succulent) plants with stem diameters  $>6$  cm, saw out a slice from the trunk at about 1.3-m height, or at one-third of trunk height if the latter is shorter than 4 m. The slice (from the bark tapering regularly into the central point) measures between 2 and 10 cm in height (depending on stem diameter and structure), its cross-section area being about one-eighth of the total cross-section area. Thus, the sample resembles a slice from a round cake. Hard-wooded samples can be stored in a sealed plastic bag (preferably cool) until measurement. Wrap soft-wooded or herbaceous samples (more vulnerable to

shrinkage) in moist tissue in plastic bags and store in a cool box or fridge until measurement.

#### *Measuring*

The volume can be determined in either of two ways, depending on the species. The philosophy is that very large spaces (in relation to the stem diameter) are considered air or water spaces that do not belong to the stem tissue, whereas smaller spaces do. Thus, the central hollow of a hollow stem is not included in the volume, but smaller xylem vessels may be.

The preferred method is the volume replacement method. Measure the volume of the fresh (but not previously immersed) stem sample by gently rubbing it dry and then totally immersing it in water for 5 s in a volumetric flask and measuring the increase in volume. (During this time interval, the larger but not yet the smaller spaces should fill with water.) Obviously, different flask sizes are needed depending on the sizes of the samples.

For very small samples or some unusual tissues this may not work. In those cases, measure the mean diameter ( $D$ ) of the cylindrical sample with a calliper (if needed the average of several measurements) and the length ( $L$ ) of the sample with a calliper or ruler. If the stem is very thin, try determining the diameter from a cross-section under the microscope. Subsequently, calculate the volume ( $V$ ) of the cylinder as:

$$V = (0.5D)^2 \times \pi \times L.$$

(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.)

After volume measurement, the sample is dried in the oven at 60°C for at least 72 h (small samples) or at least 96 h (large samples) and then weighed (oven-dry mass). (Drying at 80°C for at least 48–72 h, depending on sample dimensions, would be acceptable too.)

#### *Additional useful methods from forestry*

In forestry, tree cores are also commonly used. Although they do not always take a totally representative part of the stem volume (cores that do not taper towards the centre), similar data from tree cores are probably acceptable for use in broader comparisons where small deviations are not critical.

In the timber industry, the mass component of SSD (or ‘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. Samples are usually taken at 1.3 m (‘breast height’). There are numerous ADW data available in the forestry literature. On the basis of investigations on 379 tropical timbers from South America, Africa and Asia by

Reyes *et al.* (1992), ADW can be transformed to ODW or SSD as follows:

$$SSD = 0.800ADW + 0.0134 (R^2 = 0.99).$$

We suggest that data for ODW, directly measured or derived from ADW, can safely be used as stem specific density.

#### *Special cases or extras*

(i) For plants without a well-defined stem, for instance some rosette plants, grasses and sedges, try to isolate the central area aboveground from which the leaves grow and treat these as stems. If the plant has no recognisable aboveground support structure at all, stem density is recorded as zero. Be aware that these zero values may have to be excluded from certain types of analysis.

(ii) If a plant branches from ground level (e.g. some shrubs), select the apparent main branch or a random one if they are all similar.

(iii) If one is interested in the total carbon content of a plant, *additional* samples could be taken from other parts of the support structure (branches, twigs), along with plant volume estimates.

(iv) After immersion of the sample for 5 s, an interesting *additional* measurement would be immersion for 24 h in a cool place. The difference in volume replaced, when expressed as a percentage of fresh (short-immersed) volume, could be a useful indicator of stem water storage capacity.

*References on theory and significance:* Chudnoff (1984); Lawton (1984); Barajas-Morales (1987); Baas and Schweingruber (1987); Loehle (1988); Reyes *et al.* (1992); Chapin *et al.* (1993a); Sobrado (1993); Borchert (1994); Brzeziecki and Kienast (1994); Favrichon (1994); Gartner (1995); Shain (1995); Brown (1997); Fearnside (1997); Castro Díez *et al.* (1998); Suzuki (1999); Ter Steege and Hammond (2001).

*More on methods:* Reyes *et al.* (1992); Brown (1997); Castro Díez *et al.* (1998).

#### *Twig dry matter content (TDMC) and twig drying time*

##### *Brief trait description*

TDMC is the oven-dry mass (mg) of a terminal twig divided by its water-saturated fresh mass (g), expressed in  $\text{mg g}^{-1}$ . (It is 1 minus leaf water content expressed on a fresh mass basis). Twig drying rate is expressed in days (until equilibrium moisture).

We consider TDMC to be a critical component of plant potential flammability, particularly fire conductivity after ignition (see under *Flammability*). Twigs with high dry matter content are expected to dry out relatively quickly during the dry season in fire-prone regions. Low TDMC may be positively correlated with high potential relative growth rate

(as corresponding with LDMC and stem specific density), but this has to our knowledge not been tested explicitly.

#### *What and how to collect?*

Collect 1–3 terminal (highest ramification order; smallest diameter class), sun-exposed twigs from a minimum of five plants. Twigs (or twig sections) should preferably be 20–30 cm long. If a plant has no branches or twigs, take the main stem; in that case the procedure can be combined with that for *Stem specific density* (see above). In the case of very fine, strongly ramifying terminal twigs, a ‘main twig’ with fine side twigs can be collected as one unit.

#### *Storing and processing*

Wrap the twigs (including leaves if attached) in moist paper and put them in sealed plastic bags. Store these in a cool box or fridge (never in a freezer!) until further processing in the laboratory. If no cool box is available in the field and temperatures are high, it is better to store the samples in plastic bags without any additional moisture, then follow the above procedure once back in the lab.

#### *Measuring*

Following the rehydration procedure (see under *Leaf dry matter content*), any leaves are removed and the twigs gently blotted dry with tissue paper to remove any surface water before measuring water-saturated fresh mass. Each twig sample (consisting of 1–3 twigs) is then first dried in an oven or drying room at 40°C at relative air humidity 40% or lower. Every 24 h each sample is reweighed. Twig drying time is defined as the number of days it takes (rounding up where in case of doubt) to reach 95% of the mass reduction of the sample due to drying, 100% being the maximum mass loss until equilibrium mass. Continue until you are certain the mass is at equilibrium. TDMC is defined (analogous to LDMC) as equilibrium dry mass divided by saturated mass.

For calculating mean, standard deviation or standard error, the average TDMC for each individual plant (based on 1–3 twigs) is one statistical observation.

#### *Special cases or extras*

(i) If ignitability is determined experimentally (see above under *Flammability*), the same twigs can be used at equilibrium dry mass.

*References on theory and significance:* Bond and Van Wilgen (1996); Lavorel and Garnier (2002).

*More on methods:* Garnier *et al.* (2001b) (foliar equivalent).

#### *Bark thickness (and bark quality)*

##### *Brief trait description*

Bark thickness is the thickness (in mm) of the bark, which is defined here as the part of the stem that is external to the

wood or xylem—hence, it includes the vascular cambium. Thick bark has been shown to insulate meristems and bud primordia from lethally high temperatures associated with fire, although the effectiveness depends on the intensity and duration of a fire, on the diameter of the trunk or branch, on the position of bud primordia within the bark or cambium and on bark quality (e.g. thermal conductivity) and moisture. Thick bark may also provide protection of vital tissues against attack by pathogens, herbivores, frost or drought. It should be realised, however, that the structure and biochemistry of the bark (e.g. suberin in cork, lignin, tannins, other phenols, gums, resins) are often important components of bark defence (but partly also flammability; see above) as well.

#### *What and how to collect?*

The same type of individuals as for leaf traits and plant height should be sampled, i.e. healthy, adult plants that have their foliage exposed to full sunlight (or otherwise plants with the strongest light exposure for that species). Collect material from a minimum of five individual plants. Measure bark thickness on a minimum of five adult individuals, preferably (to minimise damage) on the same samples that are used for measurements of stem specific density (see above). For woody species with thin main stems (diameter <6 cm), take the sample at about one-third of the height or length of the main stem (see under *Stem specific density*), for woody (or thick succulent) plants with stem diameters >6 cm at about 1.3 m height. If you do not use the stem specific density sample, cut out a piece of bark of at least a few centimetres wide and long. Avoid warts, thorns or other protuberances and remove any bark pieces that have mostly flaked off. The bark as defined here includes everything external to the wood (i.e. any vascular cambium, secondary phloem, phelloderm or secondary cortex, cork cambium or cork).

#### *How to measure?*

Since fire tends to occur during dry periods, the bark piece is first air-dried at low (<60%) air humidity, unless it has reached low moisture content already at the time of sampling. For each sample, five random measurements of bark thickness are made with callipers (or special tools used in forestry), if possible to the nearest 0.1 mm. *In situ* measurement with a purpose-designed forestry tool is an acceptable alternative. Take the average per sample. Bark thickness (mm) is the average of all sample means.

#### *Special cases or extras*

(i) In addition to bark thickness, several structural or chemical components of bark quality may be of particular interest (see above). An easy but possibly important one is the presence (1) versus absence (0) of visible (liquid or viscose) gums or resins in the bark.

(ii) Bark surface structure (texture) may determine the capture and/or storage of water, nutrients and organic matter. These factors and texture itself may be important for the establishment and growth of epiphytes. We suggest the following five broad (subjective) categories: (1) smooth, (2) very slight texture (amplitudes of microrelief within 0.5 mm), (3) intermediate texture (amplitudes 0.5–2 mm), (4) strong texture (amplitudes 2–5 mm) and (5) very coarse texture (amplitudes >0.5 mm). Bark textures may be measured separately for the trunk and smaller branches or twigs, since these may differ greatly and support different epiphyte communities.

(iii) For flaking (decorticating) bark see under *Flammability*.

*References on theory and significance:* Gill (1995); Shain (1995); Bond and van Wilgen (1996); Wainhouse and Ashburner (1996); Gignoux *et al.* (1997); Pausas (1997); Pinard and Huffman (1997); Hegde *et al.* (1998).

*More on methods:* Pinard and Huffman (1997); Hegde *et al.* (1998).

## 2.4. Belowground traits

### Specific root length (SRL) and fine root diameter

#### *Brief trait introduction*

Specific root length (SRL) is the ratio of root length to mass, usually expressed as  $\text{m g}^{-1}$ . Fine root diameter is expressed in mm. SRL is considered the belowground analogue of specific leaf area (see SLA), in that it describes the amount of ‘harvesting’ or absorptive tissue deployed per unit mass invested. Plants with high SRL are able to build longer roots for a given dry mass investment and this is achieved by constructing roots of thin diameter or low tissue density. Theory based mainly on analogies to aboveground ‘leaf theory’ and empirical evidence from seedling glasshouse experiments and limited field studies suggest that species of higher SRL have (1) faster root elongation rates, (2) higher rates of nutrient and water uptake, (3) faster root turnover, (4) been linked with high relative growth rates of seedlings and (5) are less likely to be associated with mycorrhizae. Furthermore, thicker roots have been indicated to (1) exert greater penetration force on soil, (2) better withstand low soil moisture and (3) have higher rates of water transport within the root, although they are more expensive per unit length to construct and maintain.

#### *What and how to collect?*

We define absorptive roots as roots whose function is the absorption of water and nutrients. While leaves senesce and fall from a branch at the end of their lifetime as light-harvesting organs, absorptive roots may either shrivel and dry (true ‘death’), or may undergo secondary growth or subsequent thickening upon which they cease to function as absorptive organs, becoming pipes for the conductance of

water and nutrients (functional ‘death’, in terms of water and nutrient absorption) and anchors for holding the plant upright. Generally, roots <2 mm in diameter are defined as ‘fine roots’ in studies of soil occupation by roots (measuring the amount of root length per volume of soil). This does not necessarily represent a useful morphological guide to comparing roots of identical function (water and nutrient absorption) across all species of plants. With the goal of cross-species’ comparisons of SRL (of absorptive roots, befitting SRL theory), we define absorptive roots as those that display root hairs and/or healthy root caps. Be aware, though, that in ectomycorrhizal species many or all absorptive roots may be covered by a hyphal mantle (see below under *Nutrient uptake strategies*). In such cases, selecting absorptive roots is left to the judgement of the researcher.

Sample absorptive roots from at least five individuals of each species. To obtain samples, dig a hole about 20 cm deep, close to the base of the plant. (For particular large species that only have thick support roots near the surface, a deeper hole may turn out to be better.) The aim is to find a root connected to the plant and gently tease apart soil aggregations from around the smaller roots that may branch from it. If certain that there are no roots of any other species entering soil aggregates, one might consider bagging whole soil aggregates which will presumably contain some healthy absorptive roots. Place all of the small, healthier looking roots and any soil aggregates into a self-sealing plastic bag and keep cool and humid. A hand lens (magnifying glass) is useful for determining in the field whether roots appear healthy. Small plants (e.g. grasses, herbs, semi-shrubs) are more easily sampled by excavating the entire individual. More complete root systems may then be washed and the absorptive roots selected from them. Collect as much material as possible, given that samples should include 10 or more absorptive roots, depending on the time available to measure. For some semi-arid shrub species, it can be difficult to find many healthy absorptive roots at all (H. D. Morgan, pers. obs.), so the amount of material sampled will reflect the relative abundance of healthy absorptive roots on the root system of a particular species.

#### *Storing and processing*

Store humid, airtight samples of roots, including soil aggregates, in a refrigerator, for up to a week. Processing begins with washing, to remove soil from the roots. Place samples on a fine-mesh sieve (0.2 mm) and rinse until roots are as free from loose soil as possible. Remove absorptive roots from the sieve and place into a petri dish under a dissecting microscope. Using a soft brush, or fine forceps, remove as much soil as possible from the surface of the roots and amongst the root hairs. Since fine soil particles tend to lodge fast amongst root hairs, it is unlikely that roots will be entirely free of soil particles—for this reason, it is wise to

consider ashing the roots to measure the level of 'contamination' (see under *Special cases or extras*, this section and Böhm 1979).

### *Measuring*

Under a dissecting microscope, sort live, healthy roots from the recently washed sample. Live roots generally have a lighter, fully turgid appearance, compared with dead or dying roots of the same species that appear darker and floppy or deflated (Böhm 1979; Fitter 1996). It will help to observe a range of ages and colours of absorptive roots for each plant species before measurement, in order to properly identify healthy live roots. Of course, include only those roots that display root hairs and/or a root cap, or else fine roots with ectomycorrhizal hyphal mantles.

With an eyepiece graticule calibrated for each objective, record (for each plant) the length and diameter of at least 10 of the absorptive roots. For diameter measurements, use the highest-power objective possible, such that the width of each root section is maximised in the field of view. Diameter is defined as the diameter of the root not including root hairs and should be measured behind the zone of elongation, amongst the root hairs. In the case of ectomycorrhizal mantles, include these in the width measurements, since they function as part of the absorptive system of plant roots. Following measurement, dry root samples in an oven at 60°C for at least 72 h (or else at 80°C for 48 h) and weigh. Sample masses will be small (can be in the order of  $10^{-2}$  mg), so a sensitive balance must be used. Divide root length by dry mass to obtain SRL. Mean SRL of the 10 subsamples is used as one replicate (plant) for statistical analyses.

### *Special cases or extras*

(i) Since all soil particles can never be removed from the surface of roots, it is advisable to quantify the degree of soil 'contamination' present on the surface of the collected roots. This is done by ashing the roots in a muffle furnace at 650°C. Nutrients contained in the roots themselves are left as residues in the ash, so dissolve these with hydrochloric acid. Decant the acid solution and dry the remaining solid ash at 105°C. The final mass represents the mass of soil particles on the root surfaces, which is subtracted from the crude root mass obtained earlier. See Böhm (1979, pp. 126, 127) for comment on this method.

(ii) For measuring lengths (and subsequently SRL) of more extensive root systems, the line-intersect method is widely used, in which basically the number of points where roots touch a grid is recorded and translated into length via calibration (see Newman 1966 and Tennant 1975 for details). This can be mechanised with the aid of a Delta-T area meter (Cambridge, UK), set in the 'length' position, but in that case careful calibration with cotton threads of similar colour and shape (and known length) is necessary (Cornelissen 1994).

*References on theory and significance:* Nye and Tinker (1977); McCully and Canny (1989); Boot and Mensink (1990); Aerts *et al.* (1991) (implications for belowground competition); Eissenstat (1992); Ryser (1996); Eissenstat and Yanai (1997) (both: SRL and root turnover/root lifespan); Reich *et al.* (1998); Wright and Westoby (1999); Eissenstat *et al.* (2000); Wahl and Ryser (2000) (all: SRL considerations for grasses); Guerrero-Campo and Fitter (2001) (all: general theory of SRL and root diameter); Steudle (2001) (all: absorptive root length and water and nutrient uptake, change of absorptive function with age); Nicotra *et al.* (2002) (both: trait relationships with SRL among seedlings of woody species).

*More on methods:* Böhm (1979) (general root methods); Fitter (1996) (general appearance of roots); Bouma *et al.* (2000) (root length and diameter).

### *Root depth distribution and 95% rooting depth*

#### *Brief trait introduction*

Root depth distribution measures how the root biomass of an individual is distributed vertically through the soil. Depth distribution is expressed as dry root mass per volume of soil ( $\text{g m}^{-3}$ ) in relation to depth. Root depth distributions provide information about (1) where in the soil different species obtain water and nutrients, (2) the likelihood of belowground competition between species, (3) which species are likely to benefit given certain changes in resource supply, (4) distribution of carbon sequestration through the soil and (5) how aspects of atmospheric and groundwater fluxes are facilitated. A recent global analysis of root depth distributions showed that more than 90% of all root biomass profiles documented in the literature had at least 50% of root biomass in the upper 30 cm of soil and 95% of root biomass in the upper 2 m of soil (Schenk and Jackson 2002). The rooting depth of 95% is an estimate of the depth, in metres, above which 95% of the root biomass of a species is located. It was further shown to be fairly well extrapolated from incomplete root depth distributions from a range of ecosystems worldwide, using a logistic dose-response model (Schenk and Jackson 2002). Extrapolating 95% rooting depth summarises species' root depth distributions into a single value that can be compared across species. To the extent that root depth distributions do actually fit logistic curves, a single value can capture essentials of differences between species or habitats.

#### *Identity crisis*

To confirm the species identity of roots sampled by auger (apparatus to bore holes) requires anatomical or molecular comparisons with other roots of that species. This may be quickly done anatomically for the larger, non-woody roots, but to do this for all roots within a sample defeats the 'softness' of this trait. We suggest excavation of intact root

systems of whole individuals where possible (e.g. for grasses, herbs or small shrubs), making root identification simple. Further, one may determine root biomass distributions for complete root systems, while also directly measuring maximum rooting depth, which should be included in results wherever possible.

When species are too large to be excavated whole, judiciously select a grove of conspecific plants, under which presumably few roots from other species would find their way. Sample as set out below with respect to placement of cores. During washing, visual checks of roots will reveal obvious impostors in samples (but not all roots of non-target species).

#### *What and how to collect?*

Select at least five individuals of each species, given the constraints to individual selection imposed by the identification difficulties outlined previously. Using a hand auger of about 7 cm diameter (Böhm 1979), auger a vertical hole close to the base of the ramet. The hole should be dug at a random compass direction from the base of the individual. As a rough guide, sample grasses and herbs within 30 cm of the base of each ramet, for shrubs, sample 0.5–1 m from the base of the ramet, for trees, sample at a distance of 1–1.5 m from the base. Take soil from at least five separate depths of 20 cm each, to a total sample depth of *at least* 1 m. It is preferable to sample deeper, to a depth of 2 m, particularly for shrub and tree species whose roots are placed deeper; however, we realise that this will depend on the tools and time available. It does not matter that mixing of soils and roots occurs within a depth increment, but be sure to separate individual depth increments within a sample. Store individual depth increments of each sample separately in air-tight containers such as tough, self-sealing plastic bags.

If very few roots are obtained within each sample, it may be that the individual has very few roots through the soil, has not placed roots in a particular part of the soil, or both. In this case, take more samples from each individual, following the protocol set out above.

#### *Storing and processing*

*Washing roots from soil.* Roots are best removed from the soil by washing, using methods such as described by Böhm (1979). Washing by hand is effective at obtaining most roots (depending on the size of the sieves used) and does not require specialised equipment. To hand-wash, place each 20 cm deep soil sample from the core into a separate bucket and add water to form a suspension. Once all the soil aggregates have dissociated (use fingers to gently squeeze the aggregates apart) and the heavy particles have settled, tip the suspension into a fine sieve (0.2–2 mm). Water may be sprayed onto the sieve to help wash the soil particles through. Add more water to the bucket to repeat the suspension and wet-sieving process. Repeat a number of times until the

suspension contains no roots and is generally clear, leaving only the heavy roots and sand particles behind. Collect the heavy roots and add them to the washed root sample.

*Identifying and sorting roots.* Once the soil has been washed from the samples, remove any other non-root material using magnifying glass and forceps. Further separate the samples into live and dead fractions, discriminating between live and dead roots as described in *Specific root length* (above).

#### *Measuring*

Dry live and dead root biomass separately in an oven at 60°C for at least 72 h and weigh. Since it is impossible to remove all soil particles from the root surfaces, there will be some degree of contamination of root biomass with soil. To account for this, see notes on ashing (under *Specific root length*, above) and Böhm (1979). Record root biomass per soil volume for each 20-cm core section. Subsequently, estimate 95% rooting depth, for instance through regression of mass per volume on soil depth.

For guidelines for extrapolating 95% rooting depth from incomplete root depth distributions, see Schenk and Jackson (2002).

#### *Special cases or extras*

(i) Remember to always include the diameter of the sample (measured as the diameter of the core—generally the outer diameter of the auger head), so that root distribution may alternatively be calculated on a land surface-area basis (important for later syntheses).

(ii) When sampling larger shrubs and trees, the researcher will encounter thicker woody roots. The best way to deal with this is to use a specialised wood-cutting auger, such as the type shown in Böhm (1979).

(iii) If the soil is particularly clayey, aggregated, or contains calcium carbonate, consider adding a dispersal agent to the washing water. The best washing additive varies, depending on the particular condition of the soil and is discussed by Böhm (1979).

(iv) It may be possible to develop a quick, quantitative molecular technique to measure the amount of non-target species roots in a particular sample and this would be an excellent development for root research (see also Jackson *et al.* 1999).

*References on theory and significance:* Gale and Grigal (1987); Jackson *et al.* (1996); Casper and Jackson (1997) (belowground competition between individuals); Kleidon and Heimann (1998) (root depth and effects on global carbon and water cycles); Jackson (1999); Adiku *et al.* (2000); Guerrero-Campo and Fitter (2001) (both: costs and benefits of shallow and deep roots); Schenk and Jackson (2002) (all: models of root depth distribution, global syntheses of root depth distributions).

*More on methods:* Böhm (1979); Caldwell and Virginia (1989) (types of augers, separating roots and soil); Jackson *et al.* (1996); Jackson (1999); Schenk and Jackson (2000) (all: extrapolating 95% rooting depth).

### Nutrient uptake strategy

#### *Brief trait description*

The mode and efficiency of uptake of essential macronutrients is paramount for plant growth and the position of different species in ecosystems varying in nutrient availability. The Plant Kingdom has come up with a series of effective adaptive mechanisms to acquire nitrogen and phosphorus, in particular. Most of these adaptations are, logically, most common in ecosystems with low nutrient availability. Nutrient uptake strategy is a categorical trait, with the following main strategies:

- (1) *nitrogen fixer* (symbiosis with N<sub>2</sub>-fixing bacteria)—efficient N uptake;
- (2) *arbuscular mycorrhiza* (symbiosis with arbuscular mycorrhizal fungi, AMF)—efficient P uptake;
- (3) *ectomycorrhiza* (symbiosis with ectomycorrhizal fungi, EMF)—uptake of inorganic and (relatively simple) organic forms of N and P;
- (4) *ericoid* (symbiosis with ericoid mycorrhizal fungi)—efficient uptake of (simple and complex) organic forms of N and P;
- (5) *hairy root clusters* (proteoid roots)—efficient P uptake;
- (6) *orchid* (symbiosis with orchid mycorrhizal fungi);
- (7) *root hemiparasite* (green plants that extract nutrients from the roots of a host plant)—efficient capture and uptake of N and P;
- (8) *myco-heterotrophs* (plants without chlorophyll that extract carbon and probably most nutrients from dead organic matter via saprotrophic fungi or from mycorrhizal fungi associated with the roots of their host plant)—efficient C and probably efficient N and uptake;
- (9) *holoparasites* (plants without chlorophyll that extract carbon and nutrients directly from a host plant)—efficient N, P and C uptake;
- (10) *carnivorous*—efficient capture and uptake of organic forms of N and P;
- (11) *specialised tropical strategies* (mostly in epiphytes):
  - (a) tank plants (ponds)—efficient nutrient capture and water storage,
  - (b) baskets—efficient nutrient and water capture,
  - (c) ant nests—efficient uptake of nutrients,
  - (d) trichomes—efficient uptake of nutrients and water through bromeliad leaves and
  - (e) root velamen radiculum—efficient uptake and storage of water and nutrients; and
- (12) *none*: no obvious specialised N or P uptake mechanism; uptake presumably directly through root hairs

(or through leaves, e.g. in the case of certain ferns with very thin fronds).

By using the protocols below, assign preferably one category to each plant species, namely the predominant one. In cases where both a specialised N and a specialised P uptake strategy seem important (e.g. N-fixing and hairy root clusters), give both categories. N-fixing (1) takes priority over other N-related strategies. If there is good evidence to classify a species in one of the Categories 1–11, there is no need to further test for Categories 1–4. For most strategies, useful data are also available in the literature for many species, for instance Harley and Harley (1987*a*, 1987*b*, 1990) for mycorrhizal associations of many temperate European species, Sprent (2001) for a comprehensive list of N-fixing species and Maberley (1987) for general information for a huge number of genera and families.

#### *What and how to collect? (Categories 1–4)*

To check for N<sub>2</sub>-fixing capacity and mycorrhiza, dig up a minimum of five (preferably 10) healthy looking plants during the growing season, from typical sites for each of the predominant ecosystems studied. If possible, use the same plants used to determine specific root length and root depth distribution (see above). Plant roots need to be carefully washed and soil particles removed by rinsing or with fine forceps. It is important to use roots that are attached to the plant, otherwise there is the risk of mixing roots of different plant species.

#### *Storing, processing and observations (Categories 1–4)*

Washed roots can be stored at 4°C for several days before further cleaning and staining procedures start. N-fixing root nodules and ectomycorrhizal roots can be identified visually at lower magnification under a dissecting microscope (see below). Arbuscular and ericoid mycorrhizal fungi inhabit the inside of the roots and the procedures to determine root colonisation by these fungi are more elaborate. Clear and more detailed descriptions of the procedures explained below are given by Brundrett *et al.* (1996). The species belongs to one of the Categories 1–4 below if the relevant structures are clearly seen in at least a third of the plants, or in at least two plants if only five plants are sampled.

#### *(1) Nitrogen fixers*

Check for nodules on washed root systems under the dissecting microscope. The roots of most legumes (Mimosaceae, Fabaceae/Papilionaceae, Caesalpinaceae) contain mostly globose or semiglobose root nodules of diameters 2–10 mm (Corby 1988) (see Figs 3, 4). Finger-like elongated forms also occur. The number of root nodules can vary greatly: some roots are almost ‘covered’ with nodules, while on other roots they are sparsely distributed. Nodules tend to be clearly pink, or sometimes red or brown (rarely black) in colour, while active N fixation is taking place.

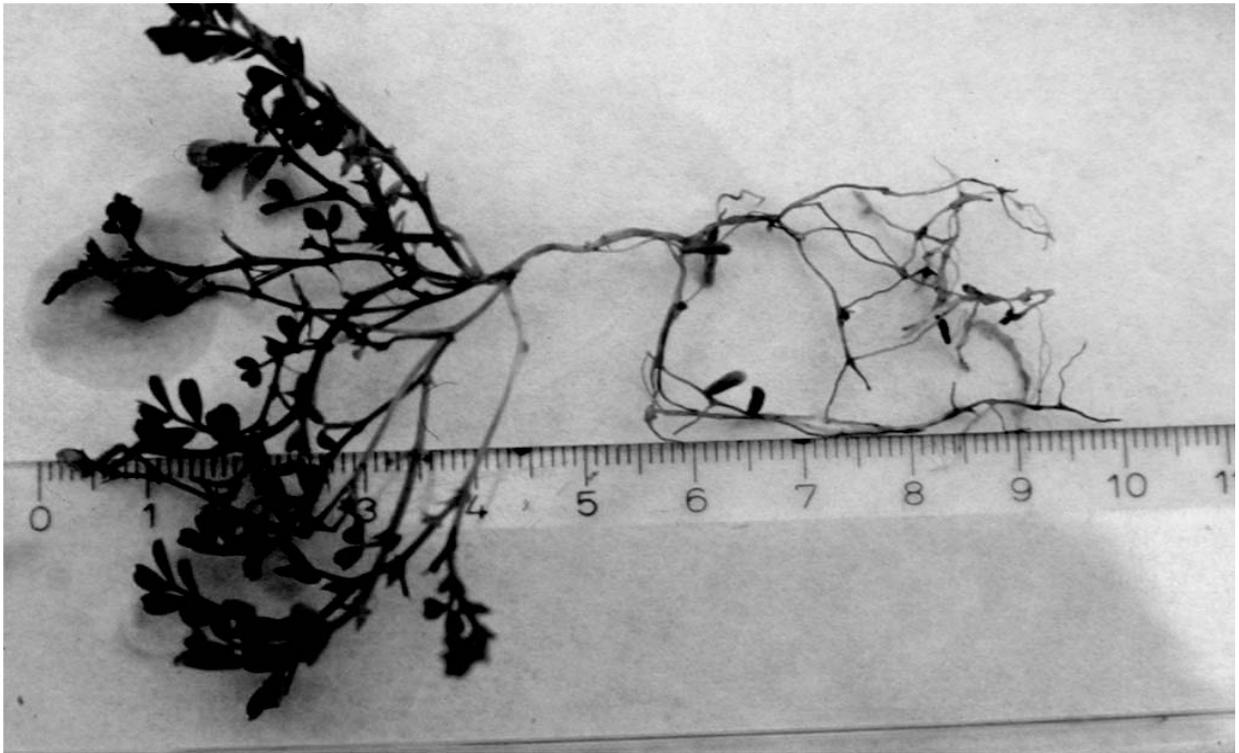


Fig. 3. Root system of *Vicia sativa*, showing N-fixing nodules.

Be aware that (i) some legume species do not form symbiotic root nodules, (ii) root nodules with symbiotic *Rhizobium* bacteria have also been reported from Ulmaceae (*Trema cannabina*) and Zygophyllaceae (*Zygophyllum* spp., *Fagonia arabica*, *Tribulus alatus*), while they have been suspected to occur in some other families as well (Becking 1975) and (iii) some legume species (e.g. *Sesbania* in tropical forests) bear the nodules on the stem.

Other root structures that host N fixers are the 'actinorhiza', found in some members of other vascular plant families (Table 7). Actinorhiza usually contain N-fixing actinomycetes, particularly of the genus *Frankia*, and they have a different morphology from legume nodules. Some taxa feature coralloid nodules (the *Alnus* type), while other taxa have upward-pointing nodules extending into upward-pointing rootlets (the *Casuarina/Myrica* type) (see Table 7). Good photographs of these types are in Becking (1975). Be aware that there are also plant taxa that feature nodule-like structures without N-fixing symbionts (Becking 1975).

Some further vascular plants host N-fixing bacteria (*Nostoc*, *Anabaena*) in looser structures, notably the water fern *Azolla*, *Gunnera* and some members of the Cycadaceae (cycads). Some tropical grasses also form loose associations with N-fixing bacteria (Wullstein *et al.* 1979; Boddey and Döbereiner 1982). The trait to look for is the presence of sheaths of sand grains on the grass roots ('rhizosheaths').

## (2) Arbuscular mycorrhiza (AMF)

(a) Clear the roots in a 10% potassium hydroxide (KOH) solution at 90°C in a water bath. Clearing time depends on root age and plant species and varies from 5 min for young herb roots collected in pot experiments, to 1 h for old roots from the field. Clearing is necessary to remove cell contents and pigments. Staining after clearing shows the fungal structures (when present) inside the root. (b) Next, wash the roots with water or hydrochloric acid to remove the potassium hydroxide. The washed roots can be stained with a trypan blue solution (0.05% trypan blue in 2:1:1 lactic acid:water:glycerol) or a chlorazol black E solution (0.03% chlorazol black E in 1:1:1 lactic acid:water:glycerol). Staining needs to be done in a water bath at 90°C for 20 min (or shorter with young fragile roots from pot experiments). (c) Wash the stained roots again with water and store and destain the roots in a glycerol solution. Trypan blue is carcinogenic and it needs to be recollected after use. Use gloves when clearing and staining! (d) Cut thin longitudinal sections of 10 root pieces per root system. (e) Examine the roots under the microscope at  $\times 100$  magnification. The degree of mycorrhizal colonisation varies depending on staining agent and plant species (Gange *et al.* 1999).

Hyphae typically spread longitudinally between cortical cells within the intercellular spaces. In some cases hyphae also penetrate cortical cells and spread from cell to cell.



Fig. 4. Close-up of a *Lotus corniculatus* nodule.

**Table 7. Non-leguminous taxa in which nitrogen-fixing, nodulated species have been reported (data from Becking 1975)**

The number of reported nodulated species is given in parentheses.

A, *Alnus*-type nodules; C, *Casuarina/Myrica*-type nodules

Family	Genus	Nodule type
Betulaceae	<i>Alnus</i> (33 spp.)	A
Casuarinaceae	<i>Allocasuarina</i> , <i>Casuarina</i> (18 spp.), <i>Gymnostoma</i>	C
Coriariaceae	<i>Coriaria</i> (12 spp.)	A
Elaeagnaceae	<i>Elaeagnus</i> (14 spp.), <i>Hippophae</i> (1 sp.), <i>Shepherdia</i> (2 spp.)	A
Myricaceae	<i>Comptonia</i> (1 sp.), <i>Myrica</i> (20 spp.)	C
Rhamnaceae	<i>Ceanothus</i> (32 spp.), <i>Colletia</i> (2 spp.), <i>Discaria</i> (1 sp.)	A
Rosaceae	<i>Cercocarpus</i> (3 spp.), <i>Dryas</i> (3 spp.), <i>Purshia</i> (2 spp.)	A
Zamiaceae	<i>Macrozamia</i>	?

Usually many hyphae can be observed in a longitudinal section of a root under the microscope (Fig. 5). AMF are characterised by arbuscules, extensively branched tree-like structures that are formed within cortical cells of young roots. Arbuscules are often difficult to detect in field roots since they have, in most cases, a limited life span. Arbuscules

have a granular appearance under the microscope. Vesicles, swollen structures of variable size and shape within the intercellular spaces, are formed by some AMF fungi and are, when present, a good indicator for AMF infection (Fig. 5). Vesicles are thought to have a storage function and contain small lipid droplets that sometimes can be detected under the microscope. It may be difficult to distinguish AMF from other root colonising fungi. Hyphae from members of the Basidiomycetes and Ascomycetes (two abundant classes of fungi in soils) contain hyphal septa at regular distances, while septa are mostly absent in AMF.

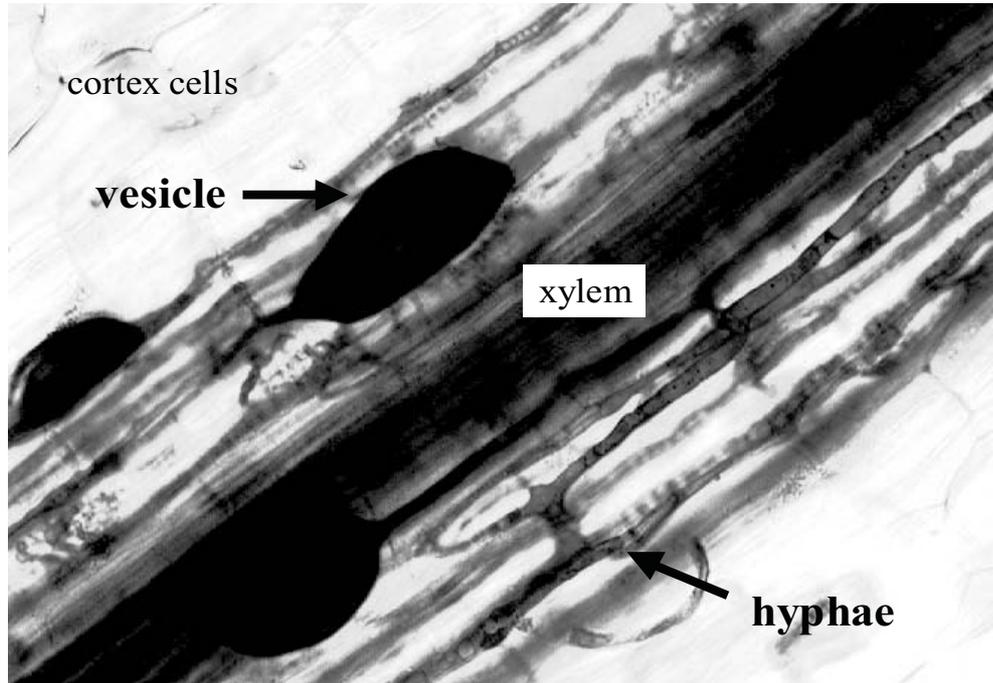
### (3) *Ectomycorrhiza* (EMF)

Parts of the root system of ectomycorrhizal plants are surrounded by a mantle of fungal hyphae, which have replaced any root hairs. Ectomycorrhizal roots are typically swollen and often dichotomously branched (Fig. 6). Ectomycorrhizal fungi differ from AMF in that the largest part of the fungus remains outside the root. Many different ectomycorrhizal structures have been observed depending on the identity of fungus and plant host. The colour atlas of ectomycorrhizae (Agerer 1986–1998) shows many types and species of ectomycorrhiza. Ectomycorrhizal structures can be further examined under the microscope. A thin cross-section of a plant root can be made with a sharp razor blade and subsequently be stained with chlorazol black (see under AMF). Such a section typically shows the mantle at the root surface and a Hartig net of fungal hyphae surrounding root cortex cells within the root. An additional useful (but not exclusive) trait is the clear ‘fungal’ smell that some ectomycorrhizal roots have. Also, many ectomycorrhizal fungi produce conspicuous epigeous fruiting bodies (including many of the well-known toadstools), which may give a first suspicion about the possible ectomycorrhizal status of neighbouring plants. Molina *et al.* (1992, table 11.1) listed the families and genera of such fungi.

Ectomycorrhizal fungi are particularly common in a range of plant families, including for instance Betulaceae, Caesalpiniaceae, Dipterocarpaceae, Fagaceae, Myrtaceae, Nyctaginaceae, Pinaceae and Salicaceae.

### (4) *Hairy root clusters* (proteoid roots or cluster roots)

Under the (dissecting) microscope, look for ‘distinct clusters of longitudinal rows of contiguous, extremely hairy rootlets’ (Lamont 1993), or ‘a region of the primary or secondary root where many short rootlets are produced in a compact grouping, giving the appearance of a bottle brush’ (Skene 1998). Examples for hairy root clusters of a sedge are shown in Fig. 7 and in Grime (2001, p. 78). These structures are a relatively recent topic of investigation and new taxa hosting them may well be found. Careful examination is especially recommended for species belonging to families known to feature members with hairy root clusters (Table 8). First get familiar with their appearance by checking roots of



**Fig. 5.** Structures of arbuscular mycorrhizal fungi (AMF) in a root of *Lotus corniculatus*. Mycorrhizal structures are stained blue (black or dark grey in picture) with trypan blue. Mycorrhizal hyphae and vesicles are growing between cortex cells. Note that the xylem is also stained.



**Fig. 6.** Ectomycorrhizal beech roots (×25 magnification).

plants known to contain them. Do check the recent literature as well!

**(5) Ericoid mycorrhiza**

Virtually all genera and species belonging to the families Ericaceae (except *Arbutus* and *Arctostaphylos*, which are usually arbutoid mycorrhizal), Empetraceae and Epacridaceae can be assumed to host ericoid mycorrhizal

fungi under natural conditions, while these mycorrhizas are not yet known from other families. Most of the genera are ericaceous (dwarf) shrubs linked with strongly organic soils such as are found in tundra, heathland, Mediterranean-type shrubland and boreal forest.

**(6) Orchid roots**

All species of orchids (Orchidaceae) appear to depend strongly on association with orchid mycorrhizal fungi for their establishment under natural conditions. Therefore, any Orchidaceae species can be assumed to form these mycorrhizas and belong to this category.

**(7) Root hemiparasites**

These are green plants whose roots tap into the roots of a host plant. Careful microscopic examination of the root system of a plant may reveal connections with a host plant, but this is very hard to verify without digging up hemiparasite and host plant simultaneously. Therefore, given that this group has been reasonably well studied, it may be wise to only check for parasite–host connections within the Scrophulariaceae and particularly the subfamily Rhinanthoideae. This is the only higher taxon that has both parasitic and non-parasitic members. Within this subfamily, *Bartsia*, *Buchnera*, *Castilleja*, *Euphrasia*, *Melampyrum*, *Pedicularis*, *Rhinanthus* and *Tozzia* are safely classified as hemiparasitic, while *Digitalis*, *Hebe* and *Veronica* are not



Fig. 7. Hairy root clusters in the sedge, *Carex flacca*.

Table 8. Known taxa with hairy root clusters (data mostly from Lamont 1993 and Skene 1998)

Family	Genus
Betulaceae	<i>Alnus</i>
Casuarinaceae	<i>Allocasuarina</i> , <i>Casuarina</i> , <i>Gymnostoma</i>
Cyperaceae	Many members
Dasypogonaceae	<i>Kingia</i>
Elaeagnaceae	<i>Hippophae</i>
Fabaceae <sup>A,B</sup>	Many members (e.g. <i>Lupinus</i> )
Mimosaceae <sup>A</sup>	Many members (e.g. <i>Acacia</i> )
Moraceae	<i>Ficus benjamina</i>
Myricaceae	<i>Comptonia</i> , <i>Myrica</i>
Proteaceae	All members (e.g. <i>Banksia</i> , <i>Hakea</i> , <i>Protea</i> ) except <i>Persoonia</i>
Restionaceae	Some members

<sup>A</sup>Previously classified under Leguminosae.

<sup>B</sup>Previously classified as Papilionaceae.

parasitic. Other known root hemiparasitic families are listed in Table 9 (Olacaceae, Opiliaceae, Santalaceae, Loranthaceae and Krameriaceae). Any species belonging to

Table 9. Angiosperm taxa with root hemiparasite members (data from Kuijt 1969 and Molau 1995)

Family	Genus	Distribution
Krameriaceae	<i>Krameria</i> only (17 spp.), possibly all root hemiparasites	(Sub-)tropical America
Loranthaceae	Few genera ( <i>Atkinsonia</i> , <i>Gaiadendron</i> , <i>Nuytsia</i> ), on shrubs or trees	Temperate-tropical
Olacaceae	25 genera, c. 250 spp. (all woody)	Pantropical
Opiliaceae	Eight genera, c. 60 spp.	Tropical
Santalaceae	35 genera, c. 400 spp.	Temperate-tropical
Scrophulariaceae	c. 90 genera, c. 1400 spp.	Cosmopolitan

Table 10. Angiosperm taxa with myco-heterotrophic members (data from Leake 1994)

Family	Genus
<i>Dicotyledons</i>	
Gentianaceae	Six genera (including <i>Voyria</i> with 19 species)
Monotropaceae	10 genera
Polygalaceae	<i>Salomonina</i> (Indo-Malesia)
Pyrolaceae	<i>Pyrola</i>
<i>Monocotyledons</i>	
Burmanniaceae	14 genera including <i>Burmannia</i> (23 spp.), <i>Gymnosiphon</i> (24 spp.) and <i>Thismia</i> (28 spp.)
Corsiaceae	<i>Arachnitis</i> (2 spp., S America), <i>Corsia</i> (c. 25 spp., New Guinea, Australia)
Geosiridaceae	<i>Geosiris</i> (Madagascar)
Lacandoniaceae	<i>Lacandonia</i> (Mexico)
Orchidaceae	37–43 genera and c. 200 species
Petrosaviaceae	<i>Petrosavia</i> (eastern Asia)
Triuridaceae	Six genera including <i>Andruris</i> (13 spp.) and <i>Sciaphila</i> (c. 50 spp.)

these families that are not shoot parasites, can safely be classified as root hemiparasites, although only 6 of the 17 Krameriaceae species have been checked (and found hemiparasitic) so far.

#### (8) Myco-heterotrophs

If a plant species does not contain chlorophyll, i.e. shows no sign of greenness, during any phase in its life cycle, it can safely be classified as a heterotroph. Myco-heterotrophs derive carbon and nutrients from dead organic matter via mycorrhizal fungi of various types. They should not be confused with holoparasites (see below). Since these plants have been studied well, we refer to a rather comprehensive overview of plant families and genera worldwide with myco-heterotrophic members (see Table 10). If a heterotrophic species belongs to any of the families in this table, it can safely be classified as a myco-heterotroph.

**Table 11. Angiosperm taxa with holoparasitic members (data from Molau 1995 and Mabberley 1987)**

Family	Genus	Type
Balanophoraceae	18 genera (subtropical and tropical)	Root parasites
Cuscutaceae <sup>A</sup>	<i>Cuscuta</i> only (c. 145 spp.)	Shoot parasites
Hydnoraceae	<i>Hydnora</i> , <i>Prosopanche</i> only	Root parasites
Lauraceae	<i>Cassytha</i> only (tropical)	Shoot parasite
Lennoaceae	<i>Ammobroma</i> , <i>Lennoa</i> , <i>Pholisma</i> only	Root parasites
Mitrastemmataceae	<i>Mitrastemon</i> (= <i>Mitrastemma</i> ) only	Root parasites
Orobanchaceae <sup>B</sup>	All members (e.g. <i>Orobancha</i> )	Root parasites
Rafflesiaceae	8 genera, c. 500 species (mostly tropical)	Root parasites
Scrophulariaceae	Some members (e.g. <i>Harveya</i> , <i>Lathraea</i> , <i>Striga</i> )	Root parasites

<sup>A</sup>Also classified as a group within Convolvulaceae.

<sup>B</sup>Also classified as a group within Scrophulariaceae.

(9) *Holoparasites*

Holoparasites directly parasitise the roots or shoots of other species. If a heterotrophic (achlorophyllous) plant belongs to any of the taxa in Table 11, it can safely be assumed to be a holoparasite.

(10) *Carnivorous plants*

Look for obvious specialised organs to capture prey, or the prey themselves, external digestive glands (often sticky), as well as showy appendages or other features to attract invertebrate animals. *Utricularia* is a specialised aquatic genus. If a plant species does not belong to one of the genera in Table 12, it is very unlikely to be carnivorous.

(11) *Specialised tropical strategies (mostly in epiphytes)*

(a) *Tank plants (ponds)*. Within the tropical Bromeliaceae family, look for rosettes of densely packed leaves that, together, create a ‘pond’ in which rain or run-off water collects. Different species may feature roots growing into these tanks or trichomes (see below) on the surface of the inner leaf bases. See Martin (1994) for details. Most tank bromeliads are epiphytes, but there are also terricolous species, for instance in salinas (where the tanks may keep salt water out).

(b) *Baskets*. Diagnostic are big leaf rosettes of epiphytic plants (often in big tree forks) that capture humus effectively. There are important representatives of this strategy within the ferns (Pteridophyta) and the Araceae family.

(c) *Ant nests*. Symbiotic relationships between epiphytic plants and ants. The ants transport seeds of ant nest plants to the ‘nests’, where these germinate and benefit from nutrients in other materials imported by the ants and their faeces. In return, the plants may offer nectar, fruit and accomodation to the ants. Ant-nest plants are found in several families, including Orchidaceae, Bromeliaceae and Asclepiadiaceae.

**Table 12. Known carnivorous plant families and genera (after Lambers *et al.* 1998)**

Family	Genus
Bromeliaceae	<i>Catopsis</i> (1 sp.)
Byblidaceae	<i>Byblis</i> , <i>Roridula</i>
Cephalotaceae	<i>Cephalotus</i>
Dioncophyllaceae	<i>Triphyphyllum</i>
Droseraceae	<i>Aldrovanda</i> , <i>Dionaea</i> , <i>Drosera</i> , <i>Drosophyllum</i>
Lentibulariaceae	<i>Genlisea</i> , <i>Pinguicula</i> , <i>Polypompholyx</i> , <i>Utricularia</i>
Nepenthaceae	<i>Nepenthes</i>
Sarraceniaceae	<i>Darlingtonia</i> , <i>Heliamphora</i> , <i>Sarracenia</i>

Some plants host ants inside special organs such as pseudobulbs, tanks or pitchers. Often more than one plant species inhabit an epiphytic ant nest. The abundance of ants in these nests is diagnostic.

(d) *Trichomes*. These are specialised epidermal water-absorbing organs on the leaves of various Bromeliaceae and members of some other families with poorly developed root systems (e.g. Malpighiaceae). Trichomes are usually recognisable as conspicuous whitish scales. Their main function is probably to absorb water and nutrients, but they may also prevent overheating by reflecting sunlight in exposed habitats, deter invertebrate herbivores and/or promote gas exchange.

(e) *Root velamen radiculum*. Look for a conspicuous spongy, white (especially when dry) or sometimes green cover of the aerial roots of certain light-exposed epiphytic orchids (Orchidaceae) and aroids (Araceae).

More information and photographs for the different specialised tropical strategies are in Lüttge (1997).

(12) *No specialised mechanism*

Only assign this category after careful checking for Categories 1–11, otherwise consider the species as a missing value for nutrient uptake strategy!

*Special cases or extras*

(i) For some rarer types of mycorrhiza, e.g. arbutoid mycorrhiza (*Arbutus*, *Arctostaphylos*), ectendomycorrhiza (certain gymnosperms) and pyroloid mycorrhiza (Pyrolaceae) consult Molina *et al.* (1992) or Smith and Read (1997).

(ii) There are also non-mycorrhizal vascular higher plant species capable of uptake of organic nutrient forms (e.g. Chapin *et al.* 1993b), but these cannot be identified without detailed investigation involving element isotopes.

(iii) Hemiparasites with haustoria tapping into tree branches are treated under *Growth forms* (see above).

(iv) The following list of plant families that are never or rarely mycorrhizal may be helpful: Aizoaceae, Amaranthaceae, Brassicaceae (Cruciferae), Caryophyllaceae, Chenopodiaceae, Comelinaceae, Cyperaceae, Fumariaceae, Juncaceae, Nyctaginaceae, Phytolacaceae,

Polygonaceae, Portulacaceae, Proteaceae, Urticaceae. Exceptions may occur, however!

*References on theory and significance:* Kuijt (1969) (parasites); Benzing (1976) (trichomes); Lüttge (1983) (carnivory); Sprent and Sprent (1990) (N fixers); Read (1991) (mycorrhiza); Lamont (1993) (hairy root clusters); Leake (1994) (myco-heterotrophs); Marschner (1995) (general); Pennings and Callaway (1996) (root hemiparasites); Lüttge (1997) (general, including specialised tropical strategies); Smith and Read (1997) (mycorrhiza); Lambers *et al.* (1998) (general); Michelsen *et al.* (1998) (mycorrhiza); Press (1998) (hemiparasites); Skene (1998) (N fixers, cluster roots); Spaink *et al.* (1998) (N fixers); Gutschick (1999) (trichomes); Hector *et al.* (1999) (N fixers); Aerts and Chapin (2000) (general); Gualtieri and Bisseling (2000) (N fixers); Cornelissen *et al.* (2001) (mycorrhiza); Grime (2001) (general, including hairy root clusters); Squartini (2001) (N fixers); Tilman *et al.* (2001) (N fixers); Van der Heijden and Sanders (2002) (mycorrhiza, myco-heterotrophs); Perreijn (2002) (N fixers); Lamont (2003) (hairy root clusters); Quedstedt *et al.* (2003) (root hemiparasites and N fixers); Read (2003) (mycorrhiza).

*More on methods:* Böhm (1979) (N fixers); Agerer (1986–1998) (ECM); Somasegaran and Hoben (1994) (N fixers); Brundrett *et al.* (1996) (mycorrhiza); Lüttge (1997) (specialised tropical strategies); Perreijn (2002) (N fixers).

### 3. Regenerative traits

#### Dispersal mode

##### *Brief trait description*

The mode of dispersal of the ‘dispersule’ (or propagule: unit of seed, fruit or spore as it is dispersed) has obvious consequences for the distances it can cover, the routes it can travel and the places it can end up in.

##### *How to classify?*

This is a categorical trait. Record all categories (listed below) that are assumed to give significant potential dispersal, in the order of decreasing importance. In the case of similar potential contributions, prioritise the one with the presumed longer-distance dispersal, for instance wind-dispersal takes priority over ant-dispersal.

(1) *Unassisted dispersal:* the seed or fruit has no obvious aids for longer-distance transport and merely falls passively from the plant.

(2) *Wind dispersal* (anemochory): includes (a) minute dust-like seeds (e.g. *Pyrola*, Orchidaceae), (b) seeds with pappus or other long hairs [e.g. *Salix* (willows), *Populus* (poplars), many Asteraceae], ‘balloons’ or comas (trichomes at the end of a seed), (c) flattened fruits or seeds with large ‘wings’, as seen in many shrubs and trees [e.g. *Acer*, *Betula* (birch), *Fraxinus* (ash), *Tilia* (lime), *Ulmus* (elm), *Pinus* (pine)]; spores of ferns and related vascular cryptogams

(Pteridophyta) and (d) ‘tumbleweeds’, where the whole plant or infrutescence with ripe seeds is rolled over the ground by wind force, thereby distributing the seeds. The latter strategy is known from arid regions, for instance *Baptisia lanceolata* in the south-eastern USA (Mehlman 1993) and *Anastatica hierochuntica* (rose-of-Jericho) in North Africa and the Middle East.

(3) *Internal animal transport* (endo-zoochory), e.g. by birds, mammals, bats: many fleshy, often brightly coloured berries, arillate seeds, drupes and big fruits (often brightly coloured), that are evidently eaten by vertebrates and pass through the gut before the seeds enter the soil elsewhere [e.g. *Ilex* (holly), apple].

(4) *External animal transport* (exo-zoochory): fruits or seeds that become attached to animal hairs, feathers, legs and bills, aided by appendages such as hooks, barbs, awns, burs or sticky substances [e.g. *Arctium* (burdock), many grasses].

(5) *Dispersal by hoarding:* brown or green seeds or nuts that are hoarded and buried by mammals or birds. Tough, thick-walled, indehiscent nuts tend to be hoarded by mammals [e.g. *Corylus* (hazelnuts) by squirrels] and rounded, wingless seeds or nuts by birds [e.g. *Quercus* (acorns) spp. by jays].

(6) *Ant dispersal* (myrmecochory): dispersules with elaiosomes (specialised nutritious appendages) that make them attractive for capture, transport and use by ants or related insects.

(7) *Dispersal by water* (hydrochory): dispersules are adapted to prolonged floating on the water surface, aided for instance by corky tissues and low specific gravity (e.g. coconut)

(8) *Dispersal by launching* (ballistichory): restrained seeds that are launched away from the plant by ‘explosion’ as soon as the seed capsule opens (e.g. *Impatiens*)

(9) *Bristle contraction:* hygroscopic bristles on the dispersule that promote movement with varying humidity.

It is important to realise that dispersules may (occasionally) get transported by one of the above modes even though they have no obvious adaptation for it. This is particularly true for endo-zoochory and exo-zoochory (e.g. Fischer *et al.* 1996; Sanchez and Peco 2002). Note that there is ample literature (e.g. in Floras) for dispersal mode of many plant taxa.

*References on theory and significance:* Howe and Smallwood (1982); Van der Pijl (1982); Bakker *et al.* (1996); Howe and Westley (1997); Hulme 1998; Poschlod *et al.* (2000); McIntyre and Lavorel (2001).

*More on methods:* Howe and Westley (1997).

#### Dispersule size and shape

##### *Brief trait description*

Of interest is the entire reproductive dispersule (=dispersal structure or propagule) as it enters the soil. The

dispersule may correspond with the seed, but in many species it constitutes the seed plus surrounding structures, for instance the fruit. Dispersule size is its oven-dry mass. Dispersule shape is the variance of its three dimensions, i.e. the length, the width and the thickness (breadth) of the dispersule, after each of these values has been divided by the largest of the three values (Thompson *et al.* 1993). Variances lie between 0 and 1 and are unitless. Small dispersules with low shape values (relatively spherical) tend to be buried deeper into the soil and live longer in the seed bank.

#### *What and how to collect?*

The same type of individuals as for leaf traits and plant height should be sampled, i.e. healthy, adult plants that have their foliage exposed to full sunlight (or otherwise plants with the strongest light exposure for that species). Of interest is the unit that is likely to enter the soil. Therefore, only parts that fall off easily (e.g. pappus) are removed, while wings and awns remain attached. The flesh of fleshy fruits is removed too, since the seeds are usually the units to get buried in this case (certainly if they have been through a bird's gut system first). The seeds (or dispersules) should be mature and alive. We recommend collecting at least five dispersules from each of three plants of a species, but preferably more (see below under *Seed mass*). The dispersules can either be picked off the plant or be collected from the soil surface. In some parts of the world, e.g. some tropical rainforest areas, it may be efficient to pay local people specialised in tree climbing (and identification) to help with the collecting.

#### *Storing and processing*

Store the dispersules in sealed plastic bags and keep in a cool box or fridge until measurement. Process and measure as soon as possible. For naturally dry dispersules air-dry storage is also okay.

#### *Measuring*

Remove any fruit flesh, pappus or other loose parts (see above). For the remaining dispersule, take the highest standardised value for each dimension (length, width and thickness), by using callipers or a binocular microscope, and calculate the variance (see under *Brief trait description*). Then dry at 60°C for at least 72 h (or else at 80°C for 48 h) and weigh (dispersule size).

#### *Special cases or extras*

We recommend complementing this trait with other direct or indirect assessment of banks of seeds or seedlings for future regeneration of a species. For seed bank assessment, there are good methods in Thompson *et al.* (1997), but (aboveground) canopy seedbanks of serotinous species of fire-prone ecosystems (e.g. *Pinus* and Proteaceae such as *Banksia*, *Hakea* and *Protea*) and long-lived seedling banks of woody species in the shaded understory of woodlands and forests may also

make important contributions. Vivipary as in some mangroves could also be part included in such assessments.

*References on theory and significance:* Hendry and Grime (1993); Thompson *et al.* (1993); Thompson *et al.* (1997b); Leishman and Westoby (1998); Funes *et al.* (1999); Weiher *et al.* (1999).

*More on methods:* FAO (1985); Hendry and Grime (1993); Thompson *et al.* (1993); Askew *et al.* (1997); Thompson *et al.* (1997b); Weiher *et al.* (1999).

#### *Seed mass*

##### *Brief trait description*

Seed mass, also called seed size, is the oven-dry mass of an average seed of a species, expressed in milligrams. Small seeds tend to be dispersed further away from the mother plant (although this relationship is very crude), while stored resources in large seeds tend to help the young seedling to survive and establish in the face of environmental hazards (deep shade, drought, herbivory). Smaller seeds can be produced in larger numbers with the same reproductive effort. Smaller seeds also tend to be buried deeper in the soil, particularly if their shape is close to spherical, which aids their longevity in seedbanks. Interspecific variation in seed mass also has an important taxonomic component, more closely related taxa being more likely to be similar in seed mass.

##### *What and how to collect?*

The same type of individuals as for leaf traits and plant height should be sampled, i.e. healthy, adult plants that have their foliage exposed to full sunlight (or otherwise plants with the strongest light exposure for that species). The seeds should be mature and alive. If the shape of the dispersal unit (seed, fruit) is measured too (see above), do not remove any parts until measurement (see below). We recommend collecting at least five seeds from each of three plants of a species, but more plants per species are preferred. Depending on the accuracy of the balance available, 100 or even 1000 seeds per plant may be needed for species with tiny seeds (e.g. orchids).

In some parts of the world, e.g. some tropical rainforest areas, it may be efficient to work in collaboration with local people specialised in tree climbing to help with the collecting (and identification).

##### *Storing and processing*

If dispersule shape is also measured, then store cool in sealed plastic bags, whether or not wrapped in moist paper (see under SLA) and process and measure as soon as possible. Otherwise air-dry storage is also appropriate.

##### *Measuring*

After dispersule shape measurements (if applicable), remove any accessories (wings, comas, pappus, elaiosomes, fruit flesh), but make sure not to remove the testa in the

**Table 13.** Examples of species' resprouting capacity on a scale of 1–10

Adults resprouting (%)	Aboveground biomass destroyed (%)	Resprouting capacity
100	100	100
100	75	75
50	100	50
50	75	38
25	100	25
25	75	19
0	75–100	0

process. In other words, first try to define clearly which parts belong to the fruit as a whole and which strictly to the seed. Only leave the fruit intact in cases where the testa and the surrounding fruit structure are virtually inseparable. Dry the seeds (or achenes, single-seeded fruits) at 80°C for at least 48 h (or until equilibrium mass in very large or hard-skinned seeds) and weigh. Be aware that, once taken from the oven, the samples will take up moisture from the air. If they cannot be weighed immediately after cooling down, put them in the desiccator until weighing, or else back in the oven to dry off again.

Note that the average number of seeds from one plant (whether based on five or 1000 seeds) counts as one statistical observation for calculations of mean, standard deviation and standard error.

#### *Special cases or extras*

Be aware that seed size may vary more within an individual than among individuals of the same species. Make sure to collect 'average-sized' seeds from each individual and not the exceptionally small or large ones.

Be aware that a considerable amount of published data are already available in the literature, while some of the large unpublished databases may be accessible under certain conditions. Many of these data can probably be added to the database, but make sure the methodology used is compatible.

For certain (e.g. allometric) questions, additional measurements of the mass of the dispersule unit or the entire infructescence (reproductive structure) may be of *additional* interest. Both dry and fresh mass may be useful in such cases.

*References on theory and significance:* Salisbury (1942); Grime and Jeffrey (1965); MacArthur and Wilson (1967); Silvertown (1981); Mazer (1989); Jurado and Westoby (1992); Thompson *et al.* (1993); Leishman and Westoby (1994); Allsopp and Stock (1995); Hammond and Brown (1995); Leishman *et al.* (1995); Saverimuttu and Westoby (1996); Seiwa and Kikuzawa (1996); Swanborough and Westoby (1996); Hulme (1998); Reich *et al.* (1998); Westoby (1998); Cornelissen (1999); Gitay *et al.* (1999); Weiher *et al.* (1999); Thompson *et al.* (2001); Westoby *et al.* (2002).

*More on methods:* FAO (1985); Hendry and Grime (1993); Thompson *et al.* (1993, 1997b); Hammond and Brown (1995); Westoby (1998); Weiher *et al.* (1999).

#### Resprouting capacity after major disturbance

##### *Brief trait description*

The capacity of a plant species to resprout after destruction of most of its aboveground biomass, is an important trait for its persistence in ecosystems with episodic major disturbance. Fire (natural or anthropogenic), hurricane-force wind and logging are the most obvious and widespread major disturbances, but extreme drought or frost events, severe grazing or browsing damage, landslides, flooding and other short-term large-scale erosion events also qualify. There appear to be ecological trade-offs between sprouters and non-sprouters. Compared with non-sprouters, sprouters tend to show major allocation of carbohydrates to belowground organs (or storage organs at soil surface level), but their biomass growth tends to be slower than in non-sprouters as is their reproductive output. The contribution of sprouters to species composition tends to be associated with the likelihood of any individual plant to be hit by a major biomass destruction event as well as to the degree of stress in terms of available resources.

##### *How to assess?*

Here we define resprouting capacity as the relative ability of a plant species to form new shoots after destruction of most of its aboveground biomass, using reserves from basal or belowground plant parts. The following method is a clear compromise between general applicability and rapid assessment on the one hand and precision on the other. It is particularly relevant for all woody plants and graminoids (grass-like plants), but may also be applied to forbs (broad-leaved herbaceous plants). Within the study site, or within the ecosystem type in the larger area, search for spots with clear symptoms of a recent major disturbance event. For herbaceous species, this event should have been within the same year, while for woody species the assessment may be done until 5 years after the disturbance, as long as shoots emerging from near the soil surface can still be identified unambiguously as sprouts following biomass destruction. For each species, try to find any number of adult plants between 5 and 50 (depending on time available) *from which as much as possible, but at least 75%, of the live aboveground biomass was destroyed, including the entire green canopy* (to ensure that regrowth is only supported by reserves from basal or belowground organs). [Note: in the case of trunks and branches of woody plants, old, dead xylem (wood) is not considered as part of the live biomass. Thus, if a tree is still standing after a fire, but all its bark, cambium

and young xylem have been killed, we record it as 100% aboveground biomass destruction.]

Make sure that enough time has lapsed for possible resprouting. Estimate (crudely) the average percentage of aboveground biomass destroyed among these plants (a measure of disturbance severity) by comparing against average undamaged adult plants of the same species. Multiply this percentage by the percentage of this damaged plant population that have resprouted (i.e. formed new shoots emerging from basal or belowground parts) and divide by 100 to obtain the 'resprouting capacity' (range 0–100, unitless) (see Table 13 for examples). When data are available from more sites, take the highest value as the species value. (This ignores the fact that great intraspecific variability in sprouting capacity may occur.) In longer-term studies, resprouting may be investigated experimentally by clipping plants to simulate 75–100% aboveground biomass destruction. (In that case the clipped parts can be used for other trait measurements as well!) If fewer than five plants with 'appropriate' damage can be found, give the species a default value of 50 if any resprouting is observed (50 being halfway between 'modest' and 'substantial' resprouting, see below). *In species where no resprouting is observed merely because no major biomass destruction can be found, it is important to consider this as a missing value (i.e. so do not assign a zero!).*

[Note: It is obvious that broad interspecific comparisons have to take into account an intraspecific error of up to 25 units due to the dependence of resprouting capacity on the severity of disturbance encountered for each species. However, within ecosystems where different species suffer the same fire regime, direct comparisons should be safe.]

Useful and legitimate data may be obtained from the literature or by talking to local people (e.g. foresters, farmers, rangers). Make sure that the same conditions of major aboveground biomass destruction have been met. In such cases, assign subjective numbers for resprouting capacity after major disturbance yourself as follows: 0, never resprouting; 20, very poor resprouting; 40, moderate resprouting; 60, substantial resprouting; 80, abundant resprouting; 100 very abundant resprouting. The same crude estimates may also be used for species (e.g. some herbaceous ones) for which the more quantitative assessment is not feasible, for instance because the non-resprouting individuals are hard to find after disturbance.

#### *Special cases or extras*

(i) In the case of strongly clonal plants, it is important that damaged ramets can resprout from belowground reserves and not from the foliage of a connected ramet. Therefore, in such species, resprouting should only be recorded if most aboveground biomass has been destroyed for all ramets in the vicinity, in other words if the disturbance covers a sufficiently large area.

(ii) Additional recording of resprouting ability of young plants may reveal important insights into population persistence (Del Tredici 2001), although this could also be seen as a component of recruitment. Thus, data on the age or size limits for resprouting ability may reveal important insights into population dynamics. It is known that some resprouting species cannot resprout before a certain age or size, or may lose their resprouting capacity when they attain a certain age or size.

(iii) Additional recording of resprouting (or regrowth, reiteration) after less severe biomass destruction may provide useful insights into community dynamics, including interspecific competitive interactions. For instance, *Quercus suber* and many *Eucalyptus* spp. can resprout from stem buds higher up after fire. Be aware that such species with efficient fire protection strategies and major stem biomass surviving severe fires, may give the false impression that an area has not been exposed to severe fires recently. Other species in the same area, or direct fire observations, should provide the evidence for that. In fire-prone systems where most individuals of a number of species have good resprouting potential, the biggest diameter of the remaining branches of a shrub or tree after a fire provides an indication for the severity of the fire, since thin branches tend to be more susceptible to fire than thicker ones.

(iv) This approach of recording resprouting after less severe biomass destruction could also include investigations of herbivory responses.

*References on theory and significance:* Noble and Slatyer (1977); Noble and Slatyer (1980); Rowe (1983); Pate *et al.* (1990); Bond and van Wilgen (1996); Everham and Brokaw (1996); Strasser *et al.* (1996); Pausas (1997); Sakai *et al.* (1997); Canadell and López-Soria (1998); Kammescheidt (1999); Pausas (1999); Bellingham and Sparrow (2000); Bond and Midgley (2000); Higgins *et al.* (2000); Del Tredici (2001); Burrows (2002).

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