Chapter 14

Manual on Vascular plant recording techniques in the field and protocols for ATBI+M sites – Inventory and Sampling of specimens

by

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Abstract

The methods applied by botanists and ecologists to record and describe the constantly changing diversity on earth are as varied as the vegetation and flora itself. Alongside this the literature covering these methods are numerous and diverse. The method used in the field is selected on the basis of the study aims, previous knowledge of geological, ecological and floristic features of the study area as well as the extent of the fieldwork.
This manual is an overview of methods and a basic introduction, aimed especially at beginners, to higher plant recording of any study area. It contains basic aspects of planning, carrying out and documenting an inventory project but focuses on practical work in the field, designing sample plots and preparation of herbarium specimens. Theoretical foundations, statistical approaches and analyses are not covered in this manual. Reference to further reading is not complete due to the extensive literature covering inventory methods.

Key words: Vascular plants, flora mapping, field work, methods

1. Introduction

Flora and vegetation (the species composition and the total plant community at a defined site) of vascular plants (ferns and spermatophytes) are the most easily recognizable results of abiotic, biotic and human impacts on the earth's surface. Vegetation on earth has an outstanding importance especially in terrestrial habitats. Plants are important primary producers, providing the basis for the food web, and habitat for numerous – sometimes highly specialized – animal and fungal communities. Due to the high value of vegetation as a bio-indicator, it is possible to use vegetation type to predict the occurrence of other organisms or abiotic conditions. These characteristics make the accurate inventory of the flora and vegetation of an area worthwhile for a broad range of issues in basic ecological and bio-geographical research. Flora and vegetation mapping has been used in the framework of scientific investigation of taxa, habitats and ecosystems as well as in the applied sciences for nature conservation and monitoring programs for round about hundred years.

In view of both the enormous diversity of flora and vegetation and the vast number of approaches and study objectives in this field of research there are innumerable methods and field study designs for, *e.g.,* selecting sampling sites, plot shape and size, recording species, as well as gathering species frequency and distribution data. Because of this it is difficult or often impossible to summarise data gathered from the literature and to compare them directly. To overcome this issue botanists should strive to improve fieldwork standards.

This chapter focuses on the fieldwork needed to carry out inventories and monitoring of vascular plant taxa. To inventory means recording every single taxon regardless of whether the taxon name is known to the fieldworker or not. For this purpose we need a specialised approach, different from those documented in the bulk of literature dealing with vegetation mapping which focus on methods to inventory dominant or frequent species or life-forms (*e.g.*, Braun-Blanquet, 1964; Ellenberg *et al.*, 1968; Müller-Dombois & Ellenberg, 1974; Daubenmire, 1968; Barbour *et al.,* 1999; Bonham, 1989; Elzinga *et al.*, 1998).

The first floristic maps, with just 13 grid squares, were produced in the Netherlands at the beginning of the last century (Goethart & Jongmans, 1902). Ostenfeld (1931) presented a combination of point and area mapping in "Danmarks Topografisk-Botaniske Undersögelse". In the last fifty years, many mapping projects have been initiated, *e.g.,* the "Atlas of the British Flora" (Perring & Walters, 1962), the "Mapping of Central Europe", which uses grid squares of 10' longitude and 6' latitude (about 12 x 10 km), (Niklfeld, 1972), or the "Atlas Florae Europaeae" on the base of 50 x 50 km grids. Over the decades, an increasing number of publications have focused on methods and standards of flora and vascular plant diversity mapping (*e.g*., Niklfeld, 1978; Magurran, 1988; Wilson, 1988; Soulé & Kohm, 1989; Økland, 1990; Peters & Lovejoy, 1992; Stohlgren, 1994; Peterson *et al.*, 1995; Dallmeier & Comiskey, 1996; Nusser & Goebel, 1997; Ashton, 1998; Krebs, 1999; Hill *et al.*, 2005; Rich *et al.*, 2005).

Widely accepted standards for fieldwork techniques for species inventory do not exist. Only a few studies have investigated the accuracy, efficiency, and validity

of different methods (see overview in Stohlgren, 2006). The detailed study to consider standards for mapping and other conservation methods was published in Germany (Plachter *et al.*, 2002). An outstanding example of a detailed manual is given by Bergmeier (1992), which is based on 20 years of experience from the Central European floristic mapping project.

Monitoring of flora and vegetation, usually based on mapping projects, is becoming more and more important, particularly in the context of increasing extinction worldwide and accelerating climate change (*e.g.*, Campbell *et al.,* 2002; Pereia & Cooper, 2006; Cleland *et al.*, 2007; Kull *et al.*, 2008). Monitoring the biodiversity of an area involves regularly recording data at a site using defined recording methods. Monitoring studies may be applied at the level of landscape, ecosystem, species, population or genetic diversity (Noss, 1999) and provides data to observe long-term changes in plant diversity. A detailed manual for monitoring standards of endangered vascular plant species in the UK with many descriptive case studies is provided by JNCC (2004), a general overview about planning, methods and realisation in Hill *et al.* (2005).

This manual aims to convey the general principles and basic methods of flora mapping and monitoring. It is written for students and other beginners in the field with basic taxonomical and ecological knowledge. We focus on the inventory and monitoring of biodiversity expressed by the composition of vascular plants species visible above ground at the time of fieldwork in a given area and recorded metrics may include species abundance, frequency, and cover. For practical reasons, the soil seed bank is not taken into consideration. Likewise, neither the genetic diversity nor the diversity of plant communities are covered in this manual.

Completing an inventory of vascular plant flora for a region includes several key activities in the field: recording taxa and related data and making herbarium specimens. The taxon list should be accompanied by herbarium specimens, as well as geographical and accurately observed ecological data from the site and metadata (collector's name, institution, expedition, ...).

2. Inventory of vascular plant taxa

2.1. General comments

When beginning fieldwork planning one should bear in mind the why this work is proposed. The following questions of particular importance should be addressed: How large is the study area? Which infraspecific taxonomic levels ought to be considered, *i.e.,* should subspecies, varieties, and microspecies be recorded? How much time and what personnel resources are available? What monitoring intervals are needed?

The sampling strategy depends on the questions posed above. In fact, one must consider if it is feasible to explore the whole area or whether representative sample plots within the investigation area or transects along ecological gradients are necessary to sufficiently survey the flora. How many sample plots are needed and where should they be located? What is the best plot size and shape? What

additional environmental data should be recorded and what methods are to be applied for this purpose? Are there locals who know the area and are willing to provide support?

Several factors increase the likelihood of a complete inventory. These include smaller and more homogeneous investigation areas or sample plots, the experience of the observer, the amount of sampling and the time invested.

Collection permit

All fieldwork, visits to conservation areas, and collections must be made legally. If you work in protected areas or need to collect endangered or protected plants do not forget to ask the responsible authorities for the collecting permission.

2.2. Investigation season

In most cases it is not feasible to completely inventory all plant species in a single excursion. In fact, for a full inventory of the vascular plant flora it is crucial to consider the different phenological aspects of the flora during the growing season. For instance, geophytes are often underrepresented in mapping projects because they appear mainly either before or after the main growing season. Therefore, selecting the time of fieldwork is an important issue. If only one visit to the study area is possible, it is obvious that this should take place at the peak of the growing season when most species are in flower ('peak phenology') so as to observe as many species as possible and to collect a maximum amount of data. To also find species which are only recognizable in early Spring or in late Autumn, several visits are crucial. As a rule, it can be stated that an area should be visited at least two times, *e.g.,* in the lowlands of Northern and Central Europe the best time for surveying the flora is in Spring and Summer, in the Mediterranean region in early Winter and late Spring, in tropical regions prior to and immediately after the rainy season. The timing of fieldwork is further dependent on the sea level of the investigation area, on predominant habitats, on the substrate, and on the local (micro)climate.

Knowledge of local experts and the study of literature and herbarium vouchers help to choose the best time, but be aware of overall weather conditions in the year when the investigation takes place. The weather influences highly the phenology of plants (*e.g.*, Pfeifer, 1996). Very hot weather accelerates the growth and flowering of plants and cold weather may retard growth by up to four weeks or more. In deserts, the majority of vascular plants are annuals which germinate and flower only after rainfall. Precipitation, and thus these annuals, may not occur for several consecutive years.

2.3. Fieldwork design

Once the aim of the fieldwork and the target area has been chosen, the method of recording data must be selected. There is no method, which is suited to every inventory or investigation region so the influence of the chosen method of sample

design, *e.g.,* the size of grids or the size, position and even the shape of sample plots (Keeley & Fotheringham, 2005) on results should be remembered.

It must be emphasized that searching, recording, and mapping taxa in a given area or region is distinct from qualitative vegetation analysis where a subjective, rather than a non-random or systematic, selection may be regarded as problematic (Daubenmire, 1968; Müller-Dombois & Ellenberg, 1974). In fact, in order to record all species, including the rarest, the selection of sample sites and transects, respectively, should not be done in a systematic or random way, but should be adapted to the heterogeneity of the terrain and the types of vegetation, respectively. Furthermore, a complete inventory requires careful attention to all microhabitats and transitions of plant communities. To record a maximum percentage of taxa in an area, all vegetation types and especially habitat borders should be visited: *e.g.* dunes, shingles, cliffs, inland surface waters, mires, bogs, fens, grasslands, forb vegetation, scrubs, heaths, woodland, forests, ruderal places, agricultural and artificial habitats. Tree falls are valuable sources of branches with leaves, flowers, and fruits as well as epiphytic and liana vegetation which are usually not easily accessible.

The flora of a small region may be surveyed completely by covering the whole area and surveying all taxa within this area. Larger areas are usually divided into grids*,* the flora of each grid being surveyed separately (see below). In the case that an area is too large for a complete exploration or else if personal, temporal or financial resources are too scarce, sample plots are assumed to represent the flora of the whole region. Before fieldwork takes place it must be decided whether and how many single scale plots, transects or nested multiscale plots are chosen. The number of plots necessary to record plant diversity most accurately strongly depends on the diversity of habitats and on the homogeneity of vegetation and must be defined in view of including all habitats and may include replications. As a rule, one has to find the balance between the completeness of the taxa inventory and time- and cost-efficiency. For benefits and drawbacks of several field methods see Rich *et al.* (2005) and Stohlgren (2006), for the tropics in particular Dallmeier (1992) or Jermy & Chapman (2002).

Data should be collected in a way that is traceable in the study area years later and fit for monitoring purposes. In order to increase efficiency and to allow accurate replications of methods fieldtrips should be well documented, *e.g.,* the number and experience of the staff involved, the time spent in the field and logistics of the fieldwork. Photographs of the sites may be helpful for monitoring purposes, provided that they contain permanent field markers, *e.g.* trees, buildings, prominent rock formations, in such a way as to easily understand the position of the photographer. Alternatively or in addition, the position of the photographer as well as the direction of the shooting should be recorded. The scale of maps used in the field should be at least 1:50.000, optimally 1:25.000 and in large areas with a homogenous flora maximally 1:100.000.

When selecting sample plots one should consider also the susceptibility of the terrain to trampling damage caused by fieldwork. If such damages are expected, access must be limited. As to the sensitivity of habitats in general, an appeal is made to common sense.

2.3.1. Flora mapping of grid cells

A widespread method for surveying plant diversity in a region is constituted by the flora mapping of grid cells whose size and position is given by the mapping project or conform to the grids used in the region (*e.g.,* UTM, 'quadrants'). Grid cells are either explored exhaustively or the flora of each cell is recorded in a representative manner by means of excursions following a fixed pathway. The results for each region and grid cell, respectively, are shown in the form of a checklist. Mapping grid cells is highly recommended. In fact, since all cells have to be explored regardless of possible logistical obstacles or the mappers' laziness, this kind of mapping provides a differentiated picture of the distribution of species in the study area. It is recommended that the investigation area is divided into grid cells which can be investigated within a day or half a day.

2.3.2. Single sample plots

Generally, the size and number of sample plots has to be adapted to the given vegetation. Several methods are available to determine the minimum size of a plot for recording a pre-assigned (high) percentage of species in different vegetation types. Best known is the 'minimum area' method used in phytosociology. It has fundamentally influenced the determination of sample-plot size (see bibliography of Tüxen, 1970; Barkman, 1989; Dietvorst *et al.,* 1982). Other, similar methods include the calculation of species accumulation curves (*e.g.,* Fisher *et al.,* 1943; Barbour *et al.,* 1980; Palmer, 1990; Palmer *et al.,* 1991; Elzinga *et al.* 1998; see also the discussion in Chong & Stohlgren 2007, Hui 2008; Gray *et al.,* 2004a, b; Keeley, 2003; Scheiner, 2003, 2004) but in the context of the fieldwork they seem rather elaborate and time consuming. Furthermore, they do not necessarily account for the presence of rare species sufficiently. Therefore, it is preferred to use empirical values which are applicable in the field (Table 1). However, in regions with an estimated rich but unknown flora, plot size determination by means of statistical methods is highly recommended. The plots were measured off in the field using tape and marked with ground stakes, coloured bands and/or small flags.

2.3.3. Transects

The transect method is recommended for large areas with one or more ecological gradient *e.g.,* humidity, sun exposition, edaphic conditions or altitude. To inventory for all taxa, all vegetation types must be considered. To set a transect means to define a plot, usually of a (long) rectangle shape, within an area comprising the ecological gradients. By doing so, the maximum range of habitat and species diversity can be covered within a minimum space and with a minimum of resources. Transect length and width largely depend on the size of the investigation area. If a transect is large, sample plots may be defined within the transect at regular distances. Transect sample distances will depend on vegetation uniformity and the overall transect size.

Vegetation types outside but in the immediate vicinity of the transect should also be investigated for new taxa but the records kept separately. For a usable

transect method in tropical forests along a precipitation and latitudinal gradient see, *e.g.,* Gentry (1982, 1995) or Clinebell *et al*. (1995).

	Müller-Dombois & Ellenberg (1974)	Dierschke (1994)
Rock vegetation, spring meadow vegetation,		up to 5 m²
Fens, pioneer lawn, and pastures		up to 10 m²
Herbs	1-2 m²	
Coast dunes, wet and dry meadows, mountain meadows, heath, bulky sedges		10-25 m²
Dry-grassland	50-100 m²	
Weed and ruderal vegetation, scrubs, rocky meadows		25-100 m²
Tall herbs-low shrubs	10-25 m²	
Tall shrubs	16 m²	
Large plants/trees/forest	200-500 m²	>100 - >1000 m²
Forest understory only	50-200 m²	100-200 m²

Table 1. Adequate single plot sizes for flora and vegetation analyses.

2.3.4. Multiscale plots

Instead of using several smaller sample plots or few large transects, multiscale plots as overlaying nested quadrats of increasing size (*e.g*., Müller-Dombois & Ellenberg, 1974; Barnett & Stohlgren, 2003) can be used. Among them, the modified Whittaker plot (Whittaker, 1977; Shmida, 1984; Stohlgren *et al*., 1995) has proven itself in practice. The modified Whittaker plot is a combination of one 1000 m² plot containing subplots of several sizes (Fig. 1). While the flora of the smaller plots is recorded exhaustively, less extensive systematic surveys are carried out in the larger plots. This design has been increasingly applied in the last years for the calculation of plant diversity (*e.g*., Keeley *et al*., 1995; Bellehumeur & Legendre, 1998; Carrington & Keeley, 1999; Brown & Peet, 2003; Byers & Noonberg, 2003; Bruno *et al*., 2004; Fridley *et al*., 2004; Davies *et al*., 2005). Multiscale-sampling is more labour- and cost-intensive but it allows estimates of species richness and plant diversity patterns to be made. This approach is based on the assumption that patterns of plant diversity can be calculated only on the basis of multiscale sample plots (Shmida, 1984). It is particularly helpful if the collected data is statistically evaluated (*e.g.,* for extrapolating species richness or total diversity) and allows diverse plant communities to be compared.

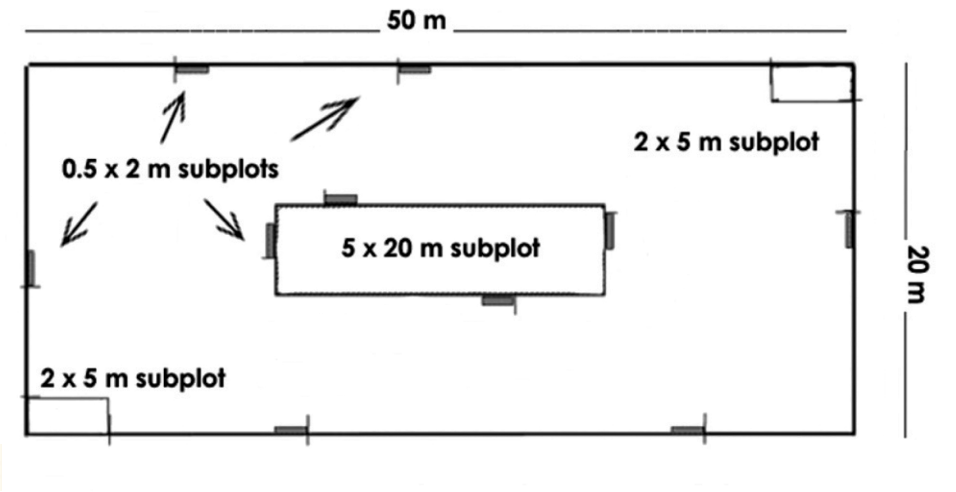

Fig. 1. An example for a modified Whittaker (Shmida, 1984; Stohlgren *et al.,* 1995) plot.

2.3.5. 'Tips and Hints'

For larger, complex areas it is recommended that several fieldtrips are undertaken during different seasons and that each utilises several plot-based-sampling techniques to record a high percentage of the vascular plant flora for checklists and to monitor plant diversity as accurately as possible.

Research can benefit from studying geological maps, biotope maps or high resolution satellite images prior to fieldwork. In fact, this will facilitate the efficient planning and implementation of fieldwork. Possible barriers and dangers in the field, like steep slopes, insurmountable streams or fens (as well as the possible appearance of wild animals) should be identified in the planning phase.

For monitoring plots it is helpful to mark the edges and the centre of each plot with magnets in order to localise the plot later by means of special detectors. Since magnets, particularly when buried several cm into the soil, may get lost, it is recommended that the plots are marked on a map and their coordinates recorded.

2.4. Taxa Recording

To inventory vascular plant taxa is to record all visible taxa – vegetative plants, bloomy plants as well as plants with fruits – by searching the whole area or representative plots for the purpose of compiling or verifying a checklist. A complete inventory includes, of course, not only dominant and frequent species but also rare and inconspicuous ones. In fact, these can make up half of the taxa in a region (Stohlgren *et al.,* 2000) yet are often only recorded after systematic, targeted and time-consuming surveys.

In the field, all plant taxa are to be noted with scientific names. Taxonomy (and preferably also nomenclature) should refer to a widely accepted modern (local)

flora. Exceptions, *e.g.,* if detected species are not (yet) treated in the reference flora or if the field worker adheres to another species concept, should also be documented. Herbarium specimens should be collected for at least those taxa that are: (i) new to the region, (ii) indicated as doubtful, (iii) belonging to taxonomically critical groups (see below). If resources allow, all taxa should be documented by at least one herbarium specimen (see below).

With a few exceptions, *e.g.,* in species-poor habitats with short growing seasons, a species inventory in a certain place and time is hardly ever complete, even when carried out by experienced botanists, and always represents a snap-shot in time. This is because species show different phenology and because the species composition of almost every habitat is subject to ongoing changes. Competent surveyors add significantly to the likelihood of a complete species list as do small survey areas and amply time available for the fieldwork. Likewise, consulting regional floras prior to the fieldwork will give an estimate of the species number to be expected, and provide a comparative list to evaluate the field results against. Statistical methods for evaluating the completeness of the taxa inventory are provided by, *e.g.*, Heltshe & Forrester (1983), Miller & Wiegert (1989), Palmer (1990), Palmer *et al.* (1991).

2.4.1. Providing additional data and metadata

The quality of biodiversity data depends on the calibre and quantity of additional data and metadata provided. Parameters include constant ones, among them mainly geographic data (see above), as well as those which are to be recorded at each collecting date and which have a considerable impact on long-term changes in plant diversity: biotic data concerning, *e.g.*, phenology or herbivory, and abiotic data concerning disturbances caused by extreme atmospheric conditions, fire, windstorms, geological processes or human impact. This is also important for monitoring. The dynamics of the populations in an area can be observed in detail over the period of monitoring more effectively if larger numbers of parameters are recorded, *e.g.*, size, extent and vitality or fitness of the population.

Record additional data separately for each region / subregion / plot / transect in a fieldbook (notebook) or on a passport data form. The documentation should include (see also methods and standards on georeferencing):

- Name and address or institution of the field workers.
- Collecting date.
- Location (country, nearest city or landmark described with cardinal direction), exact position and altitude of a record using a map or a Geographical Positioning System (GPS). Reference must be made to the map projection and geodetic datum. Avoid local terms and hints for landmarks and sites which are only known to people who know the locality.
- Ecological conditions (*e.g.,* edaphic conditions, gradient, cardinal direction, trophic level).

- Habitat type (*e.g.,* EUNIS classification), vegetation type, and human use or impact as well as predominant or characteristic species.
- Population size, vitality.

The size of a plant population (*i.e.* all individuals of a species in a region at the same time) which should be recorded wherever possible is highly influenced by environmental conditions, dispersal barriers, and specific breeding system. It is sometimes difficult or even impossible to define and delimit a population; the same holds true for an individual (*e.g*., Silvertown & Charlesworth, 2001; Gibson, 2002; Crawley, 1997; Gurevtich *et al.*, 2003).

Frequently, an exact description of population size makes sense only for clearly delimited populations such as species occurring *e.g.* in small patches of dry grassland, clearings in forests and small raised bogs. The size of a delimited population can be determined by counting or measuring the individuals, visible shoots or the area covered.

In the field, a practicable procedure is recommended and the frequency of the species in the investigation area at least should be assessed through proxy measures such as the number of individuals in samples, individual abundance, the area or through a combination of these *i.e.* the 'cover-abundance' (*'Artmächtigkeit'*) in a sample plot. The disadvantage of estimated values is that they do not represent exact measured data and may differ between field workers. However, experience has shown that they have merit for the description of the flora and vegetation of a region.

2.4.1.1. Distribution in the investigation area

The area covered by a population may serve as the base for monitoring species and populations (Jones, 1998; Brzosko, 2003), and should, in case of small populations and rare species, be estimated as accurate as possible. In the case of larger populations it is useful to map their boundaries if possible, preferably with the help of high resolution satellite or aerial images.

2.4.1.2. Abundance

Recording abundance (*i.e.* the number of individuals of a taxon in a given area) of all species occurring in the investigation area, wherever possible, is recommended. Abundance is a common parameter used to monitor rare plants and small areas. One must bear in mind, however, that recording abundance is often a difficult task insofar as it is sometimes difficult or even impossible to determine what an individual is. In fact, while individuals can easily be recognized in annual or biannual herbs or trees with one stem, this is difficult or impossible in clonal plants. In practice, it has proven useful to refer to shoots and leaf rosettes when counting 'individuals' of clonal, non-flowering or non-fruiting plants. Generally, the abundance of a taxon is recorded through rough estimation of individuals per investigation site, using a logarithmic scale as shown in the example in Table 2 (see also discussion in Barkman *et al.*, 1964). An alternative

is to use simple descriptor such as ‘rare’ or ‘frequent’ which at least give information about the representation of the species in the field.

Abundance class	Abundance in the investigated area / sample plot
1	one individual (very rare)
2	2-10 individuals (rare)
3	11-100 individuals (common)
4	101-1000 individuals (frequent)
5	> 1000 individuals (very frequent)

Table 2. Scale for rough estimation of abundance in a given investigation area or sample plot.

2.4.1.3. Cover

The amount to which plants of a species, seen from the ground (surface), cover a specific area of ground is called ‘cover’. It is often easier to assess cover than abundance, as individuals do not have to be delimited. Estimating cover is particularly useful when dealing with stoloniferous species, among them many Poaceae and Cyperaceae. A frequently used scale for cover estimation (see also Barkman *et al.*, 1964; Braun-Blanquet, 1964) is shown in Table 3.

Cover classes	Range	Midpoint
1	0-5%	2.5%
2	5-10%	7.5%
3	10-25%	17.5%
4	25-50%	37.5%
5	50-75%	62.5%
6	75-100%	87.5%

Table 3. Scale for estimation of cover.

Combined abundance / cover scale

When dealing with small plots, particularly in the framework of monitoring selected rare and endangered species or habitats, a vegetation relevé is recommended using the Braun-Blanquet’s cover-abundance scale (Braun-Blanquet, 1964) modified in the lower scale range by Reichelt & Wilmanns (1973) (Table 4). This is particularly recommended in regions where phytosociological studies, including a syntaxonomical system, have already been carried out. The vegetation relevé requires records to be taken in a specific and comparable

manner. The required records include the flora of the sample plot, the number of individuals (if feasible, see discussion above) and species cover. Furthermore, the method also provides a phytosociological survey. Relevés must correspond to the current phytosociological practice, *i.e.,* they must be based on homogeneous and sufficiently large areas.

scale	combined abundance/cover classes (Artmächtigkeit)	number of individuals
r		1
+		very few
1	0-5 %	variable
1m or 2m	< 5 %	> 100
2a	5-12,5 %	variable
2b	12,5-25 %	variable
3	25-50 %	variable
4	50-75 %	variable
5	75100 %	variable

Table 4. Cover-abundance scale (according to Reichelt & Wilmanns, 1973; Dierschke,1994).

2.4.2. Fitness Parameter

Besides data regarding size and distribution, information concerning the fitness may provide valuable hints about the status of the population. In the framework of mapping projects it is advisable to take into consideration parameters which can be ascertained quickly and easily, for example (approximate) mean height of plants, leaf size (Jones, 1998) or the proportion of flowering and fruiting plants. If monitoring includes revisiting individuals, these need to be adequate marked. Use for example rustproof metal tags fixed to a bar in the ground or fixed on branches. In addition, geo-data must be recorded. Many fitness parameters require time-consuming recording techniques and are generally used only in special monitoring projects. Such parameters include, *e.g.,* leaf size, number of seeds or fruit sets, number of seeds per fruit, germination rate, biomass, development of leaf rosettes and number of flowers (*e.g.*, Brzosko, 2003; Vitt & Havens, 2004; Willi & Fischer, 2005; Janečková *et al.*, 2006).

2.4.3. 'Tips and Hints'

In the field, it is convenient to mark off the observed taxa directly in a checklist of all taxa known from the region. Lists of critical taxa combined with knowledge from local experts point the fieldworker's attention to these taxa. Special

seasonal lists or marking checklists for, *e.g.*, Spring taxa, helps mapping in the beginning of the vegetation period.

If using a checklist to mark the species directly in the field, use one list for each grid, transect or sample plot, respectively. Before switching over to other vegetation types or new areas (*e.g.*, new grid, plot or transect) check carefully the edge of habitats, microhabitats like rocks, and inaccessible sites like the understory of (thorny) shrubs or nettle plants for tiny, prostrate species.

Record all data instantly in the field! After a long collecting trip it is impossible to remember all details.

A passport (collecting) data form is included in the appendix. It can be adapted to personal needs. Checklists and passport forms used for fieldwork should not be copied on white but on coloured or grey recycled paper, because white paper is strongly reflective on sunny days. When getting wet, absorbent paper dries faster than ordinary paper. Leave some blank lines in the fieldbook or data form between two collection notes for additional observations and comments. Bear in mind that someone else might need to read your personal comments, therefore, write legibly using a soft pencil or pen with water resistant ink and avoid any kind of (personal) abbreviation. Once lost in the field coloured notebooks and pens are easier to recover in dense vegetation! Finally, don't forget to backup all your field notes by photocopying the field notebook or the passport sheets as soon as possible.

The use of a dictation machine can be very helpful, especially in bad weather.

2.5. Making herbarium specimens

For species inventory and monitoring in particular, the collection of herbarium specimens is necessary to check field identification, especially when dealing with critical taxa. The high value of herbarium specimens as the basic of botanical research (taxonomy, morphology, phylogeny, ecology, phytosociology, ...) cannot be overemphasized.

In most herbaria, rare taxa (often from only a few well known localities!) are overrepresented, whereas common species are represented by only a few specimens. In order to set up a representative collection in herbaria, however, it is necessary to collect material from frequent and common taxa as well as from infrequent and rare taxa. The value of a herbarium voucher increases significantly with the collector's accuracy when choosing, collecting, pressing, arranging and documenting the voucher. The basic techniques of this procedure are the subject of the next paragraph. For a further in-depth study we refer to literature which offers a comprehensive introduction into the issue (*e.g.*, Savile, 1964; Radford *et al.*, 1974; Jain & Rao, 1977; Cullen, 1984; Lot & Chiang, 1986; Vogel, 1987; Stace, 1989; Walters & Keil, 1996; Bridson & Forman, 2004; Linnartz, 2007).

Numerous plant groups require special collecting techniques. Among these groups are succulent or fleshy plants (*e.g.*, Fosberg & Sachet, 1965; Jain & Rao, 1977; Leuenberger, 1982), aquatic plants (Taylor, 1977; Lot, 1986; Haynes,

1984; Rayna-Roques, 1980), Araceae (Nicolson, 1965; Croat, 1985), Balsaminaceae (Grey-Wilson, 1980), Bromeliaceae (Aguirre León, 1986), Bambusoideae (McClure, 1965; Soderstrom & Young, 1983), Lentibulariaceae (Taylor, 1977), Musaceae (Fosberg & Sachet, 1965), Palmae (Balick, 1989; Dransfield, 1986), Pandanaceae (Stone, 1983), Pteridophyta (Holttum, 1957; Henty, 1976), and Zingiberaceae (Burtt & Smith, 1976).

Beginners and students are urged to visit a herbarium prior to fieldwork. By doing so they may acquaint themselves with the most important features of a herbarium.

2.5.1. Collecting

When collecting herbarium specimens in the field, select individuals representative in size, morphology and colour. Plants should be as complete as possible and include inflorescences, fruits and seeds, as well as all types of leaves (small and large, young and older leaves, ground and stem leaves, rosette leaves, bracts), especially in heterophyllous species, and roots or rhizomes, respectively. Be aware that organs (especially rhizomes) may be cut or broken and thus overlooked easily when digging the plant. Further, keep in mind that some species are dioecious and should be represented in the herbarium by both female and male plants. All other features important for species determination that cannot be drawn from the herbarium specimen, such as stem characters, bark structure and life form, ought to be noted in the field book or the data sheet. Record colours and scents of flowers and leaves, if noteworthy, since these features may vanish or change during pressing or over time. Additionally, photographs of such details may be attached to the herbarium sheet. Avoid collecting untypical small plants solely because they fit the herbarium sheet size. Try to make them fit by using adequate techniques (see below).
When encountering populations which include only a single or few individuals no complete plants must be harvested. The same holds true for very rare and endangered species. If absolutely essential, take a small part of one plant which shows all morphological features necessary for a correct determination. In any case, take photographs of all important details.

If you collect more than one specimen, these should cover the morphological variation within the population. Collect, if possible, plant material enough to produce at least three specimens: one for an institution of the country of origin, one for the species identifier as ‘reward for determination’ and one for your institution. The locations of the duplicates should be documented.

Each specimen should be provided with a unique collection number, *i.e.* a number which, in combination with the collector’s name, unambiguously identifies a specimen. This number can be attached to the specimen with a fixed tag (*e.g.*, jeweller’s tag), labelled with pencil or water resistant ink. Use a serial number sequence which allows for unambiguous identification of all specimens (*e.g.*, Smith, 2340). Prepared tags with running numbers can help handling the vouchers. Numbers of the specimens and pictures, geo-data and detailed documentation must be noted on the collecting sheet or in the field notebook.

Plant samples can be stored in plastic bags or pressed immediately in the field. The advantage of pressing in the field is that the specimens maintain their shape to such an extent that, after the field trip, the position of flowers, stems and leaves can be arranged and corrected without difficulties before drying the specimen. Many taxa (*e.g.,* species of *Linum, Cistus*, *Hibiscus, Impatiens*) have flowers or leaves too delicate to be stored in plastic bags. Specimens of these taxa are best pressed immediately, and some of their flowers put into spirit (see below). To protect delicate flowers, press them in kitchen paper or toilet tissue, this should not be removed until the flowers are completely dry. For the field press, use a DIN A3 or A4 portfolio or two lightweight boards filled with newspaper and a few corrugated cardboards. If plastic bags are used for collecting, use separate bags for small plants and others for large, heavy plants. You can delay wilting by increasing humidity within the bag: put some water in the bag, close it, shake it and remove the surplus of water; too much water may lead to the collapse of flowers and leaves. Transport water plants in water.

Sometimes it is necessary or helpful to put collected plants or parts into chemical fixatives (*e.g*., Tomlinson, 1965). Normally, 70% alcohol is used (in emergencies high proof spirits (*e.g.,* Vodka, Gin, Rum) can be used as a substitute), optionally with a few drops of glycerine. Also common are mixtures of alcohol and glacial ethanoic acid at a ratio of 18:1 (AA) or mixtures of alcohol, formalin and glacial ethanoic acid at a ratio of 18:1:1 (FAA). After the fixation for 2-3 days in AA or FAA, the samples are transferred to 70% ethanol for storage.

In this way, delicate and tender floral characteristics relevant for a correct identification can be preserved. This is particularly important for taxa in the Aristolochiaceae, Asclepiadaceae, Balsaminaceae, Begoniaceae, Commelinaceae, Gesneriaceae, Lentibulariaceae, Orchidaceae, Orobanchaceae, Passifloraceae, and Portulacaceae. In case of tender water species plants may be fixed as a whole, in case of Gymnospermae with easily dropping needles (*e.g*., *Picea*, *Tsuga*) whole branches may be fixed.

The hermetically sealed tubes or bottles with the fixed plants should be labelled (small labels, pencil!) inside and outside, and the cap of the container should additionally be wrapped in Parafilm.

When collecting herbarium specimens, it is easy to collect silica gel samples for DNA-banks or/and seeds simultaneously (ENSCONET, 2009).

2.5.2. Pressing

Place each specimen in a newspaper sheet or between very thin, yet strong absorbent paper and arrange it as carefully as possible. Spread the leaves in such a way as to not cover the stem, flower and fruits. Leaves should overlap as little as possible. Reverse at least one leaf, in order to make both sides visible when the specimen is mounted on a herbarium sheet. Ensure all leaves are smoothly pressed. Make sure that flowers are arranged in different positions so as to make visible the calyx, stamens and carpel. Divide the flowers or cut dense inflorescences, like the capitulum of Asteraceae, in order to reveal hidden bracts. In the same way cut large fruits or thick stems.

Overlapped parts of the plant should be separated with tissue paper. If branches are too thick leaves and flowers get pressed insufficiently and become wizened. In such cases the empty space between (thinner) organs and hardboard may be filled with tissue paper so that all plant parts undergo the same pressure. If the plant is too big to fit into the press, fold the stem and big leaves, or divide the plant and press the single parts in different folders.

Palm leaves should be cut round the hastula, *i.e.* the leaf base, which is important for species identification, and further features of the palm leaves like size or the position of the inflorescence should be noted. Leaves of big ferns should be divided: press apical, mid and basal parts, and the petiole separately. Note the arrangement of the pinnae and the leaf size (Holttum, 1957; Henty, 1976).

Succulent and fleshy plants need a special pressing and drying procedure. Cut the plants and kill them by putting the parts either into boiling water, in the microwave or in alcohol (Fosberg & Sachet, 1965; Leuenberger, 1982; Womersley, 1981).

Aquatic plants need a special treatment, too (Taylor, 1977; Lot, 1986; Rayna-Roques, 1989). Arrange them on a paper floating in a tub filled with water, the paper being of the same size of the definite herbarium sheet. After the arrangement pour the water slowly and carefully out of the tub. The plant will remain attached to the paper sheet and is ready to undergo the regular drying procedure (see above).

Fig. 2. Simple equipment for pressing plants: plywood pieces or metal frames for the outsides of the press, absorbent paper, corrugated cardboard, and lashing straps.

Fig. 3. Plant press, with specimens in newspaper sheets between corrugated cardboard.

Between the papers with the specimen, put blotting paper or corrugated cardboard. Place this stack between two light boards with holes for better drying and clamp it securely with two or three straps (Figs 2 & 3).

2.5.3. The Alcohol or 'Schweinfurth' press

Sometimes, especially in the Tropics, drying equipment is not available. In such cases the use of the alcohol press (Womersley, 1981) is recommended. To conserve your collection with alcohol, bundle the newspaper with the specimen and put it into leak proof plastic bags. Make sure that the specimens are labelled with alcohol resistant ink (black china) or a soft pencil. For a pack with a high of 20 cm you need about 1 litre of 50-70% ethanol or isopropanol. Pour alcohol into the bag, turn the bag several times to disperse the alcohol and store the bundle in a horizontal position. Turn it every day until the bundle is completely saturated with alcohol. Avoid too much solution: the bundle must be completely moist, but not wet. After arriving in the lab or herbarium, dry the specimens in a drying oven as if they were fresh material. Treating the press with highly toxic formalin solutions should be avoided for environmental reasons.

The advantage of this method is that the specimens are protected against mould, but there are several disadvantages: the plants loose their colour, the specimen becomes brittle and it cannot be used as a source of DNA.

2.5.4. Drying

The faster the drying process the better the specimen will be conserved. Keep the press in a well aired warm place; if possible, expose it to the sun. If no drying sets are available, the drying paper or corrugated cardboard layered between the specimens need to be replaced every day within the first couple of days (depending on the plant material). At the first change, the correct arrangement of the whole plant must be checked, especially when dealing with delicate flowers and leaves. If the plants are very wet, replace the drying paper after three to four hours. Later, changing the paper is only necessary every second or third day until the specimens are completely dry. Coriaceous leaves need a lot of time to dry and may appear dried though still wet. To test whether they are dry bend the leaves carefully: if they are still twistable leave them in the press to continue drying.

Under humid conditions as in the tropics a drying set is recommended. Such a set is based upon air-drying forced by a fan heater or other heat sources. The warm air is conducted through the plant press, thereby drying the plant material. Botanists have competed with each other to invent (funny) drying constructions by using various heat sources like charcoal, light bulbs, kerosene or propane (which doesn't work in high altitudes due to the low oxygen content of the air!). However, exaggerated heating is to be avoided to preserve colours and to prevent browning of plant tissue (Camp, 1946; Allard, 1951).

Fig. 4. Drying set with an electric heater and a funnel of fire resistant canvas.

We suggest a simple and cheap technique by using a small electric heater. Wherever electricity is available this is a safe and quick way to dry plants. Connect the heater and the press with a funnel of fire resistant textile, *e.g.*

canvas which you can sew in the exact size of heater and press (Fig. 4). Put up to four newspaper folders containing the specimens between two corrugated boards. Piled in this way, the whole pack can be dried overnight. Pay attention that the corrugated cardboard is arranged longitudinally to the airflow and metal framed plant presses are not used.

It is also possible to dry plants with an iron by wrapping them in highly absorbent drying paper and ironing with low temperature and moderate pressure. Replace the paper when it becomes moist. Ironing with temperatures of around 30°C (but not more!) permits drying of delicate flowers and preserves colours. It is not recommendable to use an oven for plant drying because in an oven there is no exchange of air. If an oven is the only source of heat, make sure that the warm air flows through the corrugated cardboards.

If external heat sources are not available, silica gel may be used for plant drying instead. For that purpose the press, which should not be too huge, is put into an air permeable fabric bag. The bag is then placed together with silica gel inside an airtight plastic bag. The silica gel will have to be changed more often if the plants are very wet or there is only a small volume of silica gel. Indicator silica gel which changes colour when saturated with water is recommended. Silica gel can be dried in an oven and used repeatedly.

If drying systems provided with external heat sources are used, be aware of fire, especially when handling specimens conserved in alcohol! Inside of buildings do not forget to install a fire alarm in your room.

2.5.5. Herbarium sheets

Each specimen is provided with a herbarium label containing at least the following standard information: collection site including exact description of the locality (state, province, district, toponym), coordinates, altitude and information regarding the habitat (*e.g*. surrounding vegetation); the collector's name; collection date. At best, additional information may appear on the label for example the chorological status (if known or estimable) or noteworthy observations regarding *e.g.* population size, threat, …

Usually specimens are mounted on a white cardboard paper by means of gummed paper stripes or glue from hot-glue guns. Seeds and other small broken plant parts are normally stored in paper capsules which are attached to the herbarium sheet. As each large big herbarium has its own standards and methods of moulting this topic will not be covered further in this manual. See special literature (*e.g.,* Bridson & Forman, 2004; Liesner, 2009) and study label examples (Fig. 5) for that purpose.

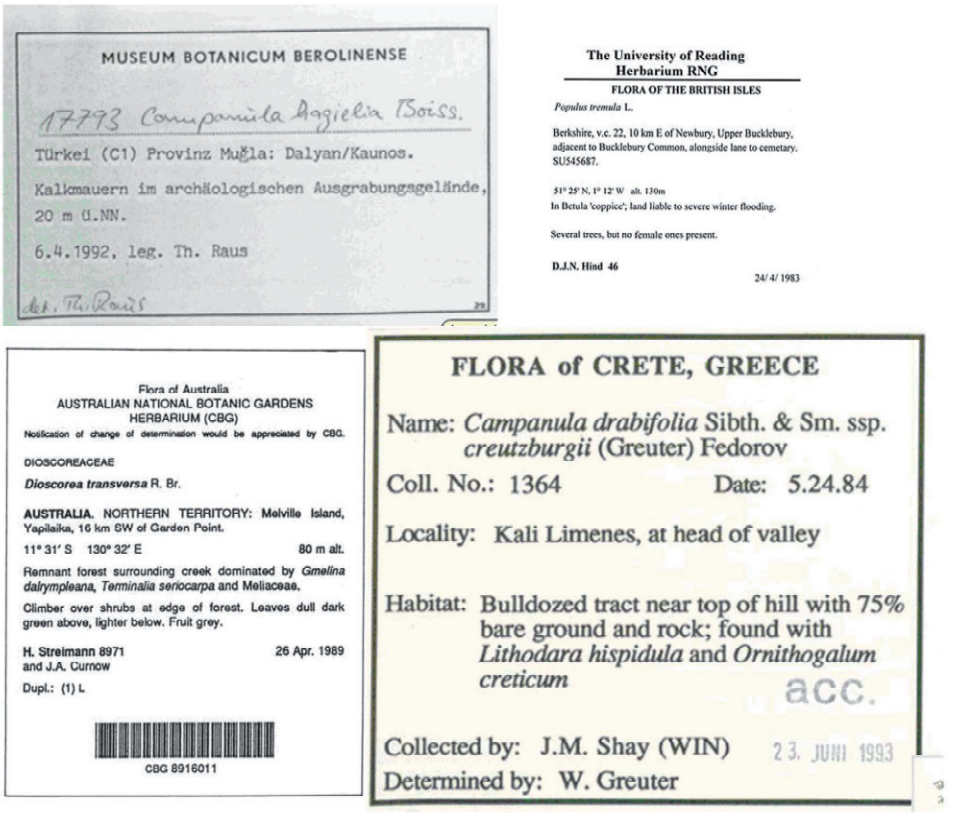

Fig. 5. Examples of herbarium specimen labels.

2.5.6. 'Tips and Hints'

Be aware of poisonous species, or plants with stinging hairs, thorns and prickles especially if you are not familiar with the regional flora, *e.g*. in the tropics!
Collect only as many plants as you can process in a day! A collection of a few well documented and preserved specimens is far more useful than a large quantity of bad and fragmentary specimens with incomplete and doubtful documentation. If it is not possible to press all plants collected in a day, store robust plants *e.g.*, succulent or lauriphyllous species in a cool moist place (*e.g*. in the fridge) overnight.

Supply yourself with newspaper whenever possible, *i.e.* before and during the field trip. The quantity of paper required is considerable!

Any kind of transport represents a serious risk of damaging the collected plant material. Wrap specimen bundles tightly to prevent mechanical damage, *e.g.* during postage. In case of long-distance shipping a treatment with insecticides may be necessary.

After the drying procedure it is recommended that the collected plant material is put in a freezer for three days at least to kill insects (including all their developmental stages) and to avoid contamination to other collections.

3. Conclusions

Recording all higher plant species of a given region is a complex task, which ought to be planned carefully. Even when satisfying scientific criteria during field work, we must bear in mind that the results of our survey always reflect reality only for a given moment in time.

The first thing to do, when carrying out a taxa inventory, is to gain a general idea of the study area and check whether any floristic data is already available. The recording itself may be accomplished either through a complete survey over the whole area or through a survey of representative plots and results in a species list. Providing additional, population specific and ecological data with the species list increases the value of the final checklist. As does an accompanying collection of representative herbarium specimens. Fieldwork should be well documented. The more (detailed) data are recorded the more valuable and significant they are and the greater the solid base for subsequent monitoring projects. It appears more reasonable to survey the flora of a limited (small) area by providing comprehensive and detailed data rather than to deal with a large area by yielding incomplete and poorly documented results.

Observing nature attentively in the field means, on the one hand, learning to understand fascinating ecological interactions and, on the other hand, experiencing the beauty and quality of nature.

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6. Appendix - Collection (passport) form

documentation of the field work

collection date ..... collection site number .....

name(s) of field worker(s) .....

institution .....

taxon data

taxon name or preliminary taxon name .....

vernacular name, language .....

herbarium voucher number ..... photos .....

colour of flower .....

additional notes (*e.g.* life-form, habit, size, type of underground organs, scent) .....

.....

phenological status: more flowers than fruits / more fruits than flowers / only fruits / fruits already dispersed

frequence: rare / few / frequent / very frequent / highly frequent *(tick)*

population and ecological notes

habitat .....

vegetation cover .....

canopy cover ..... % of bare ground .....

vegetation notes .....

associated species .....

..... EUNIS habitat code .....

human use .....

soil .....

geographical notes

country ..... region .....

location ..... coordinates .....

altitude ..... map datum .....

slope: level 0-5 % / undulating 6-10% / rolling 11-20% / moderate 21-30% / steep >30% (*tick*)

source of coordinates: topographic map / GPS / Google Earth (*tick one*)

population and site notes, circumstances of the field work (*e.g.* population size, fitness, observations) .....

regional administrations, scientists and florists .....

collection permission

used literature (national / regional flora, determination keys)