Abc Taxa

Manual on field recording techniques and protocols for All Taxa Biodiversity Inventories and Monitoring

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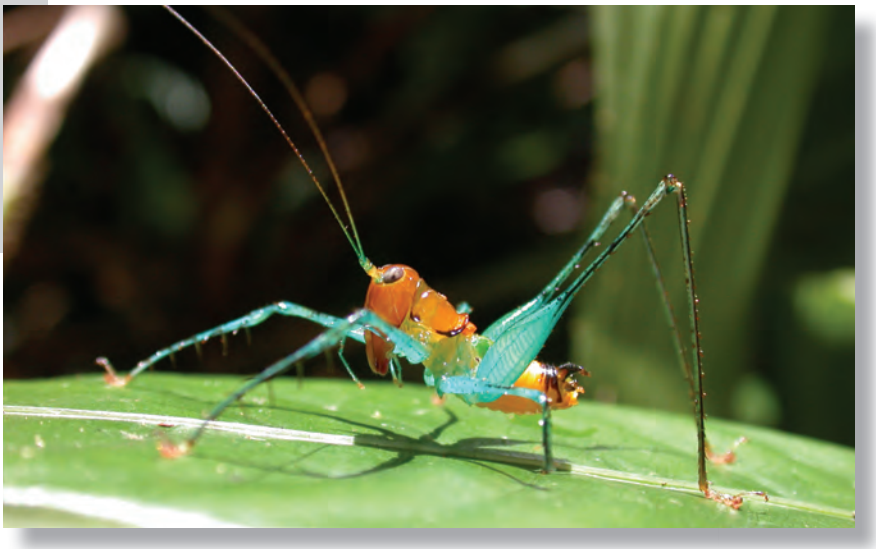
J.C. Monje, Y. Samyn and D. VandenSpiegel



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Manual on field recording techniques and protocols for All Taxa Biodiversity Inventories (ATBIs), part 2

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Cover illustration: *Arachnoscelis* sp. [Orthoptera Tettigoniidae, predator] found in San Lorenzo forest, during the large-scale biotic inventory IBISCA-Panama (Photo by Maurice Leponce).

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Chapter 13

Sampling of bryophytes

by

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Abstract

In this chapter, we provide practical guidelines for collecting and recording bryophytes. Bryophyte species exhibit a high specificity to meso- and microhabitat conditions and, although some can be observed all year-round, many are annual and/or can be identified only during a short period of the year. Completely random plot sampling (RS) or systematic sampling (SS) are therefore likely to miss important types of variation within the sampling area unless the intensity of the sampling (*i.e*. number of plots and number of visits at different seasons) is very high. Therefore, it is appropriate to use a sampling methodology, such as Floristic Habitat Sampling (FHS), that focuses on mesohabitats as the sampling unit. SS and RS offer, however, substantial advantages over FHS in terms of statistical comparisons across plots. Therefore, the combination of a systematic grid, usually of 1 to a few km², within which FHS is performed, is recommended. The size of the sampling plot is discussed depending on the goals that are followed. For recording rare species, the Area of Occupancy (AOO), defined as the area calculated by summing up all 2 x 2 km grid squares actually occupied by a taxon, is used by IUCN as a standard measure for defining species frequency. In the case of bryophytes, however, it is strongly advisable to decrease the mesh size because AOO values decline sharply as the scale of measurement reduces, as a result of the linear and frequently fragmented distribution of the species. Scientific collecting is still essential for a number of reasons, including specimen identification and herbarium collections for taxonomic studies – which is especially true for bryophytes because, although the larger species can often be named in the field, many are distinguished based on microscopic characters – and, more recently, for the constitution of DNA libraries. The collecting techniques, including information on what and how much to collect in the field, how to pack, label, dry and process specimens, are finally reviewed.

Key words: bryophyte, moss, liverwort, hornwort, floristic habitat sampling, random sampling, plot sampling, phenology, diversity

1. Introduction

Bryophyte is a generic name for plants characterized by a life-cycle of alternating haploid and diploid generations with a dominant gametophyte. They include the liverworts, mosses, and hornworts. Liverworts and hornworts comprise about extant 5,000 and 300 species, respectively. Together with mosses, which, with approximately 12,000 species, are the second most diverse phylum of land plants, bryophytes thus include a substantial proportion of the total biodiversity of land plants.

Although bryophytes are rarely the most conspicuous elements in the landscape, they play important ecological roles in terms of water balance, erosion control, or nitrogen budget, or simply by providing habitat for other organisms. Furthermore, bryophytes locally exhibit richness levels that are comparable or even higher than those of angiosperms. Lastly, and perhaps most importantly, although global biodiversity patterns tend to be congruent across taxa, especially ß diversity patterns (Schulze *et al.*, 2004; Kessler *et al.*, 2009), diversity patterns in bryophytes do not necessarily follow the patterns present in other, better-studied taxa, so that an enlarged concept of biodiversity has become increasingly necessary. As a result, there has been an increasing awareness of the necessity to include cryptogams in general, and bryophytes in particular, in conservation programs and biodiversity assessments.

In this chapter, we attempt at providing practical guidelines for collecting and recording bryophytes. From recent specialized textbooks (Goffinet & Shaw, 2009; Vanderpoorten & Goffinet, 2009), we briefly summarize the biological and ecological features of bryophytes that are relevant to their study in the field. We then review, based upon information provided in many specialized field guides, to which we refer for further information (O'Shea, 1989; Gradstein *et al.*, 2001; Wigginton, 2004), the sampling strategies and collecting techniques that are most appropriate for recording bryophyte diversity.

2. Where and when to collect bryophytes?

2.1. Where do bryophytes occur?

Bryophytes are generally seen as small plants confined to humid habitats, avoiding exposure to direct sunlight. Yet, an alert naturalist will quickly notice their presence in virtually every ecosystem. In parts of the world where short growing seasons limit plant growth, bryophytes, and especially mosses, may dominate the vegetation. Similarly, in temperate and tropical rain forests, bryophytes, and especially liverworts, compose luxuriant epiphytic communities that play important ecological functions, especially in terms of water and nutrient flow. Even in modern cities where air pollution and the man-made environment may seem unrelenting, bryophytes are able to colonize crevices in masonry.

The diversity of bryophytes is correlated with habitat heterogeneity at two spatial scales. Mesohabitats are localized physiographic (*e.g.* streams, seeps, cliffs) or physiognomic (*e.g.* forests) features. In a forested landscape, mesohabitats are

arranged into a mosaic of dominant mesohabitats (*e.g.* forests), wherein restricted mesohabitats (*e.g.* streams, seeps, cliffs) exist (Vitt & Belland, 1997). Microhabitats (*e.g.* trees, logs, rocks, stumps) are the smallest landscape units and may be unique to one type of mesohabitat. Epiphytic communities provide a classical example of microhabitat differentiation. Epiphytes typically exhibit both a vertical and a horizontal zonation, segregating vertically from the base to the crown along gradients of humidity, pH, and nutrient content (Barkman, 1958; Sillett & Antoine, 2004). Within each ecological unit, bark microtopography further generates a mosaic of microhabitats. For example, Barkman (1958) described the mosaic of species inhabiting beech bark in The Netherlands (Fig. 1). Wound exudates induce a vertical zonation of neutrophytic species, including *Orthotrichum diaphanum, Syntrichia laevipila* and *Zygodon viridissimus*, which are normally absent from acid beech bark. The last two species grow lower, presumably due to greater moisture near the ground. In contrast, acidophilous species, such as *Lophocolea heterophylla*, develop far from the wound.

Different species thus tend to utilize different portions of the resource continuum available. The competitive exclusion principle predicts that species avoid competition by occupying different niches, creating a spatial pattern that represents habitat partitioning corresponding to habitat heterogeneity. Thus, an increasing body of literature points to the strong correlation between habitat and species diversity. Some habitats are, however, more species-rich than other and hence, request a longer investigation time. Bryophytes are poikilohydric, which means that they suspend any metabolic activity upon drying. They tend therefore to be more dominant in sheltered, humid habitats than on open ground directly exposed to irradiation and desiccation.

A good trick to find species-rich habitats is to look at the extent of species cover. There is indeed a positive correlation between carpet density and species diversity for two main reasons. First, massive cover suggests that the habitat has the appropriate humidity level for many species to establish. Second, at low to moderate densities, growth is constrained by water availability. Moderately dense stands are dehydrated less rapidly than loose stands or isolated shoots because a dense packing of shoots may reduce water loss by effectively reducing the diameter of capillary spaces among close neighbours. Bryophytes growing in dense communities are therefore able to remain physiologically active for a larger part of the growing season, resulting in greater biomass and diversity.

2.2. Can we record bryophytes all year-round?

It is often believed that bryophytes occur all year-round, and this is one of the reasons why many naturalists shift to bryology in wintertime. This is definitely true for stress-tolerant species, which invest much in gametophytic development, enabling them to survive periods of stress. As a most extreme example, large cushions of the moss *Leucobryum glaucum* on forest ground or *Sphagnum* species in peat bogs, all of which occur in stable habitats and display gametophytic adaptations to store water in dead hyaline cells, can last for centuries. Thus, bryophyte species of long-lived, stable mesohabitats such as woodlands, can in fact be recorded at any time.

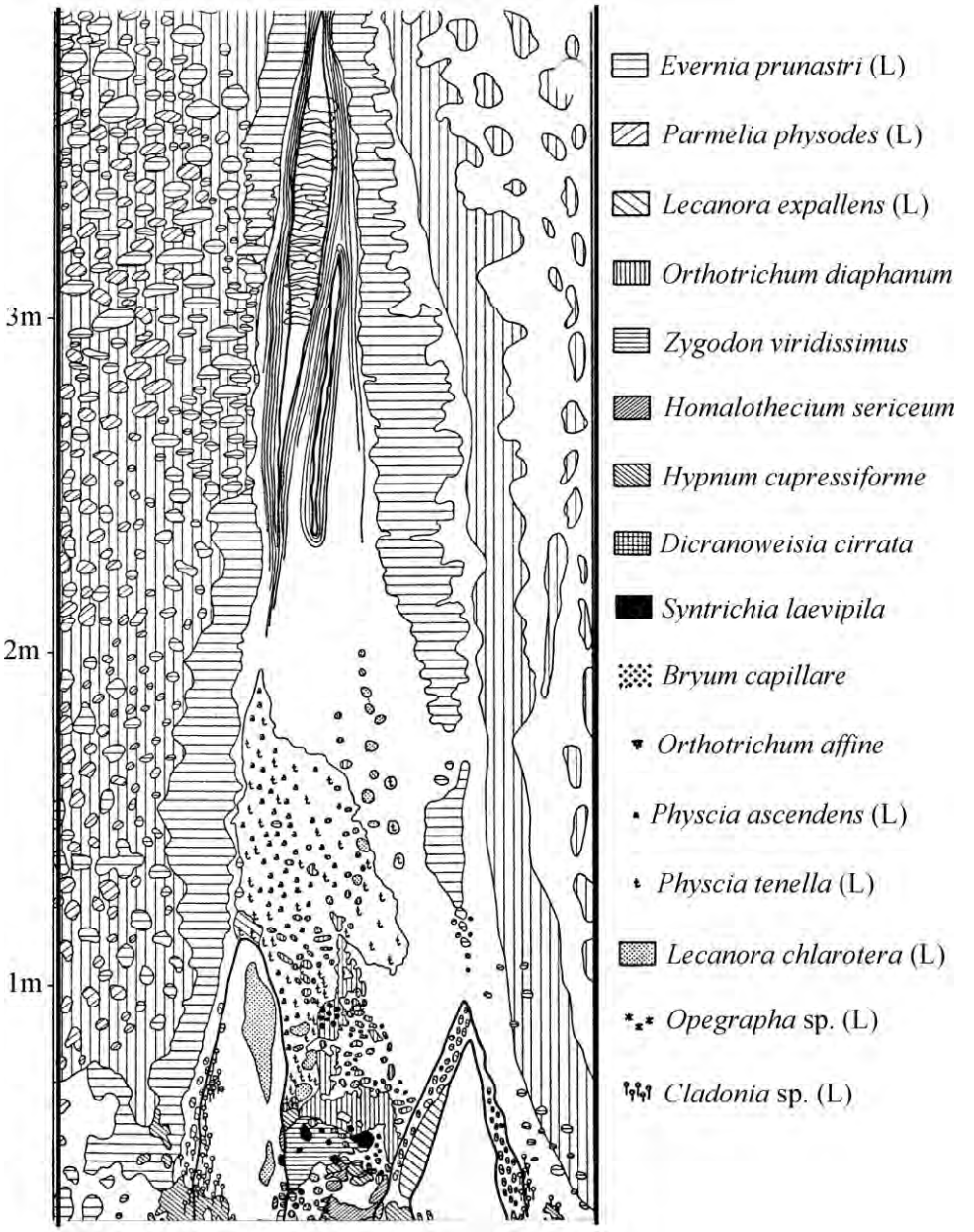

Fig. 1. Mosaic of cryptogamic vegetation comprised of lichens (L) and bryophytes along the first 4 m on an old beech trunk in The Netherlands (after Barkman, 1958).

It must be emphasized, however, that whilst perennial species can be observed regardless of the season, their identification might rely on sporophytic features that can be observed only during a short period of the year. The moss genus *Orthotrichum*, for example, includes mostly perennial epiphytic species whose identification relies on specific sporophyte features. In the northern hemisphere, the capsule reaches its full development in the spring, and taxonomically relevant characters of the peristome progressively become impossible to observe towards the summer season, during which the capsule itself eventually falls down.

In many other habitats, bryophyte species can be observed during a short period of the year only. In fact, plants have to cope with unstable habitats in time (*e.g.,* seasonal climate variations) and space (*e.g.*, habitat degradation or destruction). To face the risk of local extinction, they may either disperse in an attempt to establish new populations or remain under the form of long-lived diaspores, from which new establishment will be subsequently possible under favourable growth conditions. Parts of these diaspores may become buried into the soil, requiring light for germination, constituting a bank of diaspores. Because of the vulnerability of their gametophyte, bryophytes are, in particular, likely to rely more on stored propagules for their long-term survival than seed plants. Species of unstable habitats that recur predictably at a given site thus tend to produce a few, large spores with a low dispersal capacity but better chances of successful establishment and a longer life span in the diaspore bank. This is, for example, the case of hornworts in temperate areas, which are well adapted to regular disturbance in arable fields thanks to their diaspore bank, or of annual thalloid liverwort communities in xerotropical environments experiencing a severe drought season. On a less regular basis, habitats such as dried-out ponds are quickly recolonized thanks to the diaspore bank and their survey is often rewarded by the discovery of many specialized species.

As a result, all habitats cannot be recorded all year-round and some must be investigated during the appropriate season. During a survey of the bryophytes of arable land in Britain and Ireland for example, inventorying of the fields occurred at a time of year when the bryophytes were large enough for most of them to be identified or, in the rare cases of fields with no bryophytes, at a time of year when bryophytes would have been identifiable if present. In practice, this meant that fields were inventoried in the autumn, winter and early spring (Preston *et al.*, in press).

3. How to record bryophytes?

3.1. How to organize the sample plots?

An appropriate sampling methodology is crucial to understanding patterns of community and taxon diversity at the landscape scale. The type of sampling used for estimating diversity depends on the organism being studied, how closely that organism is associated with its substrate, and the nature of the ecological question (Krebs, 1989). In plant studies, Clements (1905) described methods for collecting plant species data using plots. Since that time, many variations of quantitative measurements using plots have been used. The bounded nature of

plots in relation to a specific sample area allows for quantitative sampling of species abundance and frequency, and later statistical analysis. This has made plot sampling a successful method for studying population and community dynamics in bryophytes and many other groups of plants.

Plots may be organized in a regular fashion, using a systematic grid, or selected at random. For instance, the combination of a systematic grid of 10 x 10 km, within which 'standard relevés' of 100 m^2 are inventoried, has been used for the standardized mapping of Swiss bryophytes (Urmi *et al.*, 1990). In each 'relevé', all bryophyte species are collected and determined, and voucher specimens are kept. This approach is most appropriate to identify the commonest species and assess their frequency and distribution, but may not allow for the recording of rare species. This is because many bryophyte species exhibit a high specificity to peculiar meso- and microhabitat conditions; a completely random plot sampling method is likely to miss important types of variation within the sampling area unless the intensity of the sampling (*i.e.* number of plots) is very high. Therefore, it is appropriate to use a sampling methodology that focuses on mesohabitats as the sampling unit. Sampling methods aimed at assessing total bryophyte diversity studies should include all of the potential habitats in an ecosystem. The method referred to as Floristic Habitat Sampling (hereafter, FHS) uses mesohabitats as the basic sampling units.

Comparisons of the efficiency of random Plot Sampling (hereafter, PS) and FHS suggested that the latter captures a greater mean species richness per stands than PS (Newmaster *et al.*, 2005). Bryophyte diversity estimates compared within the dominant forest mesohabitat were found to be much greater (*i.e.* species richness is 50% higher) when using FHS as compared to PS (Fig. 2). Although it is not made explicit, and although other data from herbarium records as well as casual observations are also included, FHS within each square of a systematic grid of one to several km is basically used in most of the European mapping programs for example in the UK (Hill *et al.,* 1991-1994), The Netherlands (van Tooren & Sparrius, 2007), Germany (Meinunger & Schröder, 2007), and Belgium (Sotiaux *et al.*, 2000; Sotiaux & Vanderpoorten, 2001, 2004).

Usually, all mesohabitats are identified from the analysis of fine-scale topographic maps. Each mesohabitat is then visited and sampled until no new species are reported. In some instance, special attention is paid to key-habitats that are identified on the basis of specific attributes, *e.g.* the known presence of rare bryophytes, special topography or soils, or, since the diversity of bryophytes most often correlates with global biodiversity patterns (Pharo *et al.*, 2000; Schulze *et al.*, 2004), the known presence of rare taxa.

The time necessary to survey an area depends of course of many factors including the number and experience of recorders, as well as the extrinsic floristic quality of the habitats. In Belgium, our experience is that the record of a grid-square of 4 x 4 km is considered complete, *i.e.* with no more than approximately 10% of missed species, takes between one (species-poor squares with low habitat heterogeneity, with approximately 50-60 species/square) and four days (species-rich squares with high habitat heterogeneity and quality with >150 species/square).

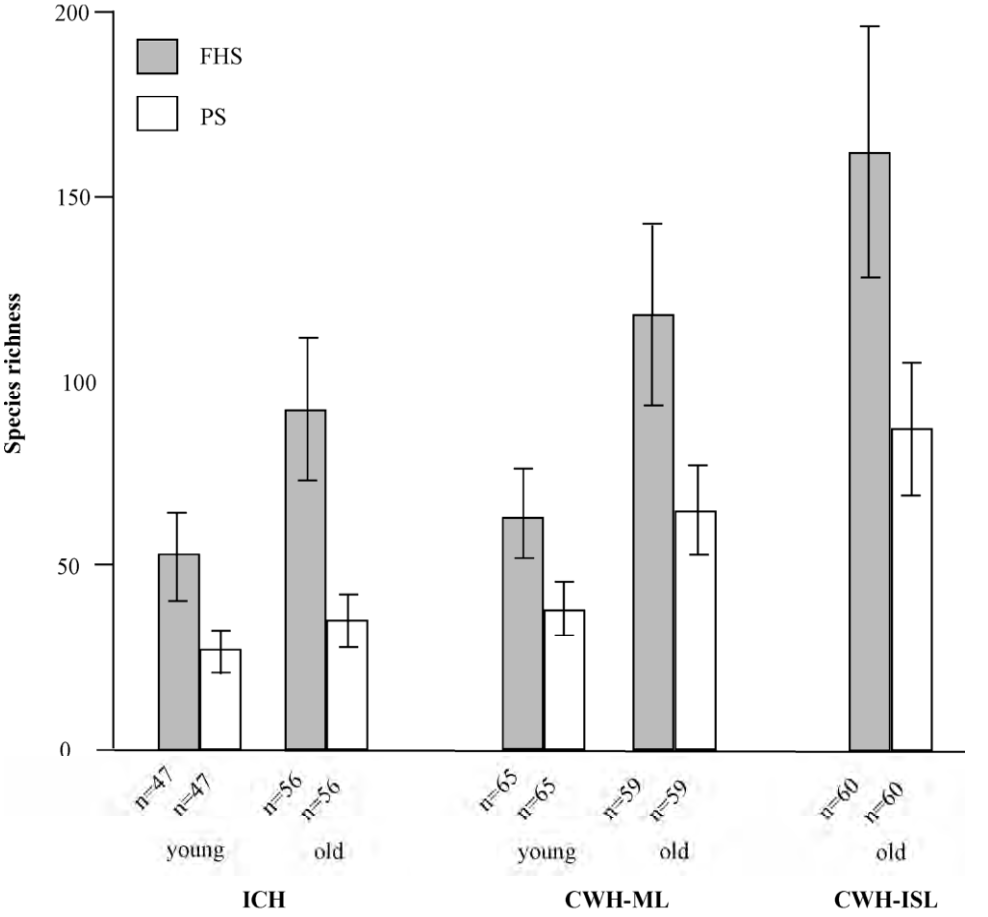

Fig. 2. Alpha diversity of stands assessed using floristic habitat sampling (FHS including all mesohabitats) and plot sampling (PS). Cedar hemlock forests are divided into inland (ICH), coastal mainland (CWH-ML), coastal oceanic (CWH-ISL), and by age classes (class 4, young = 80 years and class 9, old > 250 years). Error bars represent two standard errors on either side of the mean (reproduced from Newmaster *et al.,* 2005 with permission from Blackwell).

3.2. What size should sample plots have?

The size of the sampling plot depends on the goals that are followed. For biodiversity inventories, large plots should be favored since species richness typically increases with sample area (Fig. 3). In a comparative study of bryophyte forest diversity in Canadian forests, Newmaster *et al.* (2005) found that the 20 m-diameter plot used in the PS method sampled 314 m^2 of forest mesohabitat resulting in a mean species richness of 35 (± 5) species. Expanding sampling area to 1000 m^2 increased mean species richness by only 18 species.

Furthermore, species richness steadily increases even after 5000 m^2 has been sampled, increasing mean species richness in the dominant forest mesohabitat to just over 80 (± 6) species (Fig. 3). Using FHS, the mean species richness within the dominant forest mesohabitat was 106 (± 9) species. In fact, intensifying PS or simply sampling large areas using randomly placed plots will not necessarily include the natural variety in microhabitats. This is because PS within a mesohabitat will exclude important microhabitats and their respective bryophyte communities even after sampling unconventionally large sample areas. These results clearly suggest that the size of the sampling units depends on the sampling strategy itself, and that, in any case, the size of each sampling unit should be determined by means of species-area curves. In tropical rain forest, Gradstein *et al.* (2003) found that full sampling of 4-5 mature trees may yield 75-80% of the tree-inhabiting bryophytes in a forest stand (excluding epiphylls).

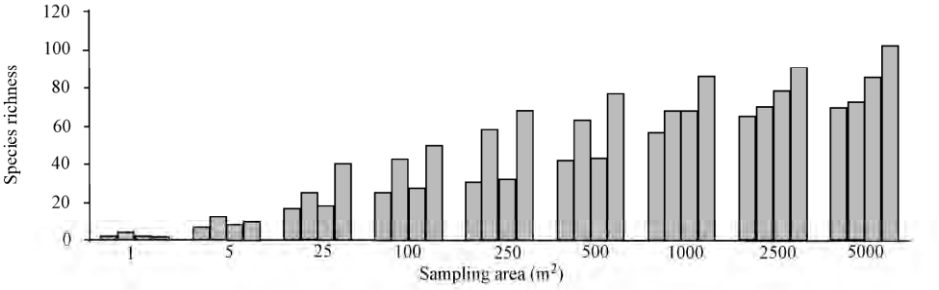

Fig. 3. Mesohabitat alpha diversity (species richness) within increasing sample size areas for 287 temperate rainforest stands (SP = seep, CF = cliff, FS = forest, ST = stream) (reproduced from Newmaster *et al.,* 2005 with permission from Blackwell).

For the record of rare species, the Area of Occupancy (AOO), which is defined as the area, calculated by summing up all grid squares with the mesh size of 2 x 2 km that are actually occupied by a taxon, excluding cases of vagrancy, is used by IUCN as a standard measure for defining species frequency. In the case of bryophytes, however, it is strongly advisable to decrease the mesh size because AOO values decline sharply as the scale of measurement reduces, as a result of the linear and frequently fragmented distribution of the species (Callaghan, 2008).

3.3. What to measure in each plot?

Depending on the time available and the goals followed, presence-absence or increasingly complex abundance indices can be used to document the frequency of each species in each sampling unit. The 'relevé' sampling method involves the attribution, to each species within the plot, of a coefficient of abundance-dominance, sometimes associated with a coefficient of sociability (see chapter on vascular plant recording), which serve to describe the cover of each species on the ground and its distribution mode, from lose, isolated plants to densely packed cushions.

In some tropical areas characterized by a very lush and species-rich bryophyte vegetation, however, this method may not be applicable and alternative

strategies must be used. One such strategy is to sub-divide each sampling unit into smaller sub-plots of a few dm², select some at random, perform complete species lists in each, and assess the frequency of each species across the sub-plots in each sampling unit. Alternatively, the same procedure of sub-division of the main sampling unit can follow a systematic scheme. This is, for instance, the method applied by the Hungarian Bryophyte Monitoring Program (Papp *et al.*, 2005) for the record of epiphytes. Within each sampling unit, each standing tree (living or dead) with a diameter of at least 19 cm at breast height is included in the sampling of epiphytic bryophyte vegetation. The sampling of epiphytic bryophytes is carried out at three levels: 10 cm (1. level), 70 cm (2. level), 140 cm (3. level) upwards from the base of the tree. A 10 cm wide cylinder is examined at each level (from the marked level 5-5 cm upward and downward), where the occurrences of the species are recorded (presence/absence data).

A protocol for rapid and representative sampling of epiphytic bryophytes growing on bark of trees in tropical rain forest was designed by Gradstein *et al.* (2003). Within a core area of one hectare, 5 mature rain forest trees (standing well apart and differing in bark structure) are sampled from the base to the outer canopy using the single rope technique (ter Steege & Cornelissen, 1988) or some other method for sampling of the forest canopy. Species are collected in 4 small plots within each of 6 height zones, the so-called “Johannson zones” (1: tree base, 2a: lower trunk, 2b: upper trunk, 3: lower crown, 4: middle crown, 5: outer crown). Plots in zones 1-3 are 20 x 30 cm and positioned in each cardinal direction, those on thin branches in zones 5-6 are ca. 60 x 10 cm long and positioned on the upper and lower surfaces of the branch. For safety reason, plots in zones 4 and 5 are sampled on the ground from cut-off branches.

A protocol for sampling of epiphyllous bryophytes in tropical rain forest was designed by Lücking & Lücking (1996).

4. Collecting techniques

Scientific collecting is essential for a number of reasons, including specimen identification, herbarium collections for taxonomic studies, and, more recently, constitution of banks of DNA. This is especially true for bryophytes because, although the larger species can often be named in the field with a 10-20x hand-lens, many are distinguished based on microscopic characters. Reference collections of specimens are thus invaluable in the study of bryology, but in order to obtain useful specimens for research, the correct techniques for collecting and processing should be employed. It must also be emphasized that, although bryophyte species rarely legally protected, it is necessary to obtain permits to collect bryophytes and an export licence if the material is to be taken out of the country. Herbarium staff can often advise on what is needed, but obtaining necessary papers and permissions can be a lengthy process, so should be investigated well in advance.

4.1. Packeting

Bryophytes are among the easiest plants to collect (Buck & Thiers, 1996). Since they lack roots, they can often be readily collected by hand, although some species closely attached to their substrate will have to be scratched using a knife. Specimens should be selected to include all the parts of the plant needed for identification. Sporophytes are often useful, if not necessary, for identification, and should be searched for. Several mosses from unstable habitats, *e.g.* riverbanks, arable fields, have rhizoidal tubers buried in the soil. As these are often diagnostic, these bryophytes should be collected with 1-3 cm of the substrate (Whitehouse, 1966; Porley, 2008).

Individual species within a collection should be packed-up separately, so far as this is possible. It is in fact generally easier when the material is still fresh than later, when several collections jumbled together in a single packet have to be separated. The specimens are normally put into envelopes. A standard envelope can be folded from an A4 paper to be (10-)12 x 14 cm in size (Fig. 4). Particularly small specimens should be wrapped separately in mini-packets before being put into normal size packets. If sporophytes or fertile structures are rare, these should also be placed in mini-packets, but attached to a piece of the gametophyte to avoid any subsequent confusion. If specimens are very wet, as is often the case with *Sphagnum*, they should be gently pressed to remove most of the water, and packed into a double or treble thickness packets. As for ground-dwelling species, it is often more appropriate to keep them in stiff boxes for transportation and storage to avoid ending up with a mixture of soil particles and plant fragments.

For collecting of epiphyllous bryophytes in tropical rain forest, whole leaves on which the epiphylls are growing are collected in new papers in a plant press, lightly pressed and dried. The epiphyllous species are subsequently sorted, and leaves cut up, in the laboratory using a dissecting microscope. For collecting of thalloid liverworts and hornworts it may also be recommendable to dry the specimens in a plant press instead of in collecting bags, in order to keep them flat and avoid them from becoming rolled inwards. Pressing of the specimens should be lightly only, to avoid damage to the plants.

4.2. How much to collect?

Collecting of specimens for scientific purposes is usually highly selective and seldom constitutes a real threat to the survival of species. The extinction of species by a targeted over-collecting has been, however, already documented. It is difficult to provide exact guidelines since everything depends on species size, local and overall abundance, etc. As a general rule, collecting enough to fill a 12 x 8 cm packet should be plenty for a robust species. On the other hand, too small specimens are of no value if there is insufficient material to allow identification and, perhaps, DNA extraction. In addition, the really important plant in a collection may not be what the collector actually saw in the field, but some minute plant sparsely mixed with it, and only discovered later in the laboratory.

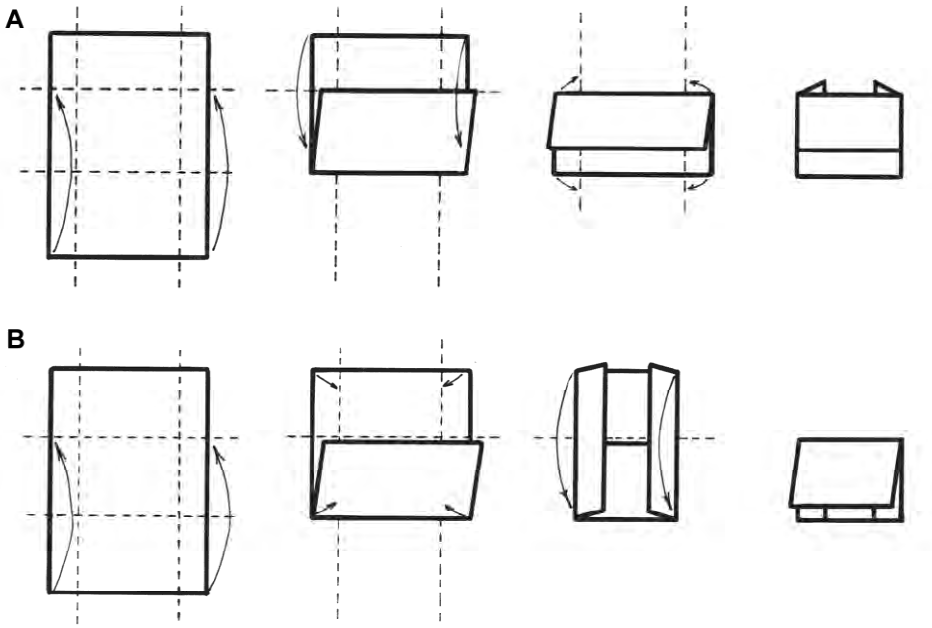

Fig. 4. Folding procedure for packing-up bryophytes.

4.3. Data and labelling

The information record is similar to that of other plants, and includes habitat information (for instance, if a species occurs on tree or rock, the tree species or rock type should be recorded), nature of the surrounding vegetation, elevation, and locality details, including GPS coordinates. For rare species, information on population size is often useful but might be difficult to assess in the case of bryophytes. Indeed, many bryophyte species are highly clonal, and several gametophytes can develop from a single protonema following the germination of a single spore.

Thus, what is the entity that best corresponds to discrete individuals like animals? For practical reasons, a purely pragmatic definition can often be used. For species that depend on discrete substrate entities (such as tree trunks or droppings), each substrate entity can be considered to contain one or two individuals. For bryophyte species growing on ground or rocks, one individual may be assumed to occupy a surface of 1 m^2. However, in some rare cases of some very small mosses (*e.g.* the genera *Seligeria* and *Tetrodontium*), one individual might be associated with a surface of 0.1 m^2.

4.4. Drying and processing

The collected specimens should be dried as soon as possible to avoid fungal damage. In most cases, the packets can be left to air-dry. In wet areas during extended expeditions, however, drying might become a major issue and

preoccupation, and the use of a plant dryer can sometimes become necessary (Frahm & Gradstein, 1986). As liverwort capsules tend open when drying, releasing their spores, it is recommended that some specimens with capsules be placed in a small paper envelope before drying together with the rest of the sample, to ensure that at least some unopened capsules are preserved.

These is no need to give a descriptive account of the plant, as one does systematically for fungi and sometimes for higher plants, since most bryophyte species recover their primary appearance upon remoistening. A special care must, however, be taken with liverworts. Indeed, the identification of many species relies on the size, shape, number, colour, and distribution of oil bodies, which are unique organelles among land plants. Because of the volatility of the oils they contain, oil bodies progressively disappear upon drying in the laboratory. In some taxa, the process takes only a few hours, so that fresh material must be studied, whereas in other, oil-bodies last for some years and can still be studied on herbarium specimens. In any case, it is advisable to take a micro-photograph of the cells to keep a record of the oil body morphology.

For preservation of DNA, fresh material should be cleaned and quickly air-dried, and subsequently kept dry. Any moistening of the material must be avoided as this might lead to degradation of the DNA, making the material unsuitable for molecular analysis.

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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.

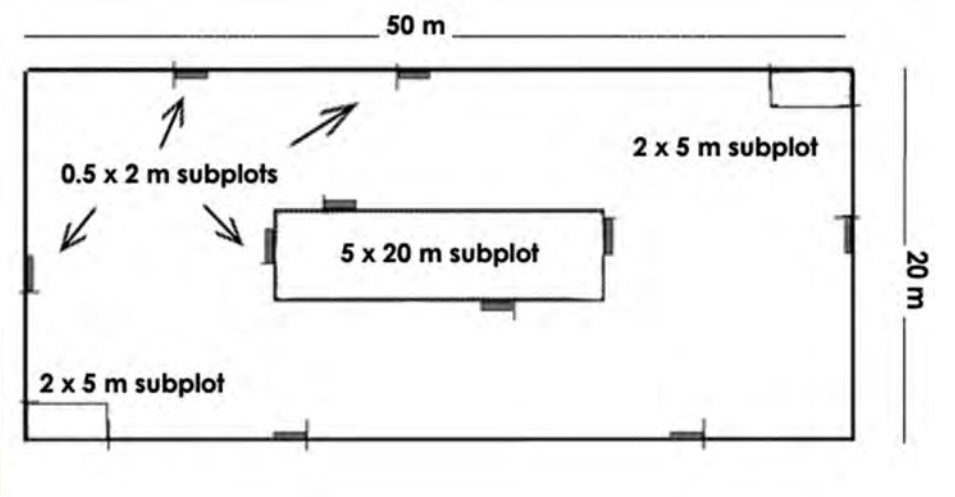

Fig. 1. An example for a modified Whittaker (Shmida, 1984; Stohlgren *et al.,* 1995) plot.

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.

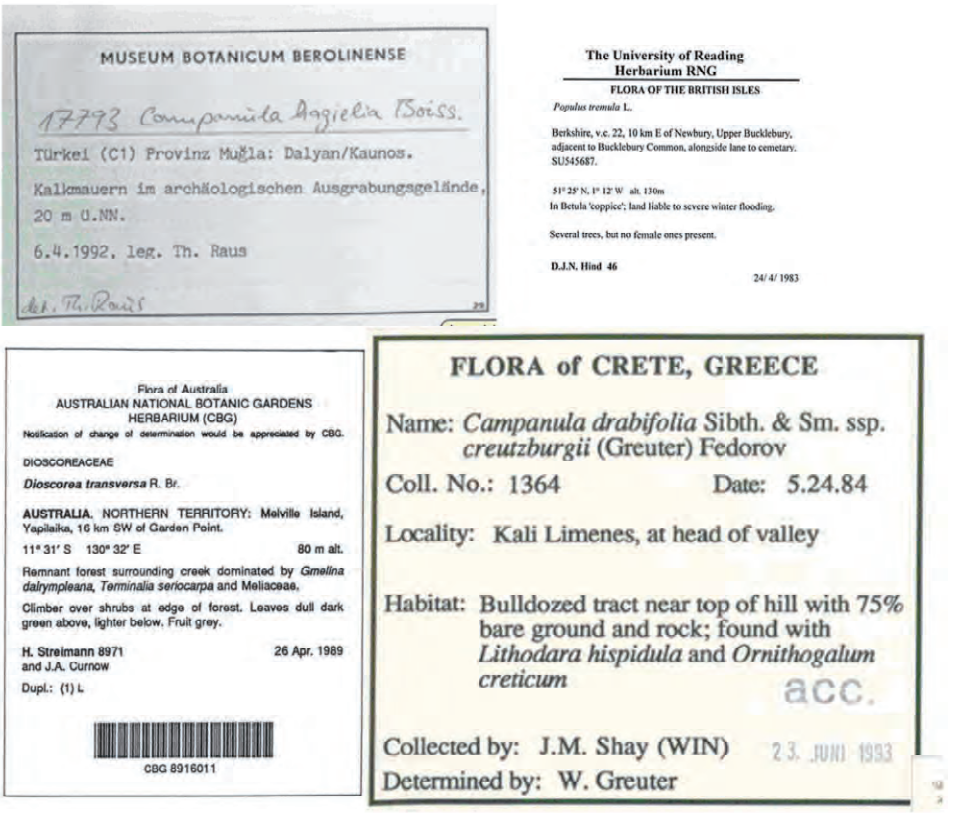

Fig. 5. Examples of herbarium specimen labels.

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)

Chapter 15

Sampling insects: general techniques, strategies and remarks

by

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Abstract

Sampling insects requires knowledge of their biology, preferred habitats and activity patterns. An overview is given of the most frequently applied collecting and recording techniques and the insect taxa that they gather in largest numbers. Sampling strategies can be deduced for each of the included taxonomic groups. Following techniques are described and recommendations and restrictions are given for them: 1. Active collecting: pooter, portable suction devices, sweepnet, visual observation; 2. Passive collecting: coloured pan traps, emergence traps, sticky traps and suction traps. For light traps, Malaise traps and pitfall traps we refer to other chapters.

Keywords: Sampling strategies, coloured pan traps, suction traps, emergence traps, sticky traps

1. Introduction

It is virtually impossible to attempt at collecting all species of one particular taxonomic group with only one sampling technique. And it is considered very unlikely to collect all of them even with several methods. This is not only due to the specific life histories of the different species, and their numbers in the field, but also to features of the recording methods used. Preferably, at least two or three collecting techniques, and visual observations in the field are mandatory to get a representative idea of the present species richness. In a canopy sampling campaign for weevils (Coleoptera: Curculionidae), the three methods applied (fogging, sticky traps, light traps) each yielded a very large number of species, but proved strongly complementary in terms of collected species (Missa, 2000) (Fig. 1).

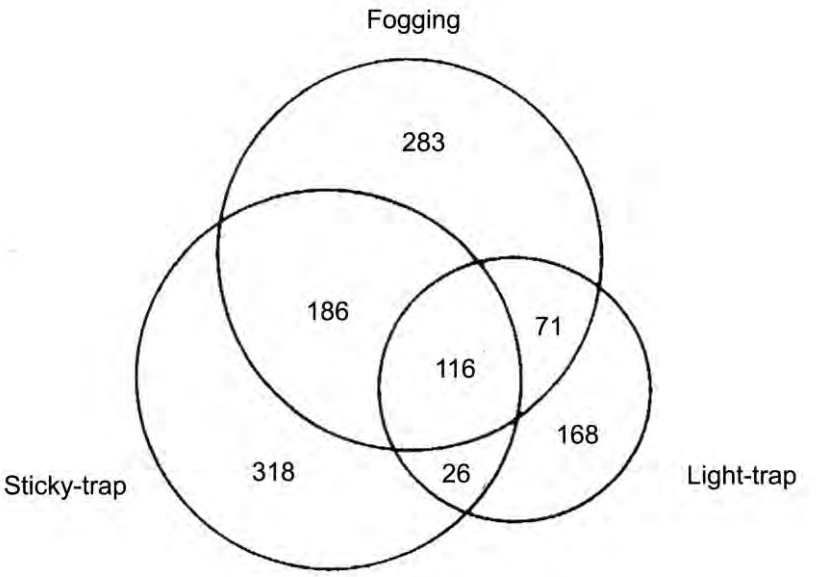

Fig. 1. Weevil species richness (Coleoptera: Curculionidae) as established by three collecting techniques in lowland rainforest in Papua New Guinea (Missa, unpublished data).

Sampling insects requires knowledge of their biology, preferred habitats and activity patterns. Like most invertebrates, many insects show oscillating population densities with cycles from 3 up to 10 years (Hunter & Price, 1998) (Fig. 2). In low density years, species' populations are difficult to measure and might give the impression that the habitat represents suboptimal conditions. In temperate and tropical climates, insects show a specific annual activity pattern, often referred to as phenology (Tauber & Tauber, 1981). In temperate regions these patterns are triggered by photoperiod in combination with temperature and humidity (van Asch & Visser, 2007), which renders species being most active during spring, summer, autumn, and even winter. Some species even have several generations per year disjunct in time. Apart from monsoon conditions it

remains unclear what exactly triggers phenology in the tropics, certainly around the equator, where photoperiod and temperature are subequal throughout the year. In tropical forests the fruiting of trees may be one of the triggers.

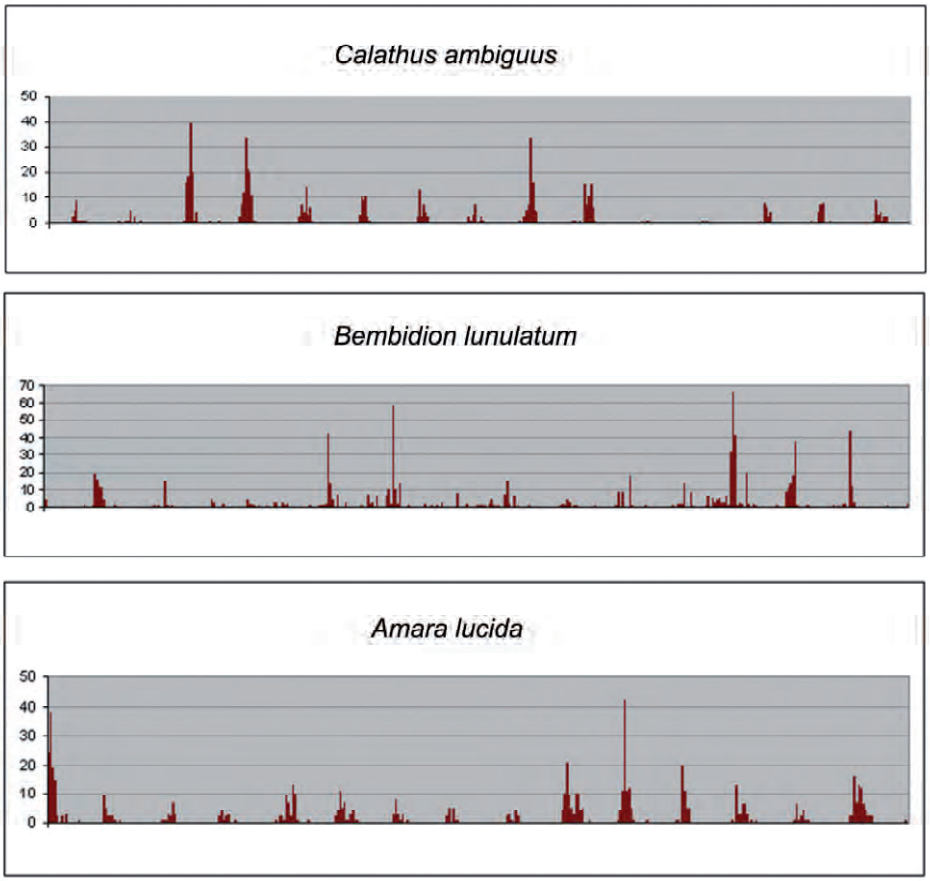

Fig. 2. Cycles of annual variations in population density of three ground beetle species (Coleoptera: Carabidae) resulting from pitfall traps over 15 years: the periodicity of population peaks varies between 5 years in *Calathus ambiguus*, and 10 years in *Amara lucida*. In low density years, populations are hard to establish (Desender, unpublished data).

An All Taxa Biodiversity Inventory (ATBI) *sensu stricto* is an illusion as well. It is not feasible to record all species at one particular site, even when sampling continuously, year-round and using different techniques. But the strategic employment of a particular combination of trapping techniques might yield a sufficiently representative portion of the species richness. Each collecting device has been constructed to gather particular taxa as efficient as possible, using species' features as mobility and attraction: *e.g.* Malaise traps collect a very diverse fauna of mainly diurnal flying insects; pitfall traps focus primarily on soil-

dwelling invertebrates, whereas coloured pan traps and light traps attract flying insects during the day and night respectively (Missa *et al*., 2009).

Before initiating a sampling campaign, the goal of the action should be very clear. Also, aspects as coverage and intensity of the sampling in time and space, practical issues, treatment of material before preparation and logistics, and the handling of possible by-catches or residue samples should be taken into account prior to the start of the campaign.

It is very important to choose the collecting method and devices according to preservational aspects. Many taxa are to be dry-mounted by pinning or gluing onto paper cards as a standard preservation method. Collecting devices using fluid fixation agents prevent satisfying results in many cases (as for all Lepidoptera, pilose and coated specimens), and require ultimate liquid specimen preservation, also dependent on fixation agent, collecting periods, temperature, etc. In these cases passive collecting devices can be used without fixation fluids, but have to be serviced in short intervals. So fixation and preservation fluids must be selected according to the final purpose of the gathered specimens (*e.g*. DNA extraction requires 100% ethanol). See chapter 18 by Krogmann & Holstein.

Traps have been designed for each stratum, from the soil surface level (to collect soil-dwelling and weak flyers), over the near-soil stratum (most of the flying insects in herb and lower canopy levels) up to the upper canopy. The canopy can hold an unprecedented biodiversity as shown by Erwin (1982) who observed that about 2/3 of the arthropods of a dry tropical forest occur in the canopy. The present chapter deals only with the near-soil stratum. Collecting strategies and techniques for soil-stratum and canopy invertebrates are treated in chapters 9 and 8, respectively.

A clear difference should be made between discontinuous or occasional, and continuous sampling techniques, and both have their advantages and shortcomings. If practically possible, continuous sampling with traps is recommended because of the relatively low service time (especially as compared to the time needed to collect the same species richness actively), and the fact that traps remain in operation regardless of weather conditions.

Trapping devices can also be separated into attraction and interception traps. Attraction traps employ the phenomenon of attraction of the species by the trap, generated by agents such as light, colour, odour and others. Interceptions traps, on the contrary, form an obstruction on the path of organisms and lead them to a collecting device. A number of traps combine both sampling methodologies.

A third way to divide sampling activities is based on the involvement of the collector himself during the collecting activity and in this frame, active and passive collecting are distinguished. The former approach implies the direct and active involvement of the collector who effectively moves (around) in search for the focal taxa. Active sampling encompasses visual observation, sweep netting and the use of pooters and related recipients. Passive collecting, on the other hand, is based on the movement of the focal taxa towards the trapping device. This methodology includes all kinds of continuous traps such as Malaise traps, pan and pitfall traps, fixed suction traps, sticky traps, light traps and emergence

traps. All of these collecting techniques are presented below, except for Malaise, light and pitfall traps, which are dealt with in chapters 17, 16 and 9, respectively.

Collecting / recording techniques relevant for ATBIs of insects

Table 1 presents an overview of the most frequently applied collecting / recording techniques and the insect taxa that they gather in largest numbers. From this table, recommended sampling strategies can be deduced for each of the included taxonomic groups. Hereunder, the different techniques are described, and recommendations and restrictions are given.

Collecting techniques (see Text)	Active collecting					Passive collecting						
	2.1 pooter	2.2 portable suction devices	2.3 sweepnet	2.4 visual observations	*fogging*	3.1 coloured pan traps #	3.2 emergence traps	3.3 light traps	3.4 Malaise traps	3.5 sticky trap	3.6 suction traps	*pitfall traps*
Collembola	□	■			□				□	□		■
Thysanura					□				□	□		■
Ephemeroptera			■					□	□			
Plecoptera			■					□	■			
Blattodea			□		■	■		□	□	□		□
Isoptera					□			■	■			
Orthoptera			■		□			□	□			
- Tettigonoidea			□	■	□			□	□			
- Acridoidea			□	■								□
- Tetrigidae			□		□			□	■			□
Embioptera					□			□	■			
Psocoptera			□		□				■			
Hemiptera			□						■			
- Cicadomorpha			□		□			■	□			
Thysanoptera			■		□				□			
Neuroptera			□		□			□	■			
Coleoptera												
- xylobionts (*e.g.* Cerambycidae, Scolytidae)			□		■		■	■	□	□		
- ground-dwelling beetles (*e.g.* Carabidae)								□				■
- phytophagous beetles (*e.g.* Chrysomelidae)			■		□			□				
- aquatic beetles (*e.g.* Dytiscidae)			■					□				

Strepsiptera							■		
Diptera		■		■ [W, Y]	□		■		
- apterous/brachyterous flies	□				□				■
- Dolichopodidae		■		■ [W, Y]	□		■	□	
- remaining Empidoidea		■		■ [W, Y]			■		
- Phoridae				■			■		□
- Syrphidae		■		□			■		
- Stratiomyidae				□			■		
- Tabanidae							■		
Mecoptera		■			□	□	□		
Lepidoptera									
- Rhopalocera		■		□ [W]			□		
- Heterocera						■	■		
Hymenoptera		■		□			■		
- Apoidea		□		■ [Y]			■		
- Cynipoidea		□	□		□		■		
- Parasitoids		□	□	□ [W, Y]	□	□	■		
- Formicidae		□	□	□ [W, Y]		□	■	□	□
- Ichneumonoidea		□		□ [W, Y]			■		
- Pompilidae		□		□ [W, Y]		○	■		
- Symphyta		□		□ [W, Y]			■		
- Vespoidea		□		□ [W, Y]		○	■		

W: white; Y: yellow pan traps

Table 1. Overview of techniques used to collect insect orders and some selected superfamilies and families. Only taxonomic groups for which at least one technique can be assigned as recommended are included. Explanation of covered collecting techniques follows the structure of the chapter; techniques not treated here are indicated in italics. Most recommended techniques are indicated as ■ (if two or more techniques are in this category, they are considered as equally recommended); useful, supplementary techniques indicated as □. If no techniques are indicated for a certain order, recommended techniques for the underlying families differ greatly.

2. Active collecting

2.1. The pooter

A pooter consists of a collecting jar closed by a cork or polymer stop with two flexible tubes inserted into it, a mouthpiece tube to aspire and a collecting tube to suck up the insect. At the inner end (in the collecting jar), the mouthpiece tube is covered by a fine gauze (Fig. 3) to avoid insects from entering the collector's mouth. Small insects are collected by positioning the collecting tube on top of the insect and abruptly sucking it up into the collecting jar. In between collecting actions, the outer end of the collecting tube must be covered or blocked by a stop to avoid the insects from escaping. Finally, the insects can be transferred to a killing jar or preservative by gently removing the stop. This method is widely used

to collect insects from all kind of surfaces (rocks, fences, tree trunks, etc.), from crevices and even from sweep net samples. This method is particularly interesting to gather insects that tend to stick to these substrates, and thus cannot easily be collected with a sweep net.

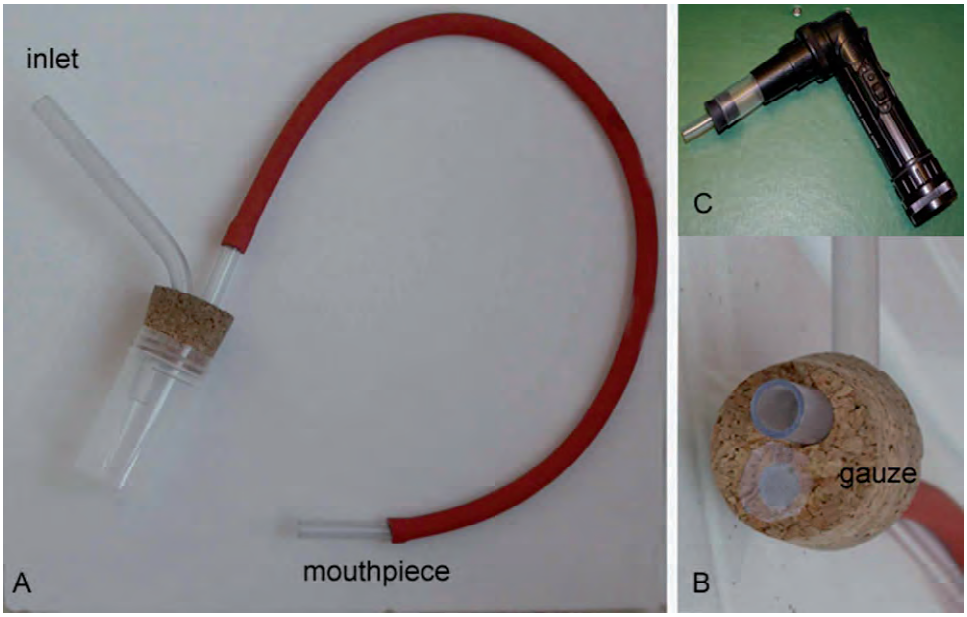

Fig. 3. A. Mouth-pooter; B. Gauze at the inner end of the mouthpiece tube prevents insects from being inhaled; C. Electric pooter (Photos A & B by Patrick Grootaert; C on http://svalbardinsects.net/index.php?id=64) .

Recommendations:

- Use a distinctive mouthpiece tube to avoid confusion with the collecting tube;
- Glass collecting jars are prone to get broken, so transparent plastic vials are safer. However, be aware that some types of polymer corrode when in contact with a killing agent;
- Transfer the collected insects regularly to the killing jar so that the pooter jar does not become too crowded. By putting a piece of paper tissue in the pooter jar, the time interval between collecting actions can be increased and collected insects do not get too damaged during the trip;
- To kill the collected insects, a piece of paper tissue with some volatile killing agent can be deposited into the pooter jar prior to their transfer into a larger killing jar. Take care that the killing agent is entirely evaporated before the pooter is used again.

Restrictions:

- While aspiring, enormous amounts of germs (fungi, bacteria, viruses, mites and their eggs, springtails, etc.) can be inhaled which might cause damage to the respiratory system. It is also highly recommended when collecting insects from excremental surfaces to use a rubber bellow on the mouthpiece tube instead or an electric pooter;
- Ants and certain beetles emit noxious products when disturbed, and in these cases an electric pooter is recommended.

In the case of tree-trunk dwelling flies, an alternative and safer method consists of a transparent vial (a recipient with a diameter of 3 cm and a depth of 7 cm is very practical) that is rinsed with some alcohol solution. This leaves a thin wet layer on the inside of the vial in which flies and other flying insects get entangled while flying up when the vial is quickly put on top of them. In this way, a surprisingly high number of specimens can be collected during one collecting action before being transferred to an alcohol solution. This method is superior to all others for collecting *Medetera* spp. (Diptera: Dolichopodidae) and other arboreal trunk-dwelling long-legged flies. This method is well suitable for specimens that are ultimately wet preserved, but only to some extent to collect dry preserved insects.

2.2. Portable suction devices

D-VAC is a portable aspirator activated by a gasoline engine and carried on the back of a person. The advantage of D-VAC vacuum sampling as compared to other sampling techniques is the more complete extraction of tiny invertebrate species, and immature forms of even larger species from the environment. Due to the pressure built up by conventional nets while sweeping, insects of low body mass simply do not enter them as they are caught in the overflow of air pressure built up as the net is sent through the air. By applying suction to the collecting bag, this inertia of air at the entrance of the net is overcome and tiny forms are collected more readily. Using a similar motion as is done while swinging an insect net, the D-VAC is also suitable to sample more heavy insects like caterpillars, beetles, etc. For fragile insects like many flies, sweep netting is preferred over suction trapping, although the latter method might be applied successfully to capture cryptic species that occur in dense vegetations, within tussocks and in *e.g.* rot-holes of trees.

2.3. The sweep net

Sweep nets come in all shapes and sizes, each designed for a particular insect group (Stubbs & Chandler, 1978). Both the net shape and sweeping technique affect the yield as commented upon by Chalcidoidea (Hymenoptera) specialists (Anonymous, 2004). While employing a sweep net, the collector not necessarily targets a specific specimen, but sometimes carries out a random sampling of the fauna present in the vegetation or on the soil surface. The species diversity in

sweep net samples often resembles that of Malaise trap yields (Guevara & Aviles, 2009).

The sweep net is by far the most widely used device to collect insects, and has been the most important one for the past centuries. Its success can be explained by its practical use and the fact that it can be employed in almost every possible habitat, except for densely vegetated or inaccessible sites (reed marshes, mangroves, etc.) and thorny vegetations. Moreover, it is ideal for short-term large scale inventories as the gathering of the separate samples is not time-consuming and several sites can be visited during the same day. Also, it does not require the collector to return to the same site more than once to collect the yields.

When using a big-sized net selected insects can be gathered with a pooter. This holds true for small specimens only and is not feasible for *e.g.* Lepidoptera and medium-sized to large arthropods. If the entire content is to be conserved, the yield is gathered in the tip of the net by sweeping the net a few times and closing it manually. If the specimens must be stored dry, the tip can be put in a jar with a knockdown agent like ethyl acetate to kill the specimens. Subsequently, the sample can be exposed on a white sheet for immediate sorting. The collector should make sure that the specimens are dead (caterpillars and beetles might be harder to kill in this way). If the specimens are stored wet, then the tip of the net with the yield can easily be emptied in a collecting jar with an alcohol solution.

Beating vegetation with a strong sweep net or with a stick and subsequently collecting the fallen insects on a sheet or in an umbrella is an alternative way to collect arthropods like spiders, beetles, bugs and caterpillars. However it is not highly recommended to maltreat vegetation in a nature reserve, especially in the presence of park guards.

***Recommendations*:**

- Use a net with the right mesh size; dipterists require a finer mesh size than *e.g.* butterfly or dragonfly collectors. Sweeping nets for sweeping through thorny vegetation must be made of a stronger fabric (*e.g*. linen), at least around the clamp to avoid ruptures;
- Transfer the sample to a collecting jar after a limited number of sweeps, depending on the size of the sample (this requires some experience). Samples collected during a long sweeping session tend to contain a high ratio of damaged specimens;
- Sweep gently (over) the vegetation; insects will fly up, end up in the net and will not be damaged, nor will the vegetation. If sweeping too severely, leaves and branches will end up in the net, damaging the specimens;
- Use an eversible stick which makes the collecting radius substantial larger;
- Take care when manipulating the sample (in the tip of the net) and watch out for stinging insects, especially when you are allergic;

- Join an experienced entomologist on one of his trips; you will learn more and much faster than studying manuals. Every entomologist has his personal technique that affects the yields.

***Restrictions*:**

- Sweep netting of vegetation cannot be done when vegetation is humid or highly thorny. Fragile insects will be severely damaged which renders them useless for identification. As insect activity only starts when the temperature is sufficiently high, collecting with sweep nets becomes only efficient when the collecting sites are exposed to the sun. In practice, collecting starts best not before 8:00 a.m., especially in strongly wooded habitats, and lasts until the late afternoon (when the weather is dry). Poorly vegetated sites like beaches, especially in the tropics, are best avoided at noon when insects escape from the soaring temperatures and hide in the soil or on the soil surface within dense vegetations.

2.4. Visual observation

Visual observation is a technique that should not be underestimated. Moreover, it is the innate feeling of most entomologists nowadays that they spend too little time in the field to learn about the whereabouts of their animals of interest. Instead, sampling is mostly done as efficient as possible, using all kinds of trapping devices which can yield very large amounts of species and specimens but only rarely uncover aspects of their life history (see further). Observing insects in their natural habitat yields information on their behaviour, commotion and preferred (micro)habitats. *E.g.* many long-legged fly species (Diptera: Dolichopodidae) in the tropics demonstrate very specific habitat affinities and are sometimes entirely confined to *e.g.* springs, waterfalls, rapids and even splash zones of rocks amid rivers.

Well-sized specimens can be collected by hand or with a jar or vial, respectively. In this way, non-flying arthropods from substrates and from under rocks, stones or bark are usually collected.

During visual observation, specimens can be photographed and pictures and related information can be stored using PDAs (personal digital apparatus), which have rather recently been developed. Recording using only visual observation is only suitable for taxonomic groups that are easily recognized in the field. In all other cases, it is strongly recommended to collect voucher specimens for confirmation of their identity in the laboratory.

3. Passive collecting

3.1. Coloured pan traps

Next to sweep nets and Malaise traps (see chapter 17), the most frequently employed technique to collect flying insects is undoubtedly pan traps. These traps were initially used in pest species sampling, but more recently have

become part of the standard biodiversity assessment instruments mainly applied by North American entomologists, but only few European researchers (Baillot & Tréhen, 1974; Pollet & Grootaert, 1987, 1991, 1996). In contrast to Malaise traps and sweep nets that are manufactured exclusively for the collecting purpose, any kind of device that holds a certain amount of (preserving) liquid and that features a colour attractive to the focal taxon is suitable as pan trap. The material can range from garbage bags, vinyl sheets, plastic food trays to aluminium roasting pans, but the most practical are definitely round plastic bowls that are weather-proof (the colour should not change over time). The specific type to be used largely depends on sampling site attributes (*e.g.* accessibility, distance to the collector's residence). Nearby sites can be sampled with large and heavy pan traps (see Pollet & Grootaert, 1987, 1991), but most recommendable in all situations are light-weight and easily stackable types such as 12 oz plastic partyware bowls (see http://www.partypro.com). These bowls that come in 41 different colours have a flat rim of 2.3 cm, an inner diameter of 15.4 cm and a depth of 3.7 cm. They were recently employed successfully during an expedition in Ecuador (Pollet, unpubl. data) (Fig. 4A, B). Unfortunately, these devices do not seem to be found easily in Europe.

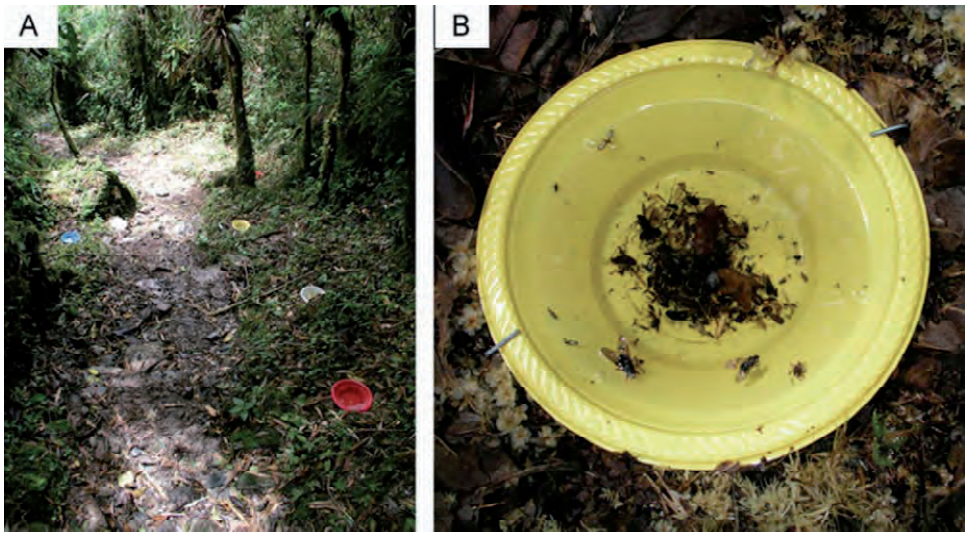

Fig. 4. A. Different coloured pan traps along a trail in a forest in Ecuador; B. detail of the insects trapped in a pan trap. (Photos by Marc Pollet).

One of the most significant advantages of the use of pan traps is their versatility: not only can the size and shape be varied infinitely but also the trap colour and its installation can be adapted greatly in order to optimise the sampling process (see Pollet & Grootaert, 1994). Traps with a bright yellow colour (often referred to as Moericke's traps) are by far the most widely used and attract a broad spectrum of low-flying insects, in particular Hymenoptera and predacious flies. Also white pan traps repeatedly proved to be excellent devices to collect certain fly families *e.g.* Syrphidae and Dolichopodidae: one trap type with a diameter and depth of approx. 9 cm yielded on average 116 and 248 dolichopodid specimens during one season in reedmarsh (Pollet, 1992) and marshland sites, respectively

(Pollet, 2001). These sampling campaigns gathered a total of 73 and 68 species using 77 and 54 traps, respectively. Moreover, a comparative study by Pollet & Grootaert (1994) involving white, yellow, and bluish green pan traps revealed that white and yellow traps collected a comparable number of species; the higher number of specimens yielded by yellow traps was explained by only one very abundant species. Most dolichopodid species thus appear to be most attracted by yellow and white and less by other colours as blue and red. This, however, does not hold true for arboreal dolichopodid species (*e.g. Medetera* spp., *Neurigona* spp., *Sciapus* spp.) that are collected in highest numbers in blue, and soil-dwelling species (*e.g. Campsicnemus* spp.) that are most numerous in red (and blue) pan traps (Pollet & Grootaert, 1987). Actually, thus far *Australachalcus melanotrichus* Pollet & Stark, a species that breeds exclusively in rot-holes of trees, has only been gathered by blue or bluish green traps in multicolour pan trap campaigns (Pollet, unpubl. data). Also other dipteran families with larvae that breed in plant tissue such as leaf miners (Chloropidae) and fruit flies (Tephritidae) are most attracted by blue pan traps.

The installation height also has a substantial impact on the yields. In general, pan traps sunk into the soil are most productive, both in terms of species and specimens (Pollet & Grootaert, 1987, 1991). Again, some species like the xerophilous *Chrysotus gramineus* (Fallen, 1823) and arboreal species are collected more abundantly in traps at 60 cm height (Pollet & Grootaert, 1987), or traps level with vegetation height (Pollet, 2001). As a result, blue or bluish green traps installed at a certain height are best employed if the research focuses on arboreal species communities. If a short-term assessment of the overall species diversity is the main aim, yellow or white pan traps are preferably used. And in case of faunas with a largely unknown ecology, a combination of yellow, white, red and blue coloured traps can be strongly recommended (as the distribution of species of the differently coloured traps holds information on their ecology).

Pan traps thus can be used in every terrestrial and semi-aquatic habitat but are most commonly installed at soil surface level. Traps that are installed on the soil only yield a fraction of the soil-dwelling fauna of *e.g.* carabid beetles and spiders, which are abundantly trapped in pan traps dug into the soil with their rim at soil surface level. In either case, they should be fixed to the soil by metal pins or any other device that prevents displacement. Pan traps can be put simply on the soil in habitats with a well developed herb layer, or sites that are subject to regular but mild flooding. In drier habitats traps are better sunk into the soil and are preferably deeper to prevent them from drying out.

Pan traps are usually filled for ¾ with water. A sufficient amount of detergent must always be added as a surfactant to break the surface tension. Depending on the servicing periodicity, salt can be added as a preservative. If traps are emptied daily or every two days, salt is not necessary, but it becomes absolutely essential with longer servicing intervals. A possible alternative that allows even longer sampling intervals is formalin solution. With a 5% solution as preservative, traps can remain in operation for at least 7 days, and for a fortnight with a 10% solution. Precipitation (rainfall) should be taken into account, especially in the tropics, which can cause a very quick and strong dilution. Deeper traps (over 5 cm) might reduce this effect, but are no guarantee for a good preservation of the

trapped specimens in the rainy season. To avoid the loss of (floating) specimens due to heavy rainfall, minute holes just below the upper rim of the pan trap work well as drainage. Further on, especially in forests and wooded habitats in general, falling leaves or branches might cover the traps largely to entirely, blocking any insect to be trapped. This can be prevented by constructing a framework of thin branches or metal wire covering the trap. As this can be rather time-consuming, it is more practical to service the traps at sampling intervals of at most 5 to 7 days.

The servicing process starts with removing large objects such as leaves, twigs, and vertebrates that might obstruct the collection of the trapped invertebrates and accelerate their decomposition. The remaining contents are subsequently scooped out with a fine mesh aquarium net while collecting the preservative liquid (in a supplementary trap) for reuse (after addition of some fresh solution if necessary). In order to recover the entire content, the net might need to be dragged several times gently near the bottom in one direction. The content of one trap can be kept separately or be pooled with the contents of other traps, depending on the specific objective of the sampling campaign. If the preservative liquid is significantly coloured (mostly by leaves), fresh solution should be used. The contents are transferred to collecting jars or (better) self-sealing plastic bags (*i.e.* whirl-pack type) and properly labelled. Preferably a 90% ethanol solution is added as preservative.

The pan trap technique holds a number of advantages as compared to Malaise traps (Pollet, 1988): (i) they are less striking in the field and as such less subject to damage or removal; (ii) yields are usually fair but not as massive as those of Malaise traps which allows processing in proper time; (iii) consequently, per sampling site a number of traps (Fig. 4A) can be installed to gather information on the heterogeneity of the fauna without jeopardizing the processing of the samples; and (iv) information on the ecology can be gathered using traps of different colours. Nevertheless, it is strongly recommended to employ both techniques in combination as they are largely complementary: a preliminary analysis of samples from Braulio Carillo National Park (Costa Rica) revealed that both trap types collected an identical number of species (26), but shared only 30% or 12 of the total number of species collected (n = 40) (Pollet, 2002). Actually, comparing the yields of both trap types also provides information on the flying activity and frequency of the trapped species.

3.2. Emergence traps

Emergence traps are based on the phenomenon that most insects move up towards the light after emerging. These traps very often reveal species that are rarely collected with other trapping techniques. This was recently illustrated by a field experiment (Fig. 5) along the Belgian coast (see further) that yielded 16 species of Diptera. Two of the species proved new to the Belgian fauna which was surprising as the same beach habitats have been sampled intensively for the past 30 years (Grootaert *et al*., in litt.). Moreover, this kind of collecting method also gathers information on larval development time and food preference.

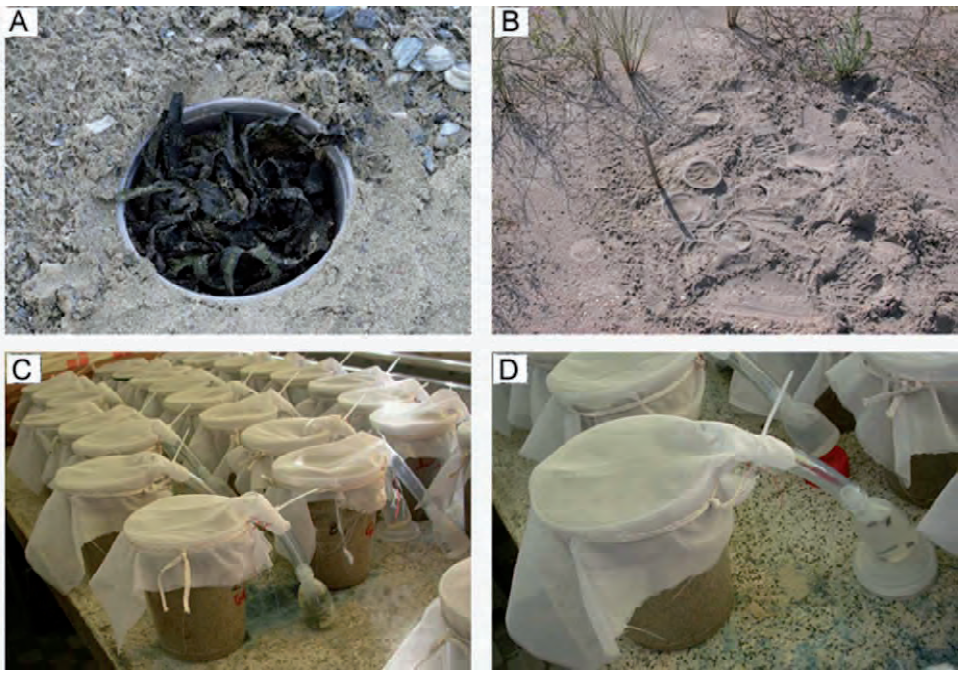

Fig. 5. Collecting insects on the beach with baited emergence traps. A. Freshly cut seaweed is put on top of a vial that is filled with sand and B. dug into the beach for two weeks; C. Subsequently the vials are transferred to the laboratory and D. a cover and collecting jar filled with 70 or 90% alcohol is attached. Emerging insects are collected weekly during a period of two months. (Photos by Wouter Dekoninck & Patrick Grootaert).

Several types of emergence traps are currently available. Some are installed for some period of time in the field, where emerging insects are gathered. Other types (see above) are baited to attract insects that deposit eggs into the intentionally provided substrate, and are returned to the lab for the larvae to accomplish their development and the adults to emerge.

Emergence traps in the field

A first type of emergence trap usually consists of a large pyramidal structure made of black fabric (nylon or other tissue) with a collecting jar on top (Fig. 6). Commercial wasp traps can be used as collecting jar and filled with alcohol. It is still unclear to what extent the climatic conditions within this trap are affected and what fraction of the present fauna eventually ends up in the collecting jar (Glen, 1976).

To collect xylobiont arthropods in the field on standing dead wood, an emergence trap can be attached to, or even constructed around the tree (Fig. 7).

Fig. 6. Emergence trap in the field. The collecting jar is a plastic commercial wasp trap filled with 70% alcohol. (Photo by Wouter Dekoninck).

Fig. 7. Emergence traps fixed around a dead tree to collect emerging xylobiont insects. (Photo by Kris Vandekerkhove).

Emergence traps in the laboratory (see also Berlese and Winkler samples, chapter 9)

Adult insects, especially Diptera, that are not easily collected with the usual sampling techniques are sometimes obtained by gathering soil, litter, dung, mushrooms, decaying fruits, wood or debris in the field, and transferring it to the laboratory for (adult) insects to emerge. Soil samples should remain undisturbed. Dead branches can be placed in large containers and can even be left for months or years as the developmental time of some xylobiont species last

several years. Xylobiont (beetle) species generally emerge in spring (April until June in northern temperate regions) and in this period, traps should be checked regularly.

- In some cases, insects are attracted by bait in order to deposit eggs. The substrates holding the eggs and larvae are subsequently transferred to the lab for the adult insects to emerge. This methodology was recently applied along the Belgian coast: jars filled with sterile beach sand were baited with freshly cut seaweed, and left in the field for about two weeks. It was assumed that fly species inhabiting the littoral zone would be attracted by the bait and deposit their eggs in the plant material. Minute holes in the bottom of the jars were provided for drainage to prevent the developing larvae from drowning. After two weeks, the jars with the soil and plant bait were brought into the lab where they were covered with a lid and a collecting jar was attached.
- A similar method is often used to collect parasitic species (mainly wasps and flies), by actively collecting the hosts in the field and rearing them in the lab. This approach enabled Dan Janzen to build an accurate idea of the tachinid parasite fauna (Diptera: Tachinidae) of caterpillars in the Santa Rosa National Park (Costa Rica) (Smith *et al*., 2006; see also Stireman *et al*., 2009).
- In each type of emergence trap, special attention should be drawn to the orientation and position of the collecting jar. As many emerging adult insects tend to be attracted by light, the jar opening is preferably on top of the trap and has a colour that is substantially lighter than the rest of the trap (Fig. 8). The collecting jar is best filled with an alcohol solution.

Recommendations:

- This method allows the collector to gather information on generation time and diet of the investigated species;
- Emergence traps in the laboratory are preferably held at room temperature (approximately 18-20°C);
- The humidity of the samples in the laboratory should be checked regularly. Samples that are too humid will cause mould and will stimulate mites to develop. An appropriate aeration is recommended in this case. Samples that dry too fast will cause a stop in the development of the insects or their death. If laboratory temperatures might be rather high (*e.g.* in summer), keeping the samples moist might be useful.

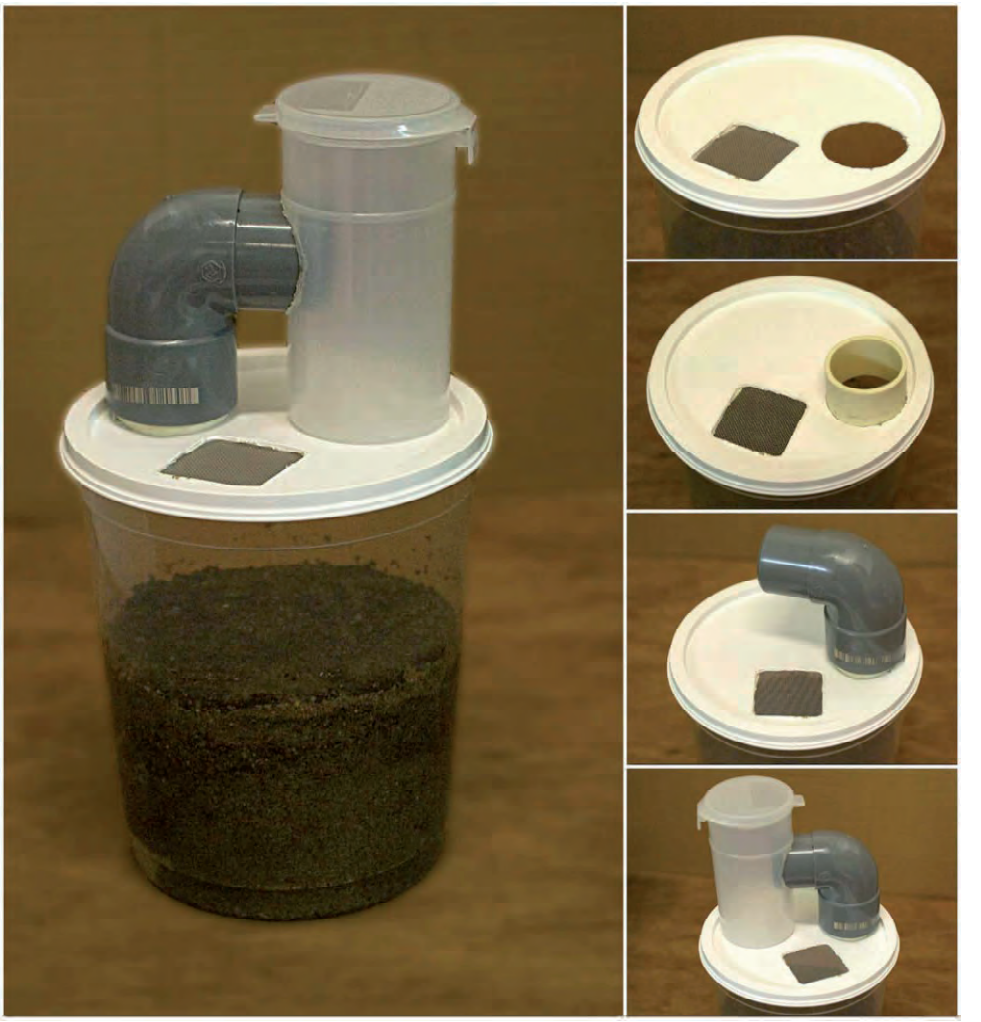

Fig. 8. Collecting jar of an emergence trap made of plumbing tubes. No glue is needed to fix the separate parts except for the mesh. (Photos by Filip De Block).

3.3. Light traps

Light traps are operated at night and are most effective from sunset till after midnight with clouded skies. Especially drizzly weather conditions are very productive, both in terms of species and specimens. This technique is generally applied for the collection of moths, scarabaeid beetles (Coleoptera, Scarabaeidae), and some Hemiptera and Hymenoptera. This trapping method is dealt with in chapter16.

3.4. Malaise traps

Next to the sweep net, Malaise traps are the most widely employed insect collecting devices since the 70'ies. They work unselective and often yield high

insect diversities with huge amounts of specimens. Sufficient time should be reserved for timely processing of these large samples. This collecting method is dealt with in detail in chapter 17.

3.5. Sticky traps

Sticky traps constitute of coloured sheets covered with a thin layer of weather-proof glue. They are made of waxed cardboard, glass, wood, plastic cups, plastic sheets or trap boards, empty milk cartons, red apple spheres or any other surfaces. The sheet's colour represents the attractive agent and depending on the applied colours, particular insect groups will be trapped. Glue types that are applied to this kind of traps are transparent. Attractants can be applied in combination with the glue to lure flying or crawling insects. Tanglefoot Tangle-Trap insect trap coating is often used as adhesive and remains sticky during the entire collecting period (Fig. 9).

Fig. 9. Sticky traps: glue-covered white wooden boards are pulled up 20-30 m high in the canopy of rain forest in Papua New Guinea in order to observe dispersal of weevils (Coleoptera: Curculionidae) between trees. (Photo by Patrick Grootaert).

***Recommendations*:**

- Unlike other traps, sticky traps can operate in inaccessible places such as the upper canopy (including tree trunks), and on top of water surfaces;
- Due to their versatility, sticky traps of different sizes and colours can be produced depending on the specific collecting purpose, similar to pan traps (see 3.1).

***Restrictions*:**

- Insects collected with sticky traps are very hard to detach without causing damage or the loss of body parts. The technique is therefore mainly used for the collection of large insects such as beetles and wasps. The glue is usually dissolved with kerosene, which is highly inflammable;
- Another type of sticky trap consists of a transparent plastic sheet with glue on both sides and attached to tree trunks. This technique should not be employed in areas with rich and endangered arboreal lizard or amphibian faunas.

3.6. Suction traps

Different kinds of suction traps are currently available: traps of the Rothampsted type are high towers that suck in air at a height of at least 10 m, and are mainly used for the monitoring of pest species like aphids (Hemiptera: Aphididae) or gnats (Diptera: Ceratopogonidae). As such, they do not seem particularly fit for ATBI purposes.

Suction traps can also be combined with attractants. The BG-Sentinel (diameter: 36 cm / 14 inches; height: 40 cm / 1.3 feet) is a simple suction trap (Fig. 11) originally designed to collect mosquitoes. Due to its white coloured packing, however, it also proved to be attractive to a large number of pollinators (Grootaert & Dekoninck, in litt.). The trap is essentially a collapsible pop-up container with a white gauze cover, and an inlet at the top. Air is sucked into the trap through a black catch pipe at the top by an electrical fan, drawing approaching mosquitoes and other insects into a collecting bag. The air then exits the trap through the white gauze, generating ascending currents (Fig 11, red arrows). These are similar to convection currents produced by a human host, both in its direction, its geometrical structure, and due to the addition of artificial human skin odours (BG-Lure), also in its chemical composition (BioGents, 2007). Insects are gathered in the collecting bag and dried. The nylon collecting bag can be placed in a cooler and later on transferred to a deep freezer. Alternatively, dried insects can be sorted and pinned immediately or transferred to an alcohol solution. Specimens collected in this way prove suitable for DNA sequencing, even when collected after one week of sampling, which is a major advantage.

Recommendations (for the BG-Sentinel trap):

- A roof should be provided in (expectedly) rainy weather to cover the trap;
- Samples are best removed every two days to prevent damage to the dried insects by large live insects; this can be combined with replacing of the batteries.

***Restrictions*:**

- While using a suction trap to investigate vegetation or the litter or soil layer, plant material and debris is collected which cause damage to the collected invertebrates;
- The working capacity of the batteries of the BG-Sentinel type is two days.

Fig. 10. The BG-Sentinel suction trap was originally designed to collect mosquitoes. The arrows indicate the convection stream with yellow arrows corresponding with the air that is sucked in, and red arrows showing the air stream carrying the odours of the lure out of the trap. Due to its white colour many pollinators are collected. (Photo by Wouter Dekoninck).

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Chapter 16

Recording insects by light-traps

by

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Abstract

Light-trapping is a general term which covers all methods of attracting and/or capturing nocturnal insects with lamps that usually have a strong emission in the ultraviolet range of the spectrum, *e.g.* mercury vapour lamps, black light lamps or fluorescent tubes. Nocturnal Lepidoptera (moths), Trichoptera and Ephemeroptera are the insect groups which can be collected most efficiently by light-trapping but many nocturnal species in several other orders are rarely recorded with other methods, *e.g.* some Coleoptera. There are various light-trap designs in common use, but they are all based on two general construction types. The advantages, limitations and performances of different trap types in relation to target group, study area, vegetation and weather conditions are briefly discussed with reference to relevant literature, and general recommendations for operations are given.

Keywords: monitoring, light trap design, light trap efficiency, abiotic factors, Lepidoptera

1. Introduction

The attraction of moths and other nocturnal insects to light is a well-known phenomenon and has been used for collecting nocturnal insects since the beginnings of scientific entomology in the 18th century. Light-trapping has become a general term which refers to all methods of attracting nocturnal insects with lamps or artificial light sources, whether they are actually connected to a trap or just being operated in front of walls or other reflective surfaces where incoming insects are then recorded or collected manually. The first purpose built devices which could be termed actual light-traps were used by the Romans in the 1st century AD (Morge, 1973; Steiner, 1991; Beavis, 1995).

While the physiological background of the attraction to light is still under discussion (see *e.g.* Hsiao, 1972, 1973; Baker & Sadovy, 1978; Sotthibandhu & Baker, 1979), attracting nocturnal insects with ultraviolet light is now in general use and presents the most effective collecting method for nocturnal species of the orders Lepidoptera, Trichoptera, and Ephemeroptera, but also for many species of Coleoptera, Hymenoptera, Diptera, Neuroptera s.l., Orthoptera, and some other insect groups. Automatic light traps have also become standard equipment for insect pest control and pest management but will not be considered here further, as these devices are purely designed to kill or even destroy the insects attracted and thereby preclude any scientific application.

The main advantage of light-trapping is the large number of species which can be recorded during a relatively short period. In Europe, for example, this can amount to 200 or more species of Lepidoptera in a single night under favourable conditions with the number of individuals running into the thousands. In the tropics the total count both of individuals and of species can be even much higher, often exceeding the available capacity for recording or collecting. On the other hand, light-trapping is still a selective method and not all taxa of a given group (family, genus) are attracted to light with the same efficiency, and females of many species are less attracted than males or not at all. For ecological studies it is sometimes seen as a drawback that light-trapping is an attraction method and it is thus not possible to directly link the species recorded to their respective (larval) habitats.

Overall there are two main approaches in the use of light traps. The qualitative approach aims at maximizing record and/or catch efficiency. For faunistic purposes, and for inventorying or assessing larger areas, it is usually preferable to use high-powered lights (*e.g.* 125 W lamps) and to chose sampling sites for maximum effect and across habitat-types, such as ridge tops, forest edges, etc. For ecological and habitat-related studies which require standardized comparisons and often target habitat- or niche-specific species it is better to use low-powered lamps (*e.g.* 8 W fluorescent tubes) placed well inside the target habitats (Wirooks, 2005).

2. Lamp types

While insects are attracted in a lesser degree to open fire, oil lamps, paraffin lamps, kerosene lamps and other light sources, the most effective lamps are those with a high emittance in the UV part of the spectrum. For most nocturnal insects the attractive part of the light spectrum lies in the ultraviolet range, somewhere between 350 nm and 550 nm (Cleve, 1954; Dufay, 1964, 1965; Mikkola, 1972; Hartstack, 1979) though spectral sensitivity varies from species to species; in a number of nocturnal Lepidoptera taxa Eguchi *et al.* (1982) reported peak sensitivities especially around 440-480 nm, and around 500-540 nm.

For field work, however, the choice of lamp type is more often determined by the actual field conditions than purely by scientific considerations. If there is access to the electricity network or if a portable generator is available, mercury vapour lamps, black-light lamps or blended (mixed light) lamps are usually the best choice because their emittance in the UV range is higher than that of standard household light bulbs (tungsten bulbs). If weight and size are an issue or in field situations without a mains power supply, fluorescent tubes are a perfect alternative which can be run from rechargeable 12 V batteries.

2.1. Mercury vapour and other UV lamps

High pressure mercury vapour lamps come in several sizes of which the 80 W and 125 W versions are those most used by entomologists. A larger 250 W version (which is no longer manufactured) is even more effective but also more trying for the human eye. All of those lamps require a separate electronic ballast (choke) to be inserted between the lamp and the power outlet. There are also 80 W versions which can be run without a ballast. The so-called black-light bulbs (125 W) produce almost no visible light; for the human eye they seem dark blue. They are thus suitable for situations where bright light is undesirable, *e.g.* in residential areas. For many groups, the 160 W blended (mixed light) lamps are less effective than the 125 W mercury vapour lamps but require no external ballast. There is also a 160 W black light bulb available, which does not need a ballast. Details can be obtained from manufacturers or from entomological suppliers via the internet.

2.2. Fluorescent tubes

The low pressure fluorescent tubes or neon tubes generally produce a bluish light and are available in a range of sizes in different lengths: 6 W (22.5 cm), 8 W (30 cm), 15 W (45 cm), 20 W (60 cm). Two special types emitting UV light are commonly used for light-trapping: the so-called "super actinic" tubes producing pale blue light, and "black light" tubes which are comparable to the black-light bulbs and are virtually invisible from a distance. While fluorescent tubes can also be operated with a voltage converter from a generator or mains power supply, in the field they are best directly run from 12 V rechargeable batteries.

A number of studies have compared the relative performance of different lamp types and their attraction on various insect orders (Williams, 1951; Bretherton,

1954; Williams *et al.*, 1955; Cleve, 1954, 1966, 1967; Lam and Stewart, 1969; Mikkola, 1972; Taylor and Brown, 1972; Taylor and French, 1974; Blomberg *et al.*, 1976; Walker and Galbreath, 1979; Leinonen *et al.*, 1998).

3. Trap design

In general, all lamps can be used without any trap or collecting vessel and incoming insects can be recorded or collected manually (Figs 1-5). This is often practised for faunistic studies and in cases when only particular species or specimens are of interest, especially if higher numbers of insects are likely to be attracted which would unnecessarily be collected by a trap or damage the desired specimens inside the collecting container. The lamp is best placed in front of a vertical white sheet, a wall or any other substrate which serves as a good reflector and also allows insects to settle near the lamp. Placing the lamp inside a larger gauze cylinder has the advantage that insects can be similarly attracted from all directions and that the lamp cannot be reached directly by incoming insects (see Figs 4 & 5). The simplest method is still to hang the lamp above a sheet lying on the ground

Fig. 1. Personal light-trapping. The sheet method. A white linen sheet mounted on a frame of aluminium poles, with two battery-powered 15 W fluorescent tubes, one actinic, one black.(Photo by A. Steiner).

Fig. 2. Personal light-trapping. A 125 W mercury vapour lamp and a sheet in a tropical rainforest. Note necessity of rain protection. (Photo by A. Steiner).

Fig. 3. Personal light-trapping. A simple set-up: A black-light bulb in a wire-frame housing at the white wall of a house. (Photo by A. Steiner).

Fig. 4. Personal light-trapping. Two battery-powered 15 W fluorescent tubes in a gauze cylinder ("tower"). (Photo by A. Steiner).

Fig. 5. Personal light-trapping. A combination of a 125 W mercury vapour lamp and two 15 W actinic fluorescent tubes in a gauze cylinder. (Photo by A. Steiner).

For actual light traps, there is a variety of individual designs in use and a vast literature available about the subject. Most designs, however, are based on the following components.

Basic features:

- Lamp
- Funnel
- Collecting container or receptacle

Additional features:

- Rain protection for light bulb
- Rain drainage
- Baffles or deflector shields
- Photoelectric switch
- Anaesthetic or killing agent

The lamp is the attractant. It is placed above or in front of a funnel which directs the insects into a collecting container, jar or receptacle. In addition, the trap can be provided with a range of useful features like a roof structure to protect the light bulb from rain and to prevent leaves, twigs, etc. from falling into the funnel. Alternately or additionally a rain drainage system can be installed, usually consisting of a small drainage funnel below the main funnel entry. A simple hole in the bottom of the trap collecting container covered with fine gauze is sometimes useful, but if a killing agent heavier than air is used the opening of the drainage funnel has to be raised above the bottom of the container.

A number of deflecting shields or baffles - usually two to four - made from Plexiglas, plastic or metal can be arranged around the lamp so that at least the larger, heavier, and faster-flying specimens fall into the funnel when hitting the baffles while circling the lamp.

Nowadays a photoelectric cell is an almost universal component of light traps. It allows the trap to be brought into the field at any time of day; the light-sensitive cell (the sensitivity can be regulated) switches the light on at dusk and off at dawn.

An anaesthetic or killing agent is often used inside the trap container to avoid damage of the specimens. Chemicals like chloroform ($CHCl_3$) or tetrachloroethane (1,1,2,2-tetrachloroethane, $C_2H_2Cl_4$) are left to evaporate from a vial or small bottle by means of a wick, whereas the often used ethyl-acetate is much less useful as it evaporates too quickly. Note that openings at the bottom of the trap have to be avoided (see caution about rain drains above).

Special features:

- Fan
- Wire mesh trays for separating insects according to size

When the trap is run without an anaesthetic it can be helpful to place a small fan inside the trap container to simulate wind which keeps the specimens inactive. Some trap designs include wire mesh or trays for automatically sorting specimens by size so that smaller insects reach the bottom trays and are less susceptible to damage by larger specimens (Common & Upton 1964; Vaishampayan, 1985a, b).

Fig. 6. Trichoptera and Lepidoptera at a gauze cylinder (Photo by A. Steiner).

Figures 7-8 illustrate two different trap designs. More information about specific designs including detailed drawings can be obtained from the literature, *e.g.* Muirhead-Thomson (1991), Fry & Waring (2001), or from individual supplier websites. For some examples of individual trap designs: Rothamsted light trap (Williams 1936, 1948; Taylor & Brown, 1972); Robinson light trap (Robinson & Robinson, 1950); Jermy trap (Jermy, 1961); Common trap (Common, 1960; Common & Upton, 1964); Heath trap (Heath, 1965). In all light traps, design significantly influences the catch especially with regard to the relative composition of different taxa, which can also be used to collect selectively specific target taxa (*e.g.*, Denmark, 1964; Lam & Stewart, 1969; Farrow, 1974; Sutton, 1979; Intachat & Woiwod, 1999).

Fig. 7. A hanging light-trap without rain-cover, showing three baffles around a 6 W actinic tube, a collapsible funnel made of thick plastic film, and a bucket as container. (Photo by A. Steiner).

Fig. 8. The same trap, disassembled. Top right: container. Right: actinic tube inside a Plexiglas cover with cable. The electronics are housed in the black top cap. Left: Plexiglas baffles and lower part of funnel. Centre: collapsible funnel with stabilising ring, screws for fastening baffles to tube housing, rubber ring for fastening lower part of funnel to container lid. (Photo by A. Steiner).

Fig. 9. A ground light-trap with a rain cover and three baffles around an 8 W black-light tube. The container is a commercially available plastic box. The black dot on the small grey box containing the electronics is the photoelectric cell. (Photo by A. Steiner).

4. Distance of light-response in nocturnal insects

In the past there was much difference of opinion about the effective range of attraction of light sources. More or less speculative values were given from around 1 m to 50 m (Daniel, 1952) or even up to 1.000 m (Koch, 1958). Various experimental studies – with different light sources and different study groups – have yielded effective distances of 3 m to 250 m (Bowden, 1982; Muirhead-Thomson, 1991). An unresolved question is whether specimens which obviously came from far outside the sampling habitat were attracted directly over a great

distance or were on a dispersal flight and at some point entered the effective range of the lamp and only then became attracted (which is more probable).

- Mark-release-recapture experiments of Sphingidae (Lepidoptera) around a 125 W mercury vapour lamp in tropical ecosystems (Borneo) suggested attraction radii (for 50% return rate within 5 minutes) of generally below 30 m (Beck & Linsenmair, 2006).
- Experiments with caged moths showed that a 15 W black light tube at a distance of 6.1 m caused 75% of *Heliothis zea* moths (Lepidoptera: Noctuidae) to move towards the light. At a distance of 69 m this response was shown by 10% of the moths. By extrapolation the maximal range of attraction was determined as 60-90 m. In *Manduca sexta* (Lepidoptera: Sphingidae) 48% of individuals showed a positive response at a distance of 4.6 m from the light source; the maximal range of attraction was determined as 120-135 m (Stewart *et al.*, 1967).
- In a similar experimental setup the threshold of attraction was calculated to be 200-250 m for *Spodoptera littoralis* (Lepidoptera: Noctuidae) (Plaut, 1971).
- Physiological studies on the eyes of *Heliothis zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) showed that 15 W blacklight tubes can trigger sensory responses from distances between 31 m and 250 m (Agee, 1972).
- Under the assumption that nocturnal insects react to wavelengths of 500-600 nm, Bowden & Church (1973) calculated the radius around a 125 W mercury vapour lamp within which the brightness of the light source is higher than the background brightness. They obtained values between 35 m (in full moon nights) and 520 m (without moonlight). On a similar basis Dufay (1964) reached results of 50 m to 700 m for another type of 125 W MV lamp, while Nowinszky *et al*. (1979) calculated distances of between 20 m (full moon) and 300 m (no moon) for a 100 W Argon bulb.

5. The role of abiotic factors

There is an abundant literature on the many abiotic and other factors which influence light trap efficiency and sample size. We can only give a basic overview and provide references of more detailed studies.

5.1. Temperature

Ambient air temperature seems to be the most important single factor influencing insect flight activity and thus the catch (Williams, 1940; Daniel, 1952; Hosny, 1959; Taylor, 1963; Pulliainen, 1965; Hanna & Atries, 1969a; Persson, 1971, 1976; Kurtze, 1974; Hanna & Hamad, 1975b; Blomberg *et al.*, 1978; Morton *et al.*, 1981; Dent & Pawar, 1988; McGeachie, 1989). Generally speaking, the higher the temperature the more insects are active, which usually translates into highest activity rates during the first hours after sunset. Rapid cooling during the night will cause inactivity sooner than slow cooling. In temperate climates cloud cover at night means less rapid cooling and thus a longer activity period of insects. Temperature dependency, of course, varies with the climate zone a

species inhabits: boreal and alpine species are adapted to lower temperatures than thermophilic, subtropical or tropical species, and specialist species having their peak activity during periods of comparatively low temperature can be found in all biomes, including the famous "winter moths" and "winter midges" of northern hemispheres.

5.2. Moonlight and starlight

Lunar periodicity plays an important role in catch efficiency and has been the subject of numerous studies (Williams, 1936; Williams & Singh, 1951; Hosny, 1959; Dufay, 1964, 1965; Hanna & Atries, 1969b; Persson, 1971, 1976; Bowden, 1973, 1981, 1982, 1984; Bowden & Church 1973; Hartstack *et al.*, 1973; Kurtze, 1974; Bowden & Morris, 1975; Hanna & Hamad, 1975a; Douthwaite, 1978; Nowinszky *et al.*, 1979; Morton *et al.*, 1981; Vaishamapayan and Verma 1982; Danthanarayana, 1986; Taylor, 1986; Dent & Pawar, 1988; McGeachie, 1989; Nag & Nath 1991). In short, the stronger the moonlight is, the less attraction a lamp has to insects. The ratio between catch in new moon nights and catch in full moon nights has been given as 2,67: 1 (Williams, 1940; a 4-year study in England) and as 2.59: 1 (Nowinszky *et al.*, 1979; 14 years of light-trapping in Hungary). While it was once suspected that insect activity in general might be lower in moon nights, it has since been shown that lamp attraction is weaker. In fact insect activity seems to be higher in bright, moonlit nights as indicated by comparisons of light-trapping with other methods such as suction traps (Bowden, 1981) and pheromone traps (Dent & Pawar, 1988). When insect activity actually diminishes in moon nights this is usually due to other negative weather factors, especially rapidly falling temperatures as commonly observed in clear nights. In subarctic regions, however, the naturally bright summer nights make lamps less attractive to insects (Blomberg *et al.*, 1978).

The relationship of background brightness (light emitted by moon and stars) and catch efficiency has been expressed in the formula:

$$\text{catch} = \text{constant} \times \sqrt{W/I}$$

where W represents lamp brightness and I is background brightness. With a constant lamp brightness there is:

$$\text{catch} = \text{constant} \times \sqrt{1/I}$$

Other weather factors can significantly influence this ratio (Bowden & Church, 1973; Bowden, 1981, 1982), while cloud cover mitigates the competing effects of moon light.

5.3. Wind

Wind speed is another important factor affecting insect activity and especially flight (Hosny, 1955, 1959; Williams, 1961; Dufay, 1964, 1965; Brown, 1970; Persson, 1971, 1976; Kurtze, 1974; Hanna & Hamad, 1975b; Douthwaite, 1978; Morton *et al.*, 1981; Tucker, 1983; Dent & Pawar, 1988; McGeachie, 1989). In stronger wind there is less insect activity: most species cease flying as soon as

they cannot any longer maintain a directional flight. The critical wind speed varies according to size and strength: larger moths (Noctuidae) cease flying to lamps at wind speeds of 10.8-13.8 m/s, smaller Diptera, Tipulidae, Limnobiidae, and Chironomidae at 8.0-10.7 m/s, Psychodidae and Trichoceridae at 6.7-9.4 m/s, and Ceratopogonidae and Cecidomyiidae at 3.4-5.4 m/s (Kurtze, 1974). A marked reduction of catch occurs at 3-4 m/s (Douthwaite, 1978) and at 4 m/s (Dent & Pawar, 1988). The highest catch rates, however, are not recorded at calm but at wind speeds between 1 and 3 m/s (Hosny, 1955; Douthwaite, 1978; Dent & Pawar 1988).

5.4. Precipitation, air humidity, and fog

Strong rainfall can reduce or prevent insect activity, especially for smaller species, while most insects are usually indifferent to light rain (drizzle, spray) unless it coincides with a drop in ambient temperature. Under certain conditions, *e.g.* in dry or semiarid areas but also in tropical regions with a pronounced rainfall seasonality, rain can induce eclosion and stimulate activity (Williams, 1940; Daniel, 1952; Hosny, 1955, 1959; Pulliainen, 1965; Harling, 1968; Brown *et al.*, 1969; Persson, 1971; Kurtze, 1974; Douthwaite, 1978; Tucker, 1983). In the tropics rain often considerably increases light trap attractivity, often leading to unusual and rare records. For running a light during tropical rain, the lamp or trap is best protected by a larger roof, which can be easily constructed with some canvas or tarpaulin (Malicky, 2002; see also Fig. 2). In addition, some drainage provisions around the position of the trap are often a helpful measure (*e.g.*, Diehl, 2001).

In temperate conditions, high air humidity can also promote insect activity unless combined with cooling. Fog in combination with falling temperatures or fog which forms in valley bottoms, basins, and wetlands, strongly reduces insect activity. Dewfall is usually a result of cooling and coincides with reduced activity. Drifting clouds and fog on slopes or in the mountains need not to lead to negative results; in certain situations they actually seem to intensify the attraction of light traps (Daniel, 1952; Hosny, 1955, 1959; Hanna & Atries, 1969a; Kurtze, 1974; Hanna & Hamad, 1975b; Esche, 1992).

5.5. Air pressure

It is sometimes said that falling air pressure improves general insect activity, *e.g.* before thunderstorms (Haase, 1929; Allan, 1947; Hosny, 1955; Lederer, 1959) while other studies claim there is no recognisable influence of air pressure (Dufay, 1964, 1965). Without quantitative studies or experimental evidence at hand, however, we also have experienced many times the highest attraction of light traps at times just before the onset of thunderstorms or heavy rainfall, both in temperate and especially under tropical conditions; whether it is specifically air pressure or other factors related to the imminent change of weather conditions which lead to high levels of insect activity remains unclear, but such situations are usually always advantageous for light-trapping.

In addition to climate and weather related factors, several locality-related conditions also play an important role in determining the most productive sites for light traps.

Forest vs. open country

Inside forests the negative effect of moonlight is less dramatic. Bowden (1982) studying trapping data from Rothamsted (England, U.K.) noted a catch ratio of *Noctua pronuba* (Lepidoptera: Noctuidae) between open habitats and forests of 1 : 3.7. Temperature change, especially nocturnal cooling is often less marked in forests, and winds are weaker. On the other hand light has a larger radius in open areas (Hosny 1955, 1959; Bowden, 1982), and results are significantly different between light traps placed in the understorey and in the forest canopy, especially in the tropics (Schulze *et al.*, 2001; Beck & Linsenmair, 2006).

Wind direction

Most insects prefer to fly against the wind when looking for food or locating females. Exceptions are migrating specimens which use wind currents and fly with the wind (Brown *et al.*, 1969; Brown, 1970). When smaller areas are to be studied it is thus advantageous to place traps at their windward side.

Terrain structure and landscape

Many insects prefer to fly upslope, also at night. Lights placed on slopes or hilltops may control a larger area; even considering that a lamp's direct effective range of attraction may be quite small, there is a higher chance that more specimens reach the neighbourhood of the trap. The landscape (and vegetation) surroundings of the light trap location also greatly influence the results, *e.g.* by offering protection from or providing exposure to local wind currents and other weather factors, and through different local microclimatic conditions, including varying albedo properties. Cold air often accumulates in even small depressions and valley bottoms, while certain terrain structures such as bare rocks can absorb heat during the day and emit part of that radiation at night. Selecting the exact placement of a light trap should also take these factors into account.

6. Concluding remarks

For any new light-trapping project, the choice of the equipment to be used is clearly an important initial step. Aside from the relevant technical and biological parameters that different lamps and trap constructions entail, the final choice should also consider more practical criteria, such as weight and transportability, durability under field conditions, and availability and cost of spare parts or repairs. It should be kept in mind that there exists no overall most effective or "best" lamp type nor "standard" light trap construction or design; all types and makes of light traps are differently selective in one way or another, and the final choice should be determined by the exact question(s) and goals to be pursued by the study. Although most equipment discussed here works well for most insect

taxa and many different habitats, no one type of light trap will equally attract all taxa. For aiming at a comprehensive inventory such as an ATBI of a local fauna or a community of different taxa, it is therefore advisable to employ a number of different lights and trap designs, if at all possible.

With standardization of methods being a requirement for many scientific approaches in order to allow for comparable and/or repeatable collection of data, especially from ecology, light-trapping provides a clear method of choice for many entomological studies. While standardization can be easily achieved for the equipment and light-trapping regime, other factors relevant for the results are much more difficult to compare or even standardize, even if the availability of fully automatic light traps allows reducing the influence of the "human factor" to a certain degree. Apart from the important effects of weather, moonlight and other factors discussed above, the exact placement of a trap in the field remains the overall most difficult and perhaps still influential parameter in making light-trapping data fully comparable, especially for highly structured habitats and landscapes such as forests and mountains. As indicated above and experienced many times, the precise placement of the light in relation to its surroundings greatly impacts the results, with sometimes a few feet or meters distance already leading to noticeably different catches. Especially for manually operated lights, finding the "best" precise location is almost always the biggest challenge in the field, for which personal experience often still provides the best guidance. All these methodological challenges should provide additional incentives for the precise recording and documenting any light-trapping session, especially for exact geographic coordinates, time, and weather conditions, which should be a common standard under all light-trapping circumstances.

7. Tips and hints – some "do-s and don't-s"

- The higher a lamp/trap is placed above the ground, the larger is the area it controls. Be sure to have sufficient possibilities to raise the light and/or trap above ground on site (*e.g.*, by carrying poles or other equipment).
- Stronger light generally means higher attraction (more specimens/species), but some species prefer to settle at some distance from bright lamps. It is often helpful to carefully check the perimeter around such a lamp to find those species.
- Small moths and other insects with a gentle flight often come to rest on the baffles of a trap or in the vegetation nearby and do not enter the collecting container. Traps should therefore be checked well before sunrise, before these specimens fly away or are eaten by birds and other predators. It is helpful to place the trap on a large white sheet or a similar background that makes it easier to find those specimens.
- Before placing light traps for longer-term studies in the field, check and record the microclimatic conditions at night at the exact location, particularly with regard to air temperature, wind strength, and wind direction.
- When using a trap without a killing agent, the container needs to be filled with materials to provide sufficient resting space for the specimens. Many authors

recommend using egg cartons, which however we find very difficult to extract resting specimens from. Instead, we recommend using rough, slightly crumpled paper, because this is easier to handle and can be more readily straightened to box specimens.

- When running light traps with a killing agent especially for specific, limited questions, try to ensure that the by-catch is also kept for / used by other researchers; all specimens collected with accurate data can be of value!
- Do not look directly into a mercury vapour lamp. Although the UV radiation from MV lamps is considered not harmful for the human eye, individual sensitivity varies and emission from strong MV lamps can be irritating.
- When going into the field, always carry sufficient torches and other additional light sources along; if for no other reason, setting up and taking down light trap equipment at night can be quite difficult without sufficient torches at hand.
- Always take some basic tool kit (screwdriver, pincer, small knife, electrical tape) along when light-trapping; equipment gets easily damaged under field conditions, and it is advantageous to be able to do basic repairs on site.

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