Collecting herbarium vouchers

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Introduction

A herbarium is a collection of plant specimens (vouchers) which is arranged systematically and stored in purpose-built cabinets. It may be housed in a special building or form part of a larger scientific institution or museum. A herbarium voucher is usually a dried and pressed plant or portion of a plant mounted on to a sheet of stiff card usually measuring 42×26 cm (frequently somewhat larger in the US). It can also be material stored in spirit or more bulky dried items such as samples of wood or large fruits. Vouchers are a permanent record of a plant. A specialist is able to name the plant or check any name previously applied to it. When named, vouchers act as a key reference point to further information on the plant. Of themselves, they are also sources of information on morphological (and possibly molecular) variation, phenology, distribution and autecology.

Germplasm collectors gather material for herbarium vouchers for various reasons:

To 'determine' particular germplasm accessions. Such vouchers may be collected either at the same time as the germplasm or before, in the course of a preliminary reconnaissance survey carried out at flowering time. This may include marking sites or even individual plants for subsequent germplasm collecting. The vouchers will most often be used in the first instance simply to identify (or 'determine') the plants accurately, though they will of course remain as a source of other information. It may, for example, be possible to extract DNA from herbarium vouchers for genetic diversity studies (e.g. Liston *et al.*, 1990). Accessions undergoing numerous regeneration or multiplication cycles, or being grown out under a different climatic regime, may change substantially in phenotype from the original population, of which it is therefore useful to have a durable record. All germplasm collections of wild species (forestry species, forages, crop relatives, etc.) should be accompanied by voucher specimens. For crops, only germplasm collections of rare or unusual types need to have accompanying voucher specimens.

- To show the range of variation in selected characteristics of the population sampled. A so-called 'mass collection' of herbarium specimens is sometimes made. This is a population sample, composed of a number of individuals large enough to be statistically useful, of critically selected corresponding plant fragments collected at a particular time and place.
- To identify pests, etc. Material showing evidence of pest or disease damage or of some abiotic problem may be collected to help in the identification of the exact cause of the problem, rather than of the plant itself (Chapter 17).
- To document the flora of a site or region. Collectors will usually gather germplasm of only a very small proportion of the plants found in an area, but may decide to collect herbarium specimens of other species, particularly if there are rare, endemic or otherwise interesting plants in the area, or if the region is poorly known botanically.

Vouchers will last indefinitely if properly prepared and stored and remain for study by future generations. This chapter will deal with how to collect, process, store and dispatch vouchers to ensure that good, welldocumented collections are made.

Collecting specimens for vouchers

A small number of well-collected and clearly annotated specimens are of much greater scientific value than a large number of badly pressed, poorly labelled scraps. Ideally, specimens should be processed immediately after gathering, using one of the methods described below. In practice, there is rarely enough time to process material in the field but specimens can be kept fresh for a reasonable length of time in large, strong polythene bags (see Box 27.1 for basic equipment). A separate bag, clearly labelled, should be used for each locality. Within these large bags, smaller bags can be used for each plant collected (certainly for samples of small or fragile plants). However, delicate plants and those that wither quickly (e.g. many Boraginaceae) or whose petals fall quickly (e.g. many Papaveraceae and Cistaceae) should be pressed immediately into a field press or portfolio. This consists of two sheets of stiff card or thin plywood, slightly larger than the card on which the specimen will be mounted, held together by webbing straps and containing folded sheets of absorbent paper.

Box 27.1

Basic equipment for herbarium collecting

The final selection of equipment will depend on the conditions under which collecting will take place and the plants of interest (Boxes 27.2 and 27.3). The following is a list of the equipment that will be necessary no matter what the method of preparation of specimens. Equipment necessary for data gathering and recording is listed in Chapter 19.

- Gardeners' gloves.
- Secateurs.
- Knife.
- Pruning saw.
- Digger or entrenching tool (for collecting underground organs).
- Polythene bags (various sizes, e.g. 100 × 75, 50 × 25 and 30 × 15 cm).
- Field press.
- Jeweller's tags.
- Paper packets for seeds, loose flowers, etc.
- Polythene bottles and spirit.

What to collect

Care should be taken to collect plants that are representative of the population (or subpopulation) from which the germplasm collection is being made, and not simply those that fit most easily into the press or are easiest to reach. In making a general collection in an area, any plant may be of interest. The very large, the very small, the spiny and the succulent are all too often undercollected for no better reason than that they are awkward to press.

In general, sterile specimens are worthless, so normally only flowering and/or fruiting material should be collected. One should try to get flowers and fruits from the same plant. If this is not possible, different collecting numbers should be given to separate flowering and fruiting specimens. For monoecious and dioecious plants, flowers of both sexes should be collected. It is important to know which specific features or organs of the plant are important for identification. For instance, in some families (e.g. Cyperaceae, Umbelliferae, Cruciferae, Dipterocarpaceae, Eleagnaceae) ripe fruit is essential, in others (e.g. Orchidaceae) flowers are necessary and fruits unimportant and in others (e.g. Gramineae) the whole plant, including underground parts, must be collected. Forman and Bridson (1992) list a selection of families with short notes not only on the features of the plants which need to be noted but also on special collecting and processing techniques. References where fuller instructions for problem families can be found are:

- Agavaceae (Sánchez Mejorada in Lot and Chiang, 1986:107);
- Araceae (Nicolson, 1965; Nicolson in Womersley, 1981:115-119; Croat, 1985);

- Balsaminaceae: Impatiens (Grey-Wilson, 1980);
- Begoniaceae: Begonia (Logan, 1986);
- Bromeliaceae (Smith, 1971:23-24; Jorgensen, 1972; Aguirre Léon in Lot and Chiang, 1986:118-119);
- Cactaceae (Sánchez Mejorada in Lot and Chiang, 1986:106-107);
- Crassulaceae (Sánchez Mejorada in Lot and Chiang, 1986:107);
- Cyclanthaceae (Hammel, 1987);
- Gramineae: bamboos (McClure, 1965; McClure in Womersley, 1981:110-114; Soderstrom and Young, 1983; Koch in Lot and Chiang, 1986);
- Lecythidaceae (Mori and Prance, 1987);
- Lentibulariaceae: Utricularia (Taylor, 1977);
- Musaceae: bananas (Fosberg and Sachet, 1965:109–110; Womersley, 1981:100–102);
- Orchidaceae (Aguirre Léon in Lot and Chiang, 1986:114-117);
- Palmae (Tomlinson, 1965; Tomlinson in Womersley, 1981:103-109; Balick, 1989; Dransfield, 1986; Quero in Lot and Chiang, 1986); rattans (Dransfield, 1979);
- Pandanaceae (Stone in Womersley, 1981:94-97; Stone, 1983);
- Passifloraceae: Passiflora (Jørgensen et al., 1984);
- Zingiberaceae (Burtt and Smith, 1976).

How much to collect

It is important to always bear in mind how much material one will need to collect, particularly if specimens are not being pressed immediately. How many duplicates will be needed? The absolute minimum will probably be three: one to be kept by the collector's institute (or an associated or nearby herbarium) for reference, one to be deposited in the country's national herbarium and one to be sent for verification to an expert, who will often be attached to a major regional or world herbarium. For each duplicate enough material should be collected to fill a herbarium sheet. This could mean several plants of a small annual or a single shoot of a shrub or tree. Of course, one should be careful not to collect so much material that the survival of the population is endangered. In certain plant groups two or even three sheets are needed for a single sample. It is often useful to collect extra flowers, which can be placed in small envelopes (sometimes called 'capsules') on the herbarium sheet. These flowers are used for dissecting, which is often necessary for identification, without damaging the main part of the voucher, which should be attached firmly to the sheet.

Processing vouchers

There are two main methods of processing herbarium vouchers:

- by drying in the field;
- by chemical treatment (the Schweinfurth method) followed by drying at a later date.

Using the first method the plants are pressed and then either they are air-dried or gentle heat is applied. In the second method the material is pressed and then stored in sealed plastic tubing with some spirit until it can be properly dried, usually back at the home institute.

Drying in the field

Drying in the field produces very good specimens and is particularly useful in dry climates. It is, however, very time-consuming and requires a lot of bulky equipment (Box 27.2). Some specimens must first be 'killed' to prevent the leaves becoming detached during drying. There is also the danger of material becoming mouldy before it is dried. Once dry it must be kept so, or mould may develop.

Plants for processing are placed directly into a flimsy. Small plants should be pressed whole and representative flowering and/or fruiting shoots with leaves should be selected from larger plants. Leaves that extend beyond the flimsy should be folded over. Pieces of stem that are too long for the flimsy can be broken and arranged in a 'V' shape; vines should be curved. Succulent plants and bulbs will dry more quickly if they are split lengthwise; spiny or intricately woody specimens can be initially flattened between boards or corrugates before being put into the plant press. Delicate flowers or flowers on thick woody stems are best pressed separately in small torn-off bits of flimsy.

The flimsy must be numbered (the collecting number) on the outside bottom right-hand (opening) corner. A jeweller's tag can also be attached to the specimen. The specimen will stay within this flimsy throughout the drying process. Notes about the plant are made in the collector's field book at this time.

The press is built up by alternating one or two drying papers with each flimsy. Corrugates added between the drying papers allow circulation of air and will speed the drying process. If enough corrugates are added the press can be as thick as the length of the straps will allow. However, for air-drying in the sun, the smaller the press the faster the plants will dry and the better the results. Finally, two straps are placed

Box 27.2

Equipment for drying specimens in the field

- Flimsies (folded sheets of thin, strong paper the same size as the press e.g. airmail newspaper or ordinary newspaper).
- Drying paper (newspaper or felt).
- Presses and straps (presses can be slatted wood or metal lattice; straps can be of webbing, leather or nylon - the last is not suitable if heat is being used).
- Corrugates (aluminium or corrugated cardboard).
- Drying frame.
- Source of heat (paraffin stove, electric heater, etc.).

around the press (breadth wise) and tightened as firmly as possible. It is important not to allow the plants to rot by using a thick press and too few corrugates.

In dry climates the presses can be placed in the sun with as much air circulation as possible-a sunny, windy spot (e.g. a car roof-rack) is ideal.

Specimens can be rearranged after a few hours but should not be removed from the flimsy until they are ready to be mounted. Drying papers must be changed daily; if this is not done, plants soon become mouldy and blackened. Damp drying papers can be quickly sun-dried by spreading them about on the ground (Davis, 1961).

Alternatively, the press can be placed over a source of artificial heat on some sort of frame. This can be a purpose-built collapsible frame or can be made from two lengths of wood supported on the backs of chairs. The heat source can be paraffin stoves (Womersley, 1981), incandescent bulbs (Lawrence, 1951), coals (Forman and Bridson, 1992) or electricity, if available. An electric heater is very effective. A skirt of suitable material (e.g. canvas) should be placed around the bottom of the frame. Care must be taken that naked flames do not ignite the specimens and that the plants are not over dried. Drying using this technique can take anything from 4 to 48 hours.

Different plants take different lengths of time to dry. Presses should be checked at least twice a day or plants can become overdried and brittle. When dried, specimens should be removed from the press as soon as possible. It can be difficult to tell when a plant is dry. A good indication is when the leaves or stems are not bendable or marked when bruised with a fingernail.

Chemical treatment (the Schweinfurth method)

Chemical treatment using alcohol is particularly useful in areas of high humidity such as tropical rain forests. The spirit prevents fungal attack and not having to dry the plants immediately saves time in the field and generally requires less equipment (Box 27.3). However, specimens often dry black and alcohol-soluble substances are lost. Also, alcohol can be difficult to obtain.

Specimens are collected into the press as described above and left overnight. Still in their flimsies, they are then removed from the press and piled into conveniently sized bundles (about 12-15 cm thick). A length of polythene tube about 1.5 m long is cut and the bundle inserted into it. One of the opened ends of the tube is then folded over and sealed with non-alcohol-soluble tape. The tube is now stood upright with the opened end uppermost and about 0.5 to 1 litre of 60-70% (higher concentrations can be used on particularly succulent material but tend to make the specimens brittle) industrial alcohol or methylated spirits or 4-6%solution of formaldehyde added. Womersley (1957, 1981) describes how to prepare formaldehyde using paraformaldehyde powder. The use of formalin is not recommended, as it is carcinogenic and is not always

Box 27.3

Equipment for the Schweinfurth method

- Flimsies.
- Drying paper.
- Presses and straps.
- Polythene tubing (heavy gauge, 0.1 mm thick and about 25 cm wide).
- Tape (insoluble in alcohol).
- Spirit and spirit containers.

successful at preventing mould developing. The opened end of the plastic tube can now be folded over and sealed.

The spirit fills the bag with vapour and if air is excluded preserves the contents for weeks. If specimens are going to be dispatched by air, excess spirit must be poured off (airlines do not allow the carriage of alcohol). The most important thing to remember is that the process depends on the exclusion of air, so great care must be taken to ensure that the polythene tube is not punctured (Fosberg and Sachet, 1965; Womersley, 1981; Forman and Bridson, 1992).

Special techniques

- Fragile plants or plants with delicate flowers should be pressed immediately or placed in a field press until they can be transfered to the main press. Particularly delicate flowers (e.g. Orchidaceae, Zingiberaceae or Bromeliaceae) should be placed in spirit (see below). The corollas of certain flowers (e.g. petaloid monocots, *Impatiens* spp. and *Hibiscus* spp.) should be taken to pieces and their parts pressed separately in tissue paper or non-absorbent toilet paper.
- Succulent or fleshy plants (e.g. species of Asclepiadaceae, Crassulaceae, Cactaceae and Euphorbiaceae) need particular attention. In general, they can be cut in half and then 'killed' in boiling water or spirit to prevent the specimen continuing to grow in the press. They can then be air-dried, although this can take several weeks, particularly if the tissue has not been killed. If a microwave oven is available, this can be used to kill the tissue (Fuller and Barber, 1981; Leuenberger, 1982). The specimen is placed in the microwave for 30 seconds to 2 minutes, depending on the toughness of the plant; it can then be air-dried in the normal way. The leaves of Aloe spp. require special treatment. They should be carefully cut around the margin and the inner tissue scraped out; the upper surface with the margin intact is pressed. With Cactaceae, as much of the fleshy inner tissue as possible is scraped away and then salt applied to draw out the water.

- Large or fleshy plants or those with complex flowers (i.e. Orchidaceae, Zingiberaceae or Bromeliaceae) can be preserved in spirit (Fosberg and Sachet, 1965:117-119).
- Bulky or cushion plants can be sectioned before they are pressed and wads of paper added to even out the pressure on leaves and flowers and thus prevent shrivelling. Some flowers should in addition be pressed separately.
- Very large leaves. Measurements and drawings should be made of the whole leaf, which should then be sectioned and labelled in such a way that it can be reassembled later. The different parts (including usually the apex, middle and basal sections) can then be pressed separately. For notes on collecting large-leaved species from selected families, see the references for Araceae, Musaceae, Palmae and Pandanaceae listed earlier.
- Aquatics with fine leaves should be floated over drying paper which is submerged under the plant and then drawn out. For detailed instructions see Fosberg and Sachet (1965:107-109), Womersley (1981:98-99), Haynes (1984), Ceska and Ceska (1986), Lot (1986) and, for Utricularia spp., Taylor (1977).
- Collecting epiphytes and flowering and fruiting specimens from the canopies of tall trees presents particular problems. A light sectional rod with interchangeable knife and grapnel at the tip is handy for reaching high branches (Fosberg and Sachet, 1965). Alternatives are climbing into the canopy and using rifles. For detailed instructions see also Hyland (1972), Mitchell (1982) and Wendt in Lot and Chiang (1986). Chapter 23 describes techniques for collecting germplasm samples from tall trees, which clearly can also be applied to the collecting of botanical voucher specimens.
- Ferns are collected in basically the same way as higher plants. Part of the rhizome attached to the stipe together with basal, middle and apical parts of the lamina should be collected. It is also important to make notes on the habit (i.e. creeping, rosette-forming, etc.). See the section on collecting large leaves above and also Holttum (1957), Stolze (1973) and Forman and Bridson (1992).
- Bryophytes can be collected into paper packets (not polythene bags), made by folding sheets of paper, and dried by laying out in the sun or by application of gentle heat (Forman and Bridson, 1992).
- For instructions on collecting *fungi and lichenized fungi*, see Duncan (1970), Hawksworth (1974) and Forman and Bridson (1992).

Recording data

Vouchers without accompanying notes are of limited value, as indeed are germplasm samples without passport information. Such notes are included on a label, which is mounted with the specimen when this is prepared for inclusion in a herbarium collection. Many of the kinds of data that need to be recorded to document germplasm samples (Chapter 19) should also be recorded on the labels of herbarium vouchers, in particular: name(s) of collector(s) (and institute), collecting number (and number of associated germplasm sample), collecting date, (preliminary) taxonomic identification, locality, specific habitat and species abundance. In addition, notes and drawings should be made of any character of the plant which would be lost or not evident in a small dried specimen. The list provided by Forman and Bridson (1992), somewhat adapted, is as follows:

- Life-form, life span and habit: plant type: tree, shrub, herb or vine: free-living, epiphyte or parasite: life span: annual, biennial or perennial; direction of stem growth: climbing, erect, geniculate, decumbent, prostrate. creeping. etc.: stem structural type: pachycaulous, succulent, bulb, corm, stolon, rhizome, etc. Underground organs: roots: type (tap, fibrous, with tubers, etc.) and extent; rhizome: depth in soil, length, spacing of shoots: bulb, corm or tuber: size and shape. Stems and trunks (for instructions on collecting bark and wood samples, see Welle, 1989): size: total height, diameter at breast height (DBH), height of trunk or stem before branches: shape in cross-section (circular, fluted, with buttressing, etc.); bark: colour, texture, thickness, lenticel colour: wood: hardness, colour, grain type; sap or latex: colour, smell, consistency: thorns and spines. Leaves: deciduous or evergreen; texture, colours, smell, glossiness, hairiness; exudate or glands: orientation in relation to petiole or stem (pendulous, horizontal, etc.): outline (if large or complex); heterophylly. Inflorescence: exudate or glands; position or form (cauliflorous, ramiflorous, etc.); colour of axis. Flowers (for instructions on collecting pollen and karyological samples, see Le Thomas, 1989, and Morawetz, 1989, respectively); monoecious, dioecious or hermaphrodyte; heterostyly:
 - scent:

exudate or glands; calyx colour and texture; corolla colour and texture; opening behaviour; pollinators.

Fruit and seeds:

smell;

fruit, seed-coat and aril: colour and texture; fruit and seed: size and shape; dispersal (animal, wind or water).

For tropical trees, Hallé and Oldeman (1970) and Hallé *et al.* (1978) have described 24 architectural 'blueprints' or 'models' for the pattern of branching and growth. Similar models have also been applied to herbs and lianes (Bell, 1991).

Photographs are often taken to complement herbarium specimens. These should show the habitat of the plant, its general habit and flowers and/or fruits in close-up.

Storing and dispatching vouchers

Field-dried specimens should be removed from the press in their flimsies, and placed in piles about 10 cm thick. These piles should be protected top and bottom by extra flimsies or cardboard corrugates and then firmly tied into bundles using thin string. Such bundles can withstand a fair degree of handling. It is essential that specimens are completely dry before they are packed, as mould can quickly spread throughout the bundle from a single damp specimen. In damp weather or in humid conditions the bundles should be placed in plastic bags. For storage or dispatch the bundles should be tightly packed into cardboard cartons or similar containers and liberally sprinkled with naphthalene or paradichlorobenzene. Screwed-up paper should be used to pad any spaces between the bundles. Dried specimens can be easily damaged if they are not packed tightly. Bundles of wet specimens packed in polythene tubing containing spirit can be stored in this form for several weeks. For dispatching they can be packed into strong hessian or plastic sacks. It is a good idea to pack specimen packages so that they can be relatively easily opened in case they need to be checked at customs. Suitable material to reseal the packages quickly in case they are opened should be taken along when dispatching vouchers. It can take a great deal of time to package specimens properly, but it is worth doing it carefully so that weeks of work are not destroyed for want of a little extra effort at the end of a long trip. For dispatch abroad, air freight is probably the best bet.

Care must be taken to ensure that the correct paper work for the export and import of duplicates has been done - delays can be very damaging to specimens. The procedures will usually be similar to those necessary for the movement of germplasm (Chapter 17). The agreement of herbaria and other institutes to accept herbarium specimens should be obtained before the start of the mission. Recipient institutes should be warned in good time that material has been dispatched.

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