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Non-vascular plants and fungi

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Introduction

This chapter describes the methods used to conserve fungi (including lichens), red and brown algae, much of the green plant Glade, except for vascular plants, including photosynthetic blue-green algae, and other microorganismic `plants', green algae, charophytes, bryophytes, including mosses, liverworts and hornworts. For horse-tails, lycopods, ferns and flowering plants see Chapter 3 on vascular plants. Organisms included as non-flowering plants do not constitute a monophyletic group but refer instead to almost any non-vascular autotrophic organisms, saprophytes and parasites maintained within a herbarium. This chapter describes a selection of techniques used in herbaria. These plants are stored in myriad ways including pressed dried specimens on flat sheets of paper, air-dried specimens in boxes or envelopes, freeze-dried specimens in air-tight containers, fixed cells on slides and as three-dimensional, liquid preserved specimens.

There is a burgeoning interest in these organisms and an increasing need to maintain and conserve voucher specimens and authentic material identified by specialists. Nonflowering plants are increasing in importance, with groups such as lichens and bryophytes being used as pollution indicators, many water-borne taxa being seen as important indicators of water quality and, conversely. various algae being toxic to fish and other wildlife. Many species have commercial significance, such as seaweeds utilized as fertilizers, alginates for chemical extracts and others used as sources of food and medicine.

Care and conservation of specimens depends on many different aspects including collecting equipment, restoration, mounting, fixing, storing, documentation and different stages of the life cycle (e.g. spores, gametophytes, sporophytes). Each group of organisms require different methods of treatment from the moment the organisms are collected to the point when they are laid out in the herbarium or stored in the slide collection. The chapter is organized so as to describe the individual groups and the methods applied to them.

Fungi

Fungi are a diverse group of organisms which are difficult to define (Ainsworth *et al.*, 1973; Alexopoulos *et al.*, 1996). Mycologists have variously described fungi as eukaryotic, spore-producing, achlorophyllous organisms with absorptive nutrition. They are usually filamentous, branched somatic structures with hyphae typically surrounded by cell walls comprised of cellulose or chitin (Alexopoulos *et al.*, 1996). Hawksworth (1991) estimates that there are 69,000 species of fungi world-wide.

Collection and preservation of fungi require specialist skills and are best undertaken by trained mycologists for successful results

(Hawksworth, 1974). Fungi are variously classified but for the purposes of this book can be considered as four major groups: Chytridiomycota (water moulds, Allomyces, etc.), Zvgomycota (bread moulds, *Rhizopus, Mucor* etc.), Ascomycota (sac fungi, yeast, *Penicillin in* etc.) and Basidiomycota (mushrooms, rusts, smuts etc.). Myxomycetes (slime moulds) are not true fungi. They lack hyphae and have an amoeboid stage as the principal part of the life cycle, but are generally associated with fungal herbaria. Mature fruiting bodies (sporangia) do take on the appearance of fungi and are filled with a powdery spore mass (Alexopoulos *et al.*, 1996).

Collecting

Fleshy fungi (fruiting bodies of ascomycetes and basidiomycetes) are best collected into flat-bottomed baskets (trugs) or open boxes so as to minimize damage during transport. Different species should be kept separate from one another in the field by wrapping them in thick, waxed paper or putting them inside open pots. Only good specimens should be incorporated into the herbarium with as little damage as possible and using as much of the structure as is available, particularly the base of the stipe and the volva in the case of fructification of agaric taxa.

Fungi deteriorate very rapidly and undergo size changes during the drying out process. Therefore it is essential to provide overall field descriptions, including details of colour, shapes of gills, teeth and pores. Special attention should be given to surface features, texture, odour and taste. However, it should be pointed out that some fungi are deadly if eaten and therefore tasting is not recommended unless identity can be guaranteed. Colour photographs and water-colour paintings are of great help for assisting later work in the herbarium. Drawings of cross-sections of fruiting caps for mushrooms and toadstools helps to provide details of gill attachment.

Spores

The necessity of obtaining spore deposits for larger fungi cannot be over-emphasized (Bridson and Forman, 1992). Spores are useful for diagnostics and descriptions of agarics, polypores and resupinate fungi. For best results the cap is placed on to a glass sheet or piece of white paper inside an air-tight vessel with a drop of water or a damp piece of cotton wool. The specimen is placed hymenial side down and left in a cool place from anything from two hours to overnight. Bridson and Forman (1992) note that polypores and resupinate fungi often have extremely short sporulation periods and indeed spores of some species have yet to be described. For these taxa, spore prints are extremely valuable. It is important to keep precise collecting numbers so as not to mix up samples. Once collected spore samples are air-dried and kept with the parent specimens.

Preservation and storage

Because fungi deteriorate very rapidly it is necessary that specimens are dried as quickly as possible to retain their shape and colour. Small specimens can be preserved intact but very large specimens need to be dissected into pieces to accelerate the drying process. Specimens are dried in a warm room in a warm stream of air around 40°C. Bridson and Forman note that higher temperatures cook the specimens whilst lower ones allow insect larvae to hatch out and damage them. For small specimens air-drying in the sun is sufficient for preservation, but field-drying can be undertaken using a combination of a fan and a portable field stove or with light bulbs in a drying box. Specimens to be dried can be arranged on to racks or suspended from lines. In the tropics and other areas of high humidity it is important to ensure that dried specimens continue to be kept dry after preparation. They can be stored in zip-top polythene bags containing silica gel or, ideally, stored in a glass desiccator over calcium chloride. Rusts and parasitic fungi living on plants can be collected and dried with the host substrate in plant presses, just as for vascular plants.

It was once believed that freeze-drying of fungi would be the panacea for the storage of larger fungi but there are no advantages for scientific investigation. It is now thought that freeze-drying might be useful for the presentation of exhibition material but such specimens are fragile and easily broken.

Lichens

A lichen is an association of a fungus with algae or cyanobacteria and the organisms are interwoven to form a single thallus. The `mycobiont' is the fungal portion of the thallus and the `photobiont' is the photosynthetic component. Nearly half of the world's described fungi are ascomycetes and about half of these live in association with algae as lichens. Of the ascomycetous lichen-forming fungi the majority are discomycetes. Of the discomycetes almost all are lecanoralean. Other groups of lichen-forming fungi are loculoascomycetes. A much smaller number of pyrenomycetes and basidiomycetes also form lichens (Alexopoulos *et al.*, 1996), and it is the fungal component that determines their systematics and nomenclature. There are estimated to be about 13,500 species (Hawksworth, 1974, 1991). Lichen thalli vary from minute crusts to large and leafy, shrub or beard-like growth forms attached, often intimately, to a variety of substrates ranging from rocks to hark, soil and leaves.

Collection

Many older specimens from early collections are inadequate for identification and study as they have deteriorated by being preserved too slowly. Lichens must be dried quickly to a low moisture content and then kept dry. Many specimens are spoiled by drying too slowly initially and then subsequently allowing the specimen to become damp again during storage. As a consequence many lichen specimens develop mould, making them difficult or even impossible to identify.

To prepare good specimens for study it is important to collect adequate material and to ensure that all relevant parts of the organism are collected. As lichens are fairly difficult to identify at the best of times, it is necessary to describe in detail the habitat and ecology, including the amount of shade, aspect, degree of moisture and the substratum. The primary difficulty in collecting lichens is detaching them from the substratum on which they grow. This may be rock, stone, bark, wood, twigs, soil, moss or leaves (especially in the tropics), as well as more unusual habitats such as bone, iron railings, concrete, glass and leather (James, 1958). It is important to collect some of the substrate to which the plant is attached but to minimize the bulk it is necessary to take a tool-kit, a geological hammer, a range of cold-steel chisels, a stout, sharp knife, secateurs, a trowel, a hand lens and soft tissue-paper for wrapping the specimens in the field.

Lichens can be grouped mainly by habit into crustose, foliose and fruticose forms, although these distinctions flow imperceptibly into one another and should in no way be interpreted as representing natural groups.

Crustose lichens

In crustose lichens the thallus spreads over the substratum forming a distinctive coating or crust which is invariably intimately attached and often immersed wholly into the substrate (James, 1958). Specimens growing on hark should be cut away with a sharp knife, although hard bark or tree stumps might require a hammer and chisel to effectively collect the material. Crustose lichens growing on soil or other soft substrates such as moss are very fragile and it is necessary to slide a knife or scraper some way below the substrate surface, before carefully lifting out the specimen. The whole sample should be wrapped in acid-free tissue-paper and stored in a cardboard box.

Foliose lichens

Foliose lichens are usually larger than crustose species with dorsiventrally flattened, spreading, leaf-like thalli, invariably attached to the substrate by thin hair-like attachments, or rhizinae. There is usually marked dimorphism in lichens, with the lower surface a different colour from the upper. Fruticose lichens are collected in much the same way as crustose lichens as they are very difficult to wholly extract from the substrate.

Fruticose lichens

Fruticose lichens consist of a thallus, radial in structure, which arises from a small holdfast or basal disc attached to the substrate. The thallus is usually spreading or pendulous and often richly branched. As the holdfast is often important in identification and classification of lichens, it should ideally be collected with the rest of the thallus. As with crustose and foliose

lichens, the entire plants can be collected with a small portion of the substrate.

Preservation and storage

All gatherings of lichens should be wrapped in tissue or soft paper and placed into tough paper packets or cardboard boxes for protection when travelling. Specimens should be dried as quickly as possible by opening the packets and placing them in a warm, dry place to avoid them becoming mouldy. Ideally, the dried specimens are stored in packets or boxes to preserve the habit as much as possible, but large foliose specimens can be pressed lightly and mounted on herbarium sheets as for vascular plants. Crustose species can be glued to cards, wrapped in tissue and then stored in paper packets or boxes (Fig. 4.1). Before mounting and placing in herbarium packets, any attached substrate should be trimmed down as much as possible, rock broken in a rock breaker if necessary and bark trimmed. Lichens on soil or other unstable substrates can either be mounted loosely in clear packets or the substrate bound together by painting with polyvinyl acetate adhesive which is allowed to dry and then glued to card (traditionally an artists material, Primal, has been used for this purpose). The mounting process is then the same as for rock and bark. Lichens attached to substrates should be fixed with polyvinyl acetate adhesive to a suitably sized piece of card to fit the standard sized capsules used. It is usual to write the collector's name and number or some unique identifier on the card to ensure that the lichen can be reunited with its label should this become separated from the specimen. The specimen and card should be placed in an archival quality paper packet.

There are two schools of thought on how leafy and shrubby lichens are best mounted. Either the specimen is mounted on a card with adhesive, similar to specimens on rock, or the specimen is placed loose in a transparent packet. Attaching material to card with adhesive obscures important diagnostic characteristic present on both surfaces. On the other hand, attachment ensures that specimen and label are easily reunited after removal from the packet for study. A compromise is to place loose material in a transparent packet (cellophane packets have been used traditionally but may be unacceptably acidic) which is then clipped to a card and annotated with a unique identifier. This allows superficial examination of material without detaching it from its identifying information. The specimen can be removed from its packet as necessary when characters need to be dissected for further study.

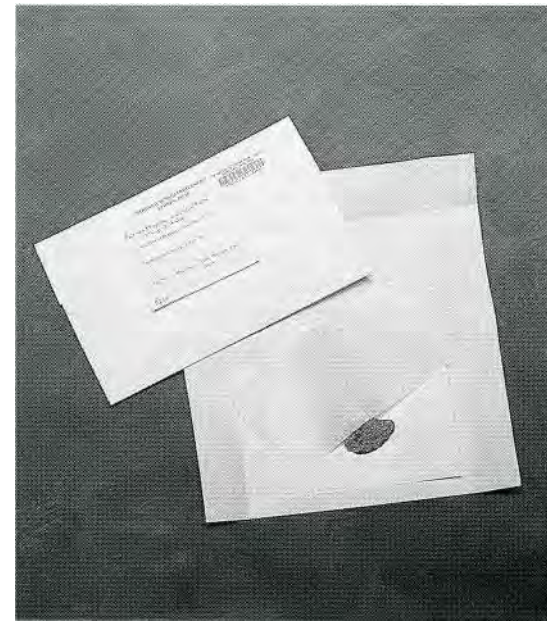

Figure 4.1 A crustose lichen specimen still attached to its rock substrate which is glued on to a card. Note the protective tissue-paper and collection detail written on the card. An unopened packet lies on top to indicate how the label is attached to the outside of a packet. Note the bar code label.

Rocks and bark that are too large or too heavy for a paper packet should be placed in appropriately sized acid-free boxes. Fragile and bound soil specimens can be given extra protection from crushing by attaching them to the base of a shallow box. This is then attached with an adhesive to a card in a similar fashion as for other specimens. This method gives additional protection while allowing easy examination of the material in the packet.

It is advisable to place a suitably sized piece of acid-free tissue over the specimens before placing them in packets to reduce abrasion

(see Fig. 4.1). Small stones and rocks should also be wrapped in this if stored loosely. Labels should be attached to the outside of the lichen packet. The irregular shape of packets makes it difficult to fix the whole label down and it is usually only attached to the narrow top fold of the packet. Determination slips and results of chemical analysis can be attached to the inside of the packet.

Storage

Lichen (and bryophyte) packets can be stored vertically in suitably sized index-card drawers which makes them easy to sort. In situations where only standard herbarium cabinets are available, loose packets can be stored in adapted herbarium folders. These have four flaps to prevent packets falling out of the folder (Fig. 4.2).

There are different opinions on whether lichen (and bryophyte) packets should be attached to standard herbarium sheets. This can be very wasteful on space and, unless the position of the bulky packets on sheets is varied carefully, then space is not used efficiently. Loose packet systems are more economical on space and make it easier to rearrange material, but many institutions still mount type specimens to sheets for additional security as sheets are less easily mislaid than loose packets.

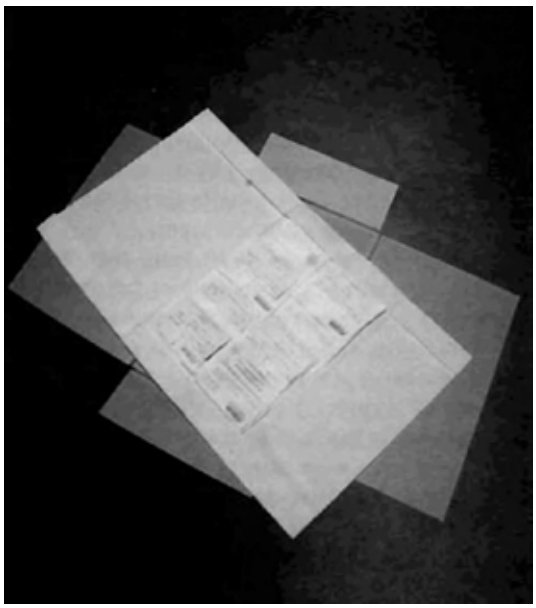

Figure 4.2 Herbarium folder opened to show a species folder holding loose packets of bryophytes. Underneath is a 'genus folder'.

`Algae'

The term 'Algae' commonly refers to a highly diverse group of 'plants' found in marine, freshwater and terrestrial habitats. They vary in form from large, attached marine plants (seaweeds) to microscopic free-floating 'phyto-plankton'. Algae also include stoneworts which are large, attached freshwater plants, slimy green epiphytic coatings on rocks and trees and symbiotic endophytes living in the tissues of other organisms.

Seaweeds

Seaweeds are stored best as dry specimens for long-term conservation and scientific study. However, some taxonomic features are studied better from fluid-preserved specimens, although these do require greater care and attention than dried specimens.

Three major groups are recognized: the Ulvophyceae (green algae), Fucophyceae (brown algae) and Rhodophyceae (red algae). They vary in form and size but the commonly collected seaweeds are pressed on herbarium sheets by the conventional method described in the previous chapter. The overall process of collection and preparation, however, is very different and is described below. Larger filamentous blue-green algae and members of less well known algal groups such as *Vaucheria* can be prepared in a similar manner (Dawson, 1956, 1966).

Collection and bulk storage

Dawson (1956) recommends that specimens should be preserved as quickly as possible to prevent unnecessary deterioration. This is best achieved by collecting the specimens in plastic bags and then immersing them in a solution of sea water with commercial 40% formaldehyde to obtain approximately a 3—4% solution, but care must be exercised when handling formalin (see Chapter 9 on policies and procedures). However, it must be pointed out that

formalin preservation is unsuitable for specimens destined for DNA analysis. All the bags can then be packed into bulk containers which also contain the preservation fluid. Dawson comments further to say that specimens can be stored temporarily in this fluid for months without deterioration. The specimens can then be removed and mounted using the flotation method after washing in sea water. Tap water can be used but has a tendency to cause cell damage by causing mucilage to swell. For indefinite storage of three-dimensional collections specimens should be transferred into alcohol.

Flotation method for mounting macroalgae (Fig. 4.3)

1. Fill a shallow plastic dish or tray (such as a photographic developing tray) with sea water and immerse the specimen.
2. Cut mounting paper to size and annotate one corner (use pencil or waterproof Indian ink) with enough data to distinguish the specimen. A reference number or date and locality should be sufficient. A heavy duty cartridge paper or similar weight of paper which is strong enough to maintain its rigidity when wet is used.

Figure 4.3 Flotation method for mounting seaweeds. Note the arrangement of the specimen using a paintbrush while it is supported with a metal gauze sheet.

3. Drain away most of the water but leave enough to allow some rigid paper to be slid beneath the specimen.
4. Lower the cartridge paper into the water and float the specimen on to the paper. A wire-mesh sheet as big as the largest specimens can be used to give extra support to the paper.
5. Tease the fronds apart with forceps, a mounted needle or small, soft paintbrush depending on the bulk and shape of the specimen.
6. Gently lift one end of the wire-mesh support or paper, bringing specimen and paper out of the water allowing the water to drain off.
7. Place the drained paper with its specimen on to absorbent drying paper.
8. Most seaweeds will adhere to the mounting paper by means of their own natural mucilage. However, any unattached parts of the specimen should be temporarily fastened with the appropriate adhesive or gummed fabric tape.
9. After excess water has drained away, place the mounted specimen on several sheets of drying paper (or newspaper) in a plant press. Cover the specimen with a fine gauze such as muslin or nylon fabric to prevent the specimen from sticking to the upper sheet of drying paper. Squares cut from nylon stockings are suitable.
10. Several specimens can be placed side by side on the drying paper, provided that they do not overlap. Lay several sheets of drying paper over one layer of specimens before starting another layer.
11. Repeat procedures 1—10 until the press is full, inserting a corrugation between the drying paper at regular intervals to ensure ventilation. The average full press should have about six ventilation levels.
12. Fasten straps around the press frames reasonably but not excessively tightly. Ideal drying is in a stream of warm air, if this is available. A box heated by a couple of light bulbs will provide enough heat. Excess heat may affect the material's usefulness for chemical analysis and prevent cells regaining their shape when rehydrated.
13. Replace all drying paper after one to two days and again after five days (the first

change may need to be earlier if drying conditions are poor). With any change. remove completely dry specimens and refasten straps. If a second press is available, it is useful to have the almost dry material in a different press. Never remove nylon fabric until the specimen is entirely dry and removed from the press.

14. Dried specimens are ready for mounting on to herbarium sheets (see Chapter 3 on Vascular plants). Extra gluing and strapping of branches may be necessary. Specimens which remain completely detached should be put in paper capsules.

Moore (1992) notes that it is important to dry the specimens quickly, but without excessive heat or they may become brittle. She also suggests that it is helpful to have some branches clumped together which can be easily removed later for microscopic examination. Dawson (1956) gives a detailed methodology on how to carefully dry the specimens by frequent replacement of wet driers with warm dry ones. This ensures that delicate specimens do not become wrinkled from shrinkage. Paper covers placed around the specimen will prevent curling of the sheet. The drying process will be more quickly and satisfactorily accomplished if the specimens are killed in formalin first.

Fluid preservation of 'Algae'

The general principles of fluid preservation and the associated health and safety features are described in Chapter 5 on fluid preservation and Chapter 9 on policies and procedures, respectively, but specific points relating to the fluid preservation of algae are mentioned here. Moore (1992) notes that any material stored in fluid for longer than 24 hours should be fixed and preserved by 4% neutralization formalin, using borax or calcium carbonate (one part 40(Ii formaldehyde diluted with nine parts water or sea water for seaweeds). Neutralization is necessary to prevent the formation of formic acid which, if allowed to build up in the solution, will eventually damage the specimens. Moore recommends that a little glycerol may be added to prevent specimens drying out.

Dried, flat specimens can be stored in herbarium cabinets in the conventional manner (see Chapter 3 on vascular plants). Fluid-preserved specimens need to be stored separately and kept in conventional sealed glass containers although a variety of plastic containers, can be used with rubber-sealed screw-top lids (see Chapter 5 on fluid preservation).

Articulated and crustose Corallinaceae (Coralline red algae) and other fragile specimens

As members of this group contain high concentrations of calcium carbonate they are exceptionally fragile when removed from their natural habitat. Specimens are damaged by conventional pressing techniques and, unlike many robust macrophytes, it is recommended that they are air-dried. Bulky crustose species which adhere to rocks or hard substrates should also be air-dried. Moore (1992) recommends that the surfaces should be first gently brushed to remove debris and small animals. Dawson (1956) notes that articulated specimens should be treated by soaking for several clays or weeks in a solution of about 40% glycerine in 3% formalin before being dried. When placed into boxes the specimens should be well packed with soft tissue-paper so as to prevent disarticulation by shaking.

Specimens should be stored in boxes or packets to give protection and not layered as in conventional herbarium storage where there is likelihood of crushing. However, small, flat boxes and well packed packets can be mounted on to herbarium sheets, making it easier to incorporate the material into the general collections.

Charophytes (Characeae; stoneworts)

These are treated with the `flotation' method (see above) but tap water should be used instead of sea water. However, it is important to note that, as several taxa are lime-encrusted, only light pressure should be applied in the press. Moreover, charophytes do not produce mucilages like marine taxa and thus do not naturally adhere to the mounting paper. They need to be strapped or glued to the sheet as with vascular plants. As charophytes are delicate and brittle, small specimens are placed into paper packets and larger specimens are covered with Mylar or Melinex

sheets as for delicate fragile vascular plants. Moore (1992) also recommends that, for research work, it is better to store specimens in formalin preservative fluid rather than keeping specimens dry to facilitate easier study.

Because dried specimens can be more difficult to identify than those preserved in liquid, Moore (pers. commun.) suggests that charophytes can be preserved in 4% formalin or 70% alcohol (three parts of 95% ethyl alcohol (IMS) to one part water). However, it is recommended never to use weak acids, such as acetic acid, in any preserving fluid as these will remove the lime encrustations (useful diagnostic features) from the specimens.

Bacillariophyta (diatoms)

Diatoms are single-celled organisms encased with silica and their collection, preservation and subsequent conservation presents few problems. Diatoms occur in two principal habitats: moist or submerged surfaces (benthic) and open water (planktonic) in freshwater, brackish and marine waters. The most easily sampled microhabitat surfaces are the fronds of macroalgae or submerged aquatic angiosperms. Shells, carapaces and skins of marine and freshwater animals support diatom communities and recently they have been found on birds' feet and on the feathers of seabirds (Round *et al.,* 1990). The sediments of streams, lakes, sandy beaches and salt marshes all have their own floras hut the collecting techniques are similar for most habitats.

Collection of benthic material

Diatoms live on and within the surface layers of most sediments and are collected easily by removing the top 5—10 mm of mud or sand (Round *et al..,* 1990). Deeply submerged sediments can be sampled by drawing a glass or plastic tube over the surface or even deeper sediments using a coring device.

Collection of terrestrial material

Similar techniques as those used for sediments have been applied to soil and sand samples for terrestrial species. However, these organisms, unlike diatoms in aquatic habitats, tend not to move towards the light. Methods such as sonification are necessary to remove the cells from particulate matter. Different species react differently to different treatments (some motile species are differentially sensitive to illumination, whilst others tend to stick and are difficult to dislodge). Consequently, only a general sample can be obtained by these methods (Round *et al.,* 1990).

Collection of phytoplankton

Planktonic diatoms are collected using bottles or a variety of different samplers. Phytoplankton nets are favoured in most lake or ocean habitats so as to concentrate samples but even within the most nutrient-rich lakes, concentrations can be as little as 10-i per ml. Fine plankton nets drawn slowly through the water can be useful for taxonomic sampling but bottle samples and sedimentation methods are required to obtain quantitative samples.

Preservation

Concentrated samples of phytoplankton deteriorate very quickly and have to be processed immediately for information on the softer parts of the cell such as protoplast and plastids (Round *at at.,* 1990). However, most taxonomy of diatoms is based on the silicon exoskeleton and therefore the cytoplasm has to be removed. This is undertaken by heating the sample in concentrated nitric acid and subsequent washing with distilled water. The early preservation technique for diatoms was to dry down' selected specimens from bulk samples on to pieces of mica or glass. The smear was allowed to dry naturally and then the pieces of mica or glass were stored in small packets fastened on to herbarium sheets which were then stored in the traditional manner. These early specimens are still in good condition and still used for scientific study. Today, bulk storage of cleared specimens is in *5%* ethanol, which gives sufficient protection from bacteria and other micro-organisms, and over time there appears to be little problem with their preservation.

Although the collection and storage of diatoms is relatively free of problems, the preparation of specimens for their study is critical. Diatoms are either studied as fresh material or processed for light and electron microscopy as follows:

1. **Fresh material.** Fresh material is essential to study plastids, morphology, colony form, overall cell symmetry, mucilage sheaths, chitin and attachment structures. Fresh material can be preserved using Lugol's iodine solution (1 g iodine crystals and 2 g potassium iodide in 300 ml water — use three drops of this solution in a 100 ml sample) to preserve the plastids and colony structures.
2. **Light and electron microscopy.** Most taxonomy of diatoms is based on the siliceous exoskeleton. Certain structures are extremely delicate and each group requires extensive knowledge about their structure to make good slide preparations. For light microscopy, either the alcohol sample is strewn on to a glass slide or individual, selected diatoms are placed on to the slide. For electron microscopy, similar strewn or individually selected specimens are placed on to a stub, or a coverslip placed on to the stub. The specimen is mounted in the synthetic medium Naphrax, which has been used for many years with good results because of its high refractive index. Canada balsam was used in the past but the refractive index of Naphrax is considered more suitable.

Bryophytes

The bryophytes include Bryopsida (mosses), Marchantiomorpha (liverworts), and Anthoceromorpha (hornworts). They have a unique life cycle with two distinct stages: a long-lived green phase, involving the gametophyte which reproduces sexually, and a short-lived dependant phase, involving the sporophyte which is not green at maturity and reproduces by means of spores (O'Shea, 1989).

Collection

Bryophytes are among the easiest plants to collect and preserve. To ensure that the entire specimen is intact it is recommended that the substrate is collected with the specimen — soil, twigs, leaf and rocks. With terricolous mosses it is important to gather some of the soil/substrate to ensure that rhizoidal gemmae or tubers are also collected. Specimens are collected directly into paper field packets. A quick and inexpensive packet to use in the field can be made before one collects the specimens (or ordinary paper envelopes serve the same purpose when available):

1. Use recycled A4 sheets of paper.
2. Fold longitudinally along the middle of the paper.
3. Place the specimen in the middle of the inside fold. If there is a considerable amount of material, ensure that it is spread evenly within the packet to assist with the drying process.
4. Fold over a 2.5 cm lip opposite the longitudinal fold.
5. Turn the paper over with the 2.5 cm folded downwards and facing towards you.
6. At each end fold the margins inwards to form a triangle.
7. Fold the pointed end of the triangle inwards to half-way and tuck the margin into the 2.5 cm lip.

Bryophytes should not be collected in plastic bags as specimens will quickly develop mould and they can also develop abnormal, etiolated shoots. It is important, whenever possible, to collect the sporophytes (see below), as they have essential characters used for identification. If leaves support the liverworts they should be dried under light pressure in a plant press (see Chapter 3 on vascular plants).

Specimens should be left to air-dry naturally in a well ventilated place and packets should be spread out and partly left open to assist with the drying process. Artificial heat is best avoided. However, should this be necessary use a fairly low temperature as a high temperature can distort the cellular structure upon rehydration for examination (Bridson and Forman, 1992). Moreover, certain liverworts contain oil bodies which are essential for identification and these are destroyed or distorted by artificial heat.

Storage

After drying the specimens should be transferred to packets made of appropriate archival paper (see Chapter 3 on vascular plants) using the appropriate standard size adopted for the

herbarium. As with lichens, packets are either stored vertically in drawers or attached to herbarium sheets and stored horizontally in cupboards (see Fig. 4.2). If bryophytes are kept dry they are seldom subject to infestation by insects or attacked by fungi. This is not so easy, however, in the tropics where dried specimens need to be kept in sealed plastic bags in the herbarium to ensure freedom from damage.

Specimens are examined by rehydration and then dissected for observation under the microscope. More detailed examination will require a compound microscope and material should be slide mounted. Any material removed but not examined should be replaced in the packet with the specimens or in a small transparent envelope attached to the card with a clip.

Summary

The key to good preservation of non-vascular plants is heavily dependent upon the collection and treatment of the specimens in the field. It is clear that different collecting techniques apply to each major group of organisms and in all groups timely treatment is essential for the production of high quality specimens because of the different deterioration rates and chemical compositions of the plants. Within certvin groups, such as seaweeds, even different species of the same genus may require slightly different handling and/or preservative techniques. The accompanying reference and extra reading lists should aid in determining the extra procedures which may be required for some species.

Once collected and dried, wherever possible herbarium specimens are stored in a dry state on paper sheets in packets or boxes within herbarium cabinets (ideal for most seaweeds, mosses and lichens). On the other hand, liquid-preserved collections of whole specimens or portions of specimens are permanently stored in individual, sequentially numbered glass vials. For some purposes permanently mounted, stained sections (crosssections, longitudinal sections, serial sections etc. of reproductive structures and/or vegetative anatomical preparations) are maintained, and either partial or whole specimens (e.g. an entire small epiphytic alga) are mounted on microscope slides. Diatom collections are primarily mounted on microscope slides. 'Bulky material' (e.g. very large specimens such as calcified, articulated and crustose lichens, coralline algae, epilithic algae on rocks, brown kelps etc.) are stored in boxes in cupboards or kept in sequentially numbered trays.

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Useful web sites

http://www.helsinki.fi/kmus/botmenu.html (Plants on the net)

http: /mycor.nancy.inra.fr/Documents/Bookmarks/MycoResources.html (fungi resources)

http://www.nmnh.si.edu/botany/projects/lichens/ (Lichens)

http://www.namh.si.eclu/botany/projects/algae/AlgCoPr.html (Algal preservation)