

## METHODOLOGY

Descriptions of various techniques and refinements are scattered through a large number of books and articles on lichens or other organisms, and most workers have their own special methods acquired through much experience and trial and error. It is desirable to make a larger portion of this extremely diffused information available in a single place, with comparisons of alternative variations, and to contribute towards eventual standardization of some of the techniques.

### EXAMINATION OF LICHENS THROUGH A DISSECTING SCOPE

Wetting the surface of the thallus will often make spermagonia easier to locate. It will also help to see the color and surface structure under pruina.

To examine the undersides of lobes or squamules and determine how they are attached to the rock, carefully detach and lift the structures using a probe needle.

### MEASUREMENTS

Measurements given in mm are made under a binocular scope on dry material. Those given in  $\mu\text{m}$  are made under a compound scope. The latter are usually made in water unless stated otherwise. Timdal (1984) made all microscopic measurements on material mounted in LCB.

Timdal (1984) generally based descriptions of the dimensions of spores or conidia on fifty or more measurements (per species). It is desirable (though not always feasible) to measure at least 10 spores (or spermatia, etc.) on each specimen. The largest one and the smallest one should be sought; otherwise the search can be either systematic or haphazard (by moving from each measured one to the closest one to it?).

Measurements can be recorded or expressed in various ways. Timdal (1984) recorded the two extreme values to the nearest 0.5  $\mu\text{m}$  (0.5 mm could be used for features measured under the dissecting scope), and calculated the arithmetic mean to the nearest 0.1  $\mu\text{m}$  (or less precisely for structures only with mean dimensions under 1.5  $\mu\text{m}$ )

### STUDYING SPORES

Do not assume that scattered spores belong to the species you are studying, unless similar ones occur in the asci. Also watch out for parasymbionts.

It is important to distinguish between mature and immature spores. In general, immature spores have granules and oil drops; mature ones often do not (Nearing, the Lichen Book). When measuring thinwalled spores, it is important not to exert pressure on the coverslip, which can increase the spore length up to one third more (Swinscow, freshwater Verrucaria).

### **MISCELLANEOUS METHODS**

Eucortex and Pseudocortex can often be distinguished in rather thick sections if they are in KOH.

To see paraphyses or other tissues clearly, let the sections absorb cotton blue for several hours, then rinse with water (Hertel, pers. comm.).

#### **SemiPermanent Slides**

Semipermanent slides, which can be rewet whenever needed, have the advantage of being easy and inexpensive to prepare, and they do not distort hyphae and tissues the way that the permanent mounting media do (Poelt, pers. comm.).

Semipermanent slides can be prepared by simply glueing one or two edges of the coverslip to the slide, and then when a dry slide needs to be reexamined, simply putting water under the coverslip again. Poelt (pers. comm.) uses "UHU Alleskleber" for this purpose; various kinds of model cement or clear nail polish also work well.

#### **Fixing Agents**

According to McWhorter (1921), fungal elements fix well in chromacetic acid, while algal elements fix well in hot bichloride of mercury.

A solution of 7% formalin or F.A.A. will preserve lichens (Sass, 1951; recipe on card).

#### **Epihymenium Chemistry**

For determination of the actual chemical contents of the

epihymenium, Leuckert (pers. comm.) recommends moistening the apothecia, blotting with a paper towel, scraping with a fine scalpel (carefully avoiding getting the margin) and doing HPLC or (if large numbers of apothecia are available) TLC, on the scrapings.

### **Reagents**

Ferrous (ic?) Chloride (FeCl<sub>3</sub>)

### **TLC**

Anisaldehyde in ethanol, mixed with sulfuric acid detects usnic acids, which turn deep violet (Leuckert, pers. comm.). When gyrophoric acid is present, at least traces of lecanoric acid are usually also present (Leuckert, pers. comm.).