14. Thallophyta and Bryophyta

This chapter brings together plants and plant organs that require essentially similar treatment. For instance, a segment of mushroom cap, a liverwort thallus and a moss gametophyte may well be prepared for sectioning by identical methods. Filamentous algae, free-floating filamentous fungi and moss prothalli also present similar problems. Fungi that live within tissues of higher plants will be discussed in terms of the responses of the fungus and the host to processing.

Algae

The most satisfactory method of studying algae is by the use of fresh living material in conjunction with well-preserved bulk material. Except for some critical cytological features, most of the life history can be worked out without stained preparations. Stages that have short duration must be preserved when available and subsequently studied from temporary mounts. However, permanent stained slides are indispensable for research and have a legitimate place in teaching to supplement bulk material. The methods of processing the most commonly used algae are outlined briefly in this chapter, with frequent references to the whole-mount methods in Chap. 10.

GREEN ALGAE

These plants exhibit a wide range of size, complexity of organization, and habitat. The following simple precautions should be observed in collecting, transporting, and storing plants:

1. Keep the plants in their natural substratum (water, soil, bark of tree) until the moment of killing.
2. Avoid subjecting the plants to excessive heat or to desiccation during storage or transportation.
3. Unless culture methods have been carefully worked out, kill the plants as soon as possible after collecting.
4. Subdivide or spread out large masses of material to promote rapid killing and hardening (fixing).
5. Keep intact the organization of the filament, or other type of colony.

The preservation of green algae for bulk material and for permanent stained slides is treated at some length in the chapter on whole-mount methods. The advanced worker will find further details in Johansen's (1940) comprehensive treatment of culture methods and processing of this group.

BLUE-GREEN ALGAE

These algae have such simple cellular and colony organization and are so easy to study in temporary whole mounts that the use of prepared slides is less justifiable than with any other group of thallophytes. Fresh cultures are easily found in a wide range of habitats; in stagnant pools, tanks, barrels, and crocks, on potted plants with stale soil, on damp, poorly drained soil, and innumerable other places. Some forms like *Oscillatoria, Rivularia, Nostoc,* and *Gloeocapsa* may be found in masses that are practically pure cultures. Collections of such materials are easy to preserve. Because of the dense undifferentiated character of the protoplast the crudest methods of preservation, such as 5% formalin, may be used. If the reagents are available, one of the fluids containing glycerin should be used. Temporary or permanent whole mounts can be made as described in Chap. 10.

THE MARINE BROWN AND RED ALGAE

The algae in these groups are available in fresh condition for a very limited number of schools. Large quantities of these plants are used by schools that are totally dependent on outside sources for their materials. Therefore, a detailed discussion of methods of collecting and preserving these plants would have but limited usefulness. For occasional casual collecting on one's travels, the simplest preservative is 5 to 10% formalin in sea water. Further refinements are the addition of 5 to 10% glycerin and ½ teaspoonful of borax to 1 liter of fluid.

For more critical preservation, nothing has been found to excel chrome-acetic, with or without addition of osmic acid. One of the best formulas is the Chamberlain formula (Table 3.1) made up with sea water. Subsequent processing of filamentous forms for whole mounts is outlined in Chap. 10.

If materials are purchased from collectors, the purchaser should indicate whether the material is to be used for temporary slides or to be processed for permanent preparations. Several reliable collectors will furnish material in specified stages of the life history, carefully fixed in a suitable fluid determined by the collector or specified by

the purchaser. Such materials will yield excellent preparations by the methods recommended in Chap. 10.

Few of the algae are microtomed for making slides. Some selected items that are customarily microtomed are discussed briefly.

CHARA AND NITELLA

The growing points of *Chara* and the sex organs of mature plants must be sectioned to show cellular organization and nuclear structures. Kill in medium I chrome-acetic or Craf II. These fluids contain enough acid to remove much of the troublesome incrustation. The condition of the material after 1 week in the fluid can be easily ascertained by examining a whole mount. If abundant material is available, several variations of these formulas should be tried, and the batch having the best fixation used for embedding. Older oögonia and zygotes are not readily penetrated by the above fluids; *FAA* should be used.

These plants become very brittle in xylene, but they section satisfactorily after the butyl alcohol process. Examine small samples during the process, thereby saving further work if a batch has undergone plasmolysis. Infiltration should be gradual, with the time interval in the oven reduced to 2 days or less. The staining of different batches is highly variable. Try iron hematoxylin and safranin-fast green.

Fucus and similar bulky forms are usually sectioned to show gametangia. Kill in medium II or strong chrome-acetic made up with sea water. Dissect out some of the gametangia to ascertain which fluid preserves them best at the given stage. Process in *TBA* or dioxan. Sections are difficult to affix to the slide. It may be necessary to use an alcoholic bulk stain with some batches. Brilliant staining of immature sperms in the antheridia of *Fucus* has been obtained with iron hematoxylin; sharp staining of nuclei during cleavage in the oögonium is very difficult.

The more massive Rhodophyceae that cannot be satisfactorily made into whole mounts may be sectioned by the method given for *Fucus*. Since the great majority of readers do not have access to fresh plants, the purchase of carefully preserved material from biological supply firms is recommended.

Fungi

The processing of fungi involves many problems that are common to other categories of previously described subject matter. For example, in processing a fungus parasitic on a leaf, the tissues of the

host must be preserved unchanged; the fungus, with an entirely different chemical and physical make-up, perhaps an alga-like siphonaceous plant body, also must be preserved intact. Another task may involve cutting a tough piece of wood bearing a delicate plasmodium, presenting a conflict between the need for drastic methods and refined methods. In order to minimize duplication of procedures in this chapter, it is proposed to use extensive cross references to appropriate sections of the text and to give detailed directions for procedures that are not adequately covered elsewhere in the manual.

SCHIZOMYCETES

The preparation of slides from cultures of bacteria is described in detail in textbooks of bacteriology. The bacteria are discussed in this manual only in conjunction with a host plant. A few typical examples of plant tissues and their bacterial invaders will illustrate the general methods of processing. *Bacterium stewartii* invades the vascular system of corn, forming a shiny yellow mass in the xylem elements. Because of the virulence and ease of dissemination of the disease, it is unwise, in regions where the disease is not normally

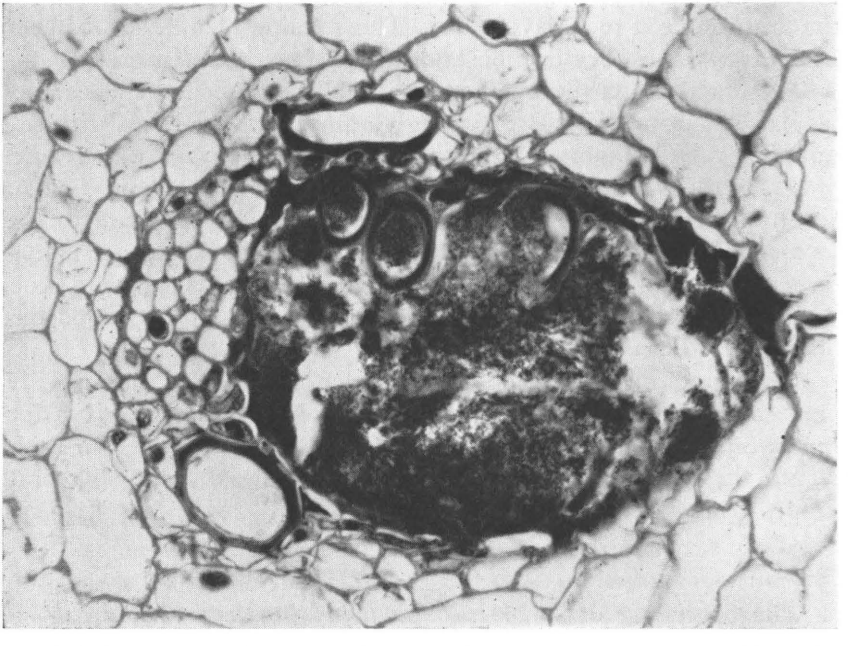

FIG. 14.1—Vascular bundle of *Zea,* with bacterial mass (*Bacterium stewartii*) in the protoxylem.

present, to infect plants to obtain diseased tissues. Preserved tissues can be purchased from the supply houses. The most satisfactory killing fluids are *FAA* and *FAA*-bichloride of mercury (Chap. 3). Chromic acid seems to become fixed in the gelatinous bacterial slime and interferes with clear staining. Process the corn stem or leaf as described in the section dealing with vegetative organs of seed plants (Chap. 13). Iron hematoxylin gives a brilliant differentiation of the bacteria. The xylem may be lightly stained with safranin or gentian violet, but the slime between the bacteria must be thoroughly destained (Fig. 14.1).

The above methods are satisfactory for the preparations of cucumber stems infected with the wilt organism, *Erwinia tracheiphila,* and succulent leaves and twigs of apple or pear infected with *E. amylovora,* the fire blight organism.

MYXOMYCETES

The slime molds are customarily studied from living cultures of the slimy plasmodium and from dried specimens of the fructification. These spore cases are exceedingly delicate and beautiful objects. Sporangia that are nearly mature can be mounted into permanent slides. Transfer directly into 95% alcohol for 10 min. Pass through three grades of anhydrous alcohol-dioxan at 10-min. intervals, then into pure dioxan, and mount in thin dioxan-balsam. A similar butyl alcohol series may also be used. The nuclei are exceedingly small, and microtoming and staining are tasks for the experienced cytologist.

PHYCOMYCETES

The saprophytic members of this group should be studied in culture whenever possible, and the use of prepared slides should be discouraged. Stages that are of short duration or difficult to obtain can be preserved, for either bulk material or permanent slides. The representatives of this group presented below are in common use for teaching, and the process for each plant has been thoroughly tested and may be regarded as type processes applicable for similar subjects. Strict taxonomic sequence is not maintained in the following discussion; organisms that are processed by similar techniques may be discussed simultaneously.

Zygomycetes.—The order Mucorales contains the best-known members of this group. Species of *Rhizopus* and *Mucor* are easily grown in culture and studied to best advantage from whole mounts. Developing and mature zygospores can be preserved by cutting out

selected pieces of the culture agar and killing in *FAA,* which also serves as a storage fluid. Such material can be used for mounts in water or lactophenol or for excellent stained permanent preparations of whole mounts (Chap. 10). Cytological preparations require such highly specialized and almost specific methods that the ambitious student should study the research publications of a given species for details of procedure.

Oömycetes.—Plasmodiophora brassicae is parasitic in the roots of cabbage and related plants. The plasmodium can be demonstrated in young roots that are just beginning to undergo distortion. Stages of cleavage and spore formation are obtained from increasingly gnarled and distorted roots. *FAA* gives good fixation, but Craf III is superior. A simple hemalum-safranin stain is adequate for most purposes, safranin-fast green is more contrasty, and iron hematoxylin gives the most brilliant differentiation of the parasite nuclei.

Synchytrium decipiens, parasitic on the hog peanut, and related parasites yield striking preparations, but poor fixation is frequent, and sectioning is unproductive, making the slides somewhat expensive. Craf II was found to give excellent fixation. Iron hematoxylin is by far the most satisfactory stain.

Saprolegnia and allied water molds are readily obtained and easily cultured, furnishing abundant vegetative and sexual material for study in the living condition. The best sources are dead fish and water insects, or steam sterilized house flies placed into a large crock of pond water. Whole mounts can be prepared by the general methods given for filamentous plants (Chap. 10). Ascertain the correct killing formula for the species being studied by testing small masses in a weak chrome-acetic or Craf I and manipulating the acetic (or propionic) acid content. Whole-mount and sectioning methods are used for cytological study, and the reader is referred to research publications for these highly specialized and difficult methods.

Pythium and *Phytophthora* are most effectively studied in culture, but preparations can be made by the methods suggested for the water molds.

Albugo (*Cystopus*), the "white rust," is an indispensable subject in teaching. Several species occur on common crops and weeds. For the conidial (zoosporangial) stage, select pustules that have just ruptured. Fully opened pustules will have most of the spores washed out during processing. The sexual stages arise after the conidial stage is on the decline, and the host tissues show evidence of hypertrophy. *A. candida* and *A. bliti* are fixed in perfect condition in Craf II.

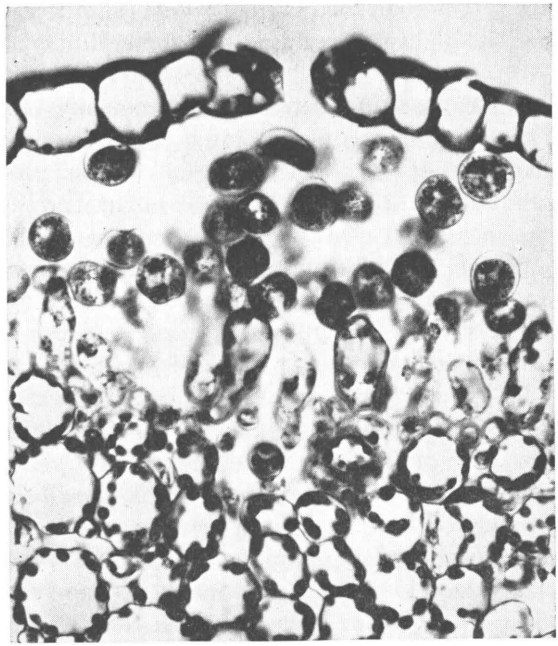

FIG. 14.2—Portion of pustule of *Cystopus candidus* on stem of *Capsella,* Craf 0.30–1.0–10.0, acetone-tertiary butyl alcohol.

Safranin-fast green and hemalum-safranin are both excellent combinations for elementary use, and iron hematoxylin is a good nuclear stain (Fig. 14.2). Mature oöspores are abundant in the older, hypertrophied stems and fruits of the host, therefore, a more vigorous killing fluid is needed; *FAA* and standard Nawaschin are both satisfactory. As might be expected, the host cells are in a distorted condition, a fact not recognized by inexperienced teachers.

Peronospora parasitica, a common parasite on crucifers, yields excellent preparations by the methods described for *Albugo.* The best compromise fluid for preserving both the host cells and the fungus is Craf II. Safranin-fast green differentiates the nuclei of the fungus, but not so sharply as iron hematoxylin.

ASCOMYCETES

The mold members of this group, such as *Aspergillus* and *Penicillium,* are so easy to culture and study from wet mounts that permanent slides are seldom necessary. Permanent slides can be made by the common whole-mount methods. Such slides are useful for

quick reference rather than for detailed study. The production of the ascigerous stage is highly uncertain, and best studied from whole mounts.

The Erysiphales are of interest because many members are parasites of considerable economic importance. *Erysiphe graminis* occurs on many grasses, from which the conidial stage is easily obtained in abundance. The conidia are studied best by freshening detached leaves in a moist chamber and examining the surface under moderate magnification, with oblique surface illumination. Microtome sections are indispensable, however. Longitudinal sections of the leaf show the clusters of long, finger-like haustoria in the long epidermal cells. Select leaves that are young and soft, kill in Craf III, and process like any young grass leaf. Iron hematoxylin is the most desirable stain, although a *good* triple stain is indeed beautiful.

Other interesting or important species are *Erysiphe polygoni* on the common weed *Polygonum aviculare, E. humuli* on the rose, *Podosphaera oxycanthae* on cherry, *Microsphaera alni* on lilac, and *Uncinula salicis* on willow. For the best slides of haustoria, collect in the conidial stage. Development of the perithecia can be studied from successive collection up to the stage in which the perithecia begin to turn gray. Kill in Craf III if the host cells are to be preserved, or in Craf I for good preservation of the young perithecium. The latter fluid does not seem to serve so well for the host cells. Stain as recommended for *Erysiphe graminis.* Mature perithecia are very brittle and difficult to section. Furthermore, this stage is studied to best advantage from dissections and macerations of bulk material preserved in one of the fluids in Chap. 10. The sectioning of the decayed, brittle, overwintered host leaves is a thankless and pointless task except for research.

Members of the Pezizales are of considerable cytological interest as well as economic importance. *Sclerotinia fructigena* occurs on cherries and plums. The conidial stage need not be sectioned, and sections of the hard sclerotia are not particularly interesting. The delicate goblet-like apothecia yield excellent sections. Very early in the spring look for the apothecia arising from mummified fruits. Collect cups of various sizes and preserve each in a separate vial of Bouin's fluid or Craf I. Successive stages of ascospore formation will be obtained in this way. Longitudinal sections through the center of the cup show numerous perfectly aligned asci and ascospores. Use iron hematoxylin for nuclear details, but safranin-fast green shows both nuclei and trama very well.

Pyronema confluens is common on steam-sterilized soil in the greenhouse. Cytological preparations of the sex organs are a task for the skilled investigator, and the reader is referred to the research literature. The apothecia are processed like those of *Sclerotinia.*

Pseudopeziza medicaginis is parasitic in the leaves of alfalfa and other legumes. Collect material when the pustules are just opening and the apothecia are bursting through the epidermis. Excellent preservation is obtainable with *FAA,* and staining presents no difficulties. Even the simple hemalum stain differentiates the ascospores.

Sarcoscypha coccinea has a brilliant red, dainty, cup-like ascocarp that can be killed entire and processed exactly like *Sclerotinia.* The larger cups like those of *Peziza repanda, Urnula,* and the familiar ascocarp of *Morchella,* the sponge mushroom, should be suitably subdivided and processed as above.

Fleshy portions of the fructifications of other Ascomycetes are handled like the foregoing types. Interesting slides are obtainable from *Hypomyces,* a parasite on mushrooms; *Cordyceps,* parasitic on insects; the fruiting head of *Claviceps,* the ergot fungus; the saprophytic *Neurospora.* Species of *Nectria* in which the stroma is moderately soft can be sectioned. Remove the stroma down to the wood, subdivide vertically into narrow strips, kill in *FAA* or Craf II, and process in *TBA.* Always examine freehand sections or smears of fleshy Ascomycetes to determine whether the desired stage of ascus formation is present.

In the Taphrinales (Exoascales) only the genus *Taphrina (Exoascus)* is of importance. *Taphrina deformans,* the casual organism of peach leaf curl, is very abundant in some localities. The malformed succulent leaves are well preserved by Bouin's solution or Craf II. Sectioning and staining present no difficulties.

Venturia inaequalis, the apple scab organism, is widely distributed and easily obtainable in the conidial stage on the leaf. A vigorous fluid like *FAA* or Craf V is necessary. A simple stain such as hemalum-safranin is adequate. The perithecia mature in early spring on last year's decayed leaves. Such material can be studied well from newly gathered soaked leaves or bulk-preserved leaves. Such material yields permanent slides of decidedly ragged appearance, and microtoming is therefore to be discouraged.

BASIDIOMYCETES

This group contains a great diversity of forms and involves a wide range of techniques. We are again confronted with saprophytes that

can be detached from the substratum and processed easily, whereas the parasitic members require adequate preservation of both parasite and host. In the following discussion the taxonomic order is subordinated to methods of preparing the material.

Ustilaginales.—These parasites occur on a wide range of hosts, but the most interesting members occur on important crop plants. *Ustilago zeae,* the corn smut, is found on all aerial parts of the corn plant. Smut galls on stems, leaves, and ovaries should be collected. Very young smut galls, that have not markedly distorted the organ being attacked, show the host cells in good condition, and contain active and rather sparse mycelium. Kill this stage in Craf III. Older galls having a milky white interior contain a great mass of mycelium and distorted host cells. Small pockets of chlamydospores occur in the white mass. This stage, which can be ascertained by freehand sections, is the latest useful stage. Kill these older galls in *FAA.*

The mycelium of corn smut has a strong affinity for hemalum, and a simple hemalum-safranin stain shows the hyphae stained blue-black, chlamydospores stained red, the thin walls of the host cells stained blue, and the lignified elements stained red. Use iron hematoxylin for nuclear studies.

Other common smuts, such as *Ustilago levis, U. hordei, U. avenae,* and the bunts, like *Tilletia tritici,* can be processed by the above methods.

The chlamydospores of many smuts and bunts germinate readily in water or carrot decoction. The promycelia and sporidia are studied best from wet mounts from culture, but the material can be made into permanent mounts by the dioxan or butyl alcohol methods (Chap. 10).

Uredinales.—The rusts rank among the most destructive and widespread plant pests, and class materials illustrating the important phases of the life cycle of the rusts are indispensable. The story of wheat rust has been so well publicized that the organism may well be the standard item representing this group.

Puccinia graminis has its red uredinial and black telial stages on wheat and many other grasses. The red summer-spore stage occurs on young leaves and is therefore easy to section. The black winter-spore pustules occur on older leaves and on the stems, both of which are difficult to section without tearing. Use the youngest leaf showing the telial stage, avoiding the use of stem material if possible. Kill in *FAA* and process like any leaf parasite. The pycnial (spermogonial) and aecial stages on barberry occur on young, tender leaves that are

preserved fairly well by *FAA,* but Craf I followed by careful embedding yields superior results. For the most critical cytological requirements, use the Flemming modifications as described in research papers. Many stain combinations give excellent results for class material, safranin-fast green is particularly good, but iron hematoxylin is by far the best as a nuclear stain.

P. coronata, the crown rust, is probably second to wheat rust in importance. The uredinia and telia on *Avena* and other grasses and the pycnia and aecia on *Rhamnus* (buckthorn) are treated like wheat rust.

Two common species of *Gymnosporangium* have the telial stage on *Juniperus,* producing woody galls of stem tissue in which the mycelium is perennial. The younger galls are soft enough to be sectioned in paraffin. Divide into wedge-shaped pieces, kill in *FAA,* and process in butyl alcohol. The pycnia and aecia of *Gymnosporangium juniperi-virginianae* occur on *Pyrus,* and those of G. *globosum* on *Crataegus.* Treat like the aecial stage of wheat rust.

Melampsora is very common on willows and poplars. The bright yellow uredinia, which may entirely cover the leaf, are handled like other leaf rusts. The coal-tar dyes do not seem to be so selective for nuclei as iron hematoxylin. The telial stage on the old leaves is a difficult problem, the host cells become very brittle in paraffin, and the nuclear staining is selective only with iron hematoxylin.

A great diversity of host tissues in which rusts are found necessitates more or less specific adjustment of the killing fluid for each problem. The foregoing recommendations are based on successful preparations and will serve as a guide for other problems in this group.

Tremellales.—The order is characterized by the small, gelatinous fructifications. Septation of the basidium differs in the several families, and some authors regard as orders some of the families incorporated here. The delicate fruit bodies must be collected in an absolutely fresh condition or the time spent in processing them is wasted. The portion near the substratum is of no interest; remove the substratum completely, and kill the entire or subdivided fruit body in weak chrome-acetic, or in Craf I. Exercise extreme care during dehydration and embedding. The best stain is iron hematoxylin, with safranin-fast green as second choice.

Agaricales.—The primary consideration in the processing of this group is to maintain intact the more or less exposed, delicate basidia and especially the exceedingly fragile sterigmata on which the

basidiospores are borne. The texture of the trama of the fruit body ranges from the soft, fragile pileus of a small *Coprinus* to the "woody" perennial pileus of *Fomes*. The softer members are difficult to preserve in normal condition but are easy to section, whereas the leathery fructifications can withstand processing but are very difficult to section.

The basidia of many species of Agaricaceae have been successfully fixed in weak chrome-acetic, in Craf III, or in Allen-Bouin II and III. The last is particularly good for cytological details. Bouin's solution has given good results, but it is rather erratic. Dehydrate in alcohol or acetone, beginning with 5% and using steps of 5% at 15- to 30-min. intervals. Iron hematoxylin and gentian violet-iodine are excellent for nuclear details. Safranin-fast green stains the nuclei well enough and also shows the gill and trama structure.

Softer members of the *Clavariaceae, Hydnaceae,* and *Polyporaceae* are processed as above; the leathery and woody forms must be dehydrated in butyl alcohol or dioxan. Fortunately, basidia mature in the soft new growth in even the toughest perennials.

Exobasidium occurs on *Vaccinium, Rhododendron,* and other members of the heath family. Kill in *FAA* or Craf III. Because of the leathery texture of the host the use of butyl alcohol is advisable.

FUNGI IMPERFECTI

This category includes fungi for which the perfect or sexual stage has not yet been found. The perfect stage, when discovered, is found to be a basidial or ascigerous stage, and the organism is then transferred to the appropriate group. Sporulation is by conidia, produced either at random on the mycelium or in closed pycnidia. The vegetative mycelium may be a superficial saprophyte, a saprophyte within dead tissues, or a parasite within tissues.

Mycelium and conidia from cultures can be prepared as whole mounts by the general methods outlined in Chap. 10. Parasitic species are handled in accordance with the properties of the organ on which they occur. Leaf parasites are the easiest to handle. The following illustrations are selected from successful preparations of important fungi.

Diplodia zeae grows readily in agar culture and produces abundant pycnidia. Cut out small pieces of agar bearing the pycnidia, fix in Craf I, and embed in paraffin. A heavy overstain in hemalum, slightly differentiated in HCl, stains the hyaline portions of the fungus very well. The pycnidia and the mature spores have considerable pigmentation.

Cercospora beticola is common on garden and sugar beets. Excise the youngest lesions to obtain sections embracing healthy tissues as well as diseased areas. If material must be killed in the field where an aspirator is not available, use *FAA,* which gives adequate fixation. Excellent preservation can be obtained with Craf III. Iron hematoxylin and safranin-fast green are the preferred stains.

The wood- and bark-inhabiting pycnidia are handled like perithecia of similar habitats. Such resistant subjects must be killed in *FAA,* and butyl alcohol is the preferred dehydrant.

LICHENS

The lichens are found in a wide range of habitats, from the mist-soaked rocks under a waterfall to the sun-baked face of a boulder. Collections should include a portion of the substratum whenever possible. Specimens usually are dried, and stored in containers that prevent breaking of the fragile dry plant. If wet preservation is preferred, use one of the fluids given in Chap. 10. Microtome sections of the vegetative thallus have little justification. The association of the green algal cells and the fungal mycelium is shown best by dissections and freehand sections of fresh or preserved material. The ascocarps should be preserved in fluid, examined with a hand lens for general organization, and teased apart for examination of bits of the hymenium under a microscope.

Microtoming of the ascocarp is a vexing problem with most species. The gelatin in the plant body becomes dry and brittle, and the sections fail to ribbon and do not adhere well to the slide. Select a species with a small, shallow cup-like apothecium. Kill in *FAA* and dehydrate in butyl alcohol. Soak the embedded blocks in warm water before sectioning. Staining presents no difficulties if selectivity for the diverse components is not demanded. Safranin-fast green is probably the best simple combination.

Bryophyta

The liverworts and mosses have such wide distribution and range of habitat that some representative member of the group is usually available for study. The most common liverworts are the aquatic *Riccia,* the well-known *Marchantia,* and two rock-inhabiting species, *Conocephalum conicum* and *Reboulia hemisphaerica. Anthoceros* seems to be less common, but it is easily overlooked if sporophytes are not present. Large and conspicuous mosses are usually preferred, the best-known ones are in the genera *Polytrichum, Mnium, Catherinia, Funaria, Rhodobryum,* and *Sphagnum.* Liverwort and moss species

that are not locally available can be purchased from supply houses, preserved either for bulk specimens or for sectioning, as specified by the purchaser.

These fragile plants must be collected and handled with care, taking precautions to keep the plants moist and undamaged until the time of killing. Entire plants preserved in fluid are indispensable for teaching. The most useful bulk preservatives are described in Chap. 10. Preservation and processing for embedding must be carried out with painstaking care, approaching cytological methods.

Hepaticae

The following recommendations, based on *Marchantia,* will apply to a wide range of liverworts. The young, actively growing thallus is usually sectioned to show the construction of the pores, the highly spongy chlorenchyma, and the gemmae. Cut out 4-mm. squares of tissue. Gemma cups should be excised with a small square of thallus. Antheridial and archegonial receptacles should be collected when they are just beginning to be elevated above the thallus. The gametangia are at their best at this stage. When the archegonial disk has been fully elevated, make a collection for the developing sporophytes. A complete series of developmental stages may be obtained by collecting at intervals. Kill in weak chrome-acetic or Craf I. A closely graded alcohol-xylene series is recommended.

The thickness of sections can be judged best at the time of microtoming. Examine a few trial sections by melting the ribbon on a slide, and decide whether the trial thickness includes the desired structures and is sufficiently thin to show internal detail. Sections will range from 6 μ for a careful examination of young antheridia, to 15 μ for maturing capsules. A simple hemalum stain, with perhaps a light counterstain of erythrosin, sets off all essential structures very well. A multicolor stain combination is quite pointless. Iron hematoxylin is the ultimate choice for cytological details.

Gemmae can be studied conveniently by dissecting them from gemma cups of fresh or preserved thalli. Permanent whole-mount slides of gemmae are of little value, but such mounts can be made by the methods outlined for preparing filamentous green algae. Microtome sections are necessary to show the initiation and development of gemmae.

Riccia and *Anthoceros* are somewhat more difficult than the foregoing type, because the sex organs are sunken in the thallus. Skillful freehand sectioning reveals the presence of sex organs and

eliminates the fruitless sectioning in paraffin of many vegetative thalli. The developing sporophytes of *Riccia* are visible within the thallus, and various stages can be classified roughly by size. Remove enough of the thallus with these organs to show some of the enveloping cells. Fruiting thalli of *Anthoceros* should be killed entire, in a vacuum jar (Fig. 3.1), and the sporophytes dissected away with a section of thallus after hardening in the fluid for several days. Both transverse and longitudinal sections of the sporophyte should be made.

The leafy liverworts are easily overlooked on collecting trips, and therefore do not receive adequate attention. *Pellia* and *Porella* are most commonly used to illustrate this group. They can be processed like the mosses as outlined below.

Musci

These plants are readily obtainable in fresh condition during the greater part of the year in all but the most severe climates, and they can be grown easily. They make usable dried specimens and can be preserved in excellent condition in the fluids given in Chap. 10. Gemmae, fully developed sex organs, and most features of the capsule can be studied from dissections. Prepared slides are needed principally for studying young sex organs, gametes, and some features of the developing sporophyte.

For the study of sex organs the large and more common species of *Mnium, Polytrichum,* and *Rhodobryum* are recommended. The proper killing fluid for sex organs and gemmae of mosses and leafy liverworts can be determined quickly. Obtain fresh turgid plants, dissect out a few short pieces of the shoot bearing the sex organs, and immerse in the fluid that is to be tried. Exhaust the air that adheres tenaciously among the leaves. After 1 hr. in the killing fluid dissect out a few gametangia, mount in a drop of the fluid, and examine with a microscope under at least 400×. If plasmolysis has occurred, adjust the formula. It is a good practice to try *FAA* and *FPA* (page 15). If these cause excessive shrinkage, try Craf I, an excellent formula. Adjustments in this formula are made by increasing the ratio of acid until no marked plasmolysis occurs. Use the stains recommended for liverworts.

Capsules of mosses are a vexingly difficult subject. Young green capsules of *Mnium cuspidatum* and *Funaria hygrometrica* are penetrated by Craf I, but for older, coloring capsules, *FAA* or *FPA* must be used. However, the interesting stages of sporogenesis take

place long before the capsules become brittle, and there is little need for slides of old capsules. The dehydrating must be gradual, and *TBA* is preferred. The embedded capsules should be oriented carefully in the microtome, and both longitudinal and transverse sections are desirable. The capsule has enough internal differentiation to justify the use of a triple stain; however, the simple combinations given on Staining Charts II and III are usually adequate.

The sporulating capsules are studied to best advantage either from fresh plants, wet-preserved plants, or dried specimens from which they can be removed and thoroughly soaked in water or lactophenol (Chap. 9). Spores can be germinated readily and the protonema held at any stage by refrigeration under weak illumination. With a little planning by the instructor there is little excuse for using permanent prepared slides of protonema, although these can be made by the methods used for delicate algae.