

On Mounting Delicate Bryophytes in Glycerol

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Abstract. *Glycerine jelly is an effective substitute microscopic mounting medium for now difficult-to-obtain gum chloral preparations. It does not collapse most thin bryophyte tissues, preserves color responses of cell walls to potassium hydroxide solution, and is made from easily obtained, non-poisonous ingredients. Like other glycerol-based mountants, it evaporates only very slowly and, with luting, is essentially permanent.*

The study slide is a valuable adjunct to research in bryology. A well labeled permanent or semi-permanent preparation allows repeated examination of morphological traits that allows serial comparisons during revisionary work and serves to reinforce poorly remembered distinctions or illuminate vaguely worded keys during routine identification. For critical species, I find two slides, each with two coverslip-protected mounts, provide space for mounting moss capsules, perichaetia, perigonia, habit (of smaller species), and sections of stem and leaves in a manner that allows subsequent illustration if needed. As anyone who has worked with microscopic mounts knows, taking the time to prepare a slide well saves much time and frustration in the long run. To this end, I have pursued (Zander 1982, 1983, 1993) a better slide medium than gum chloral preparations such as Hoyer's Solution (Anderson 1954), which have severe osmotic effects in many bryophyte species. In addition, chloral hydrate is presently governed under controlled-substance laws in the U.S.A. and good-quality powdered gum arabic is now difficult to obtain.

Glycerine jelly has been used for many years as a bryological microslide mountant (Gatenby & Beams 1950; Gray 1954; Murray 1926) but has been superseded in bryological work to a great extent by Hoyer's Solution (Anderson 1954), which is more convenient to work with since it does not need to be heated. Hoyer's Solution and its variants are likewise made with glycerol, and thus last for many years since glycerol has a very low vapor pressure (boiling point 290°C). The high index of refraction of glycerol (much higher than that of cellulose), makes mounted tissue easy to observe under the lens.

Preparations with glycerol, however, may distort thin tissues. Various authors e.g., Murray (1926) and Conard (1933), recommend transferring delicate bryophytes through a series of increasing levels of glycerol in water to avoid collapse of cells, for example, by placing a plant in a slowly evaporating

weak solution of glycerol in water. Scott and Stone (1976) suggested a preliminary soak of sensitive plants in lactic acid, then a water wash, to avoid the worst osmotic effects of gum chloral-glycerol solutions. Lightowers (1981) found a presoak in polyethylene glycol to be more effective at this than lactic acid. Lactophenol gel (Zander 1983), which uses ethylene glycol as a less damaging ingredient than glycerol, was offered as a mounting medium that allowed direct slide mounts with a minimum of osmotic effect. Ethylene glycol, however, has a high vapor pressure (boiling point 197°C) relative to that of glycerol. Slides made with lactophenol gel evaporate in a few months, and there are no sure sealants to prevent this.

I recently discovered—almost certainly a rediscovery though I find no mention of it in the literature—that heating a sensitive moss plant in glycerol or in glycerine jelly plumps up tissue nicely after an initial collapse, resulting in a quite life-like appearance. The dilating effect on tissue occurs with either hydrated or dry plants, perhaps because glycerol is hygroscopic and some water is usually present in solution. One can avoid bubbles, which are easily formed and captured in the jelly, by strongly heating a plant in a small drop of pure glycerol first, then adding glycerine jelly without boiling to make a semi-permanent mount that will set solidly. Heating in glycerol first also allows microdissection and sectioning in a more amenable medium than water, which has troublesome surface tension effects that scatter tiny sections and dissected parts.

No heat-related plumping effect on collapsed mounted tissue was found with other possible mountants I examined, including those made with gum arabic (e.g., Hoyer's and Farrant's solutions), polyethylene glycol, acrylic "floor waxes," polyvinyl alcohol, and carboxymethyl cellulose. These dissolved long-chain molecules collapse sensitive cells even when heated. Gelatin does not form a solution, but instead a dispersion which, in very

small amounts, stiffens glycerol. Unlike the case with solutions, colloidal suspensions, involving for instance the gelatin as an emulsoid, are not affected to a marked degree in vapor pressure or osmotic pressure. In the case of emulsoids, the surface tension is lower than that of the dispersion medium (D. Van Nostrand Co. 1958: 371).

A firm glycerine jelly stock is recommended here because plants can first be heated and dissected in pure glycerol, then the added glycerine jelly after dilution remains quite solid after cooling. The formula I use is:

7 g (one standard commercial packet) gelatin
5 cc water
35 cc glycerol (glycerin, glycerine, or 1,2,3-propanetriol)
a few grains of phenol or thymol

Hydrate the gelatin in the water by heating in a water bath. Add the glycerol and phenol or thymol (this counters possible fungus growth) and continue heating with occasional stirring until the liquid is clear. Pour the molten glycerine jelly into a shallow pan. After cooling, peel off the now firm sheet and cut into narrow strips with scissors and store in a waterproof box (the strips feel dry to the touch but are hygroscopic). Pinch off a small piece from the end of a jelly strip as needed.

The full technique with mosses of the Pottiaceae is to soak a plant in two percent potassium hydroxide solution, which brings out characteristic color changes and loosens opercula, blot and add a small drop of glycerol, heat with a butane lighter to steam off excess water and dilate cells, do microdissections and arrange on slide, add a small piece of glycerine jelly, heat again to melt jelly, arrange quickly with a needle, then add cover slip. The mount solidifies quickly and will be servicable for many months, or years if sealed. Quick mounts can be made by adding the jelly directly to a blotted water mount and heating.

The plants retain their KOH color reactions to some extent. Masking of anatomical structure by cell inclusions is not generally a problem with bryophytes, certainly not if older herbarium material is used, but fresh plants may be cleared by heating in a drop of lactic acid before the above procedure, which also works to rid plants of encrusted carbonates when present.

The hot glycerine technique is appropriate for mounting various delicate mosses and liverworts. Excellent long-lasting mounts were made of, for instance, such delicate bryophyte species as *Chenia leptophylla* (C. Müll.) Zand., *Mnium cuspidatum* Hedw., and *Lophocolea heterophylla* (Schrad.) Dumort. Even green algae and cyanobacteria inadver-

tently included in mounted fresh material retain their shape and slime capsules through this procedure. Only very thin-walled tissue such as immature antheridia are collapsed and somewhat degraded when heated in glycerol or glycerine jelly.

An attempt was made to find a mountant that might have an even lower vapor pressure than glycerol and yet penetrate tissue easily on heating. The "Table of Organic Solvents" given by Dean (1973) lists only four common solvents that have higher boiling points (and thus lower vapor pressures) than glycerol and therefore have lower vapor pressures. Of these, *n*-dibutyl phthalate has the highest boiling point, 340°C, 50 degrees higher than that of glycerol. My attempts to form an emulsoid with gelatin and the phthalate were unsuccessful; additionally, although this chemical is used widely in industry as a plasticizer and, for instance, an emollient in deodorants, it is a known irritant and is considered somewhat toxic (National Institutes of Health 1996). The solvents listed with lower boiling points were not examined because they were either too costly or the boiling points were too little different from that of glycerol.

Polymerizing glycerol was attempted as a way to avoid evaporation entirely. Making a glycerol phthalate (glyptal or alkyd) resin (general formula: 4 mL glycerol, 0.5 g sodium acetate, 10 g phthalic anhydride—Anderson 1996) on a microscope slide was out of the question because the heat cure took several hours, while the resin itself when made into pellets was sticky and messy to work with, had a low index of refraction, and degraded plant tissue on melting.

Murray (1926) soaked mounts in 10% formalin for 24 hours, a well known means of hardening glycerine jelly. Formaldehyde crosslinks animal glues (Houwink & Salomon 1965) such as gelatin, but if a firm jelly is used this is unnecessary. Sealing guards against hygroscopic softening of the mounts as well as eventual evaporation.

Burrells (1961) warned that expansion and contraction of mounts due to uneven heating of buildings often fractures ringing cements. He recommended gum dammar in xylene as a sealant that adheres well to glass. Murray (1926) found fine gold size (also known in the literature as "marine glue") to be an effective sealant, using three coats. Polyvinyl acetate is well known for its strong adhesion to glass (Moser 1962), but this is only true for "hot melts" (i.e., used as a thermoplastic), not in solvent applications. I continue to use poly(ethyl methacrylate) in toluene solution (Zander 1983) because it adheres well and forms a flat, highly transparent coating through which microscopic observations may be made if necessary. A related plastic, poly(methyl methacrylate), is trade-named Lucite.

One should use generous amounts of lutant and clean slides to ensure a good seal. Conard (1933) described his version of Zeller's method using two cover slips to seal with Canada balsam. This I have found to be a highly successful method that, unfortunately, is hardly a rapid technique and is extremely tedious to do well. As an alternative to lutting the coverslips, or as an additional precaution, glycerine jelly mounts should not evaporate if they are kept in a closed cabinet with an open, wide-mouthed jar of glycerol, perhaps with a wad of cotton to increase surface exposure (T. Dixon pers. comm.).

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