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NOTES ON TECHNIC

AUTOCLAVING AS AN AID IN THE CLEARING OF PLANT SPECIMENS

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Numerous recipes exist for the clearing of plant tissues prior to examination as whole mounts (see annotated bibliography by Lersten 1967). Broadly speaking, these methods may be classified into 3 groups; mild, drastic and very drastic procedures, depending upon the severity with which the tissue is extracted by the clearing procedure. For some purposes mild procedures are all that are needed for high-quality microscopy of the cleared specimen. Solvent extraction of pigments with aqueous ethanol, followed by infiltration with a liquid of appropriate refractive index (*e.g.* chloral hydrate, xylene, Farrant's medium, methyl benzoate, methyl salicylate, or glycerine) would be an example. In other cases, slightly more drastic action (*e.g.* lactic acid or lacto-phenol at 60 C or 1% KOH at room temperature) is required, especially if the tissue is rich in starch. In many cases, very drastic procedures (5% KOH for several days, followed by chlorine water bleaches to remove phenols) are necessary. In all cases, specimens rarely clear adequately in less than 48 hours, and difficult specimens may take several weeks.

In 1968, Bevege introduced the use of the autoclave to clear roots infected with vesicular-arbuscular mycorrhizas, reducing the time required to 30 min. We have found that autoclave treatment of leaves produces similar results, and suggest the following treatments.

1. Place intact leaves, flowers, or pieces of tissue in 80% ethanol in McCartney or other screw-capped autoclavable bottles. Vacuum infiltrate till tissue sinks.
2. Cap bottles tightly, autoclave for 15 mins at 15 lb/in^2
3. Follow whichever of these procedures is appropriate:
   a. *Delicate specimens* (*e.g.*, soft flowers, grass leaves): Soak in glycerine, Farrant's medium, lactic acid, or lactophenol until clear. Alternatively, dehydrate in 100% ethanol, clear in xylene, mount in DPX or other resinous mountant. If staining is required, use basic fuschin as described in any of the recipes given by Lersten (1967).

or b. *Normal specimens* (*e.g.*, clover leaves): Transfer tissue to 1% KOH in capped McCartney bottles. Autoclave at 15 lb/in^2 for 15 min. Rinse out KOH with 2–3 changes of water at room temperature. Stain if desired and mount in either an aqueous or nonaqueous medium.

or c. *Difficult specimens* (*e.g.*, leaves of Myrtaceae and most ferns): Autoclave in 5% KOH at 15 lb/in^2 for 15 min. If tissue goes black or brown, transfer to full-strength household bleach (Clorox, Javex) till nearly colorless (5–20

min). Rinse in water (× 2), stain if desired, mount in aqueous or non-aqueous medium.

Specimens put into bleach after autoclaving in KOH clear uniformly, rather than from the edges, the pattern normally observed when tissues have been exposed to KOH at room temperature. Presumably the cuticle has been strongly extracted by the KOH under autoclave conditions, permitting uniform entry of the bleach. Autoclaving in 80% ethanol does *not* have this effect. Do not attempt to speed bleaching by autoclaving; tissues are almost totally digested by such a treatment.

For soft tissues that do not blacken and where no staining is required (*e.g.*, grass leaves, leaves of most mesomorphic herbs), autoclaving the fresh tissue in 70–80% lactic acid for 15 min at 15 lb/in^2 gives beautiful results that are often enhanced by a slight discoloration of the tissue that accompanies the process.

The widespread availability of pressure-cookers in areas where autoclaves are not available encourages us to believe that many workers will find that they may readily avail themselves of considerable savings in time using these procedures.

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SUDAN IV STAINING AS A MEANS OF VISUALIZING CLEAVAGE PATTERNS IN TELOLECITHAL EGGS

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Because of the large amount of yolk, telolecithal eggs present problems not encountered in other types of eggs, especially when one tries to follow the pattern of cell division in the early stages of embryonic development. In the squid *Loligo pealei* studies on furrowing and cleavage patterns in the living egg are relatively simple because of the large size of the egg (1.0 mm by 1.6 mm),