A Technique to Facilitate the Paraffin Sectioning of Hard or Brittle Plant Material

Eric Karrfalt

ABSTRACT

Paraffin sectioning of hard or brittle plant material may often be facilitated by reimbibition of the tissue after embedding in paraffin; however, when sectioning seeds, reimbibition may cause the specimen to break out of the paraffin. If the reimbibition is carried out at a temperature just below the melting point of the paraffin, the tissue will not break loose of the paraffin and good sections may easily be obtained.

EXPERIMENTAL TECHNIQUES

Plant material with densely packed storage material such as mature seeds or woody or sclerotic tissue is often difficult or impossible to section in paraffin because the tissue shatters and falls out of the sections. A common remedy for this problem is to reimbibe the tissue after sectioning has been attempted. The uptake of water with or without the aid of a wetting agent will often soften the tissue to the extent that sections may be obtained. However, in the case of seeds, the specimen will eventually swell to the extent that it breaks out of the paraffin matrix *en toto.* This problem can be eliminated by carrying out the reimbibition at a temperature a few degrees under the melting point of the paraffin. A slide warming table set at a temperature suitable for stretching paraffin ribbons in the mounting process is ideal for this purpose if the imbibition is carried out in an open vessel such as a petri dish. Evaporation from the dish will keep the paraffin just a little cooler than it gets in the ribbon stretching process, and a warming table dedicated to reimbibition or continuing readjustment of a single warming table is not needed.

Once reimbibition is completed (a few hours to overnight), the sides of the paraffin block will bulge a bit and need to be retrimmed. The exposed surface of the embedded, reimbibed tissue must be kept wet until the tissue is sectioned, or it will begin to shrink and pull out of the paraffin. Once the ribbon is cut, however, drying does not disturb the connection of the tissue to the surrounding paraffin. Keating's adhesive (Keating, 1969) is most effective for adhering sections obtained by this (or any other) method. Keating used equal parts swine or bovine blood serum and glycerin plus a preservative (sodium salicylate, 0.06 M). I have used human or rat serum and propionic acid (0.003 M) or sodium benzoate (0.07 M) as a preservative depending upon what was readily available. Undoubtedly other substitutions would work just as well. My colleague, Dr. Marian Wilson, simply refrigerated the adhesive with no preservative with excellent results.

Equipment Needs

Equipment needed for this technique is that of routine microtomy: a rotary

Eric Karrfalt, 10261 Fry Road, McKean, PA 16426. Received 20 May 1997.

microtome, a sharp, non-disposable microtome knife, and a slide warming table.

RESULTS AND DISCUSSION

In some cases the seed coat may be too hard or separated from the rest of the seed to allow satisfactory sectioning. If this occurs, the seed coat is simply dissected from the seed prior to dehydration and infiltration, good serial sections may readily be obtained. I was able to routinely cut complete series of sections of seeds of *Adenocaulon* by this method. Razor blades are usually not sufficiently ridged to section hard or brittle material. Rigidity is not a problem with a regular, non-disposable microtome knife. A disposable knife might be adequate, but I have never used one.

REFERENCE

Keating, R. C. 1969. A serum adhesive for plant microtechnique. Turtox News 47:165.