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9. ABSTRACT		

Differential staining of paraffin embedded plant material is accomplished without removing the paraffin. Material fixed in 3% glutaraldehyde in 0.02 M phosphate buffer is dehydrated, embedded, sectioned, and mounted on glass slides using conventional methods. The slides are placed in 0.05% toluidine blue 0 in distilled water for 2-30 minutes, rinsed in water for 1 minute, and allowed to air-dry. Paraffin is removed with 2 changes of xylene and the cover slip mounted with resin. Lignified tissues, suberized tissue, and some tannins are stained blue-green, while non-lignified walls are stained red-purple. Advantages of the method are the short time needed for staining and mounting, and that critical counter-staining is not required.

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## SIMPLE METHOD FOR DIFFERENTIAL STAINING OF PARAFFIN EMBEDDED PLANT MATERIAL USING 'TOLUIDINE BLUE O'

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**ABSTRACT.** Differential staining of paraffin embedded plant material is accomplished without removing the paraffin. Material fixed in 3% glutaraldehyde in 0.02 M phosphate buffer is dehydrated, embedded, sectioned, and mounted on glass slides using conventional methods. The slides are placed in 0.05% toluidine blue O in distilled water for 2-30 min, rinsed in water for 1 min, and allowed to air dry. Paraffin is removed with 2 changes of xylene and the cover slip mounted with resin. Lignified tissue, suberized tissue, and some tannins are stained blue-green, while nonlignified walls are stained red-purple. An advantage of the method is the short time required for staining and mounting. Also, critical counterstaining is not required.

A method for differential staining of paraffin embedded plant material has been used in this laboratory for several years with good success. The method appears to work well on a variety of plant tissues from different taxa (Sakai 1970; Herbst 1972). The chief advantage of the method is the short time required for staining. Material is simply sectioned and stained without removing the paraffin, thereby saving the time needed for hydrating and dehydrating the tissue. The method also has the advantage over the standard safranin-fast green method (Sass 1961) of not requiring critical counterstaining. The only disadvantage of the method is the rapid destaining of the sections, after mounting, if the tissue has been fixed with fixatives containing OsO<sub>4</sub> or an acid chromium salt.

Toluidine blue O has been recommended for staining fresh sections (O'Brien, Feder and McCully 1964) and material embedded in polyester wax (Sidman, Mottla, and Feder 1961), glycol methacrylate (Feder and O'Brien 1968) or Epon (Hoefert 1968). Toluidine blue O works equally well for paraffin embedded material. It has been suggested for animal tissue by Lison (1960). However, to my knowledge its use has not been described for paraffin-embedded plant material.

Fixation has been best in 3% glutaraldehyde in 0.02 M phosphate buffer pH 7.2, but FAA (50 cc 95% EtOH, 5 cc glacial acetic acid, 10 cc 40% formaldehyde, and 35 cc water, Sass, 1958) or modified Karnovsky's (1965) fixative (2% paraformaldehyde and 3% glutaraldehyde in 0.02 M phosphate buffer, pH 7.2) have worked equally well. After fixation the material is dehydrated with EtOH and tertiary butyl alcohol (Sass, 1958), embedded in Tissuemat or Paraplast, sectioned, and then mounted on glass slides with Haupt's adhesive (Jensen, 1962). Slides should be dried at a temperature lower than the melting point of

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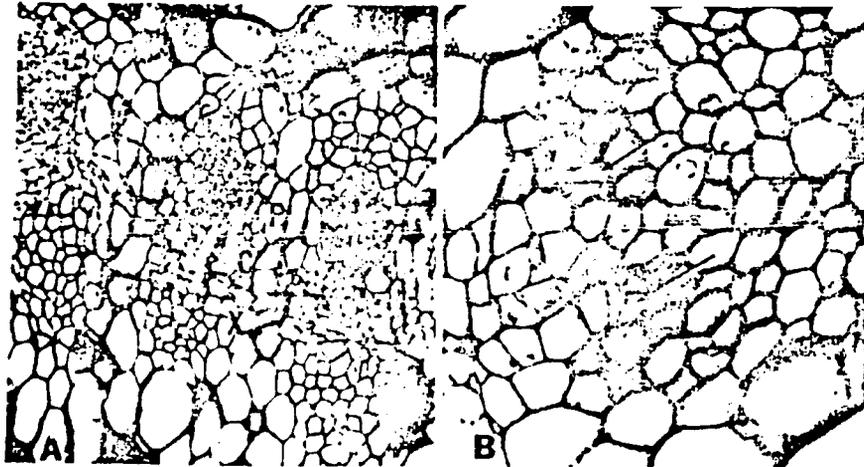


FIG. 1. Micrographs of  $10\ \mu$  thick sections, stained by the described method, from young branches of *Diagnolia grandiflora* L. A) Three vascular bundles with phloem (P), cambium (C), and xylem (X),  $\times 650$ . B) Phloem tissue showing nacreous walls of sieve elements (arrows),  $\times 1050$ .

the wax. Staining is accomplished without removing the paraffin, as in the method of Lison (1960).

1. The slides are placed in 0.05% toluidine blue O (Matheson, Coleman, & Bell, Cert. No. CU15) in distilled water or pH 4-6 citrate-phosphate buffer, for 2-30 min. The slides can be periodically removed from the stain, rinsed in water, and viewed to check the intensity of staining. Since the sections are still embedded in the paraffin there is little danger of damaging the tissues if the slides dry while being observed.

2. The slides are rinsed in water for 1 min and allowed to air dry.

3. The paraffin is removed with 2 changes of xylene and the coverslip is mounted with a synthetic neutral resin (Harleco brand was used).

Lignified tissue, suberized tissue and some tannins are stained blue-green, while nonlignified walls are stained red-purple. Starch is unstained, cytoplasm and RNA are purple, and DNA is blue or blue-green. Staining with toluidine blue O cannot be accomplished by the conventional method—removing the paraffin, hydrating, staining, dehydrating. Since the dye is extremely soluble in alcohol, metachromasia is lost and the tissues appear uniformly blue-green.

The stained material can be photographed using color or black and white film with good results (Fig. 1). The method was originally developed for staining the nacreous walls of phloem sieve elements, which show little or no affinity for many of the dyes commonly used in botanical staining (Esau and Cheadle, 1958; Mehta, 1964). However, the simplicity of the method has led to its adoption by many workers in our laboratory.

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