MITCHELL, J. W., and G. A. LIVINGSTON. 1968. Methods of studying plant hormones and growth-regulating substances. US Dep Agric, Agric Handb 336.
NIKOLAEVA, M. G. 1967. Physiology of deep dormancy in seeds. (Transl. from Russian) NSF TT68-50463. Israel Program for Scientific Translations Ltd., Jerusalem. 219 p.
PINFIELD, N. J., H. V. DAVIES, and A. K. STOBART. 1974. Embryo dormancy in seeds of *Acer platanoides*. Physiol Plant 32:268–272.
SIMMONDS, J. A., and E. B. DUMBROFF. 1974. High energy charge as a requirement for axis elongation in response to gibberellic acid and kinetin during stratification of *Acer saccharum* seeds. Plant Physiol 53:91–95.
USDA FOREST SERVICE. 1974. Seeds of woody plants in the United States. USDA, Agric Handb 450, 883 p.
WEBB, D. P., and E. B. DUMBROFF. 1969. Factors influencing the stratification process in seeds of *Acer saccharum*. Can J Bot 47:1555–1563.
WEBB, D. P., and P. F. WAREING. 1972. Seed dormancy in *Acer pseudoplatanus* L.: The role of the covering structures. J Exp Bot 23(76):813–829.
YAWNEY, H. W. 1968. Artificial regeneration. *In* Proc Sugar Maple Conf (August 20–22, 1968, Houghton, Mich.), p 65–74. USDA Forest Serv, North Cent Forest Exp Stn, St. Paul, MN.

A Tannic Acid-Ferric Chloride-Toluidine Blue Stain for Wood Amyloplasts Embedded in Epoxy Resin

Note by Thompson Demetrio Pizzolato

ABSTRACT. Starch of amyloplasts in wood can be permanently stained dark blue by treating epoxy sections of wood with a sequence of tannic acid, ferric chloride, and toluidine blue. This technique improves upon those currently available for permanently staining amyloplasts of wood. FOREST SCI. 24:49–51.

ADDITIONAL KEY WORDS. Starch, *Tilia americana*, *Ulmus americana*, *Populus tremuloides*, *Nyssa sylvatica*.

A RECENT STUDY OF STEMS of *Tilia americana* L. revealed that a sequence of tannic acid and ferric chloride permanently stained the mucilage cavities and that toluidine blue intensified the staining (Pizzolato 1977). A byproduct of that study was an indication that starch of amyloplasts might react similarly. Such a possibility deserved investigation for the following reasons: (1) Lugol's iodine test for starch is normally not permanent (Wargo 1975); (2) periodic acid-Schiff staining is troublesome (Feder and O'Brien 1968); and (3) starch is difficult to stain with other reagents (O'Brien and others 1964, Feder and O'Brien 1968). Therefore, it seemed desirable to test a tannic acid-ferric chloride-toluidine blue sequence on the amyloplasts of the wood of four local, deciduous trees.

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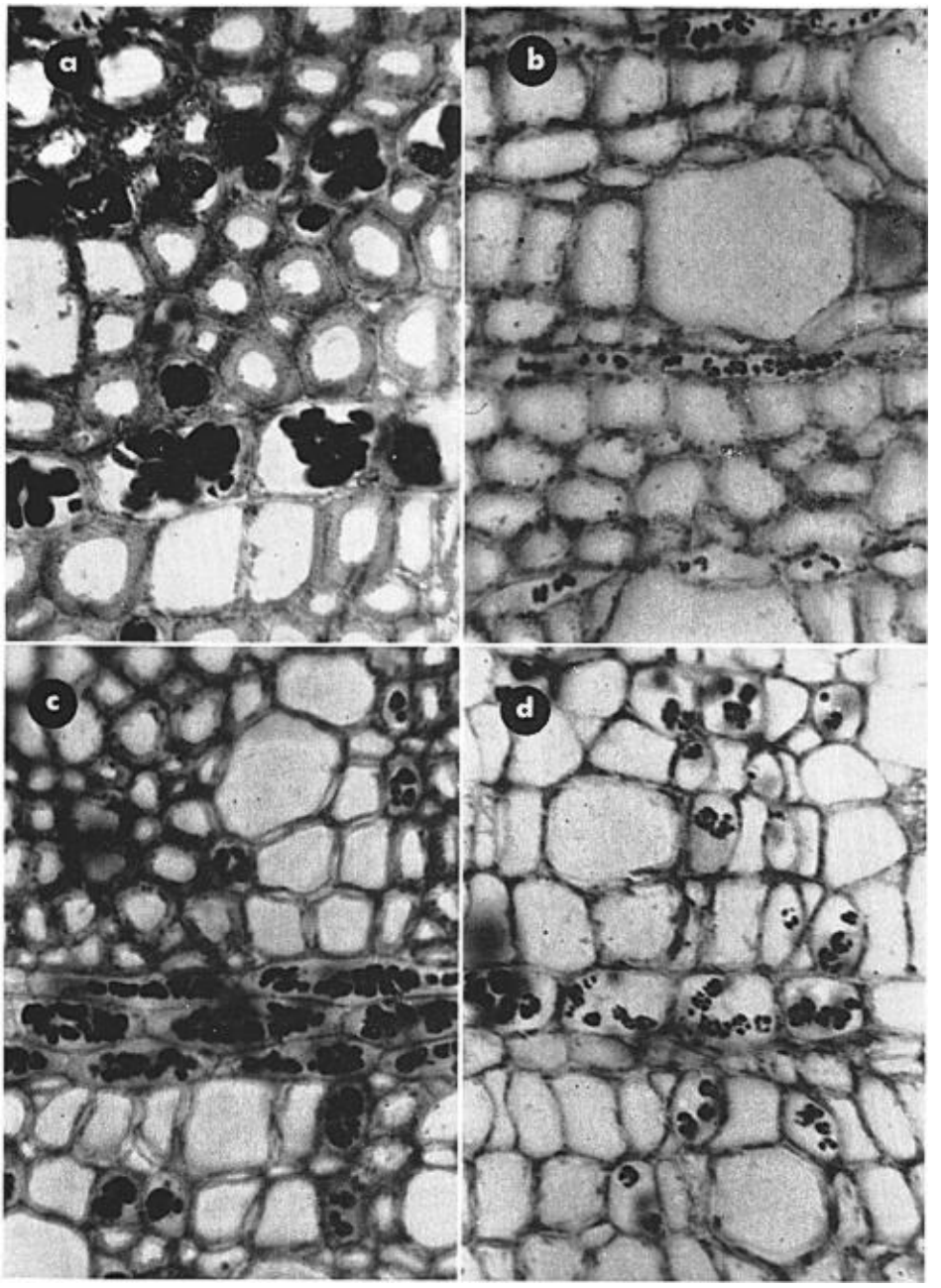

FIGURE 1. Cross sections of stem secondary xylem stained with the tannic acid-ferric chloride-toluidine blue sequence. Amyloplasts of ray and axial parenchyma are dark; cell walls of parenchyma, vessels, and fibers are lighter. ×3000. a: *Nyssa*; b: *Populus*; c: *Ulmus*; d: *Tilia*.

Materials and Methods.—Transverse internode segments, 3 mm thick, were cut with razor blades from 2 or 3 yr stems of the following trees: *Tilia americana* L. (basswood), *Ulmus americana* L. (American elm), *Populus tremuloides* Michx. (trembling aspen), and *Nyssa sylvatica* Marsh. (black tupelo). Segments were prepared for microscopy

using Pizzolato's (1976) technique altered as follows: (1) osmium fixation was omitted; (2) Spurr's (1969) modification E was used instead of his standard medium; (3) infiltration time for the specimens was doubled; and (4) sections were removed from the microtome knife with forceps and placed directly into 100 percent ethanol bypassing the xylene. Transverse sections were microtomed at 6 μm and stained with a tannic acid-ferric chloride-toluidine blue sequence (Pizzolato 1977). Specifically, the 6 μm sections were treated with 5 percent aqueous tannic acid for 10 min, washed for 15 sec, treated with 3 percent aqueous ferric chloride for 1 min, washed for 1 min, and stained with toluidine blue for 1 min. The toluidine blue used in the present study was a 0.05 percent solution in 1 percent aqueous sodium borate. The stained sections were washed, dried, and then mounted on slides in immersion oil as described previously (Pizzolato 1976). Slides were made permanent by ringing the cover slip with fingernail polish.

Results and Discussion.—The tannic acid-ferric chloride-toluidine blue sequence permanently stained starch of amyloplasts of wood a dark blue. The amyloplasts of both ray and axial parenchyma of *Nyssa* (Fig. 1a), *Populus* (Fig. 1b), *Ulmus* (Fig. 1c) and *Tilia* (Fig. 1d) were stained. Cell walls of fibers, vessels, and parenchyma stained a light blue. Amyloplasts in the extraxylary tissues were a dark blue. Pizzolato and Lillie (1973) discussed the mechanisms by which tannic acid and ferric chloride form a complex with polysaccharides and the masking of the complex by toluidine blue.

Tannic acid-ferric chloride-toluidine blue staining of plastic sections should now be recognized as an easy, reliable means of demonstrating amyloplasts in wood because it is often difficult to indicate starch with other stains (O'Brien and others 1964, Feder and O'Brien 1968). Even the classic Lugol's test (I_2KI) for starch (Johansen 1940) has limitations, particularly for wood. Lugol's stain is not absolutely specific and it fades quickly (Johansen 1940, Wargo 1975). Because of difficulty in cutting thin sections of wood unembedded in plastic, Lugol-stained wood sections are frequently too thick to allow a morphological study of their amyloplasts. This difficulty is still inherent in the technique of Hinchman (1973) although he made Lugol's stain permanent. Therefore, it is concluded that wood anatomists should consider using plastic sections (Ameele 1976, Pizzolato 1976) stained with the tannic acid-ferric chloride-toluidine blue sequence described herein when studying the amyloplasts of wood.

Literature Cited

Ameele, R. J. 1976. A method for the preparation of serial thick sections of plastic-embedded plant tissues. Stain Tech 51:17–24.

Feder, N., and T. P. O'Brien. 1968. Plant microtechnique: some principles and new methods. Am J Bot 55:123–142.

Hinchman, R. R. 1973. A permanent iodine stain-mountant combination for starch in plant tissues. Stain Tech 48:344–346.

Johansen, D. A. 1940. Plant microtechnique. McGraw-Hill, New York. 523 p.

O'Brien, T. P., N. Feder, and M. E. McCully. 1964. Poly-chromatic staining of plant cell walls by toluidine blue 0. Protoplasma 59:368–373.

Pizzolato, P., and R. D. Lillie. 1973. Mayer's tannic acid-ferric chloride stain for mucins. J Histochem Cytochem 21:55–64.

Pizzolato, T. D. 1976. Preparation and flattening of thick epoxy sections for light microscopy. Can J Bot 54:2405–2407.

Pizzolato, T. D. 1977. Staining of *Tilia* mucilages with Mayer's tannic acid-ferric chloride. Bull Torrey Bot Club 104:277–279.

Spurr, A. R. 1969. A low-viscosity epoxy embedding medium for electron microscopy. J Ultrastruc Res 26:31–43.

Wargo, P. M. 1975. Estimating starch content in roots of deciduous trees—a visual technique. USDA Forest Serv Res Pap NE-313, 9 p.