BULLETIN OF THE TORREY BOTANICAL CLUB
VOL. 104, No. 3, pp. 277–279 JULY–SEPTEMBER 1977

Staining of *Tilia* mucilages with Mayer's tannic acid-ferric chloride

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PIZZOLATO, T. D. (Harvard Forest, Petersham, MA 01366). Staining of *Tilia* mucilages with Mayer's tannic acid-ferric chloride. Bull. Torrey Bot. Club 104: 277–279. 1977.—Mayer's stain for animal mucins was found to be selective for the mucilages of *Tilia*, imparting to them a black color. Embedded stem sections and the slimy secretions of cut stems were treated with 5% aqueous tannic acid for 10 min, washed, treated with 3% aqueous ferric chloride for 1 min, washed, and dried. Because of the scarcity of histochemical techniques selective for plant mucilages, Mayer's stain for mucins merits further testing among other plants which synthesize mucilages.

During current studies on the three-dimensional stem anatomy of *Tilia americana* L. (Zimmerman 1976), the copious, mucoid product of the mucilage canals of the pith and cortex attracted attention. A literature search revealed that, although the constituents of a few plant mucilages are known, botanists recognize mucilage chiefly by its ability to swell and become slimy in water (Johansen 1940, Bonner 1950). Texts on botanical histochemistry do not include tests specific for mucilages. However, histochemical procedures for animal mucilages, the mucins, are available (see Noggle 1957). Few botanists have attempted to identify plant mucilages with techniques useful for mucins (see Hyde 1970).

Recently, Pizzolato and Lillie (1973), continuing the work of Mayer (1896), demonstrated that sequential treatment of mammalian tissues with tannic acid and ferric chloride turned mucilages gray or black. Although the tannic acid-ferric chloride method is used to stain walls of meristematic cells, it has not been tested on the mucoid tissues of plants (Johansen 1940). Because of the simplicity of this technique for characterizing mucilages and because botanists are unaware of the method, it was considered worthwhile to test Mayer's tannic acid-ferric chloride stain on the material at hand: the internodes of *Tilia americana* L., a species of a genus containing mucilage cavities (Walliczek 1893, Metcalfe and Chalk 1950).

Materials and methods. Transverse internode segments, about 3 mm thick, were cut from 2 and 3 yr branches of a forest-grown *Tilia americana* L. The segments were prepared for microscopy as previously described (Pizzolato 1976) except that osmium fixation was omitted. Sections underwent Mayer's staining for mucins (Pizzolato and Lillie 1973) at the point in the Pizzolato technique where routine staining would be done. Occasionally, after 2 adjacent sections underwent Mayer's staining, one of the pair was placed in 0.05% toluidine blue in phosphate buffer at pH 7 for 1 min. Control sections included those treated with tannic acid alone, with ferric chloride alone, and with neither tannic acid nor ferric chloride.

Additional transverse internode segments, about 5 mm thick, were cut and immersed in a few ml of water for 24 hr. A few segments were then placed on slides on a hot plate at 60°C until the segments dried. The segments were discarded. Ingredients of Mayer's stain were added with a dropper to the area on the slide where the segments had been. Washing was done with a squirt bottle of water. Tannic acid or ferric chloride or the internode segments were excluded from control slides. Slides were made permanent by covering the treated area after it dried with immersion oil and a cover slip.

Results and discussion. Internode segments of *Tilia americana* L. within 24 hr

[1] This work was done while I was a Charles Bullard Fellow. I appreciate the photography of Mr. Dale Callaham. Dr. Martin Zimmermann kindly made his laboratory available to me.

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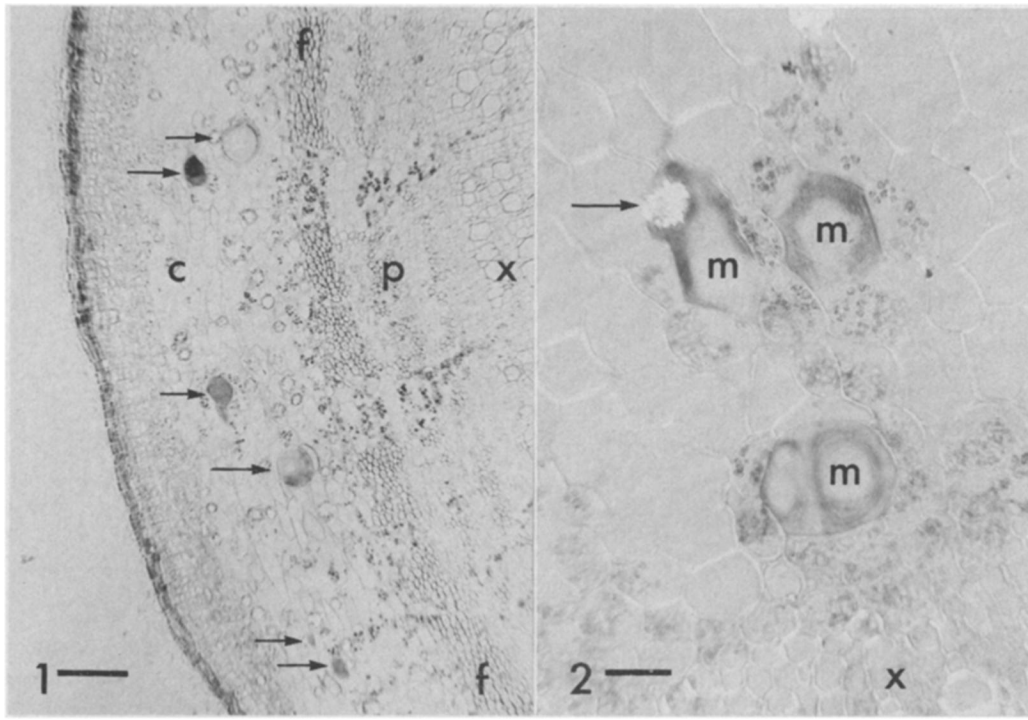
Received for publication December 20, 1976.



Figs. 1–2. Transverse sections from an internode of *Tilia americana* stained only with Mayer's tannic acid-ferric chloride.—Fig. 1. Mucilage cavities (arrows) are stained gray to black with Mayer's stain. Primary phloem fibers (f) delimit the phloem (p) from the cortex (c). The small, dark bodies in the parenchyma of the cortex, phloem, and xylem (x) are amyloplasts stained by Mayer's stain. All structures except the mucilage cavities and starch grains are not stained by Mayer's stain. Scale bar equals 1 mm. —Fig. 2. Mucilage cavities (m) in the pith adjacent to the xylem (x) stain gray to black with Mayer's stain. The only other structure stained by Mayer's stain is the starch of the amyloplasts located in the parenchyma of the xylem and pith. One of the mucilage cavities contains a crystal (arrow). Scale bar equals 40 μm.

of immersion in water were surrounded by a colorless, viscid, slimy gel. After the gel was dried on a slide and subjected to Mayer's tannic acid-ferric chloride stain for mucins, the gel gave a strong positive response by becoming black. Control slides remained colorless.

After fixed and embedded internode sections were treated with Mayer's stain, conspicuous black staining was concentrated almost exclusively within the areas of the pith and cortex designated by Walliczek (1893) as mucilage cavities (Figs. 1, 2). Control slides were unstained. The only other structures which consistently became black with Mayer's stain were the starch grains of the amyloplasts, perhaps due to the ability of some starches to bind tannic acid (Pizzolato and Lillie 1973). Occasionally, the walls of the collenchyma faintly held the stain. Because the predominant localization of the stain was within the matrix of the mucilage cavities, it appeared that Mayer's stain was nearly specific for plant mucilages.

The staining of an adjacent section with toluidine blue overcame difficulties in seeing a section stained only with Mayer's tannic acid-ferric chloride sequence. Besides staining all cell walls, toluidine blue replaced the black staining of the mucilage caused by Mayer's stain. Pizzolato and Lillie (1973) noted a similar phenomenon when toluidine blue followed Mayer's staining of mucins.

The mucilage cavities of *Tilia* internodes occurred in the cortex and pith (Figs. 1, 2). The cavities in the pith were generally larger. The pattern in which the contents of the mucilage cavities stained with Mayer's mucin stain was variable. The matrix of the mucilage cavities was sometimes uniformly dark (Fig. 1); sometimes only the periphery or one

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portion of the cavity was dark (Figs. 1, 2); and occasionally, the mucilage was stained in alternating, concentric dark and lighter bands (Fig. 2). Crystals, which were unstained by Mayer's sequence, were seen on occasion in the mucilage cavities (Fig. 2).

Conclusions. This study suggests that Mayer's tannic acid-ferric chloride stain for mucins (Pizzolato and Lillie 1973) is a selective histochemical test for the mucilages of *Tilia*. Because plant biochemistry and botanical histochemistry texts presently do not include a selective stain for plant mucilages, Mayer's stain merits further testing in the large number of families reputed to produce mucilages (Walliczek 1893, Metcalfe and Chalk 1950).

Literature Cited

BONNER, J. 1950. Plant Biochemistry. Academic Press Inc. New York.

HYDE, B. B. 1970. Mucilage-producing cells in the seed coat of *Plantago ovata:* developmental fine structure. Am. Jour. Bot. 57: 1197–1206.

JOHANSEN, D. A. 1940. Plant Microtechnique. McGraw-Hill Book Co. Inc. New York.

MAYER, P. 1896. Über Schleimfärbung. Mitth. Zool. Stat. Neapel. 12: 303–330.

METCALFE, C. R., and L. CHALK. 1950. Anatomy of the Dicotyledons. Clarendon Press, Oxford.

NOGGLE, G. R. 1957. The identification and the quantitative determination of carbohydrates. *In:* W. Pigman (Ed.), The Carbohydrates—Chemistry, Biochemistry, Physiology. Academic Press, Inc. New York.

PIZZOLATO, P., and R. D. LILLIE. 1973. Mayer's tannic acid-ferric chloride stain for mucins. Jour. Histochem. Cytochem. 21: 56–64.

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

WALLICZEK, H. 1893. Studien über die Membranschleime vegetativer Organe. Jahrb. Wiss. Bot. 25: 209–277.

ZIMMERMANN, M. H. 1976. The study of vascular patterns in higher plants. *In:* I. F. Wardlaw and J. B. Passioura (Eds.), Transport and Transfer Processes in Plants. Academic Press, Inc. New York.

The relationship of genetic polymorphism and ecological amplitude in successional species of *Erigeron* [1]

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HANCOCK, J. F. (Dept. Pomology, Univ. Calif., Davis, Calif. 95616). The relationship of genetic polymorphism and ecological amplitude in successional species of *Erigeron*. Bull. Torrey Bot. Club 104: 279–281. 1977.—Both *E. annuus* and *E. canadensis* are primary invaders after cropland abandonment, but only *E. annuus* is important after three years of succession. It was shown previously with starch gel electrophoresis and the zymogram technique that a large number of adaptively different biotypes exist in *E. annuus*. In this study, the amount of polymorphism and ecological divergence in *E. canadensis* was examined and found to be much less than that of *E. annuus*. These results suggest that a highly polymorphic species may possess ecological advantages in a changing environment.

Genetic polymorphism is quite common in natural populations, and the genotypes available to individual species may vary as to the environments for which they are best fitted. This has been indicated in a number of organisms at several enzyme loci through isozyme-property comparisons and genotype-environmental correlations

[1] This work was done under the guidance of Dr. R. E. Wilson at Miami University, Oxford, Ohio in partial fullfillment for a Master of Science degree in botany. Dr. Wilson's life was tragically taken in an automobile accident before the preparation of this manuscript. His guidance was an integral part of the research.

Received for publication January 5, 1977.