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A Berberine-Aniline Blue Fluorescent Staining Procedure for Suberin, Lignin, and Callose in Plant Tissue

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Summary

A fluorescent staining procedure to detect suberin, lignin and callose in plants has been developed. This procedure greatly improves on previous methods for visualizing Casparian bands in root exodermal and endodermal cells, and performs equally well on a variety of other plant tissues. Berberine was selected as the most suitable replacement for *Chelidonium majus* root extract after comparing the staining properties of the extract with those of four of its constituent alkaloids. Aniline blue counterstaining efficiently quenched unwanted background fluorescence and nonspecific berberine staining, while providing a fluorochrome for callose. When used with multichambered holders which allow simultaneous processing of freehand sections, this efficient staining procedure facilitates morphological studies involving large numbers of samples.

Keywords: Aniline blue, Berberine, Callose, Casparian bands, Endodermis, Exodermis, Lignin, Suberin.

Abbreviations: ISCC-NBS Inter-Society Color Council—National Bureau of Standards; UV ultraviolet light.

1. Introduction

Many procedures are available for staining lignin and suberin in plant tissues (see JOHANSEN 1940, JENSEN 1962) but the sensitivity of these techniques is often insufficient to detect small quantities of these substances. Fluorescence microscopical techniques are typically much more sensitive than their white light counterparts (see O'BRIEN and MCCULLY 1981) as a result of the enhanced contrast generated when a light object is viewed against a dark field. Casparian bands are examples of structures whose detection has been greatly facilitated by fluorescence microscopy. ELISEI (1941) first reported that an extract from the fleshy roots of *Chelidonium majus* L. contained a substance which acted as a fluorescent stain for Casparian bands. Subsequently WILCOX (1954) and PEIRSON and DUMBROFF (1969) used this extract to stain endodermal Casparian bands and lignin. This natural fluorochrome was also used by PETERSON *et al.* (1982) to identify Casparian bands in the exodermis of corn and onion roots after cell contents and phenolics in adjacent suberin lamellae were removed by clearing with hot alkali. Although their procedure allowed reasonably good visualization of exodermal Casparian bands, the alkali treatment weakened sections and made their further handling and mounting difficult. To avoid this problem, in the present procedure the clearing step has been replaced by aniline blue counterstaining which quenches both background fluorescence and nonspecific staining by the fluorochrome.

Root tissues of *C. majus* contain abundant alkaloids (STAHL and SCHILD 1981) some of which are the likely fluorochromes for suberin and lignin. These chemicals, which include berberine, chelerythrine, sanguinarine and chelidonine (along with other isoquinoline alkaloids), occur in many plant species (PHILOGENE *et al.*1984) providing numerous potential natural dye sources. However, since the composition of plant extracts varies, more consistent results should be obtained by using their active ingredient(s) alone in a staining procedure. We thus compared the staining properties of the alkaloids listed above, to those of *C. majus* extract. In the final recommended procedure, the alkaloid berberine is used to stain fresh sections of plant tissue and aniline blue, which also highlights callose, is used as a counterstain. To assess the general efficacy of this

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procedure, a wide variety of plant tissues were stained and examined.

2. Materials and Methods

2.1. Preparation and Handling of Sections

The following system facilitates the production and handling of numerous freehand sections. Fresh or alcohol-preserved roots were hand-sectioned using a modification of Frohlich's Parafilm sectioning technique (FROHLICH 1984). One coarse or many fine roots were immobilized within folded Parafilm on a plastic surface, then sectioned by drawing the corner of a sharp double-edged razor blade across them repeatedly. The sections produced in this way were transferred through various staining solutions in holders constructed for this purpose, as follows.

Mesh-bottomed, multi-chambered section holders (see Fig. 1) were assembled from 10-mm-long sections of a transfer pipette (or other polyethylene tubing) and nylon screen (50 μm mesh). A number of thin (1–2 mm) rings of tubing were first arranged in a regular pattern on a glass slide, then heated on a hotplate until they began to melt. At this time, a piece of nylon screen just large enough to cover all the rings was placed over them, followed by the tubing segments which were lined up vertically with the melting rings so that they bonded together through the screen (see Fig. 1). We have formed 1-, 4-, and 7-chambered section holders with handles attached to the screen in the same fashion; other configurations are possible. These section holders permit many separate samples to be stained simultaneously and eliminates repeated handling of sections. The holders can be used with solutions contained in shallow vessels, such as Petri dishes, to carry sections through a variety of staining procedures.

2.2. Staining Procedure

I. Transfer freehand sections into holder chambers and stain sections in 0.1% (w/v) berberine hemi-sulphate (Sigma, C.I. no. 75160) in distilled water for 1 hour.

II. Rinse by passing holders through several changes of distilled water; blot excess water from holders after each transfer.

III. Transfer holders to 0.5% (w/v) aniline blue WS (Polysciences, C.I. no. 42755) in distilled water for 30 minutes, then rinse as above.

IV. Transfer holders into 0.1% (w/v) $FeCl_3$ in 50% (v/v) glycerine (prepared by adding glycerine to filtered aqueous $FeCl_3$). After several minutes in this solution, transfer sections to slides and mount in the same solution.

2.3. Alkaloid Comparison and Controls

Chelidonium majus extract, prepared as described by WEERDENBURG and PETERSON (1983), and 0.1% (w/v) ethanol solutions of the alkaloids berberine (Sigma), chelerythrine (Accurate Chemical and Scientific Corp.), sanguinarine (Research Plus Inc.), and chelidonine (ICN Biomedicals Inc.) were used in step I of the procedure given in 2.2 above. The resultant fluorescent staining of suberin in onion root exodermal Casparian bands was compared microscopically under UV excitation. Emission colours of stained Casparian bands were categorized by comparison with ISCC-NBS Centroid Color Charts, standard sample no. 2106 (KELLY 1965). Staining intensities and rates of fading under UV illumination were also compared under standardized conditions.

Hand-sections of onion roots were used to compare combinations of stains. The fluorescence of either *C. majus* extract-stained, berberine-stained or unstained tissues of the endodermis and stele were examined alone or with aniline blue counterstaining. Differences in fluorescence intensity were documented by recording the duration of automatic photographic exposures.

2.5. Microscopy and Photography

Sections were observed using a Zeiss Photomicroscope III and a Zeiss Axiophot microscope, with UV illumination using excitation filter G 365 (365 nm peak emission), chromatic beam splitter FT 395 (395 nm) and barrier filter LP 420 (allowing wavelengths > 420 nm to pass). Most photographs were taken with 100 ASA colour slide film exposed at 50 ASA. These slides were used to make direct colour prints (colour plate) or black and white prints from internegatives. Control micrographs (Figs. 2–7) were taken with 32 ASA black and white negative film and printed at equal contrast. The $FeCl_3$ mountant prevented destaining of sections for up to 1 day, but best results were obtained when photographs were taken within a few hours of staining.

3. Results and Discussion

3.1. Aniline Blue Counterstaining Properties

A wide variety of biological stains was compared for effectiveness as quenching agents of background fluorescence and nonspecific staining by *Chelidonium majus* root extract. Aniline blue proved to be the most suitable for this purpose. A series of controls was per-

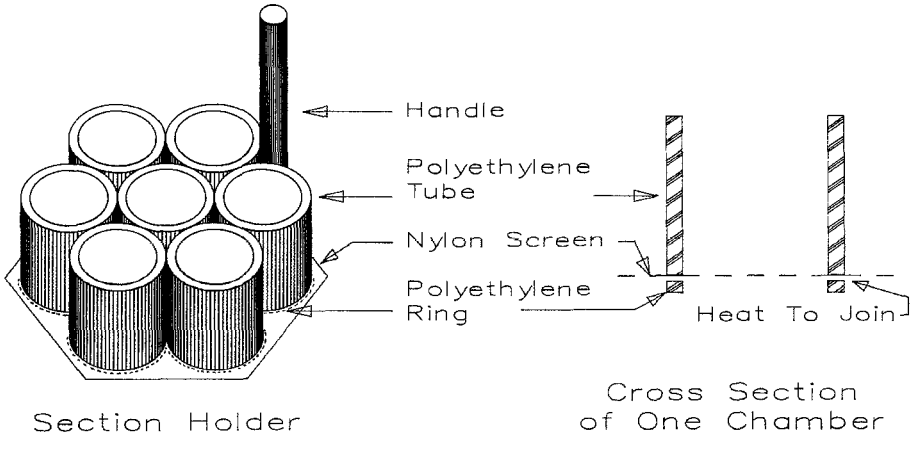
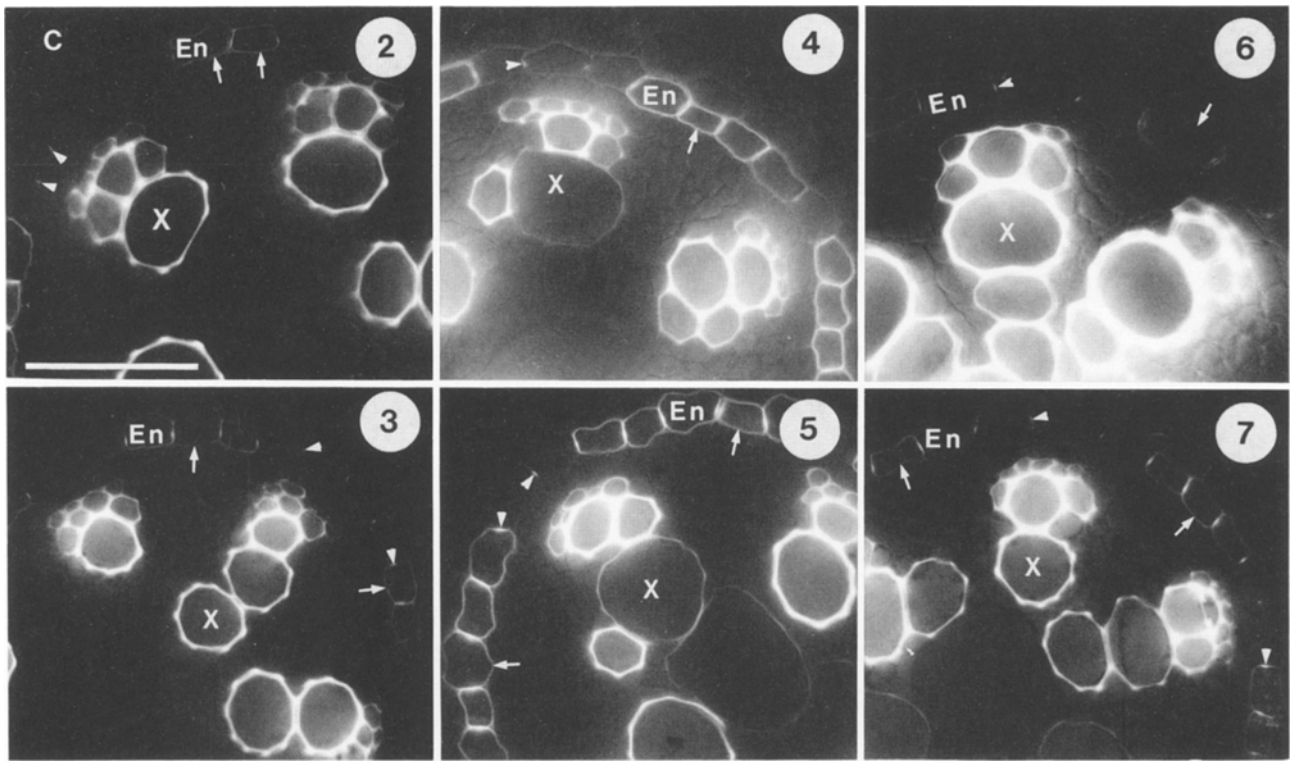


Fig. 1. Sample holder constructed by joining polyethylene rings, tubing and nylon screen together (polyethylene tubes are approx. 10 mm high)



Unless otherwise stated, all micrographs are of fresh freehand cross-sections stained with the berberine-aniline blue procedure and viewed with UV light (see text for details). *Abbreviations: Ep* epidermis, *Ex* exodermis, *C* cortex, *En* endodermis, *P* phloem, *X* xylem, ▶ Casparian bands, ➡ suberin lamellae. All scale markers = 100 μm

Figs. 2–7. Control photomicrographs illustrating the effect of aniline blue counterstaining on unstained, *Chelidonium majus* extract-stained, and berberine-stained onion root hand-sections. Photographs were taken under uniform conditions and printed at the same contrast and magnification. Photographic exposure times (s) are indicated for each figure

Fig. 2. Autofluorescence of the endodermis and xylem of an unstained section (16.6 s)

Fig. 3. Influence of aniline blue staining alone on autofluorescence. Note endodermal Casparian bands in cells with suberin lamellae (19.4 s)

Fig. 4. *C. majus* extract staining induces bright fluorescence, especially of the xylem and endodermis (6.8 s)

Fig. 5. *C. majus* staining with aniline blue counterstaining, which reduces the fluorescence of xylem and suberin lamellae, allowing endodermal Casparian bands to be seen (11.5 s)

Fig. 6. Berberine staining causes intense xylem fluorescence which overwhelms emissions from other tissues (6.1 s)

Fig. 7. Aniline blue counterstaining reduces berberine-induced fluorescence of lignin and suberin lamellae, revealing endodermal Casparian bands (11.8 s)

formed to document the counterstaining ability of aniline blue (Figs. 2–7). When completely unstained onion root sections are examined with UV light, tracheary element walls and endodermal Casparian bands and suberin lamellae autofluoresce (Fig. 2). Aniline blue staining of such a section slightly reduces the autofluorescence of the tracheary elements (Fig. 3). Suberin lamella autofluorescence was also partially quenched allowing Casparian bands to be seen in endodermal cells containing both these structures (Fig. 3). These bands are harder to distinguish by autofluorescence alone (Fig. 2). Staining with *C. majus* extract induces the most intense fluorescence in xylem cell walls (Fig. 4). Aniline blue counterstaining reduced this xylem fluorescence as well as suberin lamella fluorescence, allowing visualization of endodermal Casparian bands (Fig. 5). Staining with berberine causes fluorescence of the xylem which is so intense that it is hard to record staining of endodermal suberin in the same exposure (Fig. 6). Aniline blue counterstaining tames this excessive xylem fluorescence and reduces the intensity of suberin lamellae, but not that of endodermal Casparian bands (Fig. 7). Both *C. majus* extract and berberine stain suberized and lignified walls brightly and also

stain cell nuclei, cytoplasm and unmodified walls to a lesser degree. Fluorescence from the latter three has not registered in Figs. 4 and 6 because of the relatively short photographic exposure times dictated by the bright fluorescence of the xylem. Aniline blue quenches this weak staining so that it is not evident in Figs. 5 and 7 which had longer exposure times (see legends for Figs. 4–7). Alkaloid staining of lignified cell walls is partially quenched by aniline blue, especially in immature xylem cells which also absorbed less stain, showing stages in xylem formation (Figs. 5 and 7).

Aniline blue contains a powerful fluorochrome which stains callose in sieve elements, wound sites, etc. (CURRIER 1957, EVANS *et al.* 1984). Some newer batches of aniline blue have been purified to the point of removing this fluorochrome. It is now available in a purified form (EVANS and HOYNE 1982) and could be added to highly purified aniline blue if callose staining is required. The high concentration of aniline blue used as a counterstain (100 times that used in other callose staining procedures) and the acidic pH of the $FeCl_3$ mountant did not adversely affect callose staining. Aniline blue was used as a counterstain in all subsequent procedures, including the alkaloid fluorochrome comparisons and the survey of staining patterns in the various plant organs described below.

3.2. Alkaloid Staining Properties

The staining of onion root sections by crude *C. majus* extract and four of its component alkaloids was compared (Table 1). The crude extract induced bright yellow-orange fluorescence of exodermal Casparian bands. The individual alkaloids berberine, chelerythrine and sanguinarine had similar staining properties, producing varying hues of yellow or orange with intensities comparable to that of the *C. majus* extract (Table 1). The fourth alkaloid, chelidonine, exhibited no fluorescent staining properties (Table 1).

Table 1. *Comparison of the staining properties of Chelidonium majus root extract with those of four of its component alkaloids*

Stain	Emission colour	Rate of fading (s)
C. majus extract	very orange yellow (66)	30–60
Berberine	vivid yellowish green (97)	30–60
Chelerythrine	vivid yellow (82)	30
Sanguinarine	brilliant orange yellow (67)	20
Chelidonine	no staining	—

Standardized emission colours (and corresponding numbers) of stained onion root exodermal Casparian bands under UV excitation were determined by comparison with ISCC-NBS Centroid Color-name Charts. Approximate rates of fading to autofluorescence levels were also determined.

The intense initial fluorescence produced by staining with either crude *C. majus* root extract or a purified alkaloid faded during continuous exposure to UV light. Sanguinarine was most susceptible to fading, followed by chelerythrine, while berberine and the crude extract were most resistant (Table 1). Berberine was chosen as the most suitable replacement for *C. majus* extract as a stain for plant tissues because it had comparable staining properties and resistance to photobleaching.

3.3. Survey of Plant Tissues

Roots of numerous species were examined. The berberine-aniline blue procedure proved to be a rapid and precise method of visualizing lignified cell walls, Casparian bands, suberin lamellae and callose. In general, lignified walls stained bright yellow, Casparian bands in the endodermis and exodermis stained intense yellow-white and suberin lamellae in the endodermis and

Fig. 8. Fluorescent staining of onion root epidermal and exodermal cell walls. The latter have Casparian bands and suberin lamellae

Fig. 9. Autofluorescence of the epidermis and exodermis in an unstained onion root

Fig. 10. Thick, oblique onion root section showing wavy Casparian bands in radial exodermal and endodermal walls

Fig. 11. *Arisaema atrorubens* stele with berberine-stained xylem and aniline blue-stained phloem sieve plates. At this stage, endodermal cells have Casparian bands and unilateral suberin lamellae

Fig. 12. *Asparagus officinalis* root endodermis and stele. Sieve plate callose fluoresces blue-white, while lignified walls of tracheary elements and associated cells are yellow-blue

Fig. 13. Pine needle with berberine-stained stomata (*St*), lignified cells bordering resin ducts (*R*), endodermal Casparian bands and xylem

Fig. 14. Unstained pine needle. Note predominant red chlorophyll fluorescence and Casparian bands

Fig. 15. *Tradescantia navicularis* stem with Casparian bands in an endodermal layer surrounding a vascular bundle

Fig. 16. Unstained *T. navicularis* stem showing weak autofluorescence of Casparian bands, suberin lamellae and xylem, which can be faintly seen against a red background of chlorophyll fluorescence

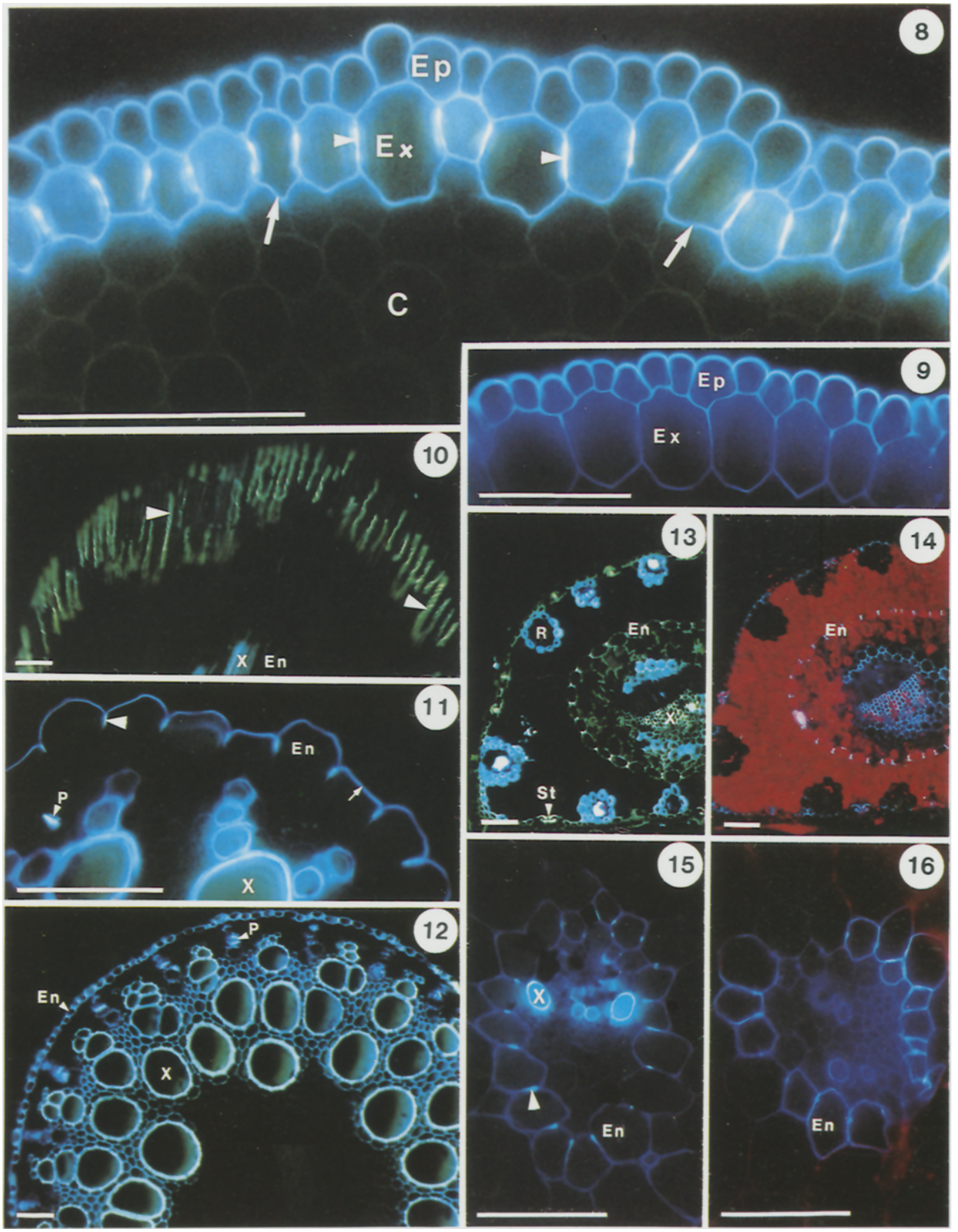


Fig. 8–16

exodermis a reduced blue-white or blue. Some of these staining patterns are illustrated by the onion root epidermis and exodermis (Fig. 8). Onion epidermal cell walls contain phenolics and diffuse suberin (PETERSON *et al.* 1978) that are autofluorescent (Fig. 9). These walls are stained by berberine but are partially quenched by aniline blue (Fig. 8). Walls of the cortical parenchyma cells are usually not autofluorescent (Fig. 9) and any berberine staining of them is almost totally quenched by aniline blue (Fig. 8). The fluorescence of the suberin lamellae in the unstained exodermis is faint (Fig. 9), but becomes more intense after staining (Fig. 8). Berberine-aniline blue induced the most intense fluorescence in exodermal cell Casparian bands (Figs. 8, 17, and 21), structures that cannot be distinguished by autofluorescence in an unstained section (Fig. 9). Exodermal and endodermal Casparian bands are also apparent as bright, wavy lines in a thick, oblique section (Fig. 10).

The berberine-aniline blue staining technique allowed different components of the endodermis and stele of roots to be readily identified. In *Arisaema atrorubens* (Ait.) Blume, Casparian bands can be seen as bright dots in the radial walls of endodermal cells (Fig. 11). Suberin lamellae have developed on the outer but not the inner tangential walls of this layer (Fig. 11). In a section of an *Asparagus officinalis* L. root, the stele is bounded by an endodermis in which all cells have a Casparian band and a suberin lamella and the majority have thickened, lignified or suberized internal walls (Fig. 12). As in onion, the lignified walls of the tracheary elements stain yellowish white, but fluorescence of the lignified walls of xylem parenchyma cells has been partially quenched by aniline blue. Callose in the sieve tubes of phloem cells fluoresces blue (Figs. 11 and 12). In the central region of a young corn (*Zea mays* L.) root, berberine has stained the endodermal Casparian bands and the lignified walls of the pericycle and protoxylem elements (Fig. 19). Note the large phloem sieve plates revealed by callose staining.

The berberine-aniline blue staining protocol can also be used for highlighting lignin and suberin in aerial plant organs. In a pine (*Pinus sylvestris* L.) needle, sclerified structures, including the epidermis with its sunken stomata and cells surrounding the resin ducts are stained (Fig. 13). Casparian bands surrounding the vascular and transfusion tissue are also clearly evident following staining (Fig. 13). In this case, the Casparian bands of the endodermis are also visible due to their autofluorescence (see unstained section Fig. 14). The predominant red fluorescence due to chlorophyll in the unstained pine needle (Fig. 14) is completely masked by aniline blue counterstaining (Fig. 13). Using the new procedure, Casparian bands were also found in the endodermal cells surrounding individual vascular bundles in the stems of several members of the *Commelinaceae*, including *Tradescantia navicularis* Ortg. (Fig. 15). In this species, the bands are much less conspicuous when observed without staining (Fig. 16). Although there have previously been conflicting reports on the presence of Casparian bands in the stems of members of the *Commelinaceae* (TOMLINSON 1969), berberine-aniline blue staining clearly demonstrated their presence in several members of this family.

Use of the staining procedure presented here has revealed previously unobserved details of exodermal Casparian band structure. In onion roots, cells in some regions have two Casparian bands, one near the outer edge of the radial wall and the other near the inner edge (Fig. 17). Such double Casparian bands were also

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Fig. 17. Onion root exodermis with double Casparian bands in its radial walls and suberin lamellae

Fig. 18. *Lilium* sp. exodermis with pronounced double Casparian band in transverse walls. Inset shows details of three exodermal cells; transverse walls are visible in two. The central cell has a double band and the cell on the right has a single, wide band (*)

Fig. 19. Endodermis and stele of a young corn root with endodermal Casparian bands, adjacent lignified pericycle (*Pe*), protoxylem, and large phloem sieve plates (*P*)

Fig. 20. *Cattleya aurantiaca* aerial root. Berberine staining reveals outer velamen layers (*V*), an intensely stained exodermis with passage cells (*Pc*), cortical phi thickenings (Φ) and idioblast cells with suberin lamellae. The walls of most cells in the endodermis and stele are heavily lignified

Fig. 21. Exodermal and endodermal Casparian bands in a young corn root

Fig. 22. Aerial root of *Monstera deliciosa*. Cells adjacent to the wound have undergone an extensive wound response (*W*). Note heavily lignified sub-exodermal sclerenchyma layer (S), trichosclereids (*T*) scattered throughout cortex, and brachysclereids (*B*) near the endodermis with Casparian bands

Fig. 23. *Asparagus setaceous* root with a multiple epidermis and biseriate exodermis. Cells surrounding an emerging lateral root are highly fluorescent. Lignified walls of the endodermis, xylem and stellar parenchyma stain intensely

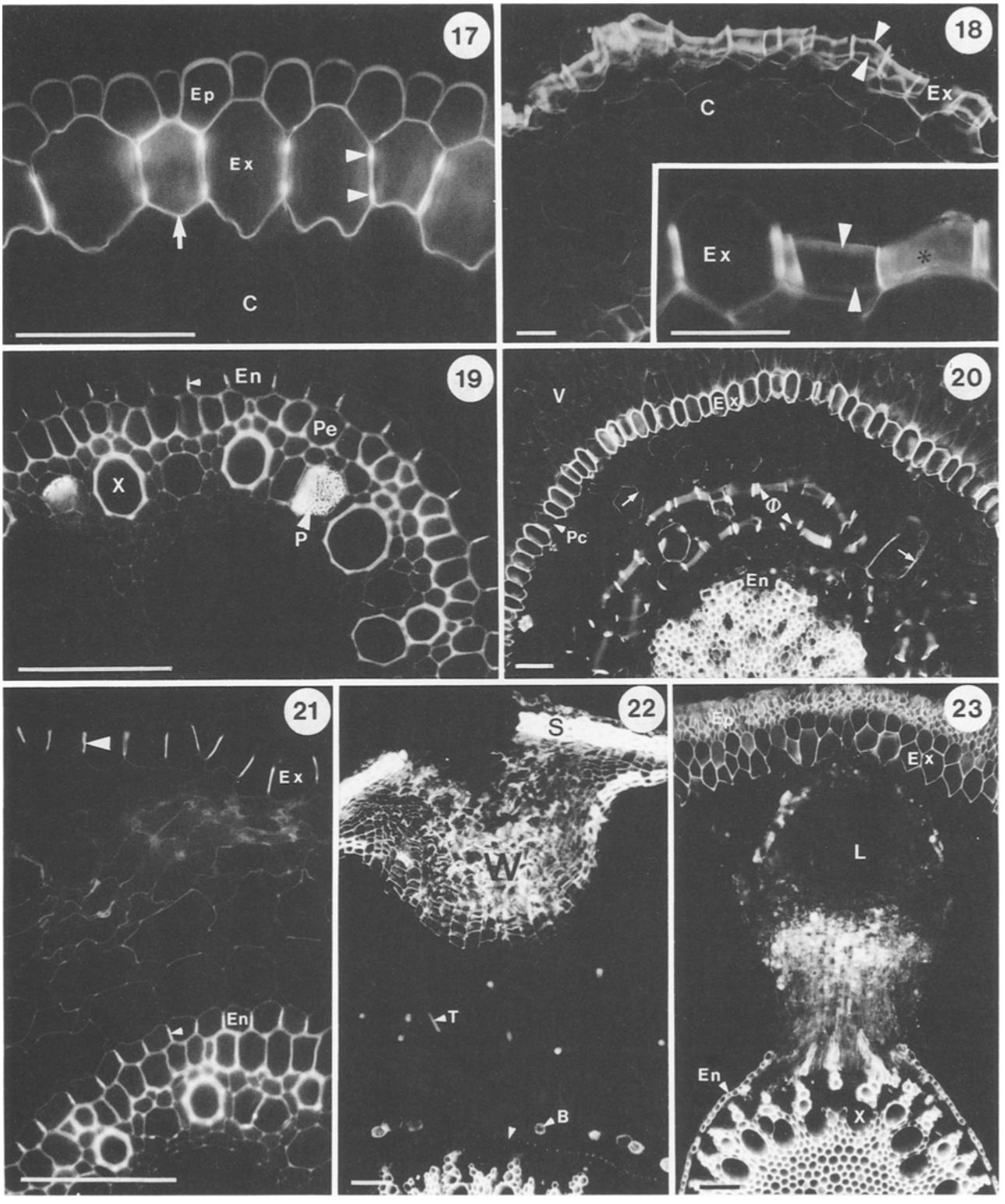
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Fig. 17–23

observed in the exodermis of *Lilium* sp. The section in Fig. 18 includes many transverse exodermal walls with double Casparian bands which appear as paired fluorescent lines. The inset illustrates variations in Casparian band structure which can occur in adjacent cells. One cell has two separate bands in its exodermis but is neighbour to the right has only one thick band.

The new staining procedure also proved useful in deciphering many aspects of the structure of roots with complicated anatomy. For example, aerial roots of the orchid *Cattleya aurantiaca* (Batem.) P. Don had a lightly stained velamen and an exodermis with very thick, intensely fluorescent walls except at passage cells (Fig. 20). The cortex of this root possessed many idioblast cells (containing raphide crystals) with suberin lamellae in their walls, and cells with irregular phi-thickenings. With the exception of phloem and passage cells, the endodermis and stele were very heavily lignified (Fig. 20). *Monstera deliciosa* Liebm. roots had a great variety of lignified and suberized structures (Fig. 22). These included a sclerenchymatous sub-exodermal layer, cortical trichosclereids and brachysclereids, as well as endodermal and xylem cells (see FRENCH 1987). The walls of cells involved in a wound reaction also fluorescence brightly. In an *Asparagus setaceous* (Kunth) Jessop adventitious root, the staining procedure, highlighted the multilayered epidermis and exodermis, the endodermis, stele and cells (probably containing secondary metabolites) surrounding a developing lateral root (Fig. 23).

3.4. Comments on the Staining Procedure

The fluorescent alkaloid, berberine, is relatively non-specific in its staining properties. It has been used to stain a variety of structures including heparin and DNA in animal cells (ENERBACK 1974) and cellulosic walls in plant cells (KARABESTOS *et al.* 1987). Berberine has several excitation bands in the UV and blue parts of the spectrum and a broad emission peak around 550 nm (ENERBACK 1974). We have found that UV excitation provides the best results with our staining procedure, since the corresponding barrier filter allows more of the visible spectrum to pass than those used with blue or violet excitation. This provides greater colour contrast between partially counterstained (blue-yellow) and berberine-stained (yellow) structures in the resulting images.

UV-induced fading prevented individual sections from being examined or photographed more than once at a high magnification. Fading rates are likely to be influenced by light intensity, wavelength and fluorochrome concentration. Longer wavelengths of exciting illumination were not used to reduce berberine fading because they reduced image contrast. Antioxidants such as paraphenylenediamine, n-propyl gallate and 1,4-diazobicyclo(2,2,2)-octane which have been successfully used to prevent fading of other fluorochromes (JOHNSON *et al.* 1982, VALNES and BRANDTZAEG 1985) did not work with berberine (unpublished results), perhaps because they did not penetrate the stained walls. Fading can be minimized by composing and focusing images with white light before switching to UV excitation.

ENERBACH (1974) reported rapid fading of heparin-bound, but not DNA-bound, berberine with UV illumination and PHILOGENE *et al.* (1984) found that berberine broke down in the presence of UV light to generate singlet oxygen. This photoactivity probably explains the fading of berberine fluorescence which we observed in stained tissue. The DNA-binding and singlet oxygen-generating properties of berberine suggest that it should be treated as a potential carcinogen, as should many other biological stains (COMBES and HAVELANT-SMITH 1982).

Berberine and *C. majus* extract have a higher affinity for cell walls containing bound phenolic components in the form of lignin or suberin than for unmodified walls. However, some nonspecific staining of cell contents and cellulosic walls also occurs at the concentrations required for efficient wall penetration. Thus, specific localization of suberin and lignin in plant tissues is enhanced by aniline blue counterstaining. Aniline blue, when used at high concentrations and mounted in $FeCl_3$, apparently reacts with excess fluorochrome (present in solution and bound to cytoplasmic components) to form a nonfluorescent complex. Aniline blue also quenches much of the primary fluorescence of plant tissues including that produced by chlorophyll and many secondary metabolites. The particular value of aniline blue in the present procedure is that it selectively quenches the fluorescence of berberine in cell walls. Berberine staining of cellulosic walls is completely quenched while walls containing different amounts of suberin and lignin remain fluorescent to a degree apparently correlated with their permeability. Thus, the quenching of immature (incompletely lignified) xylem vessel walls is greater than that of fully lignified walls. The degree of quenching of suberin lamellae is also greater than that of Casparian bands (especially if the former are relatively thin). The quenching of a particular wall component can be controlled somewhat by varying the duration of section treatment with aniline blue.

Berberine-aniline blue staining obtained in the present study is in agreement with the results of many histochemical studies including those of PETERSON *et al.* (1978) and WILSON and PETERSON (1983) in which suberin and lignin were localized in the roots of onion and other species. Conventional histochemical procedures stain suberin in the form of Casparian bands or suberin lamellae, but fail to distinguish between them. Consequently, Casparian bands cannot be detected if they are masked by suberin lamellae, as they are in the State II endodermis or exodermis. In the present study, partial quenching of berberine-stained suberin lamellae by aniline blue allowed Casparian bands in these cell types to be discerned.

Unfortunately, the differences in cell wall permeability required for this staining procedure are lost during the fixation, dehydration and infiltration processes used to embed plant tissue in plastic. Aniline blue cannot be used as a counterstain for berberine in epoxy or methacrylate embedded sections even after removal of the plastic with epoxy solvents.

When sections stained with berberine and aniline blue were mounted in glycerine, they destained rapidly. Many different mounting media were tested but only glycerine containing $FeCl_3$ inhibited destaining of both berberine and aniline blue (unpublished results). This mountant preserved differential staining, allowing sections to be observed hours after mounting.

3.5. Conclusions

1. The fluorochrome, berberine, a commercially available alkaloid, can be used as a substitute for a crude extract from the roots of *Chelidonium majus* to stain lignin and suberin in plant tissues.

2. Indiscriminate berberine staining of many cellular components, *e.g.*, unmodified cellulosic walls, nuclei and ground cytoplasm, can be quenched by counterstaining with aniline blue. Partial quenching of the fluorescence of weakly lignified walls and suberin lamellae occurs, allowing the investigator to distinguish between weakly and strongly lignified walls, and between lamellar and incrusted (Casparian band) suberin. This counterstaining method offers several advantages over a previous method which involved clearing in hot alkali.

3. The berberine-aniline blue staining procedure is efficient, especially when multichambered section holders are used to transfer many separate freehand sections through the staining series simultaneously. It thus should be of great value to developmental investigations or experiments involving correlation of anatomical and physiological data.

4. The berberine-aniline blue procedure is especially valuable for staining exodermal Casparian bands. These structures, which are located next to the root epidermis in many plants, block the apoplastic (cell wall) passage of substances (see PETERSON 1988). Unfortunately the physiological role of exodermal Casparian bands has often been neglected, perhaps because they could not easily be detected. As well as expediting their detection, the new staining procedure revealed previously unobserved details of Casparian band structure.

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