Occurrence and Location of Suberin in Wound Reaction Zones in Xylem of 17 Tree Species

A. R. Biggs

Research scientist, Agriculture Research Station, Vineland Station, Ontario, Canada L0R 2E0.
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ABSTRACT

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The occurrence and location of suberin in xylem tissues following mechanical wounding were determined using phloroglucinol HCl in conjunction with ultraviolet fluorescence microscopy. In all species examined, thin intracellular suberin lamellae were detected in ray and/or axial parenchyma present at the time of wounding and located approximately 0.8–1.2 mm centripetal to the wound surface. Suberized xylem parenchyma was associated with a visibly discolored wound reaction zone in which xylem vessels and fibers were often found plugged with amorphous phloroglucinol-positive material or occluded by tyloses. In *Castanea dentata, Hamamelis virginiana, Prunus avium, Quercus rubra, Salix nigra,* and *Ulmus americana*, tyloses were suberized. The location of suberin in xylem ray and axial parenchyma and in vessels corresponds to walls 1, 2, and 3 of the CODIT model sensu Shigo and Marx (26). However, the suberized cells per se did not form continuous "walls." Instead, cell distribution appeared discontinuous, and often suberin was restricted only to occasional cells. The normal distribution of parenchyma in nonwounded tissue precluded formation of distinct, continuous suberized boundaries following wounding. However, in most species, continuous boundaries were formed from conjoined suberized parenchyma cells and vascular elements impregnated or occluded with phloroglucinol-positive materials or tyloses.

Additional key words: *Acer negundo, Acer saccharum, Betula papyrifera, Carya cordiformis, Fraxinus americana, Morus rubra, Ostrya virginiana, Pinus strobus, Prunus persica, Prunus serotina, Tsuga canadensis.*

Injuries to bark and xylem of woody plants usually incite formation of wound reaction zones that are formed from cells present at the time of injury (15,25). Anatomy of these reaction zones has been described in detail for bark injuries (3–6,15). Investigations using light and ultraviolet epifluorescence microscopy in conjunction with histochemical methods have demonstrated the ligno-suberized nature of the cell walls in this tissue. Ultrastructural evidence for the occurrence of intracellular suberin lamellae in bark reaction zones has been presented recently (7).

Reaction zones in xylem are thought to be necrotic tissues that form after wounding or in advance of infection (25). These tissues are rich in extractives inhibitory to wood-rotting fungi. Their formation has been associated with a dynamic nonspecific mechanism of host resistance to fungi (25) and insects (2). The extractives may play a dual role in the reaction zone. In addition to their inhibitory role, these compounds and other nonextractable materials may function to make the tissue impervious to apoplastic water movement (9,14,15). In bark, resistance to fluid diffusion was associated with suberin lamellae on the inner wall surface of lignified cells (5). It seems reasonable to suspect that xylem parenchyma could respond similarly to form suberin lamellae in cells associated with the reaction zone. Suberin is thought to play several roles in wounded tissues, including toxicity of the phenolic constituent to microorganisms; barrier to outward fluid diffusion, thus preventing tissue desiccation; and barrier to inward diffusion of fungal enzymes, thus inhibiting pathogenesis (12).

This paper presents the results from an examination of twig responses to mechanical wounding and location and extent of cell suberization in wounded xylem of 17 tree species.

MATERIALS AND METHODS

Mechanical wounds were made in the field on 2- to 5-yr-old branches of *Acer negundo* L., *A. saccharum* Marsh., *Betula papyrifera* Marsh., *Carya cordiformis* (Wangenh.) K. Koch, *Castanea dentata* (Marsh.) Borkh., *Fraxinus americana* L., *Hamamelis virginiana* L., *Morus rubra* L., *Ostrya virginiana* (Mill.) K. Koch, *Pinus strobus* L., *Prunus avium* (L.) L., *P. persica* (L.) Batsch, *P. serotina* Ehrh., *Quercus rubra* L., *Salix nigra* Marsh., *Tsuga canadensis* (L.) Carr., and *Ulmus americana* L. All branches were about 5–10 mm in diameter. Four branches on each of two trees per species were wounded by applying pressure on a No. 2 cork borer (4 mm in diameter) in a twisting motion against the branch until the bark tissues separated easily from the underlying xylem. Care was taken not to injure the xylem deeper than the exposed surface. Wounded branches were removed for histological study at 7, 10, 14, and 21 days after wounding, trimmed to remove excess nonwounded tissues (to about 5 mm above and below the wound), halved radially and quartered longitudinally through the wound, then fixed in the field immediately in formalin/acetic acid/alcohol (FAA). After about 7 days' fixation, specimens were dehydrated in a *t*-butyl alcohol series (11), embedded in paraffin (4), softened (aqueous solution of glycerol [10% v/v] and sodium lauryl sulfate [1% w/v] [1]), and sectioned at 8 μm on a rotary microtome. Nonwounded control branches were collected and prepared in a similar manner. The experiment was repeated three times (8 May, 11 June, and 16 July 1985).

Suberin in xylem was detected using the autofluorescence quenching technique (3,5). Briefly, tissues mounted on slides were cleared of paraffin in two rinses of xylene, hydrated in a graded ethanol series, and mounted in phloroglucinol HCl (10). Tissues were allowed to react for 5 min, excess reagent was blotted from the slide with bibulous paper, and the sections were examined under bright field for deposition of phloroglucinol-positive material. Tissues were then examined for residual autofluorescence (suberin) using Leitz filter combination A (340- to 380-nm excitation and 430-nm barrier filters). Serial sections of tissues were treated with Sudan black B, which stained suberin blue in bright field and quenched suberin autofluorescence. Autofluorescence photomicrographs were recorded on Kodax Tri-X Pan and Plus-X Pan black and white film.

RESULTS

The response to wounding, or the deposition of suberin lamellae on the inner cell wall surface of different xylem cell types for the 17

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species examined, is summarized in Table 1. Brief descriptions of healthy xylem and the minute anatomy of xylem parenchyma distribution (using the terminology of Panshin and de Zeeuw [19]) are accompanied by specific descriptions of location and pattern of lamellar suberin deposition after wounding. Most suberized cells were limited to the wound reaction zone.

Acer negundo. Parenchyma in nonwounded xylem was sparse and restricted to occasional cells. Parenchyma cell locations were marginal, paratracheal-scanty, and apotracheal-diffuse. After wounding, suberin lamellae were visible in the xylem ray parenchyma of the previous year, the apotracheal and paratracheal parenchyma, and occasional vessels (Fig. 1). Lamellar suberin was not associated with mature, differentiated fibers or tracheids. Incompletely differentiated fibers near the wound surface often appeared suberized immediately centripetal to new callus tissue.

A. saccharum. Location and distribution of xylem parenchyma were similar to those described for *A. negundo*. After wounding, suberin lamellae were observed in all axial parenchyma cell types of the previous year's xylem and in occasional xylem ray parenchyma. Vessels lined with suberin were also observed.

Betula papyrifera. Xylem parenchyma was generally apotracheal-diffuse and in aggregates, paratracheal, and marginal. Birch xylem parenchyma formed lamellar suberin in all parenchyma cell types (except marginal) after wounding. Xylem vessel elements appeared lined with a material that retained its autofluorescence following phloroglucinol HCl (Fig. 2), was colored blue with Sudan black B (Fig. 3A), and exhibited quenched autofluorescence when treated with Sudan black B and examined under ultraviolet excitation (Fig. 3B). These xylem vessel linings appeared bright pink rather than violet when treated with phloroglucinol HCl and examined under ultraviolet excitation. In addition, some vessels in the reaction zone were partially occluded with amorphous deposits that were faintly autofluorescent (beige), phloroglucinol-negative, and Sudan-positive.

Carya cordiformis. Parenchyma in xylem appeared apotracheal-diffuse and in aggregates and marginal. Scanty paratracheal parenchyma was also observed. Lamellar suberin was observed in paratracheal parenchymatous cells, although it was not detected in ray parenchyma. Suberized parenchyma and vessels were observed in both wounded (Fig. 4) and nonwounded tissues.

Castanea dentata. Xylem parenchyma was generally paratracheal-scanty (sparse) and apotracheal-diffuse (more abundant in latewood than in spring). Following wounding, suberin lamellae were observed in ray and paratracheal parenchyma and in xylem vessel elements (Fig. 5). Xylem ray cell suberization included about three to four cells in the transverse orientation in the outer 1983 xylem.

Fraxinus americana. In healthy xylem, parenchyma was paratracheal-vasicentric and/or paratracheal-aliform to confluent with ray parenchyma in the outer latewood, and marginal. Xylem parenchyma beneath the wound was highly autofluorescent following phloroglucinol HCl, although the color (beige) and location (cell lumen) of the autofluorescence did not correspond to suberin. Suberized cells were limited to ray, paratracheal, and marginal parenchyma and tissues of the primary xylem (Fig. 6). Suberin was not observed in fibers, tracheids, or vessels of the secondary xylem.

Hamamelis virginiana. In nonwounded xylem, parenchyma was paratracheal-vasicentric, apotracheal-diffuse to banded, and marginal. Marginal parenchyma was not suberized. After wounding, a continuous band of suberized tissue was observed in springwood (which was comprised mostly of vessels and parenchyma) in the reaction zone. The tissue included suberin-lined vessels, suberized tyloses, suberized ray, and apotracheal and paratracheal parenchyma (Fig. 7).

Morus rubra. Xylem parenchyma was paratracheal-vasicentric, abundant, and conjoining with ray parenchyma. Sparse apotracheal-diffuse cells were also present. Following wounding, suberin lamellae were observed in ray, apotracheal, and paratracheal parenchyma (Fig. 8). Vessels were lined with an orangish (under fluorescence) material that retained its autofluorescence following phloroglucinol HCl treatment.

Ostrya virginiana. Healthy, nonwounded xylem was characterized by sparse marginal parenchyma and diffuse-to-banded apotracheal parenchyma. After wounding, lamellar suberin was observed in apotracheal and marginal parenchyma, and occasional vessels appeared lined with a suberinlike material similar to that described for *B. papyrifera* (Fig. 9).

Prunus avium, P. persica, **and** ***P. serotina.*** All *Prunus* spp. were similar in appearance and contained very sparse xylem parenchyma confined to occasional cells. Xylem suberin was limited to lamellar depositions in ray parenchyma one to two cells deep in the transverse orientation and about 800 μm centripetal to the wound surface (Fig. 10). A single suberized tylosis was observed in *P. avium*.

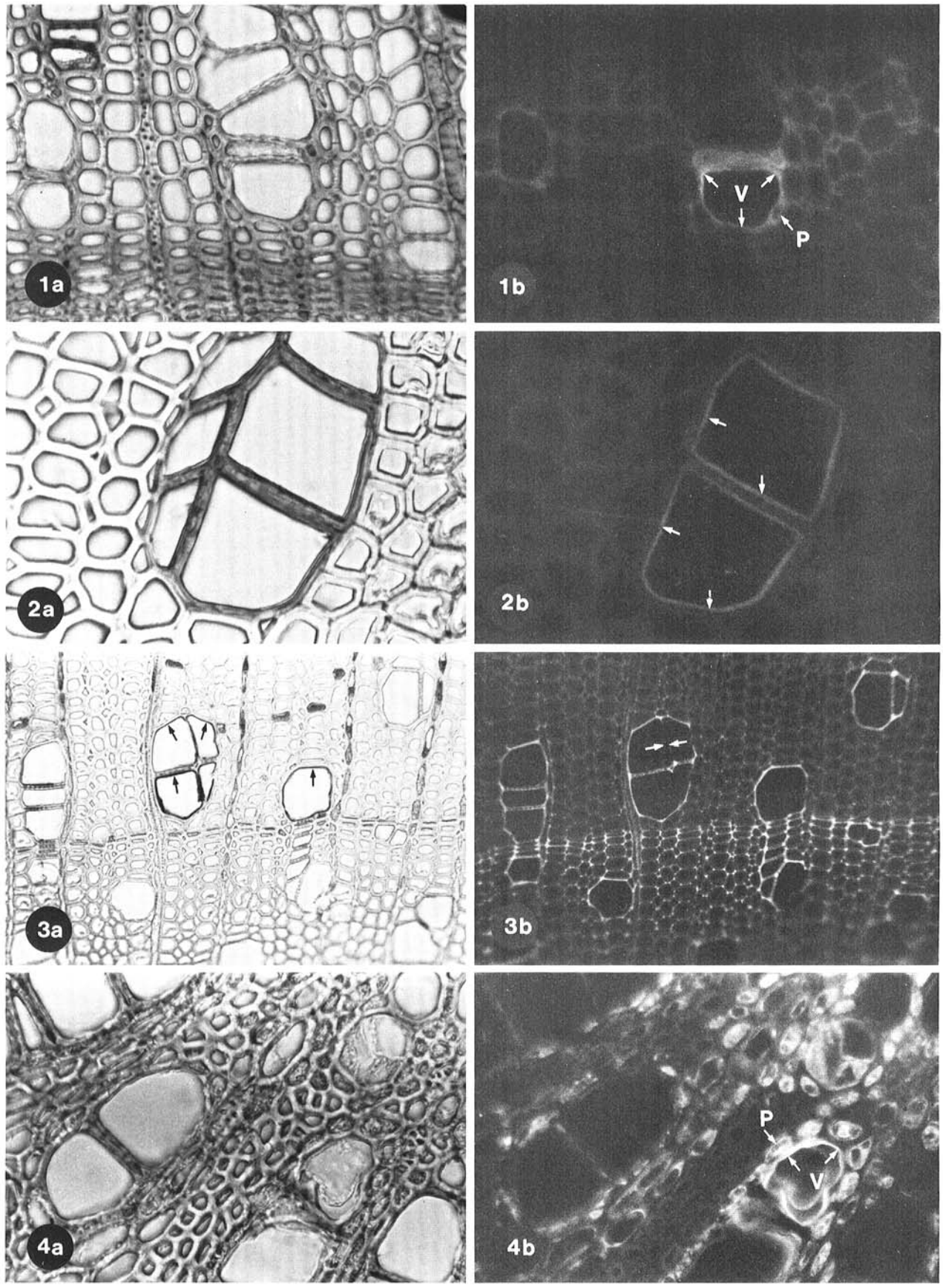
Quercus rubra. In healthy xylem, parenchyma was abundant, paratracheal, and apotracheal-diffuse with a tendency toward aggregation into concentric lines of banded parenchyma. Following wounding, all parenchyma cell types appeared suberized. Banded apotracheal parenchyma was especially noticeable (Fig. 11). Paratracheal parenchyma often formed suberized tyloses in adjacent vessel elements (Fig. 12). Incompletely differentiated fibers or tracheids formed in 1985 before wounding appeared suberized just centripetal to callus tissue.

Salix nigra. In nonwounded tissue, parenchyma was mostly

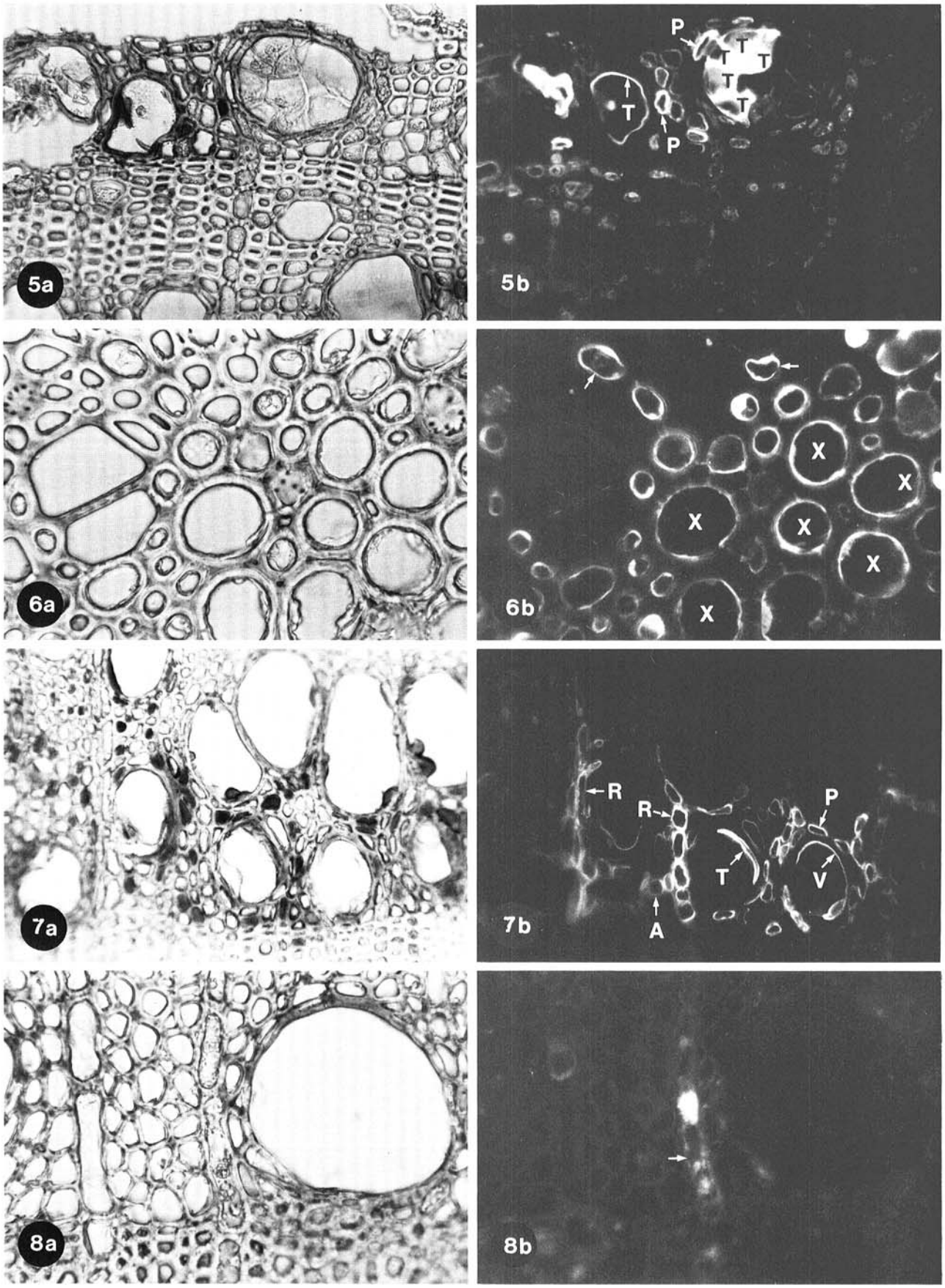
TABLE 1. Location of suberized cells in primary xylem, secondary xylem present at time of wounding, and pith 21 days after mechanical wounding of twigs of 17 tree species

	Parenchyma				Conductive/supportive elements			Occlusions	
Species	Ray	Apotracheal	Paratracheal	Marginal	Vessels	Fibers/Tracheids	Primary xylem	Tyloses/tylosoids	Pith
Acer negundo	++[a]	++	++	—	++	++	++	—	—
A. saccharum	++	++	++	++	++	—	++	—	—
Betula papyrifera	++	++	++	—	++	—	—	—	—
Carya cordiformis	—	—	++	—	++	—	—	—	++
Castanea dentata	++	—	++	—	++	—	—	++	—
Fraxinus americana	++	NA	++	++	—	—	++	—	—
Hamamelis virginiana	++	++	++	—	++	—	++	++	—
Morus rubra	++	++	++	NA	—	—	—	—	—
Ostrya virginiana	—	++	NA	++	++	—	—	—	—
Pinus strobus	++	NA	NA	NA	NA	—	++	—	++
Prunus avium	++	NA	NA	NA	—	—	—	++	—
P. persica	++	NA	NA	NA	—	—	—	—	—
P. serotina	++	NA	NA	NA	—	—	—	—	—
Quercus rubra	++	++	++	NA	++	++	++	++	++
Salix nigra	++	NA	++	++	++	++	—	++	—
Tsuga canadensis	++	NA	NA	++	NA	++	++	—	++
Ulmus americana	—	—	++	—	++	—	—	++	—

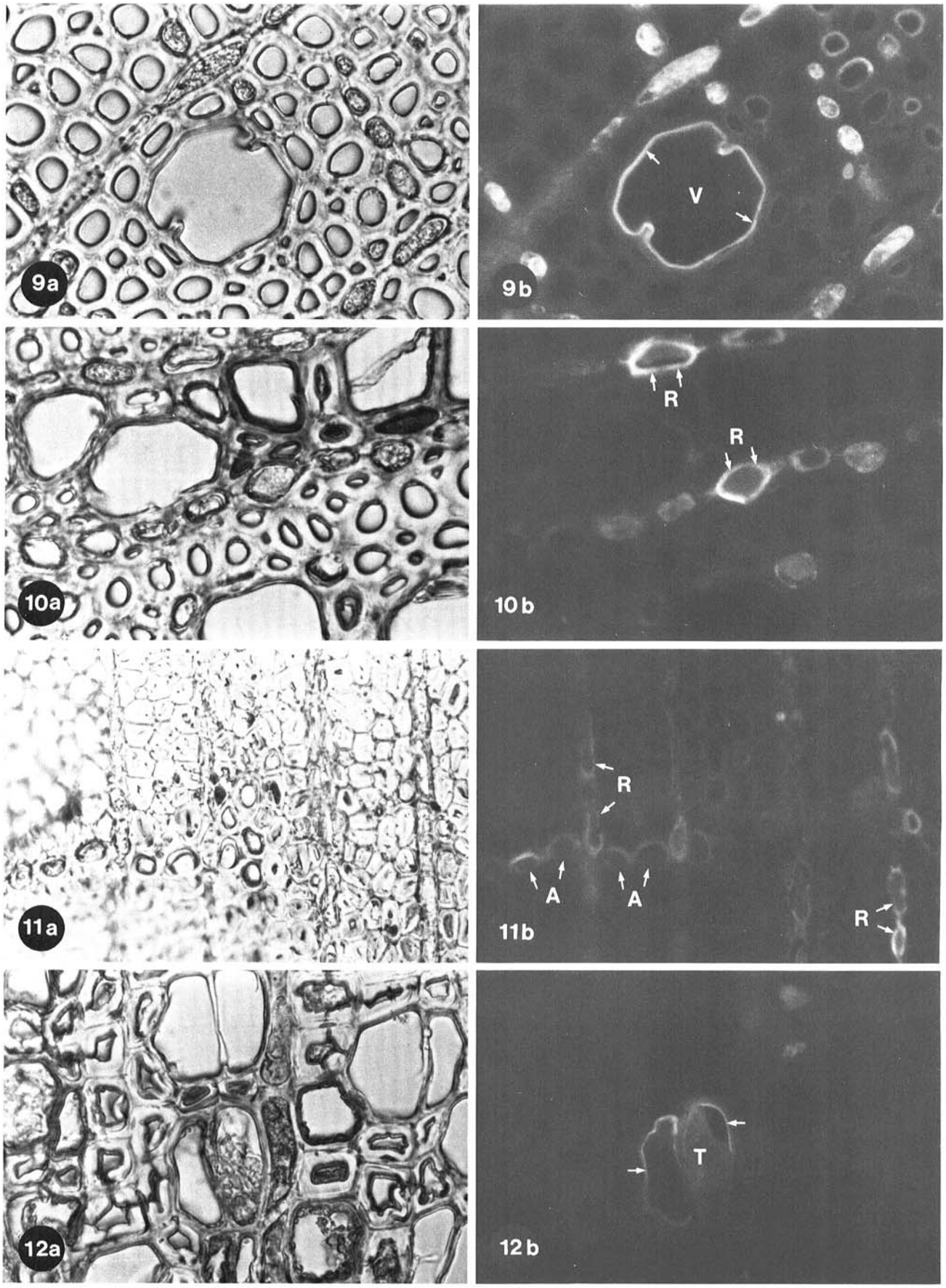
[a] ++ = cell type present and suberized, — = cell type present and nonsuberized, and NA = cell type not normally found in this species.



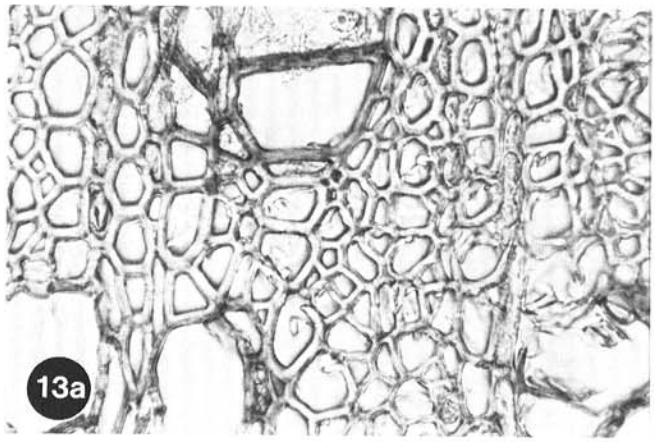
Figs. 1–4. Transverse sections through reaction zone of mechanically wounded xylem tissue 21 days after wounding. Except where otherwise noted, sections have been treated with phloroglucinol HCl to quench lignin autofluorescence and photographed under bright-field **(A)** and ultraviolet **(B)** illumination to detect suberin lamellae. **1,** Xylem of *Acer negundo* with suberin lamellae in paratracheal parenchyma (P) and vessels (V). ×450. **2,** Xylem of *Betula papyrifera* with suberin lamellae lining inner wall of two vessel elements (arrows). ×640. **3,** Xylem of *B. papyrifera* treated with Sudan black B. Note dark Sudan coloration in vessel walls in 3A (arrows). Under ultraviolet excitation (3B), Sudan-colored material exhibits quenched autofluorescence (arrows). Residual autofluorescence in cell walls is due to lignin. ×215. **4,** Xylem of *Carya cordiformis* with suberin lamellae in paratracheal parenchyma cell (P) and a vessel element (V). Also note widely dispersed intracellular autofluorescence in reaction zone. ×535.

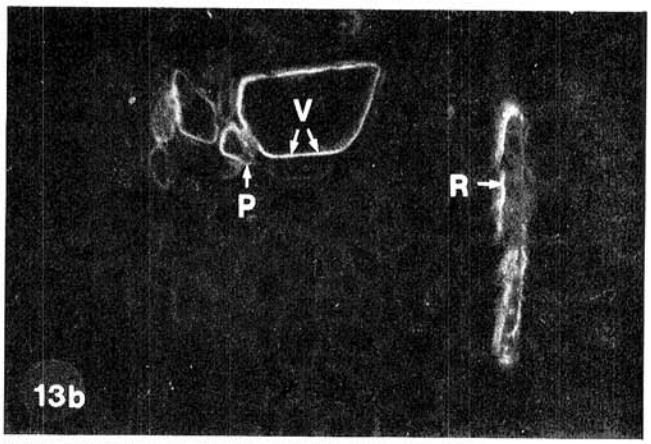


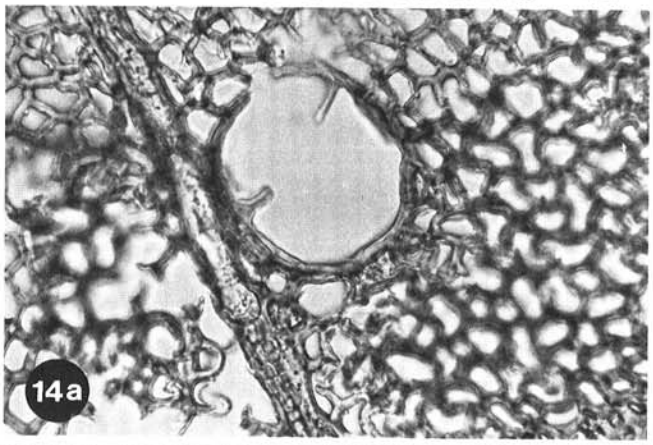
Figs. 5–8. Tranverse sections through reaction zone of mechanically wounded xylem tissue 21 days after wounding. Sections have been treated with phloroglucinol HCl to quench lignin autofluorescence and photographed under bright-field **(A)** and ultraviolet **(B)** illumination to detect suberin lamellae. **5,** Xylem of *Castanea dentata* with suberized tyloses (T) and paratracheal parenchyma (P). ×345. **6,** Reaction zone in primary xylem of *Fraxinus americana* with extensive suberized protoxylem (X) and parenchyma (arrows). ×535. **7,** Reaction zone of *Hamamelis virginiana* in springwood showing suberin lamellae in vessels (V), tyloses (T), ray (R), paratracheal (P), and apotracheal (A) parenchyma. ×215. **8,** Xylem of *Morus rubra* with single suberized ray cell (arrow). ×535.

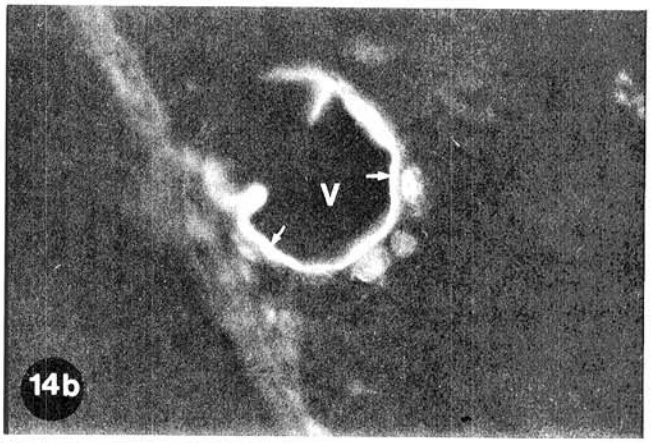


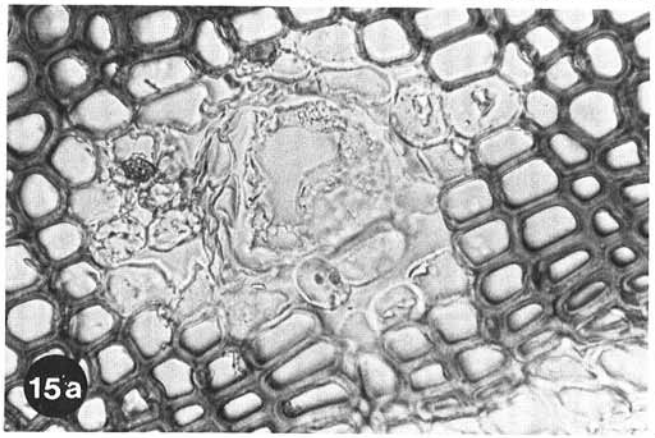
Figs. 9–12. Transverse sections through reaction zone of mechanically wounded xylem tissue 21 days after wounding. Sections have been treated with phloroglucinol HCl to quench lignin autofluorescence and photographed under bright-field **(A)** and ultraviolet **(B)** illumination to detect suberin lamellae. **9,** Xylem vessel (V) of *Ostrya virginiana* with suberin lamellae (arrows). Note intracellular autofluorescence of xylem parenchyma. ×740. **10,** Reaction zone in *Prunus avium* with suberized ray parenchyma (R, arrows). ×820. **11,** Xylem of *Quercus rubra* with suberized ray parenchyma (R) and banded apotracheal-diffuse parenchyma (A). ×483. **12,** Xylem vessels of *Q. rubra* with suberized tyloses (T). ×687.

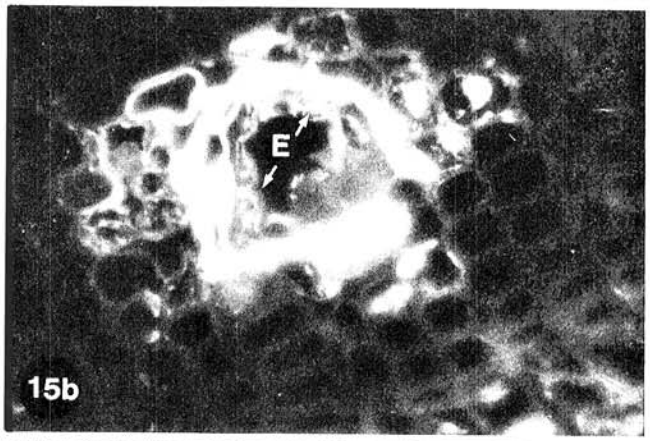


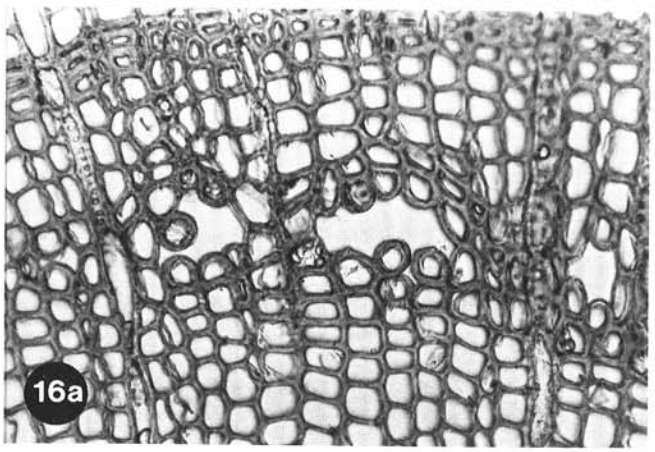


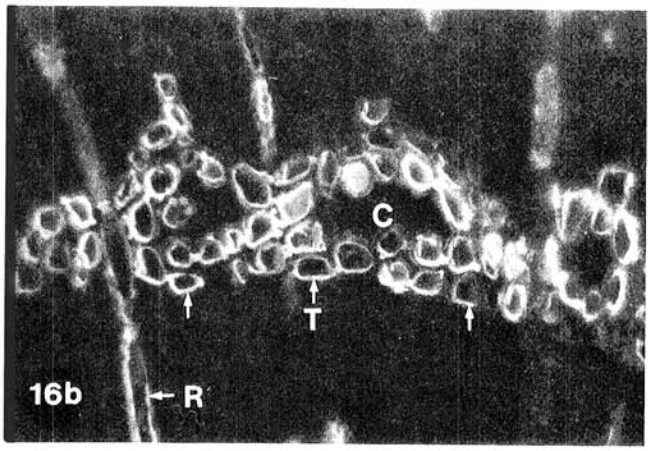












Figs. 13–16. Transverse sections through reaction zone of mechanically wounded xylem tissue 21 days after wounding. Sections have been treated with phloroglucinol HCl to quench lignin autofluorescence and photographed under bright-field **(A)** and ultraviolet **(B)** illumination to detect suberin lamellae. **13,** Xylem of *Salix nigra* with suberized vessel (V), paratracheal parenchyma (P), and ray (R). ×513. **14,** Xylem vessel (V) of *Ulmus americana* with suberin lamellae. ×530. **15,** Resin canal in nonwounded xylem of *Pinus strobus* showing highly fluorescent suberized cells surrounding epithelium (E, arrows). ×450. **16,** Traumatic resin canals (C) of *Tsuga canadensis* with suberized tracheids (T) and ray parenchyma (R). ×330.

marginal, forming a narrow, continuous or interrupted, one- to two-seriate line. Some paratracheal parenchyma was also observed. Lamellar suberin formed after wounding in ray parenchyma across the transverse plane of the wound and in axial parenchyma centripetal to new callus growth. Vessels appeared plugged with distinct suberized tyloses (Fig. 13). Occasionally, vessels were lined with suberin in the absence of tyloses. Immature fibers or tracheids centripetal to the new callus also appeared suberized.

Ulmus americana. Healthy, nonwounded xylem parenchyma was paratracheal, scanty to vasicentric, and apotracheal-diffuse. After wounding, suberin lamellae were observed in paratracheal parenchyma and in tyloses (Fig. 14).

Pinus strobus. In healthy xylem, axial parenchyma was generally absent. Suberized cells were abundant in nonwounded tissue and included crystalliferous and tanniferous ray parenchyma and parenchyma bordering resin ducts (Fig. 15). Resin-duct epithelial cells were not suberized. Suberin deposition associated with xylem wound response was not observed in parenchyma or tracheids.

Tsuga canadensis. Longitudinal parenchyma in nonwounded xylem was usually marginal and very sparse. In healthy xylem, both ray and axial parenchyma cells appeared suberized. Suberization of marginal parenchyma appeared more or less continuous beneath the wound as opposed to its interrupted appearance elsewhere. Some tracheids appeared suberized. Cells bordering traumatic resin canals, perhaps induced by a previous injury, also appeared highly suberized (Fig. 16).

Influence of time. It was difficult to make species comparisons of the timing of suberin deposition in wounded xylem because of the many confounding variables associated with field sampling. However, wound events over time were observed and recorded. In May, suberization of xylem proceeded more slowly than in June or July. In those months, suberized xylem cells were observed in some species 7 days after wounding and in all species 10 days after wounding; no increases or changes in suberized cell numbers, location, or autofluorescence intensity were noted from 14 to 21 days after wounding. This suggests that wound boundaries were complete within 2 wk after wounding. In May, however, progressive changes in suberin autofluorescence intensity and cell distribution were recorded between 14 and 21 days after wounding. Distribution of suberized cells and suberin intensity in tissues examined 21 days after wounding in May appeared similar to those examined 14 and 21 days after wounding in June and July.

DISCUSSION

The occurrence and location of suberized xylem parenchyma, vessels, and tyloses, as part of the wound reaction zone, are described. There are few previous reports on the presence of suberized cells in xylem, and none of these addresses reaction zones specifically.

Pearce and Rutherford (22) and Pearce and Holloway (21) described for the first time the occurrence of suberin in tyloses and vessel-coating material in sapwood and heartwood vessels of English oak (*Quercus robur* L.). Suberin was especially apparent in cells of the barrier zone (CODIT wall 4 [26]) formed in new tissues produced after wounding (22). These authors attributed increased resistance to decay by *Stereum gausapatum* (Fr.) Fr. to suberization of the barrier zone. In addition, they observed suberized ray parenchyma near the junction of the ray with the barrier zone and suggested a possible involvement of suberin in wall 3 of the CODIT model. Parameswaran et al (20) described the ultrastructure of suberized tyloses in undecayed sap wood and heartwood of *Q. robur* and European beech (*Fagus sylvatica* L.), thus confirming the findings of Pearce and Holloway (21). Suberized vessel occlusions correspond to CODIT wall 1.

Robb et al (23,24) have reported lipid material, which stains with Sudan dyes, associated with tyloses and vessel linings in several herbaceous species infected with vascular wilt pathogens. Modified cell wall layers also have been observed in xylem parenchyma (16), fibers (18), and tyloses (17) of elm infected with *Ceratocystis ulmi* (Buism.) C. Moreau. These cells were thought to be suberized because of the lamellar structure of the modified layer (18).

The present study has demonstrated that xylem parenchyma cells in various locations have the ability to form lamellar suberin on their inner cell wall surface. However, the distribution of suberization is not necessarily matched with the distribution of parenchyma in the reaction zone. For example, marginal parenchyma of most species did not become suberized, although ray parenchyma suberization appeared regularly with few exceptions.

It appears that suberin should be considered as a de novo component of the wound reaction zone. In CODIT model terminology, it may play a role in delimiting necrotic tissue as part of walls 1, 2, and 3 (26). Wall 1 (responsible for vertical delimitation) would consist of suberized tyloses derived from paratracheal parenchyma or from marginal parenchyma adjacent to vessels. The presence of nonlamellar forms of suberin must also be considered. In *B. papyrifera*, amorphous Sudan-positive (phloroglucinol-negative) materials were often found occluding vessels in the reaction zone. Other studies have reported nonlamellar suberin produced after wounding (8,13,23,24).

In wall 2 of the CODIT model (the wall that resists centripetal spread of pathogens), suberin lamellae were found in marginal apotracheal parenchyma either as single cells or continuous bands, banded apotracheal parenchyma, paratracheal parenchyma, and the transverse wall of xylem ray parenchyma. Where ray cell suberization was observed, considerable variability in the extent of tissue suberization was found between species. In *Prunus* spp., suberized parenchyma in a ray ranged from one to two cells in the reaction zone, compared with *F. americana*, where up to 22 cells in one ray (in the cross-sectional plane) appeared suberized.

Extensive ray suberization, as noted above for *F. americana*, was most likely to occur in the position of CODIT wall 3, the wall responsible for lateral delimitation of wounds or pathogens. This wall was comprised of suberized radial walls of the xylem ray parenchyma, suberized fibers beneath the developing bark callus (these suberized fibers were probably not completely differentiated into fibers at the time of wounding), and suberized paratracheal parenchyma.

In summary, the present study has demonstrated the de novo formation of lamellar suberin in xylem following mechanical wounding. Suberized xylem parenchyma and vessels were usually associated with a visible wound reaction zone, although a few species exhibited lamellar suberin in apparently healthy, nonwounded wood. Additional studies using older tissues—heartwood, for example—may reveal that suberin is a normal component of healthy structural xylem (21). More detailed studies are required to determine the importance of suberin deposition in conifer xylem. The general lack of longitudinal parenchyma in most conifers, and the extensive suberization in nonwounded tissues, suggest that suberin formation in conifer wounds is of limited extent. Cell wall structural changes in reaction zones probably represent only one of many components in the response of xylem to wounds or infections.

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