Histological Changes Induced in Scotch Pine Needles by Sulfur Dioxide

H. J. Smith and D. D. Davis

Graduate student and Associate professor, respectively, Department of Plant Pathology and Center for Air Environment Studies, The Pennsylvania State University, University Park, PA 16802. Current address of senior author: Argonne National Laboratory, Argonne, IL 60439.

Contribution 1023, Department of Plant Pathology, The Pennsylvania Agricultural Experiment Station. Authorized for publication 10 March 1978 as Journal Series Paper 5465, and Contribution 498-78, Center for Air Environment Studies. The Pennsylvania State University, University Park.

Accepted for publication 6 July 1978.

ABSTRACT

SMITH, H. J., and D. D. DAVIS. 1978. Histological changes induced in Scotch pine needles by sulfur dioxide. Phytopathology 68: 1711-1716.

Scotch pine cotyledons, primary needles, and secondary needles of various ages were exposed to 2,620 $\mu g/m^3$ (1.0 ppm) SO_2 for 1, 3, or 5 hr. Needles from unexposed (control) and exposed plants were embedded in paraffin, sectioned, stained, and examined under a light microscope. Mesophyll collapse was the most common structural change induced by SO_2 in all three needle types. Resin canal occlusion also was observed in primary and secondary needles exposed to SO_2. Increased dosages of SO_2 did not induce additional symptoms but intensified those already present.

Additional key words: air pollution, sulfur dioxide, *Pinus*.

Linzon (10) reported severe mesophyll collapse and distortion of stelar tissue (5) in needles of eastern white pine (*Pinus strobus* L.) after exposure to sulfur dioxide (SO_2). Stewart et al (14) observed that mesophyll cells in the foliage of several conifer species, including Scotch pine (*P. sylvestris* L.), generally are the first cells injured following exposure to 2,620 μg of SO_2/m^3 (1.0 ppm) for 5 hr. Epithelial cells exhibit hypertrophy that results in complete occlusion of the resin ducts. Epidermal and endodermal tissues are not directly affected and remain intact until after the collapse of underlying cells. Occasional hyperplasia and hypertrophy are observed in transfusion tissue and vascular parenchyma.

In general, previous studies with SO_2 have been conducted on secondary needles of mature trees or mature needles of seedlings older than 1 yr. Information is needed regarding anatomic changes induced by this air pollutant on the foliage, including cotyledons and primary needles, of seedlings younger than 1 yr. In addition, few studies illustrate the anatomy of healthy conifer cotyledons and primary needles (8,9). The purpose of this study was to examine the normal anatomy and to document histological changes induced by SO_2 in the cells and tissues of young cotyledons, primary needles, and secondary needles of Scotch pine.

MATERIALS AND METHODS

Scotch pine seedlings (selection Spanish Sierra Guadarrama) with cotyledons, primary needles, or secondary needles were grown in the greenhouse and then exposed to SO_2 in controlled environment chambers for 1, 3, or 5 hr. At 1, 3, 5, and 7 wk after emergence, seedlings were exposed to 2,620 $\mu g/m^3$ (1.0 ppm) SO_2. Needle emergence was considered to be the stage at which most of the needles extended 1–2 mm beyond the fascicle sheath. Exposure techniques and conditions were as described previously (16, 17).

Needle samples representing the three needle types, four needle ages, three SO_2 dosages, and appropriate unexposed control needles were collected 0 and 48 hr after exposure to SO_2 and fixed in formalin/acetic acid/alcohol (FAA) for 24 hr (7). Then samples were dehydrated in a tertiary butyl alcohol series (6,7) and embedded in Paraplast (7). Embedded specimens were softened in a mixture of 90 ml of 1% sodium lauryl sulfate (Dreft) and 10 ml of glycerol for 6.5 hr (1) prior to sectioning at 10 μm with a rotary microtome. Longitudinal and transverse serial sections were floated in a 4% formalin solution on chemically cleaned microscope slides covered with a thin layer of Haupt's adhesive and allowed to set for 24 hr on a warming tray at 40 C. Mounted slides were air-dried for 24 hr and stained with a modified quadruple stain (7). Stained slides were examined with a light microscope. Representative sections of exposed and unexposed needles were photographed on Kodak Plus X Pan film with a Leitz Ortholux microscope fitted with a Leitz Aristophot camera and a 10.2 × 12.7 cm (4 × 5 in.) Graflex back.

RESULTS

Cotyledons.—The number of cotyledons per seedling ranges from five to nine and they are generally triangular in transverse section. The periphery of the leaf is constructed of a single continuous row of thin-walled epidermal cells interrupted only by occasional stomata. The number of stomata varies and rows of stomata are found on all surfaces of the cotyledon. Unlike secondary

0032-949X/78/000309$3.00/00

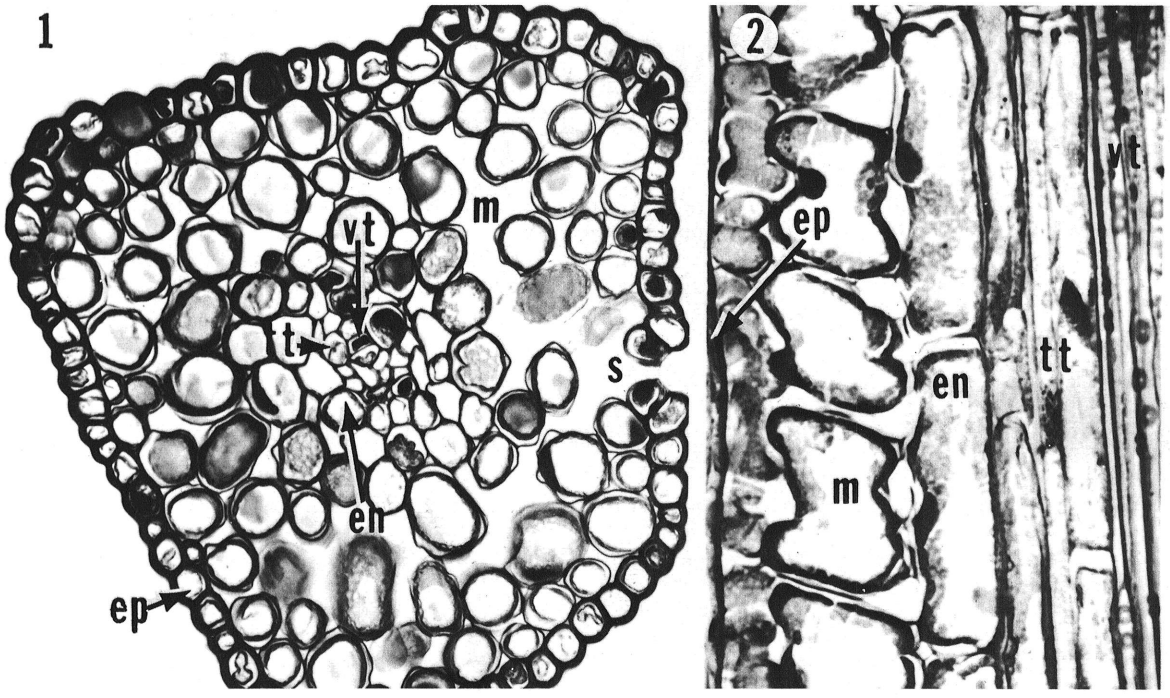

Fig. 1–2. Normal (unexposed to SO_2) Scotch pine cotyledon tissue in **1)** transverse and **2)** longitudinal sections showing the arrangement of the epidermal cells. Legend: epidermis, ep; mesophyll, m; endodermis, en; transfusion tissue, tt; vascular tissue, vt; and stoma, s. Magnifications: Fig. 1 ×378 and Fig. 2 ×337.

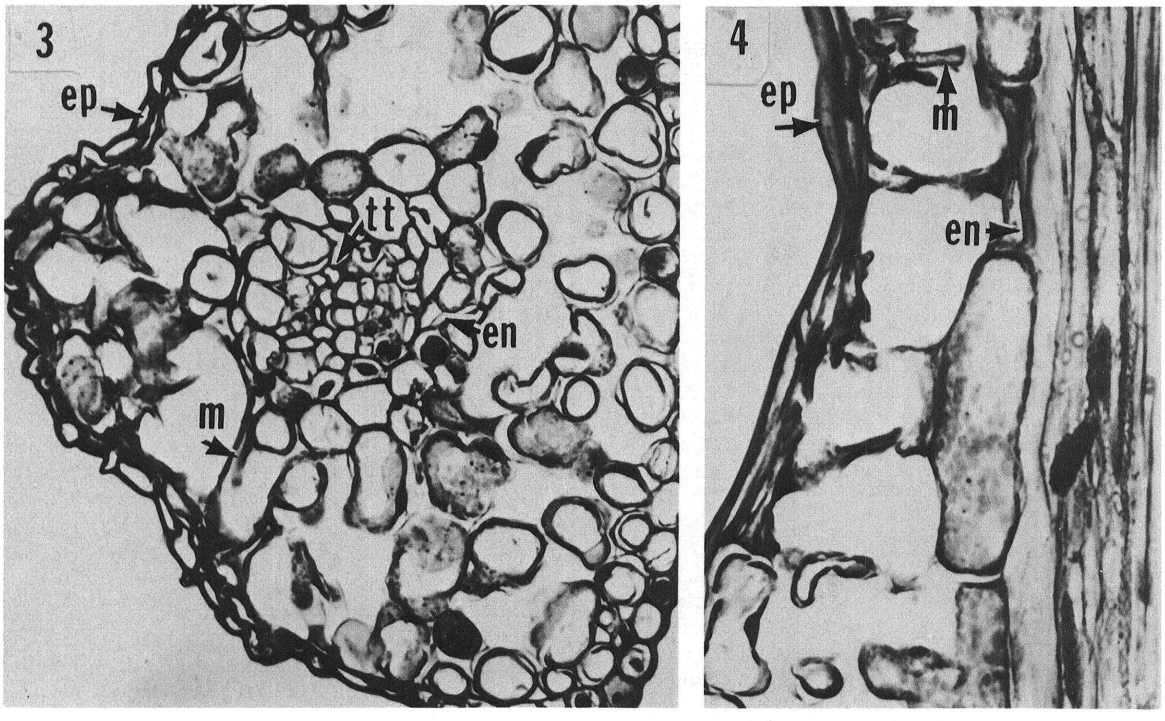

Fig. 3–4. Scotch pine cotyledon tissue exposed for 5 hr to 2,620 μg SO_2/m^3. **3)** Transverse section showing collapse of mesophyll (m), distortion of epidermis (ep) and endodermis (en), and compression of the transfusion tissue (tt) (×314). **4)** Longitudinal section showing collapse of necrotic mesophyll tissue and distortion of the epidermis and endodermis (×362).

needles, cotyledons do not contain a hypodermal layer or resin canals. The mesophyll is composed of loosely-packed, spherical cells that lack the typical internal ridges of primary and secondary needles (Fig. 1-2). A circular, single row of thin-walled cells forms the endodermis. A central single vascular bundle of primary xylem and primary phloem tissue is surrounded by one or two layers of transfusion parenchyma.

The most common anatomic change in cotyledons exposed to SO_2 was collapse of mesophyll cells (Fig. 3-4). Occasional hypertrophy of these cells was observed in the abrupt 1–2 mm transition zone between necrotic and apparently uninjured tissue. The epidermis did not appear to be directly affected by SO_2 but eventually became distorted after the underlying mesophyll cells collapsed. Distortion of the stelar region was observed only after the initiation of mesophyll collapse and hypertrophy. This response was observed in the endodermis, transfusion tissue, and phloem cells. Xylem distortion occurred only after all other structures collapsed.

Changes were not observed in any cells of cotyledons exposed to SO_2 for 1 hr or in any samples exposed at the time of seed coat shedding regardless of the dosage used. The types of changes recorded were similar for all ages from 1 to 7 wk for both 3-hr and 5-hr exposures. The only variation noted was with the total amount of tissue injured.

Primary and secondary needles.—As the cotyledons, the periphery of the primary needle is composed of a single continuous row of thin-walled epidermal cells and is devoid of a hypodermis (Fig. 5-6). Typical plicate mesophyll cells (4,15) are present in a closely-packed arrangement (Fig. 6). Resin canals are present and are circumscribed by a well-defined single layer of sclerenchyma cells. The endodermis is formed by a single row of thin-walled cells arranged in a circle when viewed in cross section (Fig. 5). As observed in the cotyledons, primary needles contain only one vascular bundle that is surrounded by several layers of transfusion tissue.

Secondary needles are semicircular in cross section with a periphery constructed of a single row of thick-walled epidermal cells and a single row of sclerified, fibrous hypodermal cells (Fig. 7–8). Mesophyll cells are plicate and contain several deep internal ridges. The endodermis of the secondary needles consists of one row of thick-walled cells. Two vascular bundles are present and surrounded by several layers of transfusion parenchyma and tracheids. Each needle has six or seven peripheral resin canals. These canals are surrounded by a single row of sclerenchyma cells attached to the hypodermis and are lined with a single row of thin-walled epithelial cells.

Both primary and secondary needle types responded similarly regardless of age of SO_2 dosage (Fig. 9–10,11-12). As with the cotyledons, the most common change in needle anatomy was collapse of the mesophyll tissue with hypertrophy (Fig. 8, 12). Hypertrophy was limited to the

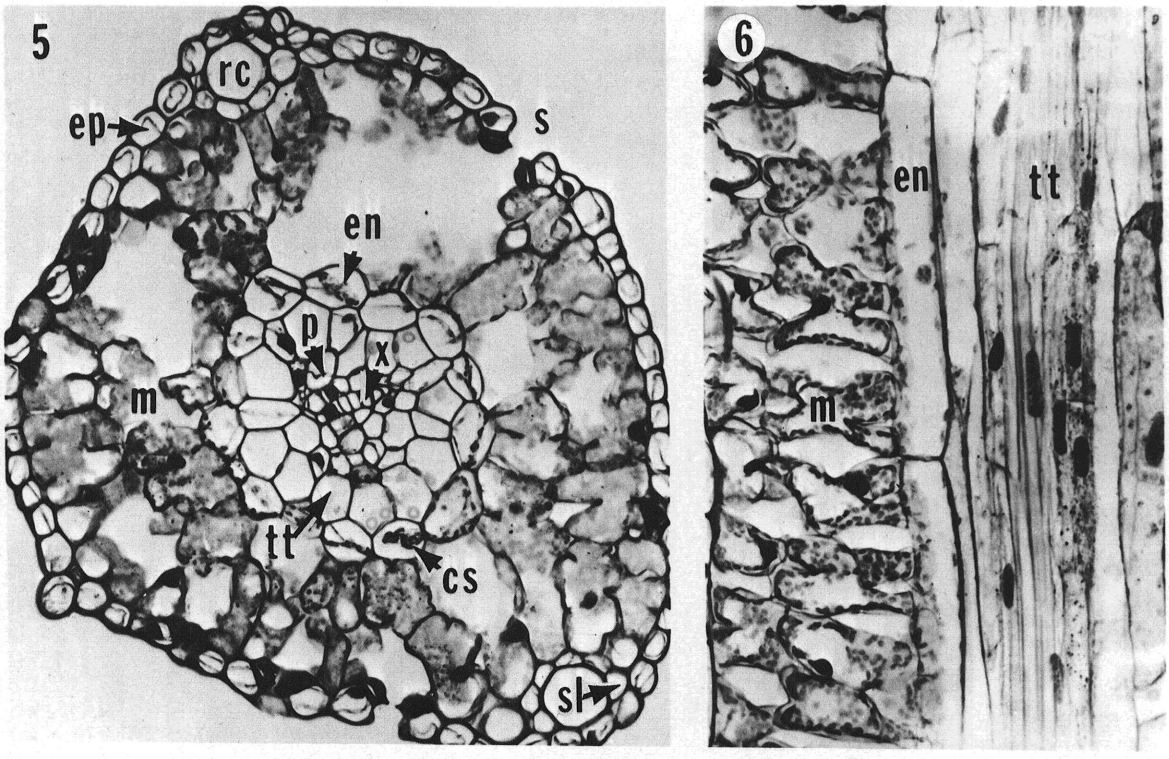

Fig. 5–6. Normal (unexposed to SO_2) Scotch pine primary needle tissue. **5)** Transverse section illustrating the arrangement of epidermis (ep), plicate mesophyll (m), resin canals (rc), stoma (s), endodermis (en) with Casparian strips (cs), transfusion tissue (tt), xylem (x), phloem (p), and sclerenchyma layer (sl) (×244). **6)** Longitudinal section illustrating the arrangement of plicate mesophyll, endodermis, and transfusion tissue (×272).

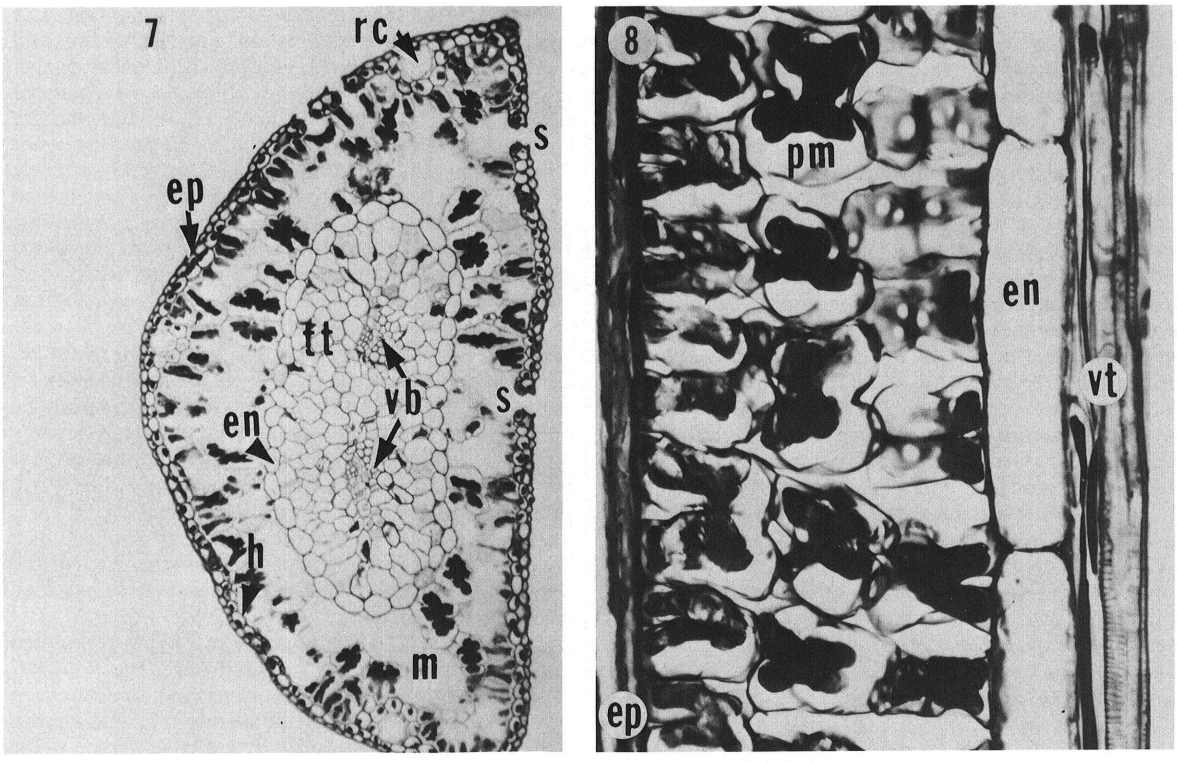

Fig. 7–8. Normal (unexposed to SO_2) Scotch pine secondary needle tissue. **7)** Transverse section showing needle shape and internal structure including epidermis (ep), hypodermis (h), mesophyll (m), resin canal (rc), stomata (s), endodermis (en), transfusion tissue (tt), and vascular bundles (vb) (×106). **8)** Longitudinal section showing epidermis, plicate mesophyll (pm), endodermis, and vascular tissue (vt) (×270).

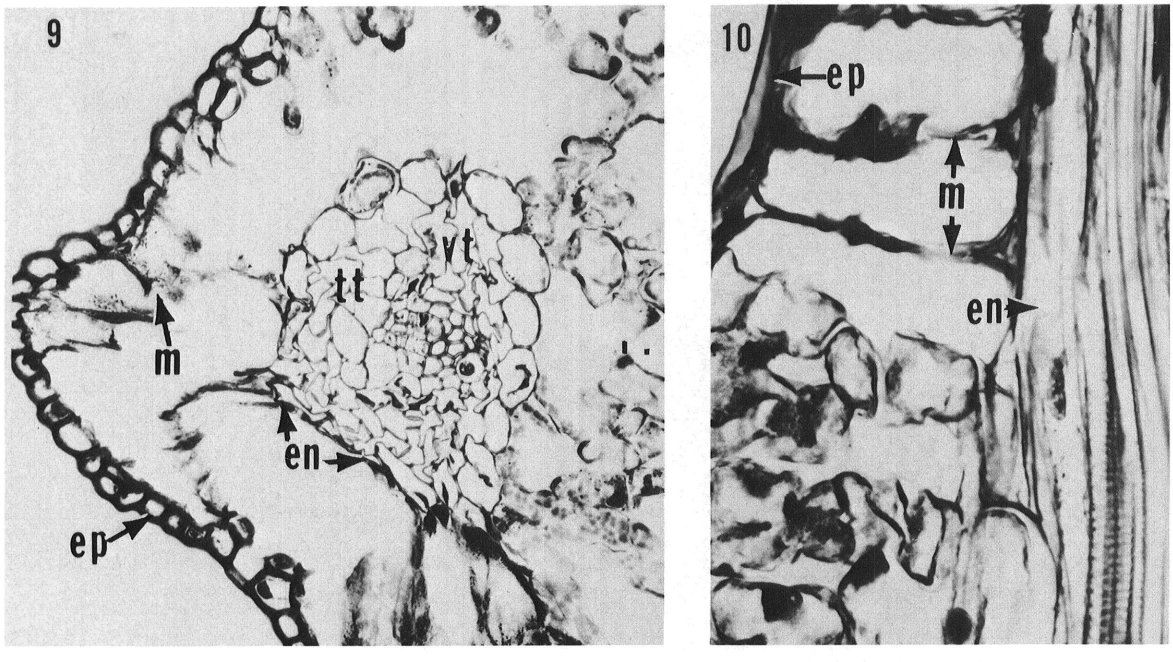

Fig. 9–10. Scotch pine primary needle tissue exposed for 3 hr to 2,620 μg SO_2/m^3. **9)** Transverse section showing collapsed mesophyll (m) and the resulting distortion of the epidermis (ep), compression of endodermis (en), transfusion tissue (tt), and vascular tissue (vt) (×266). **10)** Longitudinal section illustrating mesophyll (m) collapse, distortion of epidermis, and compression of the endodermis (×430). Section is from the transition zone between asymptomatic and necrotic (tip necrosis) tissue.

transition zone between healthy and injured tissue and did not vary appreciably between primary and secondary needles. However, the extent of collapse appeared to be slightly greater in the secondary needles. Again, the epidermis was not directly affected by SO_2 but became distorted after the underlying cells collapsed. Epithelial cells showed severe hypertrophy resulting in total occlusion of the resin canals (Fig. 11). The layer of sclerenchyma cells surrounding the canals was compressed as a result of simultaneous mesophyll and epithelial cell hypertrophy. This occlusion of the resin ducts continued far into the portion of the needle that did not show macroscopic symptoms. As with the epidermis, the endodermis did not generally show direct injury as a result of exposure to SO_2. Compression and collapse of the endodermis became evident as a result of the hypertrophy of adjacent mesophyll and transfusion tissue. Changes in the vascular region were characterized by hypertrophy and associated compression of transfusion and phloem parenchyma. Xylem tissue was the last of the vascular tissues to collapse (Fig. 7).

As with the cotyledons, no variation in the type of structural changes was observed from one age to another or between the 3-hr and 5-hr doses of SO_2. The primary needles did not exhibit changes when exposed to SO_2 at the time of seed coat shedding (0 wk); secondary needles exposed at the time of needle emergence from fascicle sheaths (0 wk) showed anatomic changes for both 3-hr and 5-hr dosages. No significant changes in anatomy were observed in cotyledons, primary needles, and secondary needles collected immediately after exposure to SO_2. Detectable changes were induced only after 3 or 5 hr of exposure to SO_2. Microscopic injury was observed only on needles that exhibited macroscopic symptoms. The most common macroscopic symptom on Scotch pine cotyledons, primary needles, and secondary needles was a tan necrosis, extending from the needle tip toward the base. Necrotic areas were separated from the uninjured green base by an abrupt line of demarcation.

DISCUSSION

Sutherland (15) and others (2–5,10,12–14) have provided information on the anatomy of mature conifer foliage, but few reports deal with the structure of cotyledons and primary needles. Such a lack of basic information can create problems, as illustrated by comparing the study conducted by Kozlowski and Clausen (8) with that of Krugman and Critchfield (9).

The anatomy of healthy cotyledons, primary needles, and secondary needles of Scotch pine were quite similar to those reported for red pine by Krugman and Critchfield (9). Both studies reveal histological differences among needle types. Both primary and

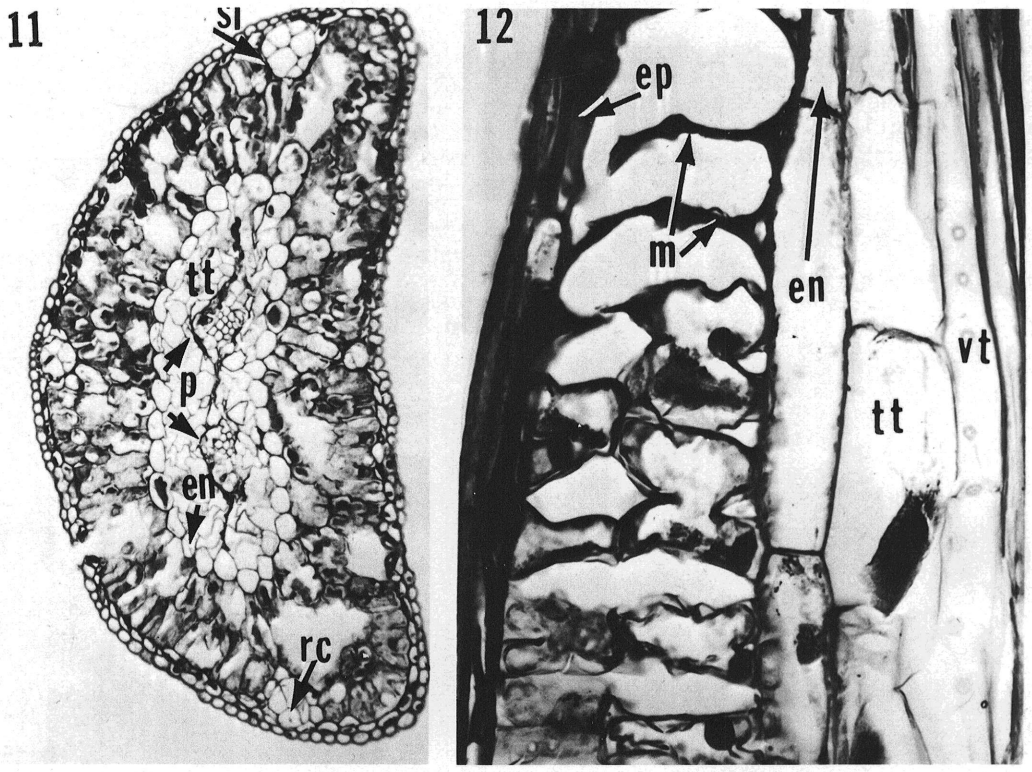

Fig. 11–12. Scotch pine secondary needle tissue exposed for 5 hr to 2,620 μg SO_2. **11)** Transverse section showing occlusion of resin canals (rc), compression of the sclerenchyma layer (sl) surrounding the resin canal, compression of the endodermis (en) and transfusion tissue (tt), and distortion of the phloem tissue (p) (×116). **12)** Longitudinal section showing hypertrophy and collapse of mesophyll (m), compression of endodermal cells, and distortion of epidermis, transfusion tissue, and vascular tissue (vt) (×383). Section was taken in the transition zone between asymptomatic and necrotic (tip necrosis) tissue.

secondary needles contain resin canals, but the cotyledons do not. The secondary needles have a thick-walled epidermis accompanied by a fibrous sclerified hypodermis, and the cotyledons and primary needles have a thin-walled epidermis and lack a hypodermis. Such differences are uniform, and the needle types do not vary with age. These findings are similar for Scotch pine (14) and red pine (9) and may be true for the genus *Pinus* in general.

In this study most changes on needles injured by SO_2 were similar to previously described alterations of secondary needles of Scotch pine and other conifers exposed to this pollutant (2,10,11,14). The extent of most changes did not vary appreciably with needle types or ages. The most frequent change was the severe collapse of the mesophyll cells that may have been responsible for the distortion of the epidermis and stelar region in the endodermis. An interesting observation was the extension of the resin canal occlusion far into the apparently uninjured portion of symptomatic primary and secondary needles; all other responses terminated at the end of the 1–2 mm transition zone. A similar response was reported in mature secondary Scotch pine needles exposed to SO_2 (14).

Stewart et al (14) reported that SO_2 induces severe hypertrophy of the tissues of Scotch pine needles in the transfusion and vascular regions. In this study only minor swelling was observed on the needles and the most common change in those areas was compression or collapse but not hypertrophy. In addition, Stewart et al (14) observed granulation in both mesophyll and transfusion tissue cells. No evidence of granulation was observed in this study. Some of these differences, however, may reflect the differences in needle age in the two studies or a genetic difference in the selections investigated.

LITERATURE CITED

1. ALCORN, S. M., and P. A. ARK. 1953. Softening paraffin-embedded plant tissue. Stain Technol. 28:55-56.
2. EVANS, L. S., and P. R. MILLER. 1972. Comparative needle anatomy and relative ozone sensitivity of four pine species. Can. J. Bot. 50:1067-1077.
3. EVANS, L. S., and P. R. MILLER. 1972. Ozone damage to ponderosa pine: A histological and histochemical appraisal. Am. J. Bot. 59:297-304.
4. ESAU, K. 1960. Anatomy of seed plants. John Wiley & Sons, New York. 376 p.
5. ESAU, K. 1965. Plant anatomy. John Wiley & Sons, New York. 767 p.
6. JENSEN, W. A. 1962. Botanical histochemistry. W. H. Freeman and Co., San Francisco. 408 p.
7. JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill, New York. 523 p.
8. KOZLOWSKI, T. T., and J. J. CLAUSEN. 1966. Anatomical responses of pine needles to herbicides. Nature 209:486-487.
9. KRUGMAN, S. L., and W. B. CRITCHFIELD. 1968. Red pine needle structure. Nature 217:685-686.
10. LINZON, S. N. 1967. Histological studies of symptoms in semimature-tissue needle blight of eastern white pine. Can. J. Bot. 45:133-143.
11. SCURFIELD, G. 1960. Air pollution and tree growth. For. Abstr. 21:339-347, 517-528.
12. SOLBERG, A., and D. F. ADAMS. 1955. Some effects of hydrogen fluoride on the internal structure of Pinus ponderosa. Natl. Air Pollut. Symp. 4:164-176.
13. SOLBERG, A., and D. F. ADAMS. 1956. Histological responses of some plant leaves to hydrogen fluoride and sulfur dioxide. Am. J. Bot. 43:755-766.
14. STEWART, D., M. TRESHOW, and F. M. HARNER. 1973. Pathological anatomy of conifer needle necrosis. Can. J. Bot. 51:983-988.
15. SUTHERLAND, M. 1933. A microscopic study of the structure of the leaves of the genus Pinus. N. Z. Inst. Trans. Proc. 63:517-568.
16. SMITH, H. J. 1976. Influence of SO_2 on conifers with emphasis on morphology and histology of Scotch pine. M.S. Thesis, The Penn. State Univ., University Park. 74 p.
17. SMITH, H. J., and D. D. DAVIS. 1977. The influence of needle age on sensitivity of Scotch pine to acute doses of SO_2. Plant Dis. Rep. 61:870-874.