Structure of Panax quinquefolius L. (Araliaceae)
Roots with Emphasis on Secretory Duct Formation

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Abstract. Primary and first-order lateral roots of Panax quinquefolius L. (American ginseng) were collected from plants in an experimental garden during their second year of growth and processed for light and transmission electron microscopy. Roots in primary growth had either a diarch or triarch primary xylem pattern, a pericycle, an endodermis with Casprian bands and subsequently a suberized cell wall, and a cortex of variable thickness with a suberized hypodermal layer. Both root types underwent rapid secondary growth and the primary root particularly formed a fleshy storage organ. The secondary phloem and secondary xylem had abundant parenchyma and few conducting elements. Secretory ducts differentiated in tissue derived from the pericycle and in the secondary phloem. Each schizogenous duct consisted of six to eight epithelial cells, which possessed dense, globular deposits but lacked starch. A phellogen, which produced several layers of suberized phellem, was initiated in the periphery of tissue derived from the pericycle. The results of this study clarify the anatomical localization of secretory ducts in roots of this species.

American ginseng (Panax quinquefolius L.) is a perennial understorey herb of eastern North America (Anderson et al., 1993) that has been harvested extensively for its fleshy root, since, like the Asian species, Panax ginseng C.A. Meyer, it is used for various medicinal purposes (Charron and Gagnon, 1991; Li, 1995). Habitat destruction throughout its range has also led to a decline in natural populations of this species (Charron and Gagnon, 1991). Panax quinquefolius is cultivated in the provinces of Ontario and British Columbia in Canada and in several states in the United States and has become an important cash crop in these areas (Small et al., 1994).

Roots of P. ginseng and P. quinquefolius produce a variety of triterpene saponins known collectively as ginsenosides that are the reputed chemicals of use medicinally (Li, 1995; Proctor and Bailey, 1987; Smith et al., 1996). The seven most common ginsenosides are grouped into two classes: 20(S)-Protopanaxadiol and 20(R)-Protopanaxatriol (Smith et al., 1996). In P. ginseng, ginsenosides have been localized histochemically to “oil canals” in tissue external to the vascular cambium (Kubo et al., 1980). A brief description of the origin and anatomical characteristics of resin ducts in roots of this species has indicated that primary ducts formed in the pericycle and secondary ducts in the secondary phloem (Mei et al., 1990). Hu (1976) illustrated “resin ducts” in line drawings of roots of P. ginseng and P. pseudoginseng Wallich., another Asian species. Holm (1917) has reported on oil ducts in primary and secondary root tissues in P. quinquefolius in a discussion of medicinal plants of North America. A transverse section of a P. quinquefolius root illustrates resin ducts in what is labelled as cortex (Proctor and Bailey, 1987). In spite of the commercial importance of roots of P. quinquefolius (Small et al., 1994), there are few studies on the development and structure of this organ. Thompson (1987) has reported some anatomical features of roots in primary and secondary growth in his overall description of botanical aspects of P. quinquefolius. Additional information on root structure is needed as economic interest in American ginseng as a medicinal plant has increased. Roots have been studied in terms of the tissues containing the highest levels of ginsenosides (Smith et al., 1996) and the effect of root age on ginsenoside quantity (Court et al., 1996). The objective of the present study is to characterize the anatomy of P. quinquefolius roots in primary and secondary growth, particularly in terms of the initiation and location of secretory ducts.

Materials and Methods

Light Microscopy. Plants of P. quinquefolius were collected during their second growing season from cultivated experimental plots at the Agriculture and Agri-Food Canada Pest Management Research Centre, Delhi, Ontario. Primary and first-order lateral roots from 35 plants were cleaned and 0.5-cm segments beginning from the root apex and continuing to 10 cm basipetal to the apex were fixed overnight in 3% glutaraldehyde in 0.025 M potassium phosphate buffer, pH 6.8 at 4°C. Tissue was rinsed three times in buffer, dehydrated in a graded ethanol series, and embedded in LR White resin. Sections (0.5 to 1.0 μm) were cut with glass knives on a Reichert ultramicrotome and stained either with 0.5% toluidine blue O (TBO) in 1.0% sodium borate for 1 to 2 min or in periodic acid-Schiff’s using the protocol of O’Brien and McCully (1981) followed by TBO as above. Sections were viewed with a Leitz Orthoplan microscope, and Ilford FP4 (ASA 125) film was used for all photomicrographs.

Roots from another 25 plants were removed, cleaned and sectioned with a sharp, two-sided razor blade either fresh or after being stored in 30% ethanol. Sections were viewed either unstained or after staining with berberine-aniline blue to intensify suberin and lignin (Brundrett et al., 1988), using a Leitz SM-LUX microscope with an epi-illumination system coupled with broad band ultra violet (UV) light (excitation filter providing 340 to 380 nm wavelengths combined with barrier filter 400K-430; UG1/TK 400/K430 filter system). The presence of suberin in endodermal and hypodermal cell walls was verified by Sudan IV staining (Biggs, 1984). Ilford HP5 (ASA 400) film was used for fluorescence microscopy.

Transmission Electron Microscopy. Two-year-old plants were harvested and roots were washed, segments of primary and first-order lateral roots fixed overnight in 2.5% glutaraldehyde in 0.1 M
Caspian band. With increasing distance from the apical meristem, each endodermal cell deposited suberin throughout the wall (Fig. 1D). The pericycle was uniseriate next to the primary xylem and biseriate around the remaining circumference of the vascular cylinder (Fig. 1A and B). The walls of the uniseriate hypodermis and older epidermal cells fluoresced under UV light (Fig. 1D). The hypodermal cell walls stained with Sudan IV indicating that they were likely suberized. Epidermal cell walls showed primary fluorescence and did not stain with Sudan IV; these walls likely contained phenolic substances. Secretory ducts were absent in roots during primary growth (Fig. 1A–D).

Secondary growth was initiated early in all primary and first-order lateral roots examined. The first periclinal divisions occurred between primary xylem and primary phloem (Fig. 1A and B), and a cambial zone was evident within 1.0 cm of the root apical meristem. The vascular cambium formed many derivatives that differentiated into secondary phloem and secondary xylem (Fig. 2).

sodium phosphate buffer, pH 6.8, rinsed in buffer, and postfixed in 2% O₂O₃ in the same buffer for 2 h. Tissue was dehydrated in a graded acetone series, and infiltrated over several days with Spurr's resin (Spurr, 1969). After polymerization, sections were cut with glass knives and stained for 10 min in uranyl acetate followed by 5 min in lead citrate (Venable and Coggeshall, 1965) before observation with a JEOL-100CX transmission electron microscope.

**Results**

Primary roots and first-order lateral roots had a vascular cylinder consisting of either a diarch primary xylem and a corresponding two groups of primary phloem elements (Fig. 1A) or a triarch primary xylem with three distinct primary phloem regions (Fig. 1B). Roots with diarch xylem generally had fewer rows of cortical cells than roots with triarch xylem (compare Fig. 1A and B) although quantitative data were not collected. Roots had a uniseriate endodermis (Fig. 1A and B) that was best observed using fluorescence microscopy (Fig. 1C). Initially, endodermal cells had a

Fig. 1. Transverse sections of *Panax quinquefolius* roots. (A and B) Sections of roots embedded in LR White stained with toluidine blue O. (C and D) Hand sections of fresh roots stained with berberine–aniline blue. (A) Section of first-order lateral root taken 0.3 cm from root apical meristem. A diarch primary xylem strand (X) and two groups of primary phloem elements (arrowheads) are present. Cortex (C), endodermis (En) and pericycle (P) have differentiated. Periclinal divisions (double arrowheads) are present between the primary xylem and primary phloem. Scale bar = 10 μm. (B) Section of primary root taken 0.3 cm from root apical meristem with triarch xylem (X), three groups of primary phloem elements (arrowheads), pericycle (P), endodermis (En) and cortex (C). Periclinal divisions (double arrowheads) are evident between primary xylem and primary phloem. Scale bar = 10 μm. (C) Section of primary root taken 0.2 cm from root apical meristem with triarch xylem (X) and a uniseriate endodermis. Caspian bands (arrowheads) are evident in some endodermal cells and other cells are in State II (double arrowheads). Scale bar = 25 μm. (D) Section of primary root taken 0.4 cm from root apical meristem with endodermal cells in State II (double arrowheads) and a modified hypodermis (*). Epidermal cells (arrowheads) also have modified walls. Scale bar = 25 μm.

Fig. 2. Transverse sections of *Panax quinquefolius* roots either embedded in LR White resin (A, B, and D) or hand sectioned fresh (C). All scale bars = 25 μm. (A) Root in early stage of secondary growth. Section taken 0.5 cm from root apical meristem. The vascular cambial zone (VC) surrounds the triarch primary xylem (X). The pericycle has proliferated to form several rows of parenchyma (P), and several secretory ducts (arrowheads) and a phellogen (double arrowheads) have initiated in this multilayered tissue. (B) Root in secondary growth. Section taken 0.6 cm from root apical meristem showing development of air lacunae (*) in secondary phloem. Groups of small sieve tube elements (arrowheads) have differentiated in the secondary phloem and several large tracheary elements (double arrowheads) have differentiated in the secondary xylem. (C) Fresh root showing air lacunae (*) in secondary phloem and a multilayered phellogen (arrowheads). (D) Section of primary root 5.0 cm from root apical meristem with considerable amount of secondary growth. Air lacunae (*) are present in the secondary phloem (Ph). Parenchyma cells in the secondary phloem and secondary xylem (SX) have stored starch (arrowheads). Secretory ducts (double arrowheads) are present in the tissue derived from the pericycle (P) and secondary phloem. VC = vascular cambial zone.
(Fig. 3A); fully formed ducts were surrounded by six to eight epithelial cells that contained spherical dense bodies in tissue processed for light (Fig. 3 B and C) and electron microscopy (Fig. 4 A–C). Unlike surrounding parenchyma cells, epithelial cells lacked starch grains (Fig. 2D). Droplets of secretory substances were often apparent within ducts in hand sections of fresh roots (Fig. 3D). Druse crystals were frequently deposited within parenchyma cells derived from the pericycle (Fig. 3D) and sometimes in vascular parenchyma.

At the ultrastructural level, the cytoplasm of epithelial cells adjacent to newly formed secretory ducts contained mitochondria, endoplasmic reticulum, osmiophilic deposits, and a large nucleus (Fig. 4A). Epithelial cells surrounding older secretory ducts had large central vacuoles with peripheral cytoplasm (Fig. 4B). Osmiophilic spherical deposits were present and epithelial cell walls adjacent to the secretory duct often showed loose fibrillar organization (Fig. 4C).

**Discussion**

The fleshy perennial storage roots of _P. quinquefolius_ (American ginseng) have anatomical characteristics similar to storage roots of many other species in that secondary growth is pronounced and the bulk of the secondary phloem and secondary xylem is comprised of storage parenchyma (Esau, 1965). In many aspects, the basic anatomy of _P. quinquefolius_ resembles that of _Daucus carota_ L. (carrot) storage roots. In both, the cortex is shed early during secondary growth, the pericycle proliferates and a phellogen is initiated from its periphery, the secondary phloem and secondary xylem have groups of small conducting elements interspersed with large amounts of storage parenchyma, and schizogenous oil ducts occur in the pericycle and the secondary phloem (Esau, 1940). Major storage sites of starch are parenchyma cells within the secondary phloem and secondary xylem. Characteristics of secondary growth in _P. quinquefolius_ roots can not be used to ascertain plant age (Anderson et al., 1993).

Although Thompson (1987) has reported presence of an endodermis with Casparian bands in _P.
**Literature Cited**


