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

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ADDITIONAL INDEX WORDS. ginseng, *Panax*, roots, secretory ducts, anatomy

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 Casparian 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 understory 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)-Protopanaxadiols and 20(S)-Protopanaxatriols (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-Schiffs 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

Received for publication 2 Sept. 1997. Accepted for publication 6 Apr. 1998. We thank the Natural Sciences and Engineering Research Council of Canada and Human Resources Development Canada for financial support. We thank Lewis Melville for help with the plates and Laurie Winn for typing the manuscript. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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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 μ m. (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 μ m. (C) Section of primary root taken 0.2 cm from root apical meristem with triarch xylem (X) and a uniseriate endodermis. Casparian bands (arrowheads) are evident in some endodermal cells and other cells are in State II (double arrowheads). Scale bar = 25 μ m. (D) Section of primary root taken 0.4 cm from root apical meristem with endodermal cells in State II (double arrowhead) and a modified hypodermis (*). Epidermal cells (arrowheads) also have modified walls. Scale bar = 25 μ m.

sodium phosphate buffer, pH 6.8, rinsed in buffer, and postfixed in 2% O_3O_4 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

Casparian 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

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 μ m. (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. 3. Transverse sections of *Panax quinquefolius* roots, all taken 7.5 cm from root apical meristem. (A–C) Tissue embedded in LR White resin. (D) Hand section of fresh root viewed with differential interference contrast optics. (A) Primary root with considerable secondary growth. Several layers of phellem (*), a secretory duct (arrow) in the tissue derived from the pericycle, several secretory ducts (arrowheads) in the secondary phloem, groups of sieve tube elements (double arrowheads), and tracheary elements (Tr) are present among abundant vascular parenchyma cells. Scale bar = 25 μ m. (B) Enlargement of secretory duct (*) within tissue derived from the pericycle. The duct has elongated radially with the increase in root diameter. Dense, globular deposits (arrowheads) are present within epithelial cells. Scale bar = 10 μ m. (C) Secretory ducts within secondary phloem. Epithelial cells have spherical, dense deposits (arrowheads). Scale bar = 10 μ m. (D) Secretory duct within cells derived from the pericycle. Secretory substances (arrowheads) are present in the duct lumen and within epithelial cells (*). A druse crystal of calcium oxalate (double arrowhead) is evident. Scale bar = 10 μ m.

A and B). As cambial activity progressed, a multilayered tissue was derived from the pericycle and a phellogen was initiated in this tissue (Fig. 2A). Frequently, large air lacunae developed in the secondary phloem and these were apparent in embedded roots (Fig. 2B) as well as in fresh, hand-sectioned roots (Fig. 2C). In the latter, some of the space was probably due to tearing during sectioning. Groups of small sieve tube elements and associated parenchyma differentiated among the large number of storage parenchyma cells in the secondary phloem (Figs. 2B and D and 3A). Following initial differentiation of several large secondary xylem tracheary elements (Fig. 2B), small-diameter secondary xylem tracheary elements differentiated among storage parenchyma (Figs. 2D and 3A). Starch was deposited in storage parenchyma in secondary phloem and secondary xylem (Fig. 2D). Following the initiation of a phellogen, a multilayered phellem formed around the circumference of the root (Fig. 2C). The epidermis, cortex and endodermis were sloughed in an early stage of secondary growth. Peripheral secretory ducts formed by schizogen in cells derived from the pericycle (Figs. 2A and D and 3A) and persisted in this tissue but became elongated radially as further secondary growth occurred (Fig. 3A and B). Several rows of secretory ducts also formed by schizogen in the secondary phloem

(Fig. 3A); fully formed ducts were surrounded by six to eight epithelial cells that contained spherical dense bodies in tissue processed for light (Fig. 3B and C) and electron microscopy (Fig. 4A–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.*

Fig. 4. Transmission electron microscopy of roots of *Panax quinquefolius*. All scale bars = 1.0 μ m. (A) Early stage of the formation of a schizogenous duct (*) showing remnants of middle lamella material (double arrowheads). A large nucleus (N) is present in one of the epithelial cells and electron dense deposits (arrowheads) and mitochondria (M) are present in several epithelial cells. (B) Portion of secretory duct with adjacent vacuolated epithelial cells (*). Dense deposits (double arrowheads) are evident within the peripheral cytoplasm. (C) Portion of epithelial cell showing dense deposits (*) and part of a nucleus (N). The epithelial cell wall adjacent to the duct lumen has loosely organized cellulose microfibrils (arrowheads).

quinquefolius, there is no mention of the hypodermis. This is the first report of the existence of a uniseriate suberized hypodermal layer in *P. quinquefolius* roots. Modified hypodermal layers are common features in angiosperms (Perumalla et al., 1990).

The terminology used to describe the anatomy of *P. quinquefolius* roots is frequently inaccurate. For example, Proctor and Bailey (1987), have labelled phellem as epidermis and the pericycle as cortex. Likewise, the cortical tissue used for various portions of 4-year-old *P. quinquefolius* roots processed for the ginsenoside content determination (Smith et al., 1996) is incorrect. Roots of this age lack a cortex. Similarly, the medullary tissue which supposedly includes xylem and pith is also incorrect as these roots lack a pith as found in this study and by Proctor and Bailey (1987). The cortical tissue most likely includes periderm, pericycle, and secondary phloem; the medullary tissue is most likely secondary and primary xylem. It is interesting that Smith et al. (1996) report a high level of specific ginsenosides in the latter tissue. In our study secretory ducts have not been observed in this tissue and this may suggest that ginsenosides are synthesized and/or stored in cells other than secretory ducts. In other reports on the distribution of ginsenosides in roots of *P. ginseng* reference is also erroneously made to the cortex and pith of 6-year-old roots (Kubo et al., 1980; Tani et al., 1981). However, Kubo et al. (1980) have found that ducts localized by histochemical reactions of their secretory compounds are present only in tissues external to the vascular cambium which concurs with anatomical features of *P. quinquefolius* roots. Since roots of *P. quinquefolius* and *P. ginseng* are commercially important, understanding development and structure of these storage organs is important.

Secretory ducts in roots of *P. quinquefolius* have been referred to by Holm (1917), Proctor and Bailey (1987), and Thompson (1987). A brief account of their formation and structure has been published for *P. ginseng* (Mei et al., 1990), also. The location of secretory ducts in tissue derived from the pericycle and in secondary phloem is a common feature of both species but their location in cortex (Proctor and Bailey, 1987; Thompson 1987) is erroneous. As roots increase in diameter there is an increase in the number of secretory ducts in both species. This is due to continued initiation of secondary phloem and the differentiation of secretory ducts within the abundant parenchyma found in this tissue. It may be possible to alter the balance of growth regulators to enhance secondary phloem and secretory duct formation. If these ducts are important for synthesis of ginsenosides (Kubo et al., 1980) then levels of these substances may be increased.

This study shows that the secretory ducts of *P. quinquefolius* are schizogenous in origin, a feature of the secretory ducts in *P. ginseng* (Mei et al., 1990) and in many other species (Fahn, 1979). Mature secretory ducts have variable numbers of epithelial cells surrounding the duct lumen. The wall of epithelial cells lining the duct is modified and is composed of loosely arranged cellulose microfibrils, similar to epithelial cells of resin ducts in *Pinus pinea* L. (Wooding and Northcote, 1965). Further research, particularly at the ultrastructural level, is needed for a better understanding of the role of secretory ducts in *Panax* species.

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