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Needle anatomy changes with increasing tree age in Douglas-fir

MARTHA APPLE,[1–3] KEN TIEKOTTER,[4] MICHAEL SNOW,[4] JAMES YOUNG,[5] AL SOELDNER,[6] DONALD PHILLIPS,[7] DAVID TINGEY[7] and BARBARA J. BOND[1]

[1] *Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA*

[2] *Present address: Department of Environmental and Resource Sciences, University of Nevada, Reno, NV 89557, USA*

[3] *Author to whom correspondence should be addressed (mapple@unr.edu)*

[4] *University of Portland, Portland, OR 97203-5798, USA*

[5] *Environmental Molecular Sciences Laboratory, DOE, Richland, WA 99352, USA*

[6] *Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA*

[7] *U.S. Environmental Protection Agency, Corvallis, OR 97333, USA*

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Summary Morphological differences between old-growth trees and saplings of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) may extend to differences in needle anatomy. We used microscopy with image analysis to compare and quantify anatomical parameters in cross sections of previous-year needles of old-growth Douglas-fir trees and saplings at the Wind River Canopy Crane site in Washington and at three sites in the Cascade Mountains of Oregon. We also compared needle anatomy across a chronosequence of 10-, 20-, 40- and 450-year-old Douglas-fir trees from the Wind River site. Anatomy differed significantly between needles of old-growth trees and saplings at all sites, suggesting a developmental change in needle anatomy with increasing tree age. Compared with needles of old-growth trees, needles of saplings were longer and had proportionately smaller vascular cylinders, larger resin canals and few hypodermal cells. Astrosclereids, which sequester lignin in their secondary cell walls and occupy space otherwise filled by photosynthetic cells, were scarce in needles of saplings but abundant in needles of old-growth trees. Needles of old-growth trees had an average of 11% less photosynthetic mesophyll area than needles of saplings. The percentage of non-photosynthetic area in needles increased significantly with increasing tree age from the chronosequence of 10-, 20-, 40- and 450-year-old trees at the Wind River site. This reduction in photosynthetic area may contribute to decreased growth rates in old trees.

Keywords: astrosclereids, cells, lignin, needles, structure.

Introduction

Old-growth Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) trees in the Pacific Northwest are venerable giants that inhabit ancient forests (Franklin and Waring 1980, Franklin and Spies 1991), often living longer than 500 years and reaching heights of over 75 m (Burns and Honkala 1990). The morphology of old-growth Douglas-fir trees differs dramatically from that of saplings (Franklin and Waring 1980, Franklin and Spies 1991). These morphological differences may extend to anatomical differences between same-aged needles of old-growth trees and saplings (Apple et al. 1999).

Douglas-fir needles are relatively ephemeral and small compared with the longevity and stature of entire trees, but they have the important role of interacting with the atmosphere in the processes of transpiration and photosynthesis. Needle anatomy influences needle physiology (Pachepsky et al. 1995), and leaves within species are known to exhibit structural variations with associated metabolic costs and benefits (Oleksyn et al. 1997, Gutschick 1999). Variations in needle anatomy between old and young Douglas-fir trees may contribute to the reduced photosynthetic rates that have been demonstrated in old trees (Yoder et al. 1994).

Astrosclereids are large, non-living cells in the mesophyll of Douglas-fir needles. Astrosclereids function as structural entities and develop from photosynthetic mesophyll cells that differentiate to form lignified secondary cell walls with stellate extensions (Owens 1968, Esau 1977). Astrosclereids occupy space in the mesophyll that would otherwise be filled by photosynthetic mesophyll cells. The presence of astrosclereids decreases the mesophyll area available for photosynthesis. Little is known about the distribution of astrosclereids with respect to tree age and canopy location, although there is evidence that they occur with high frequency in needles of old-growth Douglas-fir (Apple et al. 1999).

Extensive lignified tissue may reduce the photosynthetic efficiency of needles of old-growth Douglas-fir but may in turn confer greater structural integrity and increase needle longevity. In contrast, rapidly growing saplings, which must maximize photosynthetic efficiency and growth, may not invest in lignified structural tissue, but may replace needles frequently as branches are being shed during establishment of tree architecture (Romberger et al. 1993).

Water that is transpired reaches the atmosphere by way of the needles. Therefore, needles of tall, old-growth trees may have xeromorphic structural features such as an extensive hypodermis and proportionately larger vascular cylinders that could be instrumental in countering the increased water stress associated with greater tree height (Ryan and Yoder 1997, Bauerle et al. 1999).

We used microscopy and image analysis to compare needle anatomy in saplings and old-growth Douglas-fir trees at the Wind River Canopy Crane site in Washington and at three sites in the Cascade Mountains of Oregon. We also compared the anatomy of Douglas-fir needles across a chronosequence of 10-, 20-, 40- and 450-year-old trees at the Wind River Canopy Crane site. The results of these comparisons were used to determine whether anatomical differences exist between needles of young and old trees, and if so, when such differences begin to appear. Such knowledge will be useful in interpretating physiological changes that occur with increasing tree age.

Materials and methods

Plant material and field sites

Previous-year needles were collected in 1998 from old-growth Douglas-fir trees and adjacent saplings at three Oregon Cascade Mountain sites (Toad Creek, 1198 m, 44°42′ N, 122°03′ W, 200 ± 5-year-old trees; Soapgrass, 1190 m, 44°34′ N, 122°29′ W, +450-year-old trees; and Falls Creek, 528 m, 44°39′ N, 122°37′ W, 105 ± 5-year-old trees). Total soil nitrogen content (Kjeldahl NO_3^- and NH_4^+) of the A horizon was similar at Falls Creek (0.47%) and Soapgrass (0.48%) and was substantially less at Toad Creek (0.17%). Needle longevity was determined by counting bud scale scars. We examined 10 mid-crown needles from five saplings and 10 lower-, mid- and upper-crown needles from five old-growth trees at the three Oregon sites.

In 2000, we examined 10 previous-year, upper-canopy needles from each of five trees that were about 10, 20, 40 and 450 years old (50 needles per tree age class) at the time of sampling. Needles were collected from dominant trees at even-age sites in the vicinity of the Wind River Canopy Crane site near Carson, Washington; sites were chosen because they were typical for these age classes at the Wind River Canopy Crane site and because complementary research is being conducted at these locations. The sites have been described in detail by Phillips et al. (2002) and Chen et al. (2002). Briefly, the site supporting the 450-year-old trees (elevation 368 m) originated from a natural disturbance. The tallest trees at this site average 55 to 65 m with a maximum height of 67 m. At the time of sampling, the 10-, 20- and 40-year-old trees averaged 2.5, 16 and 32 m in height, respectively. The stands of 10-, 20- and 40-year-old trees (elevation 500 m) were established by planting after clear-cutting of old-growth stands (Chen et al. 2002, Klopatek 2002). Nutrient availability was highest at the site supporting 40-year-old trees (Klopatek 2002). Needles were collected from canopy tops utilizing existing canopy access.

Light microscopy and image analysis

Fresh unfixed needles were hand-sectioned transversely at mid-needle (Ewers 1982). Sections were observed with polarized light to visualize birefringent lignin or were stained for lignin with 0.1% (w/v) berberine hemisulfate in deionized H_2O for 1 h, rinsed three times in deionized H_2O (Peterson 1994), mounted with Cytoseal and examined by UV fluorescence (480–530 nm). Needles were photographed through a Nikon (Melville, NY) Diaphot microscope with a Kodak (Rochester, NY) DCS 200 digital camera. We used NIH-Image software (http://rsb.info.nih.gov/nih-image) to quantify the area and perimeter of entire cross sections, and of the astrosclereids, resin canals, vascular cylinder, epidermis, hypodermis and mesophyll. Measurements were made from lines drawn around the outer edges of these structures. Needle thickness was measured from the upper to the lower center of the cross section, and width was measured at the widest lateral extension of the mesophyll. Needle roundness was evaluated in cross sections of needles as the ratio of the shortest to the longest axis, with a value of 1.00 representing a circle. The percentage of non-photosynthetic area was calculated by subtracting hypodermal, vascular, resin canal and astrosclereid areas from the cross-sectional area. Tannins in astrosclereids were stained with the nitroso reaction (Jensen 1974). Whole needles were cleaned in bleach, washed twice in H_2O (4 h), placed in 0.025% tannic acid (30 min), washed twice in H_2O (20 min), dehydrated to 100% EtOH, placed in 1:1 EtOH: methyl salicylate (20 min) and in methyl salicylate (12 h), and photographed with a Carl Zeiss (Thornwood, NY) Photomicroscope I under crossed polarization or with the addition of a first-order red compensator plate.

Confocal and scanning electron microscopy

A Leica Microsystems (Wetzlar, Germany) TCS confocal microscope with an argon/krypton laser and a 488-nm FITC filter was used to optically section needles stained with berberine hemisulfate at 5 μm intervals. Air-dried and sputter-coated needles were observed with an AmRay (KLA–Tencor, Bedford, MA) 3300 field emission scanning electron microscope (SEM). Freeze-fractured and carbon-coated needles were probed with a Leo (Thornwood, NY) 982 SEM with energy dispersive X-ray spectroscopy (EDS) to determine the elemental composition of astrosclereids.

Statistical analyses

At the three Oregon Cascade sites, measurements of needle characteristics were compared in three groups: (1) between saplings and old-growth trees, (2) among lower, mid- and upper canopy needles of old-growth trees, and (3) among upper canopy needles from old-growth trees. For the Oregon Cascades data, two-way analyses of variance (ANOVA) were used, with site as the additional factor. At the Wind River sites, measurements were compared among needles from 10-, 20-, 40- and 450-year-old trees with a one-way ANOVA performed with the Statview statistical software package (SAS Institute, Cary, NC). If significant differences within groups were found with the ANOVA *F*-test, Fisher's protected LSD test was used

to identify pairwise differences. Because we examined needle anatomy in the context of tree age, trees were considered to be the experimental units, with individual needles as the subsamples. Because trees within an age class were from a single stand, age effects may be confounded with stand effects (e.g., soils, history). Given the limitations on availability of canopy access, such confounding was unavoidable.

Results

Needle structure and tree age

Needles of saplings and old-growth trees had many significant ($P \leq 0.05$) structural differences (Tables 1 and 2) at the three Oregon Cascade Mountain sites and at the Wind River sites (Table 3). Differences between needles from old-growth trees and saplings were greater than differences among needles from different canopy positions of old-growth trees at the three Oregon Cascade sites, and there were no significant site × age interactions. At the Oregon Cascade sites, needles of old-growth trees were shorter with larger vascular cylinders, smaller resin canals, and a more extensive hypodermis than needles of saplings. Astrosclereids were found in the majority of needles of old-growth trees, but were scarce in needles of saplings. Photosynthetic mesophyll area was 12.1% less in needles of old-growth trees than in needles of saplings (65.3 versus 77.4%). Needles were retained 4–7 years on saplings and 6–11 years on old-growth trees (n = 20 trees per age class).

Needle anatomy also differed significantly among tree age classes at Wind River (Table 2, Figure 1). Differences between needles of 10- and 450-year-old trees were similar to those between saplings and old-growth trees from the Oregon Cascades. Photosynthetic area decreased by 9.6% (78.2 to 68.6%) between 10 and 450 years. The decrease in photosynthetic area was greatest between 40- and 450-year-old trees (4.4%). The percentages of photosynthetic area were similar within age classes at the Oregon Cascades sites in 1998 and at Wind River in 2000 (Tables 1 and 2). At the Oregon Cascades sites, photosynthetic area was 77.4% in saplings and 65.3% in old-growth trees, whereas at Wind River, photosynthetic area was 78.2% in 10-year-old trees and 68.6% in 450-year-old trees.

Needle dimensions changed with tree age. At Wind River, needle length, thickness (height), width, cross-sectional area and perimeter, vascular cylinder area and mesophyll area were maximal in 40-year-old trees. All of these factors except needle height decreased significantly between 40 and 450 years. The needle height/width ratio increased with increasing tree age, whereas needle roundness increased sharply between 10 and 20 years but not between 20 and 450 years. The percentage of vascular cylinder area increased by 2.5% between 10 and 450 years, with an abrupt gain of 2% between 10 and 20 years. Resin canal area and percentage of area decreased with increasing tree age. The increased percentage of hypodermal and epidermal area with increasing tree age may have occurred as a function of changing needle shape. Although the percentage of needles with astrosclereids did not differ significantly between 20 and 450 years, needles of 450-year-old trees had the greatest number of astrosclereids per section and the greatest area and percentage of area occupied by astrosclereids. Astrosclereid perimeter reached 0.812 mm in 450-year-old trees, indicating that astrosclereid surface area is extensive.

Table 1. Comparison of anatomical characteristics of needles from mid-canopy old-growth trees and saplings of Douglas-fir (10 needles/5 trees/3 sites) for all three of the Oregon Cascades sites combined. Values are averaged for each tree at each site and expressed as means (standard error). Lengths are expressed in mm and areas in mm^2. Asterisks denote significant differences ($P \leq 0.05$) between needles of saplings and old-growth trees.

Characteristic	Saplings	Old-growth trees
Needle length	23.983 (1.066)	20.217 (0.575)*
Needle roundness	0.384 (0.009)	0.406 (0.017)
Cross-sectional perimeter	2.954 (0.066)	3.140 (0.099)
Cross-sectional area	0.490 (0.026)	0.550 (0.038)
Mesophyll area	0.430 (0.024)	0.443 (0.030)
Vascular cylinder area	0.037 (0.006)	0.052 (0.005)*
Resin canal area	0.013 (0.003)	0.003 (0.000)*
Hypodermal cells/section	16.417 (4.073)	106.600 (10.543)*
Hypodermal–epidermal area	0.059 (0.002)	0.106 (0.007)*
Astrosclereid area	0.001 (0.001)	0.040 (0.017)*
Astrosclereid perimeter	0.072 (0.059)	1.294 (0.231)*
Astrosclereids/section	0.067 (0.051)	1.600 (0.272)*
Astrosclereid presence (%)	12.0 (7.0)	77.3 (10.5)*
Percent cross-sectional area occupied by:		
Vascular cylinder	7.1 (0.9)	9.4 (0.3)*
Resin canal	2.9 (0.9)	0.6 (0.1)*
Astrosclereid	0.2 (0.2)	6.5 (2.4)*
Hypodermis–epidermis	12.3 (0.4)	19.2 (0.3)*
Non-photosynthetic cells	22.6 (0.5)	34.7 (2.4)*
Photosynthetic mesophyll	77.4 (0.5)	65.3 (2.4)*

Astrosclereid composition

In cleared, whole needles of old-growth trees, astrosclereids were evenly distributed along the length of the mesophyll. Scanning electron and confocal microscopy revealed that stellate branches of astrosclereids extended into intercellular space and to points near the vascular cylinders and resin canals without attaining contact (Figures 2 and 3). Cell walls of astrosclereids fused with those of adjacent mesophyll cells. The astrosclereid lumen was connected to mesophyll cells or to intercellular space via channels in the cell walls and contained granular masses that stained red for tannin.

Needle anatomy and canopy level

There were no site × canopy-level interactions for needles from old-growth trees at the Oregon Cascade sites. The sole significant difference in needle anatomy with canopy level in old-growth trees was that upper canopy needles showed greater roundness than lower canopy needles. Lower-canopy needles of old-growth trees were similar in shape to needles of saplings.

Table 2. Comparisons of anatomical parameters in upper canopy needles from 10-, 20-, 40- and 450-year-old Douglas-fir trees at the Wind River Canopy Crane site (10 needles/5 trees/4 age classes). Values are means with standard error in parenthesis for each tree at each site. Needle length, width, thickness and perimeter are in mm; areas are in mm^2. Letters denote *P*-values ≤ 0.05 in pairwise comparisons as follows: a = 10,20; b = 10,40; c = 10,450; d = 20,40; e = 20,450; and f = 40,450.

Characteristic	Tree age (years)			
	10	20	40	450
Needle length	21.875 (2.282)	23.340 (1.056)	25.400 (1.616) f	18.880 (0.668) f
Needle thickness	0.430 (0.019) abc	0.562 (0.017) a	0.573 (0.030) b	0.516 (0.016) c
Needle width	1.282 (0.057) c	1.265 (0.016) e	1.297 (0.045)	1.095 (0.038) cef
Needle thickness/width	0.341 (0.021) abc	0.435 (0.012) a	0.446 (0.027) b	0.476 (0.022) c
Needle roundness	0.683 (0.018) abc	0.840 (0.019) ad	0.792 (0.014) bd	0.836 (0.006)
Cross-sectional perimeter	3.060 (0.135)	3.069 (0.045)	3.210 (0.115)	2.810 (0.756)
Cross-sectional area	0.493 (0.039)	0.565 (0.018) e	0.598 (0.046) f	0.446 (0.031) ef
Mesophyll area	0.388 (0.039)	0.428 (0.015) e	0.455 (0.045) f	0.319 (0.024) ef
Vascular cylinder area	0.035 (0.003) ab	0.052 (0.003) a	0.059 (0.006) bf	0.043 (0.004) f
Resin canal area	0.007 (0.002) abc	0.003 (0.002) ae	0.003 (0.001) b	0.001 (0.001) ce
Hypodermal cells/section	42.177 (6.103) abc	112.920 (7.871) a	133.497 (7.929) b	123.812 (6.499) c
Hypodermal–epidermal area	0.068 (0.003) abc	0.082 (0.003) a	0.093 (0.006) b	0.084 (0.003) c
Astrosclereid area	0.006 (0.003) ac	0.006 (0.001) ae	0.004 (0.002) f	0.011 (0.002) cef
Astrosclereid perimeter	0.082 (0.041) ac	0.461 (0.108) ae	0.328 (0.141) f	0.812 (0.128) cef
Astroscleroids/section	0.200 (0.105) ac	0.900 (0.152) ae	0.580 (0.211) f	1.940 (0.266) cef
Astrosclereid presence (%)	22.0 (10.2) ac	80.0 (8.4) ad	48.0 (13.9) df	96.0 (4.0) cf
Percent cross-sectional area occupied by:				
Vascular cylinder	7.1 (0.1) abc	9.1 (0.5) a	9.8 (0.4) b	9.6 (0.4) c
Resin canals	1.4 (0.3) abc	0.6 (0.02) a	0.5 (0.1) b	0.2 (0.02) c
Astroscleroids	0.1 (0.1) c	0.8 (0.3) e	0.8 (0.4) f	2.5 (0.3) cef
Hypodermis–epidermis	13.2 (0.5) bc	14.5 (0.4) e	15.7 (1.0) bf	19.0 (0.8) cef
Non-photosynthetic cells	21.8 (0.5) abc	25.3 (0.7) ae	27.0 (1.3) bf	31.4 (1.2) cef
Photosynthetic mesophyll	78.2 (0.5) abc	74.7 (0.7) ae	73.0 (1.3) bf	68.6 (1.2) cef

Table 3. Comparisons of site versus mid-canopy needle parameters in old-growth Douglas-fir trees. The comparison is based on a sample of 10 needles/5 trees/3 sites. Values are means with standard error in parenthesis for each tree at each site. Lengths are expressed in mm and areas in mm^2. Letters denote *P*-values ≤ 0.05 in pairwise comparisons as follows: a = Falls Creek, Soapgrass; b = Falls Creek, Toad Creek; and c = Soapgrass, Toad Creek.

Characteristic	Site		
	Falls Creek	Soapgrass	Toad Creek
Needle length	19.540 (0.778)	18.660 (2.049)	20.620 (1.111)
Needle roundness	0.392 (0.026)	0.394 (0.039)	0.462 (0.014)
Cross-sectional perimeter	2.956 (0.087)	2.730 (0.324) c	3.558 (0.233) c
Cross-sectional area	0.482 (0.016) b	0.489 (0.079) c	0.668 (0.045) bc
Mesophyll area	0.380 (0.020) ab	0.392 (0.049) ac	0.534 (0.030) bc
Vascular cylinder area	0.043 (0.003) b	0.047 (0.007) c	0.072 (0.007) bc
Resin canal area	0.004 (0.001)	0.003 (0.001)	0.003 (0.001)
Hypodermal cells/section	84.853 (6.848) b	86.253 (14.211) c	125.080 (12.691) bc
Hypodermal–epidermal area	0.086 (0.003) b	0.102 (0.008) c	0.131 (0.013) bc
Astrosclereid area	0.021 (0.009)	0.010 (0.005) c	0.044 (0.012) c
Astrosclereid perimeter	1.200 (0.361) a	0.645 (0.301) ac	1.753 (0.223) c
Astroscleroids/section	1.425 (0.586)	0.483 (0.295) c	1.950 (0.157) c
Astrosclereid presence (%)	70.8 (16.2) a	38.3 (23.2) ac	100.0 (0.0) c
Percent cross-sectional area occupied by:			
Vascular cylinder	9.2 (0.3)	9.1 (0.8)	11.1 (0.8)
Resin canal	1.3 (0.7)	0.5 (0.1)	0.4 (0.1)
Astrosclereid	4.3 (1.7)	2.0 (0.8)	5.8 (1.6)
Hypodermis–epidermis	18.2 (0.5)	17.7 (1.5)	18.9 (0.7)
Non-photosynthetic cells	32.9 (2.4)	29.3 (2.3)	34.5 (2.4)
Photosynthetic mesophyll	67.1 (2.4)	64.0 (4.5)	65.5 (2.4)

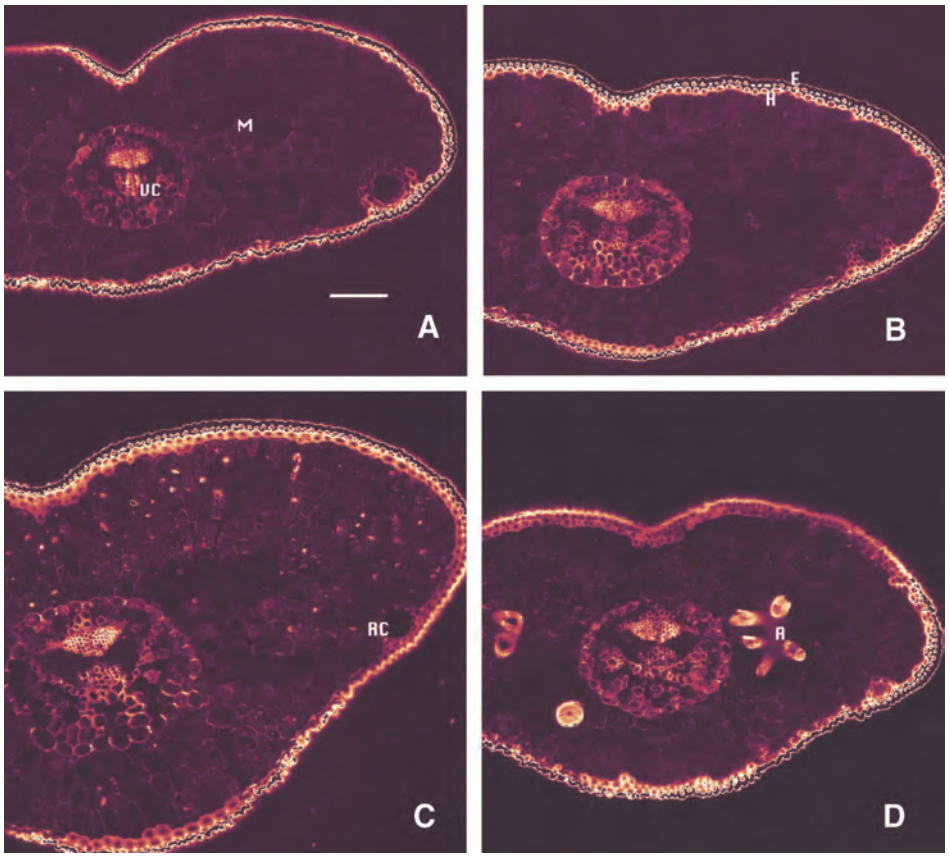

Figure 1. Confocal images of cross sections of previous-year, upper canopy needles of Douglas-fir from a chronosequence at Wind River. Ten-year-old (A), 20-year-old (B), 40-year-old (C) and 450-year-old (D) trees. Abbreviations: A = astrosclereids; E = epidermis; H = hypodermis; M = mesophyll; RC = resin canal; and VC = vascular cylinder. Scale bar = 100 µm.

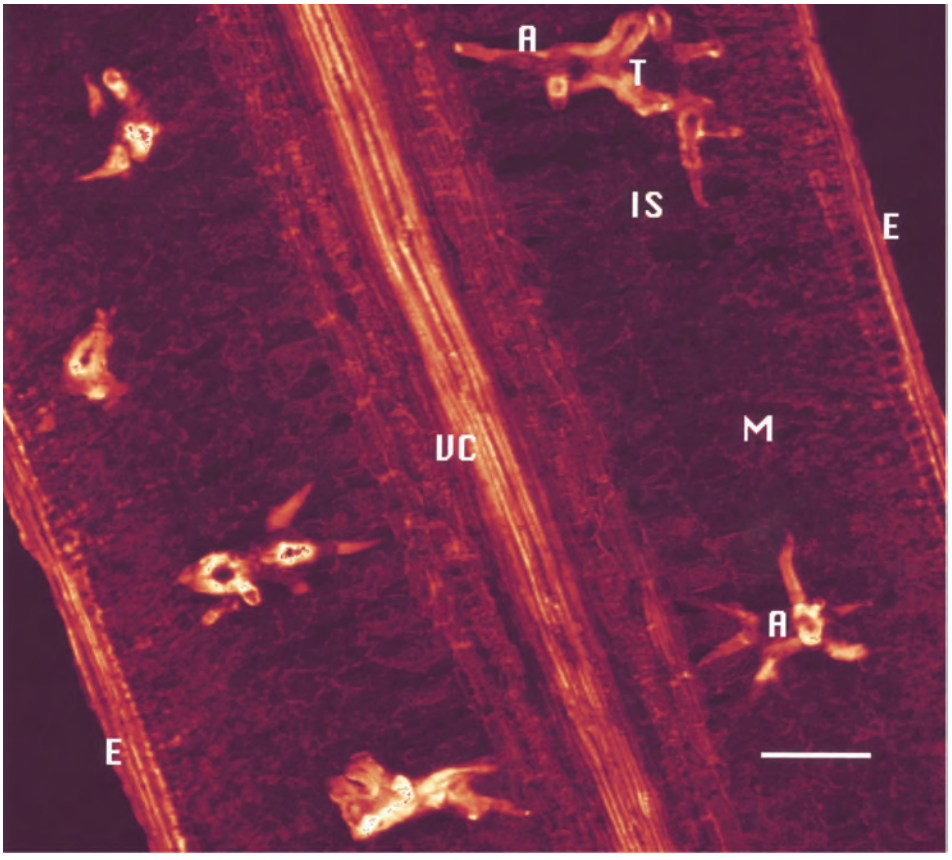

Figure 2. Confocal image of astrosclereids in the mesophyll of a longitudinally sectioned upper canopy needle from an old-growth Douglas-fir tree. Abbreviations: A = astrosclereid; E = epidermis; IS = intercellular space; M = mesophyll; T = tannin; and VC = vascular cylinder. Scale bar = 100 µm.

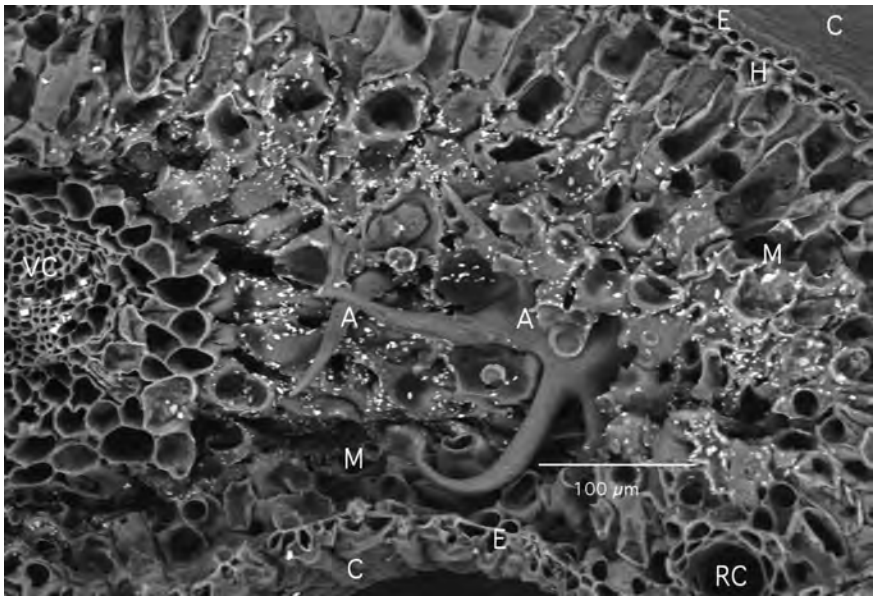

Figure 3. Scanning electron micrograph of a freeze-fractured and carbon coated upper canopy needle of a 450-year-old Douglas-fir tree. Abbreviations: A = astrosclereid; C = cuticle; E = epidermis; H = hypodermis; M = mesophyll; RC = resin canal; and VC = vascular cylinder. Scale bar = 100 µm.

Needle anatomy and site

Needle anatomy of old-growth trees, but not of saplings, varied significantly among the Oregon Cascade sites (Table 3). Among sites, needles on trees at Toad Creek had the highest number of astrosclereids per section, the greatest cross-sectional area and perimeter, mesophyll and vascular cylinder areas, the greatest number of hypodermal cells, and the highest percentage of cross-sectional area composed of the vascular cylinder. The number of astrosclereids per section decreased with site as follows: Toad Creek > Falls Creek > Soapgrass.

Discussion

Needle anatomy changed with tree age at all sites. Differences between needles of saplings and old-growth trees at the Oregon Cascades sites in 1998 were similar to those found between needles of 10- and 450-year-old trees at Wind River in 2000. Anatomical differences between needles of saplings and old-growth trees were more pronounced than differences occurring with canopy level in old-growth trees. This suggests a developmental change in needle anatomy with increasing tree age and indicates that the influence of tree age on needle anatomy is greater than that of canopy position or, in the Oregon Cascades, site differences.

Needles of Douglas-fir saplings had a significantly greater percentage of photosynthetic mesophyll tissue than needles of old-growth trees. The proportionately smaller vascular cylinders and low numbers of hypodermal cells and astrosclereids, as well as the change in needle shape, contributed to the larger photosynthetic area of needles of saplings compared with needles of old-growth trees. In contrast, the shorter needles of old-growth trees have proportionately larger vascular cylinders, many hypodermal cells, abundant astrosclereids, and thus a smaller percentage of photosynthetic area per needle cross section. The decrease in photosynthetic area (12.1% between saplings and old-growth trees at the Oregon Cascades sites, 9.6% between 10- and 450-year-old trees at Wind River) could affect leaf gas exchange, although direct measurements of gas exchange at these sites have not revealed significant differences between 20-year-old and 450-year-old trees (N.G. McDowell, Oregon State University, Corvallis, OR, unpublished results). These differences in photosynthetic mesophyll area suggest that saplings and old-growth trees may have contrasting ecological strategies (Lambers et al. 1998, Van Volkenburgh 1999). Old-growth trees have a high structural investment in lignified hypodermal cells and in astrosclereids, which may function as protectants against damage by abiotic and biotic factors. Old-growth trees benefit from the longer retention of these metabolically expensive needles at the cost of a reduction in growth. In contrast, saplings retain their needles for shorter periods, which reflects the capacity for rapid growth in young trees during the stand establishment phase.

In the chronosequence of 10-, 20-, 40- and 450-year-old trees at Wind River, needles from the 20- and 40-year-old trees had the greatest length, thickness (height), cross-sectional area, mesophyll area and vascular cylinder area. These dimensions, with the exception of needle thickness, decreased significantly in trees between the ages of 40 and 450. Although we did not examine needles of trees between the ages of 40 and 450 at Wind River, we did examine needles of 105-, 200- and 450-year-old trees in the Oregon Cascades at Falls Creek, Toad Creek and Soapgrass, respectively. Needles at Falls Creek were similar to those at Soapgrass, suggesting that needle development shifts toward production of anatomical characteristics associated with needles of old-growth trees between the ages of 40 and 100 years, and needle dimensions may increase with tree age until that point.

However, each point in a chronosequence reflects conditions at the time of origin and the influence of subsequent events. Conditions were different between the natural disturbances (usually stand-replacing fires) that led to establishment

of the 450-year-old trees and the management practice of clear-cutting and planting that resulted in the 10-, 20- and 40-year-old stands. Thus, there were different conditions at the origin as well as in age between young and old trees. Therefore, the passage of time is not necessarily the only variable in a chronosequence (Yanai et al. 2000, Bond and Franklin 2002).

Nevertheless, a major difference between needles of old-growth trees and saplings was the frequent presence of astrosclereids in the former and their rarity in the latter. Astrosclereids fill space that would otherwise be occupied by photosynthetic mesophyll cells, thus reducing the amount of photosynthetic tissue available for carbon fixation. Stellate extensions of astrosclereids occupy intercellular space and block mesophyll cell surface area that would otherwise be available for gas exchange and carbon uptake. Therefore, astrosclereids are likely to influence transpiration, photosynthesis and growth. The increase in astrosclereid number per needle cross section with tree age suggests that astrosclereids may have a greater influence on these physiological processes in older trees than in younger trees. Moreover, because astrosclereids develop from what would otherwise have become photosynthetic mesophyll cells, these old trees are converting more of their potentially photosynthetic cells into structural cells, which should influence photosynthesis. In pine, $CaSO_4$ formations apparently block stomatal apertures and alter the diffusive pathway, thereby contributing to increased xeromorphy (Pritchard et al. 2000). This suggests that astrosclereids impart a xeromorphic character to needles, because they are likely to alter the diffusive pathway in Douglas-fir. The presence of an extensive hypodermis, astrosclereids, proportionately larger vascular cylinders and reduced mesophyll area all suggest that needles of old-growth Douglas-fir trees are more xeromorphic than needles of saplings.

Water transport efficiency may be increased by the proportionately larger vascular cylinders in needles of old-growth trees compared with saplings. In the Wind River chronosequence, vascular cylinders were smallest and occupied the smallest percentage of cross-sectional area in 10-year-old trees. This percentage increased sharply (2.0%) between 10 and 20 years, by 0.7% between 20 and 40 years, and then decreased slightly (0.2%) between 40 and 450 years. Therefore, vascular cylinder area may increase in proportion to mesophyll area during the early, rapid phases of tree growth but remain constant in established trees.

Astrosclereid and hypodermal secondary cell walls are lignified. Lignin provides structural support (Esau 1977) and is an insoluble polymer of phenylpropane residues (Goodwin and Mercer 1983). Lignin is more energetically expensive to synthesize than cellulose (1.9 g glucose per 1.0 g lignin versus 1.2 g glucose per 1.0 g cellulose) (Chung and Barnes 1977). Construction costs of astrosclereids may be offset by increased structural integrity in needles of old trees. Carbon in lignin is sequestered until needles decompose. If heavily lignified old-growth needles require more time to decompose in the litter layer than needles of saplings, this may influence the rate of nutrient cycling in Douglas-fir forests.

Oleksyn et al. (1997) state that increases in structural tissue and chemical defense are associated with higher structural costs that may reduce photosynthetic activity in older needles. However, the needles from our old-growth trees were the same age as the corresponding needles from saplings, suggesting that the changes in needle anatomy were a result of tree age. Therefore, the whole tree, and not just the older needles, may incur a reduction in photosynthetic activity as the tree ages. This does not, however, preclude the possibility that older needles of old-growth trees have a lower photosynthetic capacity than younger needles of old-growth trees.

Differences between needles of old-growth trees and saplings were consistent at all sites, but differences were found among needles of old trees from the three Oregon Cascade sites. More astrosclereids per needle cross section were found in trees at Toad Creek, which had a lower soil mineralization rate and nitrogen content than at Soapgrass. When carbon availability is high but nitrogen is limited, as is the case with trees growing on volcanic soils of the Cascade Mountains (Perry 1994), more carbon is metabolized by the shikimic acid pathway to produce secondary metabolites such as lignin and tannin (Gershenzon 1984). Mesophyll cells may be more likely to differentiate into astrosclereids when nitrogen is limited. Therefore, it appears that needle development is influenced by nitrogen availability as well as by tree age. However, as growth rates decrease in old trees (Yoder et al. 1994) and the trees become less active as carbon sinks, tissue carbon may increase in needles. Such retention of carbon may trigger differentiation of mesophyll cells into astrosclereids. Slow growth, therefore, may be the cause of changes in needle anatomy.

Chemical defense strategies may differ with tree age. Tannin synthesized in mesophyll cells may reach the astrosclereid lumen through channels in the astrosclereid wall. Tannin and lignin are both phenolic compounds and may therefore both contribute to chemical defense, which may in turn be related to the longer lives of needles of old-growth trees (Oleksyn et al. 1997). However, the isolation of antimicrobial tannin (Bennett and Wallsgrove 1994) in the astrosclereid lumen may limit its function as a chemical defense agent. Alternatively, the synthesis and accumulation of tannin in mesophyll cells may serve as a signal for differentiation of mesophyll cells into astrosclereids. Tannins may function in chemical defense before differentiation. In contrast, needles of saplings may rely on phenolic monoterpenes carried in larger resin canals (Langenheim 1994). Seasonality of monoterpene production varies with phenology and nitrogen availability in Douglas-fir (Lerdau et al. 1995). Monoterpenes act against vertebrate herbivores (Bryant et al. 1991, Snyder 1992), and in conifers their presence may reduce herbivory by terrestrial vertebrates such as deer (Iason et al. 1996).

Needle anatomy influences needle physiology (Pachepsky et al. 1995). Changes in needle anatomy such as increased astrosclereid abundance with increasing tree age are therefore likely to influence physiological processes such as photosynthesis, growth and carbon allocation in Douglas-fir. Characterization of needle anatomy of Douglas-fir as well as other trees, combined with measurements of needle and whole-crown physiology along chronosequences, may prove useful in

predicting physiological and morphological change.

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